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Study on invasive alien species Development of risk assessments to tackle priority species and enhance prevention: final report (and annexes)

Wolfgang Rabitsch, Helmut Kudrnovsky, Helen Roy, Björn Beckmann, Jodey Peyton, Beth Purse, Siobhan Edney, Riccardo Scalera, Marc Kenis, Lukas Seehausen, et al.

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Wolfgang Rabitsch, Helmut Kudrnovsky, Helen Roy, Björn Beckmann, Jodey Peyton, et al.. Study on invasive alien species Development of risk assessments to tackle priority species and enhance prevention: final report (and annexes). [Technical Report] European Commission. 2022. hal-03579685

HAL Id: hal-03579685

<https://hal.science/hal-03579685>

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Study on invasive alien species

**Development of risk assessments
to tackle priority species and enhance prevention**

Final Report
(and annexes)

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PDF ISBN 978-92-76-46920-9 doi:10.2779/302048 KH-09-22-032-EN-N

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Study on invasive alien species

Development of risk assessments
to tackle priority species and enhance prevention

Contract No
No 07.0202/2020/834529/ETU/ENV.D.2

(1st renewal of Study Contract No
07.0202/2019/812602/ETU/ENV.D.2)

Final Report
(and annexes)

EUROPEAN COMMISSION

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**STUDY ON INVASIVE ALIEN SPECIES
DEVELOPMENT OF RISK ASSESSMENTS TO TACKLE PRIORITY
SPECIES AND ENHANCE PREVENTION**

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There are 28 annexes to this report:

- Eight new Risk Assessments (Annex 1a-8a)
- Eight new documents on measures and their implementation cost and cost-effectiveness for these eight species (Annex 1b-8b)
- Twelve revisions of existing Risk Assessments (Annex 9-20).

Annex 1a: Risk assessment for *Bipalium kewense* (Kew Flatworm)

Annex 1b: Evidence on measures and their implementation cost and cost-effectiveness for *Bipalium kewense* (Kew Flatworm)

Annex 2a: Risk assessment for *Cervus nippon* (Sika Deer)

Annex 2b: Evidence on measures and their implementation cost and cost-effectiveness for *Cervus nippon* (Sika Deer)

Annex 3a: Risk assessment for *Cherax destructor* (Common Yabby)

Annex 3b: Evidence on measures and their implementation cost and cost-effectiveness for *Cherax destructor* (Common Yabby)

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Annex 6a: Risk assessment for *Mulinia lateralis* (Dwarf Surf Clam)

Annex 6b: Evidence on measures and their implementation cost and cost-effectiveness for *Mulinia lateralis* (Dwarf Surf Clam)

Annex 7a: Risk assessment for *Obama nungara* (Obama Flatworm)

Annex 7b: Evidence on measures and their implementation cost and cost-effectiveness for *Obama nungara* (Obama Flatworm)

Annex 8a: Risk assessment for *Pycnonotus jocosus* (Red-whiskered Bulbul)

Annex 8b: Evidence on measures and their implementation cost and cost-effectiveness for *Pycnonotus jocosus* (Red-whiskered Bulbul)

Revised Risk Assessments on

Annex 9: *Asterias amurensis* (North Pacific Sea Star)

Annex 10: *Axis axis* (Axis Deer)

Annex 11: *Broussonetia papyrifera* (Paper Mulberry)

Annex 12: *Callinectes sapidus* (Atlantic Blue Crab)

Annex 13: *Castor canadensis* (North American Beaver)

Annex 14: *Celastrus orbiculatus* (Staff-vine)

Annex 15: *Cortaderia selloana* (Pampas Grass)

Annex 16: *Faxonius immunis* (Calico Crayfish)

Annex 17: *Misgurnus anguillicaudatus* (Oriental Weatherfish)

Annex 18: *Pycnonotus cafer* (Red-vented Bulbul)

Annex 19: *Vespa mandarinia* (Asian Giant Hornet)

Annex 20: *Xenopus laevis* (African Clawed Frog)

Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention

Contract No 07.0202/2020/834529/ETU/ENV.D2

Period: 14 October 2020 – 14 October 2021

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Abstract

Providing evidence-based and scientifically robust risk assessments, as foreseen under Article 5 of the Regulation (EU) 1143/2014 on the prevention and management of the introduction and spread of invasive alien species (IAS), is one step in the process of developing the list of IAS of Union concern. The aim of the current study is to develop such risk assessments alongside evidence on management measures and their implementation cost and cost-effectiveness to help inform policies and prioritise actions.

This study includes eight new risk assessments and associated management annexes, for the following species: *Bipalium kewense* (Kew Flatworm), *Cervus nippon* (Sika Deer), *Cherax destructor* (Common Yabby), *Delairea odorata* (Cape Ivy), *Marisa cornuarietis* (Colombian Ramshorn Apple Snail), *Mulinia lateralis* (Dwarf Surf Clam), *Obama nungara* (Obama Flatworm), and *Pycnonotus jocosus* (Red-whiskered Bulbul).

This study further includes ten risk assessments from previous contracts, revised according to the comments from the Scientific Forum and other stakeholders, concerning the following species: *Asterias amurensis* (North Pacific Seastar), *Axis axis* (Axis Deer), *Broussonetia papyrifera* (Paper Mulberry), *Callinectes sapidus* (Atlantic Blue Crab), *Cortaderia selloana* (Pampas Grass), *Faxonius immunis* (Calico Crayfish), *Misgurnus anguillicaudatus* (Oriental Weatherfish), *Pycnonotus cafer* (Red-vented Bulbul), *Vespa mandarinia* (Asian Giant Hornet), and *Xenopus laevis* (African Clawed Frog). In addition, two risk assessments submitted by a Member State were revised in this contract: *Castor canadensis* (North American Beaver) and *Celastrus orbiculatus* (Staff-vine).

Résumé

Comme le prévoit l'article 5 du règlement (UE) n° 1143/2014 sur la prévention et la gestion de l'introduction et de la propagation des espèces exotiques envahissantes (EEE), la production d'évaluations de risques sur base d'éléments probants et solides scientifiquement est une étape dans le processus d'élaboration de la liste des EEE préoccupantes pour l'Union. L'objectif de la présente étude est d'élaborer de telles évaluations de risques, ainsi que des informations probantes sur les mesures de gestion, leur coût de mise en œuvre et le rapport coût-efficacité pour informer les politiques et définir les priorités d'action.

Cette étude comprend huit nouvelles évaluations des risques et les annexes de gestion associées, pour les espèces suivantes: *Bipalium kewense* (Vers plat à tête de marteau), *Cervus nippon* (Cerf sika), *Cherax destructor* (Ecrevisse de Murray), *Delairea odorata* (Lierre d'Allemagne), *Marisa cornuarietis* (Marise corne-de-bélier), *Mulinia lateralis* (Palourde naine), *Obama nungara* (Vers plat Obama), et *Pycnonotus jocosus* (Bulbul orphée).

Cette étude comprend également dix évaluations de risques issues des contrats précédents et révisées sur base des commentaires du Forum scientifique et des autres parties prenantes. Elles portent sur les espèces suivantes: *Asterias amurensis* (Etoile de mer du Pacifique Nord), *Axis axis* (Cerf axis), *Broussonetia papyrifera* (Mûrier à papier), *Callinectes sapidus* (Crabe bleu de l'Atlantique), *Cortaderia selloana* (Herbe de la Pampa), *Faxonius immunis* (Ecrevisse calicot), *Misgurnus anguillicaudatus* (Loche asiatique), *Pycnonotus cafer* (Bulbul à ventre rouge), *Vespa mandarinia* (Frelon géant), et *Xenopus laevis* (Xénope lisse). En outre, deux évaluations des risques proposées par un Etat membre ont été révisées dans ce contrat: *Castor canadensis* (Castor nord américain) et *Celastrus orbiculatus* (Bourreau des arbres).

Contributors

Project team, affiliations and tasks

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Additional experts, affiliations and tasks

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Executive Summary

Disclaimer: “*The information and views set out in this study are those of the authors and do not necessarily reflect the official opinion of the Commission. The Commission does not guarantee the accuracy of the data included in this study. Neither the Commission nor any person acting on the Commission’s behalf may be held responsible for the use which may be made of the information contained therein.*”

On 1 January 2015 the Regulation (EU) 1143/2014 on the prevention and management of the introduction and spread of invasive alien species (IAS) entered into force. The center piece of the Regulation is a list of IAS of Union concern (the Union list), for which different measures must be taken, depending on presence/absence/distribution in the EU Member States. Providing evidence-based and scientifically robust risk assessments, as foreseen under Article 5 of the Regulation, is one step in the process of developing the Union list. The aim of the current project is to provide such risk assessments and further information that then can inform whether the IAS should be considered for inclusion on the list of IAS of Union concern.

This Final Report is part of the fulfilment of the contract “Study on invasive alien species – Development of risk assessments to tackle priority species and enhance prevention” 07.0202/2020/834529/ETU/ENV.D.2 (1st renewal of contract 07.0202/2019/812602/ETU/ENV.D.2). It includes all deliverables and further information on the background and processes within the project tasks completed since the Kick-off Meeting on the 12th November 2020, which was held online (Zoom). Specifically, it includes eight new Risk Assessments and Risk Management Notes along with a documentation of the peer-review process before, during and after the Quality Assurance Workshop held online on the 15th and 17th September 2021, as well as ten revised Risk Assessments from a previous contract of the wider project team and two revised Risk Assessments from a third party. The results of the study are presented according to the five tasks defined in the contract:

Task 1: Revision of existing Risk Assessments

Task 2: Selection of new species to be assessed

Task 3: Preparation of new Risk Assessments

Task 4: Preparation of new Risk Management Notes (Evidence on measures and their implementation cost and cost-effectiveness)

Task 5: Quality assurance workshop

A few minor corrections have been made in the templates for the Risk Assessments and the Risk Management Notes from the version used in the first year of the contract 07.0202/2019/812602/ETU/ENV.D2, based on discussions within the project team and the Commission. Modifications include *inter alia* the correction of typographical errors, an update of one hyperlink that was not functional anymore, clarifications regarding the risk assessment area after Brexit, the abstinence of providing a response and confidence score in case of “no information has been found” and that a higher potential future impact score due to climate change needs an explicit justification in the provided comment. More specifically:

1) Whenever the instructions state that In the case of lack of information the assessors are requested to use a standardized answer: “No information has been found.” it is suggested to add the following clarification: In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable).

2) In Qu. 4.3, it is suggested to add the following clarification to the instructions: A potential increase in the distribution range due to climate change does not per se justify a higher impact score. A reference to this is also added to Qu. 4.5 and 4.20.

The current versions of both documents are provided as supplements at the end of this report.

Task 1: The Commission provided twelve Risk Assessments for revision that have been developed under previous contracts by the wider project team or by a Member State. These revisions consider comments from the Scientific Forum (representatives of Member States) and stakeholders and generated updated versions of the documents, which are included here as Annexes 9-20 to this Final Report.

The twelve species are:

#	Group	Scientific Name	Common Name
1	Marine	<i>Asterias amurensis</i>	North Pacific Sea Star
2	Vertebrates	<i>Axis axis</i>	Axis Deer
3	Plants	<i>Broussonetia papyrifera</i>	Paper Mulberry
4	Marine	<i>Callinectes sapidus</i>	Atlantic Blue Crab
5	Vertebrates	* <i>Castor canadensis</i>	North American beaver
6	Plants	* <i>Celastrus orbiculatus</i>	Staff-vine
7	Plants	<i>Cortaderia selloana</i>	Pampas Grass
8	Freshwater	<i>Faxonius immunis</i>	Calico Crayfish
9	Freshwater	<i>Misgurnus anguillicaudatus</i>	Oriental Weatherfish
10	Vertebrates	<i>Pycnonotus cafer</i>	Red-vented Bulbul
11	Invertebrates	<i>Vespa mandarinia</i>	Asian Giant Hornet
12	Vertebrates	<i>Xenopus laevis</i>	African Clawed Frog

*Risk Assessment submitted by a Member State

Task 2: The objective of this task was to agree on a selection of priority species for subsequent Risk Assessments in task 3 and development of Risk Management Notes in task 4. The project team was divided into five expert thematic groups: Freshwater animals, Marine species, Plants (including freshwater), Terrestrial invertebrates and Vertebrates. Proposals from the team, and the Commission, were subsequently discussed before and during the online Kick-off Meeting on the 12th November 2020. Emphasis was placed on IAS that are not yet present in the European Union (or have a limited distribution) and have the potential to have an adverse impact on biodiversity. From this consultation process, the following eight species were selected:

#	Group	Scientific Name	Common Name
1	Invertebrates	<i>Bipalium kewense</i>	Kew Flatworm
2	Vertebrates	<i>Cervus nippon</i>	Sika Deer
3	Freshwater	<i>Cherax destructor</i>	Common Yabby
4	Plants	<i>Delairea odorata</i>	Cape Ivy
5	Freshwater	<i>Marisa cornuarietis</i>	Colombian Ramshorn Apple Snail
6	Marine	<i>Mulinia lateralis</i>	Dwarf Surf Clam
7	Invertebrates	<i>Obama nungara</i>	Obama Flatworm
8	Vertebrates	<i>Pycnonotus jocosus</i>	Red-whiskered Bulbul

Task 3: The Risk Assessments were developed by experts from the project team, along with additional experts acting as lead authors, co-authors and/or peer-reviewers. For all eight species dedicated new species distribution models were developed to provide a more robust knowledge base (acknowledging the caveats of such models) for the relevant questions in the Risk Assessments. All Risk Assessments were subject to an internal quality control review, an external peer-review by at least two independent experts according to the requirements of COM Delegated Regulation (EU) 2018/968 and a Quality Assurance Workshop (task 5). The final Risk Assessments of the eight species are included as Annex 1a-8a to this Final Report.

Task 4: The Risk Management Notes were developed in parallel with the risk assessments by experts from the project team, along with additional experts as lead authors, co-authors and/or peer-reviewers for the eight species. All Risk Management

Notes, as for the Risk Assessments, were subject to an internal quality control review, an external peer-review by at least two independent experts and were discussed at the Quality Assurance Workshop (task 5). The final Risk Management Notes of the eight species are included as Annex 1b-8b to this Final Report.

Task 5: Due to the on-going COVID-19 situation the Quality Assurance Workshop was organized as an online meeting on 15th & 17th September 2021. The aim of the workshop was to provide authors, peer-reviewers and the Commission the opportunity to discuss any unclarified issues, eliminate remaining uncertainties as far as possible, and assure the highest possible quality of the Risk Assessments and Risk Management Notes. In preparation for the workshop, authors have considered and incorporated comments from the peer-reviewers before the workshop. At the workshop, authors presented their work in plenary sessions, while open questions were discussed in virtual breakout groups. The comments from the peer-reviewers and the considerations from the authors are included in this Final Report. After the workshop, authors finalized the Risk Assessments and Risk Management Notes for this Final Report.

This Final Report outlines the work achieved for each task in detail. It is accompanied by 28 Annexes: eight new Risk Assessments (Annex 1a-8a), eight new Risk Management Notes (Annex 1b-8b), and twelve revised Risk Assessments (Annex 9-20).

Résumé exécutif

Avertissement: "Les informations et les opinions présentées dans cette étude sont celles des auteurs et ne reflètent pas nécessairement l'opinion officielle de la Commission. La Commission ne garantit pas l'exactitude des données contenues dans cette étude. Ni la Commission ni aucune personne agissant au nom de la Commission ne peuvent être tenues responsables de l'utilisation qui pourrait être faite des informations contenues dans cette étude. "

Le 1er janvier 2015, le règlement (UE) 1143/2014 sur la prévention et la gestion de l'introduction et de la propagation des espèces exotiques envahissantes (EEE) est entré en vigueur. La pièce maîtresse du règlement est une liste d'EEE qui préoccupent l'Union (la liste de l'Union), pour lesquelles différentes mesures doivent être prises, en fonction de leur présence/absence/distribution dans les États membres de l'UE. Comme prévu à l'article 5 du règlement, la production d'évaluations de risques sur base d'éléments probants et solides scientifiquement est une étape du processus d'élaboration de la liste de l'Union. L'objectif du projet actuel est de fournir de telles évaluations des risques et d'autres informations qui pourront ensuite indiquer si on doit envisager d'inclure ces EEE dans la liste des EEE de préoccupation européenne.

Ce rapport final s'inscrit dans le cadre de l'exécution du contrat " Étude sur les espèces exotiques envahissantes - Développement d'évaluations des risques pour lutter contre les espèces prioritaires et renforcer la prévention " 07.0202/2020/834529/ETU/ENV.D.2 (1er renouvellement du contrat 07.0202/2019/812602/ETU/ ENV.D.2). Il comprend tous les livrables et des informations supplémentaires sur le contexte et les processus dans le cadre des tâches du projet réalisées depuis la réunion de lancement du 12 novembre 2020, qui s'est tenue en ligne (Zoom). Plus précisément, il comprend huit nouvelles évaluations des risques et notes de gestion des risques ainsi qu'une documentation sur le processus d'examen par les pairs avant, pendant et après le workshop portant sur l'assurance qualité qui s'est tenu en ligne les 15 et 17 septembre 2021, ainsi que dix évaluations des risques révisées provenant d'un contrat précédent de l'équipe de projet élargie et deux évaluations des risques révisées provenant d'un tiers. Les résultats de l'étude sont présentés en fonction des cinq tâches définies dans le contrat:

Tâche 1: Révision des évaluations de risques existantes

Tâche 2: Sélection de nouvelles espèces à évaluer

Tâche 3: Préparation des nouvelles évaluations de risque

Tâche 4: Préparation de nouvelles notes relatives à la gestion de risque (Données probantes sur les mesures, leur coût d'implémentation et leur rapport coût-efficacité).

Tâche 5: Workshop portant sur l'assurance qualité

Quelques corrections mineures ont été apportées aux modèles pour les évaluations des risques et les notes de gestion des risques par rapport à la version utilisée au cours de la première année du contrat 07.0202/2019/812602/ETU/ENV.D2, sur la base des discussions au sein de l'équipe de projet et de la Commission. Les modifications comprennent notamment la correction d'erreurs typographiques, la mise à jour d'un lien hypertexte qui n'était plus fonctionnel, des clarifications concernant le domaine d'évaluation des risques après le Brexit, l'abstention de fournir une réponse et un score de confiance en cas de « aucune information n'a été trouvée » et le fait que le score d'impact potentiel futur plus élevée en cas de changement climatique nécessite une justification explicite dans le commentaire fourni. Plus précisément:

1) Lorsque les instructions indiquent qu'en cas de manque d'information, les évaluateurs sont invités à utiliser une réponse standardisée: "Aucune information n'a été trouvée", il est suggéré d'ajouter la précision suivante: Dans ce cas, aucun score et aucune confiance ne doivent être donnés et le "score" standardisé est N/A (non applicable).

2) Au point Qu. 4.3, il est suggéré d'ajouter la clarification suivante aux instructions: Une augmentation potentielle de l'aire de répartition due au changement climatique ne justifie pas en soi un score d'impact plus élevé. Une référence à ce point est également ajoutée aux questions 4.5 et 4.20.

Les versions actuelles des deux documents sont fournies comme suppléments à la fin de ce rapport.

Tâche 1: La Commission a fourni douze évaluations de risques pour révision qui ont été développées sous des contrats précédents par l'équipe de projet plus large ou par un État membre. Ces révisions prennent en compte les commentaires du Forum scientifique (représentants des États membres) et des parties prenantes et ont généré des versions mises à jour des documents, qui sont incluses ici en tant qu'annexes 9-20 au présent rapport final.

Les douze espèces sont:

#	Groupe	Nom scientifique	Nom vernaculaire
1	Marines	<i>Asterias amurensis</i>	Etoile de mer du Pacifique Nord
2	Vertébrés	<i>Axis axis</i>	Cerf axis
3	Plantes	<i>Broussonetia papyrifera</i>	Mûrier à papier
4	Marines	<i>Callinectes sapidus</i>	Crabe bleu de l'Atlantique
5	Vertébrés	* <i>Castor canadensis</i>	Castor nord américain
6	Plantes	* <i>Celastrus orbiculatus</i>	Bourreau des arbres
7	Plantes	<i>Cortaderia selloana</i>	Herbe de la Pampa
8	D'eau douce	<i>Faxonius immunis</i>	Ecrevisse calicot
9	D'eau douce	<i>Misgurnus anguillicaudatus</i>	Loche asiatique
10	Vertébrés	<i>Pycnonotus cafer</i>	Bulbul à ventre rouge
11	Invertébrés	<i>Vespa mandarinia</i>	Frelon géant
12	Vertébré	Xénope lisse	Xénope lisse

*Évaluation de risques produite par un Etat membre

Tâche 2: L'objectif de cette tâche était de convenir d'une sélection d'espèces prioritaires pour les évaluations de risques ultérieures de la tâche 3 et le développement de notes de gestion des risques de la tâche 4. L'équipe de projet a été divisée en cinq groupes thématiques d'experts: animaux d'eau douce, espèces marines, plantes (y compris d'eau douce), Invertébrés terrestres et Vertébrés. Les propositions de l'équipe et de la Commission, ont ensuite été discutées avant et pendant la réunion de lancement en ligne du 12 novembre 2020. L'accent a été mis sur les EEE qui ne sont pas encore présentes dans l'Union européenne (ou dont la distribution est limitée) et qui ont le potentiel d'avoir un impact négatif sur la biodiversité. Sur base de ce processus de consultation, les huit espèces suivantes ont été sélectionnées:

#	Groupe	Nom scientifique	Nom vernaculaire
1	Invertébrés	<i>Bipalium kewense</i>	Vers plat à tête de marteau
2	Vertébrés	<i>Cervus nippon</i>	Cerf sika
3	D'eau douce	<i>Cherax destructor</i>	Ecrevisse de Murray
4	Plantes	<i>Delairea odorata</i>	Lierre d'Allemagne
5	D'eau douce	<i>Marisa cornuarietis</i>	Marise corne-de-bélier
6	Marines	<i>Mulinia lateralis</i>	Palourde naine
7	Invertébrés	<i>Obama nungara</i>	Vers plat Obama
8	Vertébrés	<i>Pycnonotus jocosus</i>	Bulbul orphée

Tâche 3: Les évaluations des risques ont été élaborées par des experts de l'équipe du projet, ainsi que par d'autres experts agissant en tant qu'auteurs principaux, co-auteurs et/ou réviseurs. Pour les huit espèces, de nouveaux modèles de distribution ont été développés afin de fournir une base de connaissances plus solide (tout en reconnaissant les limites de ces modèles) pour les questions pertinentes des évaluations des risques. Toutes les évaluations des risques ont fait l'objet d'un examen de contrôle de qualité interne, d'une révision externe par au moins deux experts indépendants conformément aux exigences du règlement délégué (UE) 2018/968 de la COM et d'un workshop d'assurance qualité (tâche 5). Les évaluations finales des risques des huit espèces figurent à l'annexe 1a-8a du présent rapport final.

Tâche 4: Les notes de gestion des risques ont été élaborées parallèlement aux évaluations des risques par des experts de l'équipe de projet, ainsi que par d'autres experts en tant qu'auteurs principaux, co-auteurs et/ou pairs pour les huit espèces. Toutes les notes sur la gestion des risques, comme pour les évaluations des risques, ont fait l'objet d'un contrôle de qualité interne, d'une révision externe par au moins deux experts indépendants et ont été discutées lors de l'atelier sur l'assurance qualité (tâche 5). Les notes finales de gestion des risques des huit espèces sont incluses dans l'annexe 1b-8b de ce rapport final.

Tâche 5: En raison de la situation COVID-19 toujours en cours, le workshop d'assurance qualité a été organisé sous forme de réunion en ligne les 15 et 17 septembre 2021. L'objectif était de fournir aux auteurs, aux pairs examinateurs et à la Commission l'opportunité de discuter de tous les problèmes non clarifiés, d'éliminer les incertitudes restantes autant que possible, et d'assurer la meilleure qualité possible des évaluations des risques et des notes de gestion des risques. En préparation de l'atelier, les auteurs ont pris en compte et intégré les commentaires des pairs examinateurs avant l'atelier. Lors de l'atelier, les auteurs ont présenté leurs travaux lors de sessions plénières, tandis que les questions ouvertes ont été discutées dans des groupes de discussion virtuels. Les commentaires des évaluateurs et les considérations des auteurs sont inclus dans ce rapport final. Après l'atelier, les auteurs ont finalisé les évaluations des risques et les notes de gestion des risques pour ce rapport final.

Le présent rapport final décrit en détail le travail accompli pour chaque tâche. Il est accompagné de 28 annexes: huit nouvelles évaluations des risques (annexe 1a-8a), huit nouvelles notes de gestion des risques (annexe 1b-8b) et douze évaluations des risques révisées (annexe 9-20).

Overview of Tasks

Task 1: Revision of existing Risk Assessments

Task lead: Wolfgang Rabitsch

Over the project period, the Commission selected and provided twelve existing risk assessments of species for revision that had been developed under previous contracts or submitted by a Member State. The aim of these revisions was to consider and incorporate – if appropriate – comments from the Scientific Forum and stakeholders and subsequently generate updated versions of the risk assessments along with a documentation of the revisions. The twelve species identified for this task are:

Annex	Group	Scientific Name	Common Name	Delivery date
Annex 9	Marine	<i>Asterias amurensis</i>	North Pacific Sea Star	October 15
Annex 10	Vertebrates	<i>Axis axis</i>	Axis Deer	March 4
Annex 11	Plants	<i>Broussonetia papyrifera</i>	Paper Mulberry	October 15
Annex 12	Marine	<i>Callinectes sapidus</i>	Atlantic Blue Crab	October 15
Annex 13	Vertebrates	* <i>Castor canadensis</i>	North American beaver	January 14
Annex 14	Plants	* <i>Celastrus orbiculatus</i>	Staff-vine	January 14
Annex 15	Plants	<i>Cortaderia selloana</i>	Pampas Grass	October 15
Annex 16	Freshwater	<i>Faxonius immunis</i>	Calico Crayfish	October 15
Annex 17	Freshwater	<i>Misgurnus anguillicaudatus</i>	Oriental Weatherfish	October 15
Annex 18	Vertebrates	<i>Pycnonotus cafer</i>	Red-vented Bulbul	March 4
Annex 19	Invertebrates	<i>Vespa mandarinia</i>	Asian Giant Hornet	October 15
Annex 20	Vertebrates	<i>Xenopus laevis</i>	African Clawed Frog	March 4

*Risk Assessment submitted by a Member State

According to the Terms of Reference, the delivery of up to ten revised risk assessments is part of the Progress Report. Due to the delayed submission of such documents to the project team, it was approved by the Commission to decouple the revisions from the Progress Report and deliver these with the Final Report, due by October 2021.

Following discussions in the context of the COVID19-pandemic approval was given by the Commission to organize the Quality Assurance Workshop as an online meeting (see Task 5). To compensate for the reduced costs (for T&S), it was discussed and approved that the project team will revise two additional existing Risk Assessments from previous contracts, i.e. twelve revisions in total.

Comments from the Scientific Forum and stakeholders have been received and addressed by the project team in relation to two Risk Assessments submitted by a Member State. In these cases, a separate, stand-alone document with the suggested amendments and clarifications was produced (Annex 13 & 14). For the other Risk Assessments, a clean and track-changes version together with a cover note addressing the changes made were returned to the Commission within the given deadlines.

Task 2: Selection of new species to be assessed

Task lead: Alan Stewart, Jodey Peyton

The objective of this task was to agree on a selection of priority species for subsequent risk assessments in task 3 and development of risk management notes in task 4. As in previous contracts, groups of experts were convened with expertise in five taxonomic/ecological groupings. The membership of these groups was as follows (group co-leaders in bold):

Freshwater animals: **Frances Lucy, Elena Tricarico**, Hugo Verreycken

Marine species: **Marika Galanidi, Jack Sewell**, Argyro Zenetos, Paraskevi Karachle

Plants (including freshwater): **Rob Tanner**, Jodey Peyton, Helmut Kudrnovsky

Terrestrial invertebrates: **Marc Kenis, Richard Shaw**, Wolfgang Rabitsch, Alan Stewart, Helen Roy

Vertebrates: **Riccardo Scalera, Tim Adriaens**, Peter Robinson, Yasmine Verzelen

The Commission was sent a draft list of priority species in advance of the kick-off meeting, with indications of the species of highest priority for Risk Assessments according to the views of the project team. The Commission also provided species to be considered. The species were discussed and reconciled at the kick-off meeting on 12th November 2020.

The following working procedure was adopted:

Leaders of each expert group were asked to propose four species per group. This resulted in 20 species to put forward, from which to select the eight to suggest to the Commission for formal Risk Assessment. Experts were requested to prepare a single paragraph for each species explaining why it is being proposed (Annex II). The instructions for Task 2 in the project tender were also circulated among the experts, along with the Excel spreadsheet to be used as the starting point for discussion within each group. The above mentioned Excel spreadsheet (the same that was used for the selection during the previous contract year) included a sheet with the list of species arising from the 2015 horizon scanning exercise, and separate sheets for each of the groups. Experts were invited to add new species as appropriate. As in previous years, the following criteria were considered:

- Not yet arrived in EU or very small populations
- Likely to arrive in next 10 years
- Likely to impact on:
 - Biodiversity
 - Economy
 - Human health & wellbeing
- Practicality of management, probability of success, cost

Once experts from each group had suggested their species, Task 2 co-leaders combined them into a single list of 20 species from which they selected 2 top priority species for each group (Figure 1) in consultation with other members of the team. This list was sent to the Commission on 3 November 2020, in advance of the kick-off meeting.

After preliminary feedback from the Commission, the plant and marine groups added two more species for further discussions (Fig. 1).

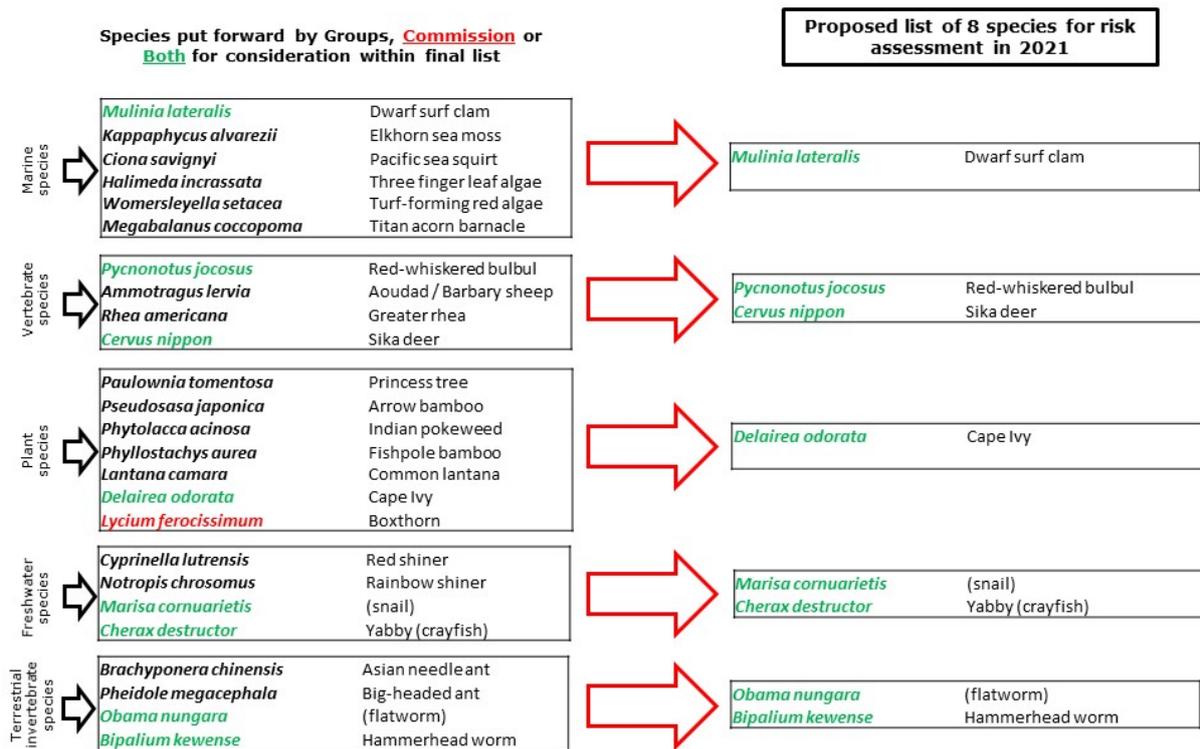


Figure 1. Process of selection of species for risk assessments in 2021 (Suggestions by both Expert Groups and European Commission in green, suggestions by European Commission in red).

All species proposed by the project team were presented and discussed at the kick-off meeting, from which a final list of 8 species was agreed for risk assessments in Task 3 and management notes in Task 4 (Table 1).

For *Mulinia lateralis*, the Commission remarked that they were hesitant but tried to give at least one species per group and this one seemed the most interesting marine species of those proposed.

For the plants, the Commission pointed out that although the plant experts insisted on *Paulownia* they were not convinced that anything could be done about it in the framework of the IAS Regulation (since beyond horticulture that is mentioned in the summary, it is widely used in plantations for timber). It was also mentioned, however, that the species will be re-assessed next year.

The bamboos seemed problematic as these genera have 20–60 species each and potential listing would be impossible to implement. The *Phytolacca* would be interesting but at the previous round the experts dismissed it. In the final report of 2019 under task 2 it is indeed mentioned that “there is very little available information on its current or potential impacts”.

Table 1: Final list of eight species for risk assessment in 2021, agreed following the kick-off meeting with the European Commission and leading author(s) from the project team.

#	Group	Scientific Name	Common Name	Leading Author(s)
1	Invertebrates	<i>Bipalium kewense</i>	Kew Flatworm	Leigh Winsor
2	Vertebrates	<i>Cervus nippon</i>	Sika Deer	Riccardo Scalera
3	Freshwater	<i>Cherax destructor</i>	Common Yabby	Elena Tricarico
4	Plants	<i>Delairea odorata</i>	Cape Ivy	Rob Tanner
5	Freshwater	<i>Marisa cornuarietis</i>	Colombian Ramshorn Apple Snail	Frances Lucy
6	Marine	<i>Mulinia lateralis</i>	Dwarf Surf Clam	Christine Wood
7	Invertebrates	<i>Obama nungara</i>	Obama flatworm	Archie Murchie
8	Vertebrates	<i>Pycnonotus jocosus</i>	Red-whiskered Bulbul	Tim Adriaens

With respect to other species discussed for inclusion, it was concluded to keep the African boxthorn, *Lycium ferocissimum*, and the green macroalgae *Halimeda incrassata* for further discussions next year.

Task 3: Preparation of new Risk Assessments

Task lead: Riccardo Scalera

Before the external peer-review an internal quality check was made by the task 3 and 4 leaders and the project lead, with the aim to ensure that all questions were addressed in line with the template guidelines and instructions, and that all Risk Assessments and Risk Management Notes were consistent between and across the documents.

The "Checklist for peer-review", which was developed in the first year of the contract (see Final Report 2020) to support a coherent summary of the reviewing process, was distributed to the peer-reviewers with information on the guidelines and the criteria for each score. Peer-reviewers were specifically asked to consider these elements within their assessment.

Contact was made with designated peer-reviewers, explaining the rules of procedure and expected deadlines. Table 2 provides an overview of the peer-reviewers. Risk Assessments and Risk Management Notes were distributed to the peer-reviewers along with the before mentioned checklist with a deadline of four weeks for execution of the peer-review. For each risk assessment, it was ensured that the author(s) and the peer-reviewers are not affiliated to the same institution, as required under Article 2(9) of the Delegated Regulation 2018/968 on risk assessments.

Table 2: Peer-reviewers for the eight Risk Assessments and Risk Management Notes.

#	Scientific Name	Common Name	Peer-Reviewers
1	<i>Bipalium kewense</i>	Kew flatworm	Brian Boag Richard Shaw
2	<i>Cervus nippon</i>	Sika deer	Wojtek Solarz Alastair Ward
3	<i>Cherax destructor</i>	Common yabby	Antonin Kouba Francisco Oficialdegui
4	<i>Delairea odorata</i>	Cape ivy	Giuseppe Brundu Johan van Valkenburg
5	<i>Marisa cornuarietis</i>	Colombian ramshorn apple snail	Elena Tricarico Hugo Verreycken
6	<i>Mulinia lateralis</i>	Dwarf surf clam	Francis Kerckhof Argyro Zenetos
7	<i>Obama nungara</i>	Obama flatworm	Brian Boag Richard Shaw
8	<i>Pycnonotus jocosus</i>	Red-whiskered bulbul	Thomas Evans Enrique Murgui

In addition to the "Checklist for Peer-Review", the authors were provided with the short "Checklist after Peer-Review", which was also developed in the first year of the contract (see Final Report 2020). The purpose of this Checklist is to streamline and document any substantial changes from the peer-review, which is requested according to Commission Delegated Regulation (EU) 2018/968 of 30 April 2018 and forms an integral part of this Final Report. Commission Delegated Regulation (EU) 2018/968 of 30 April 2018 requests in Art. 2(7) that "A quality control process shall be an integral part of the risk assessment and shall include at least a review of the risk assessment by two peer reviewers. The risk assessment shall include a description of the quality control process."

EU IAS risk assessments 2020/21: Summary of modelling approach

Björn Beckmann & Dan Chapman

Species Distribution Models (SDMs) were created for all eight species for which risk assessments were conducted this year: *Delairea odorata*, *Bipalium kewense*, *Obama nungara*, *Mulinia lateralis*, *Marisa cornuarietis*, *Cherax destructor*, *Pycnonotus jocosus* and *Cervus nippon*. No recent models with a suitable geographical scope existed for these species, and we considered that sufficient information was available to build models in terms of distribution data, and information about ecological and ecophysiological requirements of the species.

Species distribution records were obtained from eight large online databases – the Global Biodiversity Information Facility (GBIF), the Biodiversity Information Serving Our Nation database (BISON), iNaturalist, the Berkeley Ecoinformatics Engine database, the VertNet databases, the Integrated Digitized Biocollections (iDigBio), the Ocean Biogeographic Information System (OBIS), and the Atlas of Living Australia. Additional records were supplied for several species by the respective risk assessment teams, for example museum records and records extracted from publications. For all species, records were scrutinised and any removed which did not refer to populations established outdoors (e.g. captive records), as were those where the georeferencing was too imprecise (e.g. records referenced to a country or island centroid).

A presence-background (presence-only) ensemble modelling strategy was employed using the BIOMOD2 R package version 3.4.6 (Thuiller et al. 2009, 2020). These models contrast the environment at the species' occurrence locations against a random sample of the global background environmental conditions (often termed 'pseudo-absences') in order to characterise and project suitability for occurrence. This approach has been developed for distributions that are in equilibrium with the environment. Because invasive species' distributions are not at equilibrium and subject to dispersal constraints at a global scale, we took care to minimise the inclusion of locations suitable for the species but where it has not been able to disperse to (Chapman et al. 2019). Therefore the background sampling region included:

- Regions where we have an a priori expectation of high unsuitability for the species so that absence is assumed irrespective of dispersal constraints; AND
- The area accessible by native populations, in which the species is likely to have had sufficient time to disperse to all locations; AND
- The area accessible to non-native populations, defined as a buffer around the non-native occurrences, encompassing regions likely to have had high propagule pressure for introduction by humans and/or dispersal of the species.

Within the "unsuitable" background region, 10 samples of 5000 randomly places pseudo-absences were obtained. In the "accessible" background regions, 10 samples of the same number of pseudo-absences were taken as there were occurrence points, weighting the sampling by recording effort (reflecting a higher confidence in the absence of the focal species in better-surveyed regions). We used the density of records held by GBIF for the higher taxonomic group of each species as a proxy for recording effort.

Climate data for modelling terrestrial species were selected from the 'Bioclim' variables contained within the WorldClim database (Hijmans et al. 2005), and for marine species from the 'Bio-ORACLE' set of GIS rasters providing geophysical, biotic and environmental data for surface and benthic marine realms (Tyberghein et al. 2012, Assis et al. 2017). For *Mulinia lateralis*, climatic data were supplemented by a "Growing Degree Days" measure (a sum of biologically useful warmth) calculated from MARSPEC monthly sea surface temperature data (Sbrocco & Barber 2013). For species where climatic moisture availability was considered important, a Climatic moisture index (CMI) was calculated as the ratio of mean annual precipitation to potential evapotranspiration by estimating

monthly potential evapotranspirations from the WorldClim monthly temperature data and solar radiation using the simple method of Zomer et al. (2008) which is based on the Hargreaves evapotranspiration equation (Hargreaves 1994). For *Cervus nippon*, as a proxy for potential depth and duration of snow cover, the inverse of the mean temperature of the coldest quarter (Bio11) was multiplied with mean precipitation of the coldest quarter (Bio19), to produce a number that is greatest in areas where winters are both cold and have high precipitation. The numbers were scaled to values between 0 and 1.

To estimate the effect of climate change on the potential distribution, equivalent modelled future climate conditions for the 2070s under the Representative Concentration Pathways (RCP) 2.6 and 4.5 were also obtained. These represent low and medium emissions scenarios, respectively.

Additional non-climatic predictor layers were used for several species where these were considered relevant: Tree cover, estimated from the MODerate-resolution Imaging Spectroradiometer (MODIS) satellite continuous tree cover raster product, produced by the Global Land Cover Facility (<http://glcf.umd.edu/data/vcf/>), and a Human influence index (HII), since many non-native invasive species associate with anthropogenically disturbed habitats. We used the Global Human Influence Index Dataset of the Last of the Wild Project (Wildlife Conservation Society & Center for International Earth Science Information Network, Columbia University, 2005), which is developed from nine global data layers covering human population pressure (population density), human land use and infrastructure (built-up areas, night-time lights, land use/land cover) and human access (coastlines, roads, railroads, navigable rivers). For *Mulinia lateralis*, the distance to the nearest large river mouth was used as a proxy for presence of brackish water (in addition to the Bio-Oracle salinity layer mentioned above). The location of rivers and their average discharge was taken from the "HydroRIVERS" dataset, version 1.0 (Lehner et al. 2008). Point locations of river mouths were "rasterized" to the model grid, summing discharge per 0.25 x 0.25 degree grid cell in case there were several river mouths per cell. Endorheic rivers (not flowing into the oceans) were excluded. For each model grid cell, distance-by-sea (in meters) to the nearest cell with average discharge > 10 m³/s was then calculated using "gridDistance" in the R package "raster", version 3.4.5 (Hijmans 2020). For *Delawarea odorata*, we calculated distance to the sea as a proxy for oceanic climate; values were ln+1 transformed for modelling to improve normality.

Each dataset (i.e. combination of the presences and the individual background samples) was randomly split into 80% for model training and 20% for model evaluation. With each training dataset, seven statistical algorithms were fitted with the default BIOMOD2 settings (Generalised linear model, Generalised boosting model, Generalised additive model with a maximum of four degrees of freedom per smoothing spline, Artificial neural network, Multivariate adaptive regression splines, Random forest, and Maxent). Since background samples tended to be larger than the number of occurrences, prevalence fitting weights were applied to give equal overall importance to the occurrences and the background. Normalised variable importance was assessed and variable response functions were produced using BIOMOD2's default procedure.

Model predictive performance was assessed by the following three measures: AUC, the area under the receiver operating characteristic curve (Fielding & Bell 1997, Manel et al. 2001), Cohen's Kappa (Cohen 1960), and TSS, the true skill statistic (Allouche et al. 2006). Ensemble models were created by first rejecting poorly performing algorithms with relatively extreme low AUC values and then averaging the predictions of the remaining algorithms, weighted by their AUC.

Projections were classified into suitable and unsuitable regions using a "lowest presence threshold" (Pearson et al. 2007), setting the cut-off as the lowest value at which 98% of all presence records are classified correctly under the current climate. In order to express the sensitivity of classifications to the choice of this threshold, thresholds at which 95% and 99% of records are classified correctly were used in the calculation of error bars in

the figures illustrating suitability per bioregion and country, in addition to taking account of uncertainty in the projections themselves.

We also produced limiting factor maps for Europe following Elith et al. (2010). For this, projections were made separately with each individual variable fixed at a near-optimal value. These were chosen as the median values at the occurrence grid cells. Then, the most strongly limiting factors were identified as the one resulting in the highest increase in suitability in each grid cell.

Finally, we calculated the proportion of the area of each EU member state and the UK, and of each Biogeographical region of Europe, projected to be suitable for establishment of each terrestrial species, both for the current climate and for projected climate for the 2070s under the two climate change scenarios mentioned above. For marine species, we calculated the proportion of the areas projected to be suitable of the coastal territorial waters of European Union countries and the UK, and of the marine subregions of Europe.

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The peer-review

The comments of the peer reviewers have been taken into account in the documents discussed during the workshop and any pending issue was further discussed before the finalization of the documents. As such, the final versions of the risk assessments and risk management notes represent the consensus reached between authors and peer reviewers. A summary of key points in response to the peer-reviewer comments and the discussions at the quality assurance workshop is provided below by the authors of the assessments. Also, authors were invited to provide pending research needs that would improve the knowledge base for the species.

***Bipalium kewense* (Kew flatworm)**

Both the internal and external reviews were most helpful, and the reviewers are thanked for their comments, suggestions, and advice, all of which were greatly appreciated. There were no substantial disagreements with the reviewers, and their valuable suggestions to clarify and to expand on certain topics were adopted. Some additional useful information was supplied by the reviewers regarding the outdoor establishment of the species. Following discussions at the Workshop the Risk Management Notes were expanded slightly to ensure inclusion of health, safety, and environmental precautions where relevant, and an indication of a time line for remedial action upon the discovery of the species.

Where the reviewers suggested amending scores, this was only one stage up or down, and was done to ensure consistency of approach across all the risk assessments, especially in the "Impacts" section. Also, where responses to a question were "No information has been found on this issue", and the responses scored as N/A, they were checked to ensure that a confidence level was NOT provided. In all cases, scores were amended without disagreement, and were justified to provide clarity to the response.

Have there been substantial changes to the scores of the probabilities of introduction, entry, establishment, and spread or the magnitude of impacts?

No substantial changes. Some scores were amended but only one step up or down to be consistent with other risk assessments.

Have there been substantial changes to the scores in the risk summary and the conclusion of the risk assessment (overall risk)?

As above, no substantial changes.

Have there been substantial changes to the levels of confidence?

As above, no substantial changes.

Have there been important additional references and other sources of information included that were previously missing?

Yes. Some additional references supporting statements in those sections that were expanded or clarified subsequent to the advice from the reviewers.

Have there been clarifications to the content that improved readability and understanding?

Yes. The reviewers provided many queries to the risk assessment to improve clarity and understanding. In some cases, they suggested alternative wording or asked for

certain topics to be expanded. They also checked the text to make sure that conclusions were consistent with the evidence supplied.

Identified data gaps and research needs

As an experimental animal regarded as an example of a triclad flatworm, *Bipalium kewense* has been the subject of numerous scientific studies. However, the few ecological studies conducted on the species were largely concerned with the seasonal occurrence and survival of the species. Although *B. kewense* is solely vermivorous, there are no studies concerned with the impact of the flatworm upon earthworm populations in the urban situations worldwide in which the flatworm is encountered outside its natural distribution. Possibly the presence and behaviour of *B. kewense* in urban gardens and parks, in contrast say, to *Arthurdendyus triangulatus*, the New Zealand flatworm, has not been such so as to raise concerns by gardeners and horticulturalists over its possible impacts on the garden environment that might otherwise have led to further investigations. As is the situation with other alien species of flatworms, little is known about how to specifically control *B. kewense*. Rather, incursions of all land planarians are likely to be controlled in much the same way as they all share a similar biology.

The evidence gaps with respect to the Risk Assessment are therefore:

- What are the food preferences of *B. kewense* for various species of earthworms?
- What is the impact of *B. kewense* upon urban populations of earthworms?
- Depending upon the results of the foregoing two points, what risk does the flatworm pose to native / other earthworm populations should it become established outside of urban situations?
- What is the effectiveness of hot-water treatment against flatworm zooids (fragments), adults and egg capsules, and also
- What is the possible cost-effectiveness of such treatment?

Cervus nippon (Sika Deer)

Have there been substantial changes to the scores of the probabilities of introduction, entry, establishment, and spread or the magnitude of impacts?

No. Following the peer-review and the discussion held during the workshop, only some changes on the level of confidence were made (see below).

Have there been substantial changes to the scores in the risk summary and the conclusion of the risk assessment (overall risk)?

No. In general peer-reviewers agreed with the scoring and relevant justifications. Scores were double checked during the workshop for many points throughout the document, but only a few changes were made, i.e. Qu. 1.6a, 1.6d, 3.12, and on some small remark to the comments (but they did not affect the final result). In particular, a discussion was held on whether a "major" score on impact to biodiversity was sufficiently justified, and this reached general consensus given the impact of the species due to the risk of hybridisation with the native red deer.

Have there been substantial changes to the levels of confidence?

The levels of confidence were double checked for many points throughout the document to ensure they correctly reflect a proper interpretation/understanding of the current knowledge on the relevant issues (some minor changes were made following agreement by all authors during the workshop). However, in general this did not lead to any substantial changes to the overall assessment.

Have there been important additional references and other sources of information included that were previously missing?

A few references were added, e.g. Dvořák and Palyzová 2016, González-Suárez et al 2015, Goodman et al. 1999, Liu et al. 2020, Osterholm et al. 2019, Polaina et al. 2021, Wyman et al. 2016, but in general the assessment was already well covered and supported by all key literature.

Have there been clarifications to the content that improved readability and understanding?

Yes. Some minor changes were made to the text throughout the document, based on the correct interpretation of the available literature and the knowledge on the issue (thanks to the specific skills of the peer-reviewers). In many cases, the points raised by the peer reviewers, and preliminarily addressed by the authors, were (re)discussed (and relevant justification fine tuned, if needed), so to achieve consensus among co-authors and peer-reviewers. Among the other things, the importance of the occurrence of hybrids was extensively discussed. It was decided that hybrids must be explicitly addressed by the RA, and that while it is not clear whether the species has to be considered established in countries where only hybrids are present, all relevant information has to be clearly reported/discussed in the replies. Also, the need to consider more clearly both introduction and entry was stressed, particularly in relation to the issue of detection. In general, it was agreed that any information that would appear too generic or speculative, e.g. based on the knowledge of other cervids, would be not included, unless strictly necessary because of evident knowledge gaps for the target species. The SDM did not require any revisions as it was considered by the peer-reviewers of very good quality already.

Cherax destructor (Common Yabby)

The SDM has been recalculated after discussion at the workshop, based on new records provided for France and Ireland. The model results are similar to before, but the projected suitabilities are larger for some bioregions and countries, with north-western France now considered suitable, and Ireland very nearly suitable under current climate, and with large suitable areas under future climates.

Have there been substantial changes to the scores of the probabilities of introduction, entry, establishment, and spread or the magnitude of impacts?

No substantial changes to the scores of probabilities of introduction, entry, establishment, and spread or the magnitude of impacts. Some minor adjustments based upon by additional information and references provided by the reviewers.

Have there been substantial changes to the scores in the risk summary and the conclusion of the risk assessment (overall risk)?

No substantial change to the scores in the risk summary and the conclusion of the risk assessment (overall risk).

Have there been substantial changes to the levels of confidence?

No substantial changes to the levels of confidence. Some minor adjustments based upon additional information and references provided by the reviewers.

Have there been important additional references and other sources of information included that were previously missing?

Some additional references were added for the new records used in the model, for better illustrating the interspecific crayfish species interactions and the other diseases potentially carried by the species.

Have there been clarifications to the content that improved readability and understanding?

Some minor clarifications in order to improve readability and understanding. The issue of alien American crayfish possible acting as a barrier for the species has been better explained.

Delairea odorata (Cape Ivy)

Have there been substantial changes to the scores of the probabilities of introduction, entry, establishment, and spread or the magnitude of impacts?

No. Only some editorial changes were made to the text.

Have there been substantial changes to the scores in the risk summary and the conclusion of the risk assessment (overall risk)?

No. Only some editorial changes were made to the text.

Have there been substantial changes to the levels of confidence?

No. though additional text has been added for some questions to further justify the confidence level.

Have there been important additional references and other sources of information included that were previously missing?

The reviewers did supply some additional references that have been included in the risk assessment.

Have there been clarifications to the content that improved readability and understanding?

Yes, some text has been amended to further clarify answers. In particular, the reviewers suggested the removal of the pathway 'Landscape/flora/fauna important in the wild' as the species is generally described as growing poorly in full sunlight, and there is no evidence that the species has been used for this purpose. In the risk assessment, it is stated that the pathway was considered but not evaluated further due to the points above.

Marisa cornuarietis (Colombian Ramshorn Apple Snail)

There were no substantial disagreements with the reviewers on the Risk Assessment document. There were valuable suggestions for clarification and to expand on certain topics. For example, two extra pathways of spread suggested by one reviewer were added to the Risk Assessment. Some additional clarifications and references were also sought. The keypoint, with regard to availability of references, was that in some cases the response changed, for example for economic cost, the response was changed to, 'No information has been found on the issue'. The Risk Management Notes were expanded at the reviewers' request to more clearly address the questions asked in the guidelines, for example those relating to acceptability, cost and potential environmental damage of

any control measures. Where the reviewers suggested amending response scores, they were amended without disagreement. At the workshop one member state record on the distribution map was questioned and after discussion, was removed. The model was subsequently recreated and the new version added to the risk assessment.

Have there been substantial changes to the scores of the probabilities of introduction, entry, establishment, and spread or the magnitude of impacts?

No.

Have there been substantial changes to the scores in the risk summary and the conclusion of the risk assessment (overall risk)?

No.

Have there been substantial changes to the levels of confidence?

No.

Have there been important additional references and other sources of information included that were previously missing?

Yes. Some important references were included in both the RA and the Management Annex. The reassessed model was included, but did not change the predicted spread to new member states or regions.

Have there been clarifications to the content that improved readability and understanding?

Yes, in some cases copy-editing improved the quality and comprehension of the document.

***Mulinia lateralis* (Dwarf Surf Clam)**

Have there been substantial changes to the scores of the probabilities of introduction, entry, establishment, and spread or the magnitude of impacts?

Following the peer-review and the discussion held during the workshop, some reductions of a single level, were made to the scores. These changes primarily related to the probability of spread via ballast water. In addition, the possible points of introduction score was changed from widespread to isolated. These changes followed a discussion as to whether the habitat surrounding major transshipment ports in the Mediterranean would be suitable for establishment of *M. lateralis*. As there is no good evidence available either way, it was agreed to adopt the lower score and the relevant confidence levels were reduced.

Have there been substantial changes to the scores in the risk summary and the conclusion of the risk assessment (overall risk)?

Only a reduction in the probable rate of spread from rapidly to moderately.

Have there been substantial changes to the levels of confidence?

Some changes to the levels of confidence were made as mentioned above, and to provide consistency across the assessment.

Have there been important additional references and other sources of information included that were previously missing?

No. One reviewer provided details of additional evidence of establishment and predator behaviour resulting from personal observations, which was included.

Have there been clarifications to the content that improved readability and understanding?

Some minor changes were made to the text to provide clarification.

Identified data gaps and research needs

- Research on the presence of *Mulinia lateralis* in ballast water and sediments
- Research on non-native species (including *M. lateralis*) present in dredging waste, in hopper water, and on dredging equipment
- Data on international dredging activities including: number of operational vessels, dredging locations, transit points, current biosecurity measures, any current legislation in member states
- Research on the presence of *Mulinia lateralis* in bivalve transfers in the Greater North sea
- Research on the presence of *Perkinsus chesapeaki* in the Greater North Sea clam population
- Research on its impact on native species, including species it interacts with through competition and predation

Obama nungara (Obama flatworm)

The input provided by both the internal and external reviewers was positive and very valuable. There were no major disagreements and virtually all suggestions were accepted. The only suggestion not incorporated in the RA was a request to include more pathways for spread (e.g. as a 'contaminant of plants'), as suggested in the internal review. We did not add this in as the existing evidence for spread was via the pathways 'contaminant nursery stock' and 'transportation of habitat material'. Therefore, with limited data and to avoid duplication and speculation, we discussed the 'contaminant of plants' pathway in the section introduction rather than as a full section. The risk management notes were expanded on the reviewers' recommendations to include more assessment of stakeholder/consumer acceptance, environmental impact and health and safety implications of any management measures.

Have there been substantial changes to the scores of the probabilities of introduction, entry, establishment, and spread or the magnitude of impacts?

No, there were no substantial changes for the majority of the scores, with only a one step amendment in some cases to keep uniformity with other RAs. There was discussion about the impact score of *Obama nungara* and the lack of data to substantiate a 'major' score. This was discussed in detail with the reviewers and at the workshop. It was agreed that 'absence of evidence' was not 'evidence of absence' and the purpose of the RA was to be proactive in preventing damage caused by invasive alien species. Since the flatworm is a relatively recent introduction to Europe, clear evidence of impact may take time to be seen and there are no studies to date. However, delaying scoring until evidence of damaging impact is apparent would be a strategic management mistake. Therefore, scoring was done on the basis of the

biological similarities of *O. nungara* with other invasive terrestrial flatworms and the damaging impact caused by them.

Have there been substantial changes to the scores in the risk summary and the conclusion of the risk assessment (overall risk)?

No, there were no substantial changes bearing in mind the above.

Have there been substantial changes to the levels of confidence?

No substantial changes. For the impact of *O. nungara*, confidence levels were rated low due to its recent introduction to Europe and lack of data.

Have there been important additional references and other sources of information included that were previously missing?

The reviewers supplied additional records that became available during the period of the risk assessment exercise.

Have there been clarifications to the content that improved readability and understanding?

Yes, there were several instances where the experience of the reviewers assisted in making the text clearer and more understandable to non-specialists.

Identified data gaps and research needs

Obama nungara is established and spreading in Europe. There is an urgent need to assess the impact of this predatory flatworm on prey species. First, the prey species range in Europe needs to be clarified. Second, the impact of *O. nungara* predation on said prey populations needs to be investigated. Without these data, confidence in assessing the impact of *O. nungara* will remain low. For detection of flatworms in general, eDNA techniques need to be investigated. Similarly, for control of alien terrestrial flatworms, phytosanitation using hot water treatments needs to be trialled.

***Pycnonotus jocosus* (Red-whiskered Bulbul)**

Have there been substantial changes to the scores of the probabilities of introduction, entry, establishment, and spread or the magnitude of impacts?

No. Apart from some small changes in confidence levels (e.g. Qu 4.1) that were flagged during the review process, there were no substantial changes in scores across the different modules. At the workshop however, all suggestions for alternative scores raised by the two reviewers were collectively discussed until consensus was reached on the scores:

The likelihood of introduction and entry into the RA area under current (Q1.9) and future (Q1.10) climate was scored as very likely with high confidence. Likewise, the likelihood of establishment in the RA area under current (Q2.9) and future (Q2.10) climate was scored as very likely with high confidence. The score for introduction/entry was challenged on the basis of the EU bird ban which reduced the number of wild birds kept in captivity as pets and therefore one introduction per year could probably be too much. Likewise, the score for establishment was challenged arguing that in 20 years only one population managed to establish in the RA area and therefore this would correspond with a score of likely (1 in 10 years) or lower. The decision was to stick to the scoring guidance for introduction/entry and establishment,

which stipulates a score of very likely should be given for already introduced and established species.

There was some discussion on raising the score for impact outside the RA area to massive, considering the documented extinction and declines in *Nephila* spiders and native bulbul respectively. Revisiting the scoring ranges for biodiversity impact, the group decided to stick with major impact. The report on the Mauritius spider extinction is not presenting spider population monitoring data and most probably other factors contributed to the extinction (e.g. habitat loss). This is certainly also the case for Nicobar bulbul (cf. Birdlife factsheet information). Therefore, the group considered the criterion "widespread impact" not fulfilled and we agreed on major impact.

4.3 future impact in the RA area. Although the landscape matrix around the currently established population in Valencia might not provide perfect bulbul habitat, there is scope for the bulbul to spread to the adjacent natural park which is a pretty vast area (~3000ha + the city of Valencia). Second, we should not consider the current case of invasion as the sole representative model for other invasion events (e.g. on Mediterranean islands). Kept current score.

Concerns were raised by one of the reviewers about potentially over-scoring the current and potential economic impact of the species (Q 4.9, 4.11, 4.12), making parallels with overstated damages to orchards and agricultural assets in the Valencia area done by native species like thrushes and starlings. It was however noted that agricultural assets vary greatly across the risk assessment area therefore solely considering the area of current establishment is not representative for a potential future invasion scenario. Also, there is direct evidence outside the RA area of agricultural damages on a variety of crops (e.g. California, Hawaii), although there is a lack of quantified studies of production losses. This is already mentioned in the argumentation and reflected in low confidence. The group decided to reiterate these arguments in the relevant questions with direct reference to the monetary impact scale. It is noted that actually a conservative approach was taken but still a score of moderate (>100,000 euros) is easily reached even in case of small agricultural damages across the entire RA area.

Revisit confidence scores across the impact section of the PRA (quite a few times a score of low while overall the confidence was scored medium). After discussion, the current confidence scores stand except for 4.1 (impact outside RA area). Here, the confidence score was increased to high considering the direct observational evidence and the relatively good body of evidence of impacts across the non-native range - impact is not inferred from other species but there is direct evidence for the species itself. As a consequence, the overall confidence on impact is more balanced but it was noted that in principle, the overall confidence has no direct link with the composite questions.

Have there been substantial changes to the scores in the risk summary and the conclusion of the risk assessment (overall risk)?

No. There was a discussion on one reviewer comment suggesting lowering the score for impact and for overall risk (from major to moderate and high to moderate respectively). However, after having reviewed scoring ranges en impact information, it was concluded that a high risk score was justified, especially considering the presence of sensitive island biota, documented ecosystem-level effects and competitive exclusions and local extinctions of native species.

Have there been substantial changes to the levels of confidence?

Some minor changes to confidence levels were made following agreement by all authors and reviewers present at the workshop. Most of these were upgrades of the level of confidence, for instance because impacts were not derived from other species

but based on actual evidence for *P. jocosus*. The changes in confidence did not lead to any substantial changes to the risk assessment.

Have there been important additional references and other sources of information included that were previously missing?

Yes. At the suggestion of the reviewers some additional references were included. Most notably, some relevant literature on factors explaining invasion success in birds related to specific species traits were included that helped justify scores for establishment and spread (e.g. Callaghan et al. 2019, Cassey et al. 2004, Sol et al. 2002). Also the atlas of breeding and wintering birds of Valencia was in press and is now properly referenced. A few extra references were added on prayer release. Additionally, it was decided not to cite the red-vented bulbul risk assessment but to refer to primary references.

Have there been clarifications to the content that improved readability and understanding?

Yes, there were several clarifications throughout the PRA suggested by the reviewers and discussed at the workshop. Minor changes were made throughout, based on reviewer's knowledge on the issue, one particular area for instance concerns the situation with the Valencia population where clarifications and additions were provided about landscape context, conservation value, other species present in the area and details on invasion history.

The relevance of inclusion of the pathway "prayer release" (CBD: Other intentional releases) was discussed at the workshop. Although it is widely practiced in buddhist cultures in the native range, and there is growing concern reported worldwide in the literature about the conservation implications of this phenomenon, it is probably quite unlikely this is an issue in the RA area with birds (there are however examples in other taxa e.g. lobsters, butterflies). As there are documented cases in western countries (e.g. UK, Canada), it cannot be excluded. Evidence however reports that the chances of birds surviving these releases are low. Decision is to keep it in as a pathway, as very unlikely, also to be consistent with the red-vented bulbul PRA which mentions it.

One particular issue was discussion around the concept of "detectability" (how likely is the species to be introduced/enter the environment undetected). Reviewers raised the issue that it is not clear who is concerned in this detection. For introduction, this should refer to official control and inspection services, for (post-border) entry this is more related to the public (naturalists, landowners, gardeners, etc.). The group's decision was to consider introduction and entry separately in the score argumentation but not to go for a "confounded" average score to keep the focus on the pathway and how it is controlled in line with the nature of the question. The group also decided that a lower likelihood (to become detected) for the pet pathway, as opposed to zoo escapes, was justified, even if probably there are a lot of facilities that are not up standard with respect to housing and do represent a risk of escapes of a similar level as pets kept by private people. Additional information on reporting obligations for zoos in the framework of the Zoo Directive was provided by the Commission and was included in the RA. There is formal reporting of the number of birds kept in -zoos, therefore any birds missing could be noticed, even if zoos may have more birds. In case of private collections, there is no such mechanism and the likelihood of escapes not being detected is higher. As for entry into the environment, the species is conspicuous and would most probably be reported by the birding community fairly rapidly.

The species distribution model was discussed following comments from one reviewer who had particular concerns with counterintuitive results of the model for Iberia, identifying some areas (e.g. Galicia, Extremadura and Andalucia) which are probably

less suitable for the species because of harsh winters or unsuitable landscape context, as relatively higher risk than the area where the species is currently established in Iberia. In part, this may be due to the lack of a detailed habitat layer. The group decided to try including this layer and explore whether the model results are any better, and to point to the limitations of the model in relevant questions. It is also noted that the response curves of some of the models in the ensemble deviate for bio6 (minimum temperature of the coldest month) which might be an important variable, it is unclear where this is coming from. In response to the workshop discussion, a new version of the model was produced by Bjorn Beckmann (October 2021, version 6) which included a habitat layer and removed predictors with little influence in the model and/or correlated with others. More specifically, forest cover, which had minimal influence in the model was removed and instead a "modified Enhanced Vegetation Index", was used in the model. However, this still only had a marginal influence in the model (2%). As the projected suitability was similar to the current version (slightly higher in the Atlantic and Mediterranean regions) and had similar uncertainties, in line with a decision at the workshop, we stick to the original model and better outline uncertainties on model predictions in some areas.

A few small reviewer comments were taken into account for the risk management annex, notably additional information on public acceptance of management was included in the document.

Identified data gaps and research needs

The following knowledge gaps were identified: data on the size and geographic spread of captive populations in the RA area, the suitability of (semi)natural habitats in the RA area, studies on the current biodiversity impact of the species in the RA area and quantified data on production losses caused by the species.

Task 4: Preparation of new Risk Management Notes (evidence on measures and their implementation cost and cost-effectiveness)

Task lead: Peter Robertson

Task 4 uses a template for data collection based on the requirements from the Commission. This requires the collection of information in relation to management techniques and their costs. This is compiled as a separate Annex to each of the Risk Assessments and provides information that would help inform management decisions. The format of these templates was reviewed at the onset of the project, including opportunities to increase the level of structured detail required for completion, and to provide a summary assessment of the availability of methods suitable for different management objectives. A new section requiring details of monitoring and surveillance methods was introduced in the previous year of the contract.

The Annex describes methods for prevention, eradication and management, and supporting methods for monitoring and surveillance. These include a description of the method, evidence for its cost and cost-effectiveness, a measure for the confidence in the available information in each case and a list of bibliographic references.

For the eight species assessed under this study, there was increased emphasis on the inclusion of quantitative information on the costs of management and the maximum areas (in m², km², ha) over which successful management, particularly eradications, have been conducted. These were considered to be particularly useful to inform possible management decisions, however, it was accepted that the availability of published information on these topics, for selected species or their close relatives, was often limited. In cases where there are no methods available to meet a particular objective, or the available methods are only useful in limited circumstances, this should be clearly highlighted in the summary and in the text.

First drafts were produced by authors of the risk assessments and/or members of the project team, followed by an internal quality review and then external peer review. Revised drafts were then discussed at the Quality Assurance Workshop (task 5). This included a check on completeness, a simple analysis of content, and whether this was of a quantitative or qualitative nature. This highlighted a short-list of issues to consider in relation to the individual species accounts in subsequent break-out sessions. Following this, final drafts were produced and these are included as Annex 1b-8b to this Final Report.

Task 5: Quality Assurance Workshop

Task lead: Helen Roy

As a consequence of the COVID19-pandemic it was approved by the Commission to organize the Quality Assurance Workshop on 15 and 17 September 2021 as an online meeting. The aim of the workshop was to provide authors, peer-reviewers and the Commission the opportunity to discuss any unclarified issues, eliminate remaining uncertainties as far as possible, and assure the highest possible quality of the Risk Assessments and Risk Management Notes.

In preparation for the workshop, authors considered and incorporated comments from the peer-reviewers submitted in advance of the workshop. The comments from the peer-reviewers and the considerations from the authors are also documented within this report (see Task 3). At the workshop, all lead authors presented a summary of their work and open questions were discussed in virtual breakout groups and in the plenary. After the workshop, authors finalized the Risk Assessments and Risk Management Notes taking into account also further comments provided by the Commission.

The Agenda of the meeting was adjusted to the shorter duration compared to previous physical meetings in Brussels. After a short introduction and overview of the project by Wolfgang Rabitsch (Project lead), Björn Beckmann provided an overview of the species distribution modelling approach, followed by Pete Robertson providing an overview of the Management Annex. Then, lead authors of four of the eight Risk Assessments gave short summaries of the major sections and overall assessments of their species and explained any peculiarities and difficulties of the work. On the second day, the remaining four Risk Assessments were presented as on the first day.

Agenda of the virtual Quality Assurance Workshop:

Day 1 (15th September)		
Time	Agenda Item	Lead
0930	Registration / Log-in Zoom	
0945	Welcome and introduction	EC
1000	Overview of the project	Wolfgang Rabitsch
1015	Overview of modelling approaches	Björn Beckmann
1030	Overview of RMN	Pete Robertson
1045	Overview of selected (4) RA+RMN (10 minutes talk + 5 minutes general discussion per assessment)	RA lead authors
	<i>Bipalium kewense</i> (Kew Flatworm)	Leigh Winsor
	<i>Cervus nippon</i> (Sika Deer)	Riccardo Scalera
	<i>Cherax destructor</i> (Common Yabby)	Elena Tricarico
	<i>Mulinia lateralis</i> (Dwarf Surf Clam)	Christine Wood
1145	Break	
1200	Breakout groups to finalise RA+RMN	Groups

1330	Feedback from breakout groups including general discussion	
1415	Wrap-up and End of Day 1	Wolfgang Rabitsch

Day 2 (17th September)

Time	Agenda Item	Lead
0930	Registration / Log-in Zoom	
0945	Welcome	Wolfgang Rabitsch
0950	Overview of selected (5) RA+RMN (10 minutes talk + 5 minutes general discussion per assessment)	RA lead authors
	<i>Delairea odorata</i> (Cape Ivy)	Rob Tanner
	<i>Marisa cornuarietis</i> (Colombian Ramshorn Apple Snail)	Frances Lucy
	<i>Obama nungara</i> (Obama Flatworm)	Archie Murchie
	<i>Pycnonotus jocosus</i> (Red-whiskered Bulbul)	Tim Adriaens
1100	Breakout groups to finalise RA+RMN	Groups
1145	Break	
1200	Breakout groups to finalise RA+RMN (cont.)	Groups
1245	Feedback from breakout groups including general discussion	
1330	Wrap-up and End of Workshop	Wolfgang Rabitsch

Attendees of the Workshop:

Tim	Adriaens
Björn	Beckmann
Giuseppe	Brundu
Bram	D'Hondt
Siobhan	Edney
Thomas	Evans
Marika	Galanidi
Darren	Garland
Jean-Lou	Justine
Francis	Kerckhof
Antonin	Kouba
Helmut	Kudrnovsky
Frances	Lucy

Leonardo	Mazza
Archie	Murchie
Francisco	Oficialdegui
Juan	Perez-Lorenzo
Jodey	Peyton
Wolfgang	Rabitsch
Peter Andrew	Robertson
Riccardo	Scalera
Richard	Shaw
Wojtek	Solarz
Alan John	Stewart
Anthony	
Robert Anton	Tanner
Elena	Tricarico
Johan	Van Valkenburg
Hugo	Verreycken
Alastair	Ward
Leigh	Winsor
Christine	Wood

Conclusions

A few modifications were made to the Risk Assessment and Risk Management Note templates, but essentially they remained the same as in the first year of the contract.

The level of detail and consistency across the eight Risk Assessment and Risk Management Annexes has benefitted from the internal quality control and this feedback loop will be applied again in the third year of the project. Also, the use of structured “checklists” for peer-reviewers and authors after peer-review supported an effective and structured approach for collecting the necessary information for this Final Report and will be continued in the third year of the project.

Of the eight species assessed in this year of the project, four were deemed to constitute an overall “High risk”, three with “Medium” and one with “High” confidence. The overall risk for the four other species was deemed to be “Moderate”, with “Medium” and “Low” confidence for two species each (Tab. 3).

Table 3: Compilation of the responses (High, Moderate, Low) and confidence (High, Medium, Low) assigned within the conclusion of the risk assessments

#	Scientific Name	Common Name	Overall Risk	Confidence
1	<i>Bipalium kewense</i>	Kew flatworm	Moderate	Low
2	<i>Cervus nippon</i>	Sika deer	High	High
3	<i>Cherax destructor</i>	Common yabby	High	Medium
4	<i>Delairea odorata</i>	Cape ivy	Moderate	Low
5	<i>Marisa cornuarietis</i>	Colombian ramshorn apple snail	Moderate	Medium
6	<i>Mulinia lateralis</i>	Dwarf surf clam	Moderate	Medium
7	<i>Obama nungara</i>	Obama flatworm	High	Medium
8	<i>Pycnonotus jocosus</i>	Red-whiskered bulbul	High	Medium

Supplement Risk Assessment Template

Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention" Contract No 07.0202/2020/834529/ETU/ENV.D.2¹

Name of organism:

Author(s) of the assessment: *name, affiliation, city, country*

Risk Assessment Area: The risk assessment area is the territory of the European Union 27 and the United Kingdom, excluding the EU-outermost regions.

Peer review 1: *name, affiliation, city, country*

Peer review 2: *name, affiliation, city, country*

Document version history:

Version	Date	File Name	Author(s)	Status and description
0.1				First draft for internal review
1.0				First draft for peer-review
1.1				Draft for Workshop
2.0				Final RA within project year for submission to Scientific Forum
2.1				Updated version after comments from Scientific Forum and Stakeholders
3.0				Final RA for submission to Committee

¹ This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA). A number of amendments have been introduced to ensure compliance with Regulation (EU) 1143/2014 on IAS and relevant legislation, including the Delegated Regulation (EU) 2018/968 of 30 April 2018, supplementing Regulation (EU) No 1143/2014 of the European Parliament and of the Council with regard to risk assessments in relation to invasive alien species (see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>).

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General instructions:

- Completing risk assessments can be time consuming. Risk assessors and peer reviewers should read all questions before completing each assessment to determine where most detail needs to be provided.
- Responses and justifying comments should be concise and directly answer the question being asked.
- The risk assessment shall be based on the most reliable scientific information available, including the most recent results of international research, supported by references to peer reviewed scientific publications. In cases where there are no peer reviewed scientific publications or where the information provided by such publications is insufficient, or to supplement the information collected, the scientific evidence may also include other publications, expert opinions, information collected by Member States' authorities, official notifications and information from databases, including information collected through citizen science. All sources shall be acknowledged and referenced.
- All responses in the risk assessment shall be backed up by primary references. However, as the risk assessment is not a comprehensive review of the biology or ecology of the species but rather needs to assess the relevant information, references to major monographic reviews are acceptable for these points.
- The scoring of the magnitude of impacts (see Annex II) is not identical with the scoring of other risk assessment protocols. For example, the score “Major” in EICAT has a different meaning than “Major” in the present template. Assessors should not copy-paste scores from other protocols without explanations, specifically with regard to the assessment area, and follow the definitions as given in Annex II (see Qu. A.3).
- Questions in the risk assessment should be answered even where there is little information to support a response, with uncertainty in the response clearly discussed. Where there is such a lack of information, the assessor shall state this explicitly.
- The scoring of impacts does not take possible management actions into account.
- Certain questions are not accompanied by specific instructions or explanatory comments as these are sufficiently self-explanatory. Authors should not consider any such questions as less important. In case of doubt or uncertainty, authors may contact wolfgang.rabitsch@umweltbundesamt.at for clarification.
- Each answer provided in the risk assessment shall include an assessment of the level of confidence attached to that answer, reflecting the possibility that information needed for the answer is not available or is insufficient or the fact that the available evidence is conflicting. See Annex III for the documented method.
- The author(s) of the risk assessment and the peer reviewers shall not be affiliated to the same institution.

SECTION A – ORGANISM INFORMATION AND SCREENING

A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?

including the following elements:

- the taxonomic family, order and class to which the species belongs;
- the scientific name and author of the species, as well as a list of the most common synonym names;
- names used in commerce (if any)
- a list of the most common subspecies, lower taxa, varieties, breeds or hybrids

As a general rule, one risk assessment should be developed for a single species. However, there may be cases where it may be justified to develop one risk assessment covering more than one species (e.g. species belonging to the same genus with comparable or identical features and impact). It shall be clearly stated if the risk assessment covers more than one species, or if it excludes or only includes certain subspecies, lower taxa, hybrids, varieties or breeds (and if so, which subspecies, lower taxa, hybrids, varieties or breeds). Any such choice must be properly justified.

Response:

A2. Provide information on the existence of other species that look very similar [that may be detected in the risk assessment area, either in the environment, in confinement or associated with a pathway of introduction]

Include both native and non-native species that could be confused with the species being assessed, including the following elements:

- other alien species with similar invasive characteristics, to be avoided as substitute species (in this case preparing a risk assessment for more than one species together may be considered);
- other alien species without similar invasive characteristics, potential substitute species;
- native species, potential misidentification and mis-targeting

Response:

A3. Does a relevant earlier risk assessment exist? Give details of any previous risk assessment, including the final scores and its validity in relation to the risk assessment area.

Response:

A4. Where is the organism native?

including the following elements:

- an indication of the continent or part of a continent, climatic zone and habitat where the species is naturally occurring
- if applicable, indicate whether the species could naturally spread into the risk assessment area

Response:

A5. What is the global non-native distribution of the organism outside the risk assessment area?

Response:

A6. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species been recorded and where is it established? The information needs to be given separately for recorded (including casual or transient occurrences) and established occurrences. “Established” means the process of an alien species successfully producing viable offspring with the likelihood of continued survival².

A6a. Recorded: List regions

A6b. Established: List regions

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

- Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea.

Comment on the sources of information on which the response is based and discuss any uncertainty in the response.

For delimitation of EU biogeographical regions please refer to <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2> (see

² Convention on Biological Diversity, Decision VI/23

also Annex VI).

For delimitation of EU marine regions and subregions consider the Marine Strategy Framework Directive areas; please refer to <https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions/technical-document/pdf> (see also Annex VI).

Response (6a):

Response (6b):

A7. In which biogeographic region(s) or marine subregion(s) in the risk assessment area could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A7a. Current climate: List regions

A7b. Future climate: List regions

With regard to EU biogeographic and marine (sub)regions, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (7a):

Response (7b):

A8. In which EU Member States has the species been recorded and in which EU Member States has it established? List them with an indication of the timeline of observations. The information needs be given separately for recorded and established occurrences.

A8a. Recorded: List Member States

A8b. Established: List Member States

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland,

France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden; provide information for the United Kingdom if relevant

The description of the invasion history of the species shall include information on countries invaded and an indication of the timeline of the first observations, establishment and spread.

Response (8a):

Response (8b):

A9. In which EU Member States could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A9a. Current climate: List Member States

A9b. Future climate: List Member States

With regard to EU Member States, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (9a):

Response (9b):

A10. Is the organism known to be invasive (i.e. to threaten or adversely impact upon biodiversity and related ecosystem services) anywhere outside the risk assessment area?

Response:

A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea

Response:

A12. In which EU Member States has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden; provide information for the United Kingdom if relevant

Response:

A13. Describe any known socio-economic benefits of the organism.

including the following elements:

- Description of known uses for the species, including a list and description of known uses in the risk assessment area and third countries, if relevant.
- Description of social and economic benefits deriving from those uses, including a description of the environmental, social and economic relevance of each of those uses and an indication of associated beneficiaries, quantitatively and/or qualitatively depending on what information is available.

If the information available is not sufficient to provide a description of those benefits for the entire risk assessment area, qualitative data or different case studies from across the risk assessment area or third countries shall be used, if available.

Response:

SECTION B – DETAILED ASSESSMENT

Important instructions:

- In the case of lack of information the assessors are requested to use a standardized answer: “No information has been found.” In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable).
- With regard to the scoring of the likelihood of events or the magnitude of impacts see Annexes I and II.
- With regard to the confidence levels, see Annex III.
- Highlight the selected response score and confidence level in **bold** but keep the other scores in normal text (so that the selected score is evident in the final document).

1 PROBABILITY OF INTRODUCTION AND ENTRY

Important instructions:

- **Introduction** is the movement of the species into the risk assessment area (it may be either in captive conditions and/or in the environment, depending on the relevant pathways).
- **Entry** is the release/escape/arrival in the environment, i.e. occurrence in the wild
- Introduction and entry may coincide for species entering through pathways such as “corridor” or “unaided”, but it also may differ. If different, please consider all relevant pathways, both for the introduction into the risk assessment area and the entry in the environment.
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used (see Annex IV). For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document³ and the provided key to pathways⁴.
- For organisms which are already present (recorded or established) in the risk assessment area, the likelihood of introduction and entry should be scored as “very likely” by default.
- Repeated (independent) introductions and entries at separate locations in the risk assessment area should be considered here (see Qu. 1.7).

Qu. 1.1. List relevant pathways through which the organism could be introduced into the risk assessment area and/or enter into the environment. Where possible give details about the specific origins and end points of the pathways as well as a description of any associated commodities.

For each pathway answer questions 1.2 to 1.8 (copy and paste additional rows at the end of

³ <https://circabc.europa.eu/sd/a/7e5f0bd4-34e8-4719-a2f7-c0cd7ec6a86e/2020-CBD-pathways-interpretation.pdf>

⁴ <https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf>

this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 1.2a, 1.3a, etc. and then 1.2b, 1.3b etc. for the next pathway.

In this context a pathway is the route or mechanism of introduction and/or entry of the species.

The description of commodities with which the introduction of the species is generally associated shall include a list and description of commodities with an indication of associated risks (e.g. the volume of trade; the likelihood of a commodity being contaminated or acting as vector).

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 1.2-1.9.

Pathway name:

Qu. 1.2a. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional	CONFIDENCE	low
	unintentional		medium
			high

Response:

Qu. 1.3a. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely	CONFIDENCE	low
	unlikely		medium
	moderately likely		high
	likely		

	very likely		
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Response:

Qu. 1.4a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Qu. 1.5a. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Qu. 1.6a. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Qu. 1.7a. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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Response:

Qu. 1.8a. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

End of pathway assessment, repeat Qu. 1.3 to 1.8 as necessary using separate identifier.

Qu. 1.9. Estimate the overall likelihood of introduction into the risk assessment area or entry into the environment based on all pathways and specify if different in relevant biogeographical regions in current conditions.

Provide a thorough assessment of the risk of introduction in relevant biogeographical regions in current conditions: providing insight in to the risk of introduction into the risk assessment area.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Qu. 1.10. Estimate the overall likelihood of introduction into the risk assessment area or entry into the environment based on all pathways in foreseeable climate change conditions?

Thorough assessment of the risk of introduction in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of introduction (e.g. change in trade or user preferences)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely introduction within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

2 PROBABILITY OF ESTABLISHMENT

Important instructions:

- For organisms which are already established in parts of the risk assessment area or have previously been eradicated, the likelihood of establishment should be scored as “very likely” by default.
- Discuss the risk also for those parts of the risk assessment area, where the species is not yet established.

Qu. 2.1. How likely is it that the organism will be able to establish in the risk assessment area based on similarity of climatic and abiotic conditions in its distribution elsewhere in the world?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Qu. 2.2. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area? Consider if the organism specifically requires another species to complete its life cycle.

RESPONSE	very isolated isolated moderately widespread widespread ubiquitous	CONFIDENCE	low medium high
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Response:

Qu. 2.3. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely	CONFIDENCE	low medium high
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	likely very likely		
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Response:

Qu. 2.4. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Qu. 2.5. How likely is the organism to establish despite existing management practices in the risk assessment area? Explain if existing management practices could facilitate establishment.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Qu. 2.6. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Qu. 2.7. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?

including the following elements:

- a list and description of the reproduction mechanisms of the species in relation to the environmental conditions in the risk assessment area
- an indication of the propagule pressure of the species (e.g. number of gametes, seeds, eggs or propagules, number of reproductive cycles per year) of each of those reproduction mechanisms in relation to the environmental conditions in the risk assessment area.
- If relevant, comment on the likelihood of establishment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in establishment whereas for others high propagule pressure (many thousands of individuals) may not.
- If relevant, comment on the adaptability of the organism to facilitate its establishment and if low genetic diversity in the founder population would have an influence on establishment.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Qu. 2.8. If the organism does not establish, then how likely is it that casual populations will continue to occur?

Consider, for example, a species which cannot reproduce in the risk assessment area, because of unsuitable climatic conditions or host plants, but is present because of recurring introduction, entry and release events. This may also apply for long-living organisms.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Qu. 2.9. Estimate the overall likelihood of establishment in the risk assessment area under current climatic conditions. In addition, details of the likelihood of establishment

in relevant biogeographical regions under current climatic conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in current conditions: providing insight in the risk of establishment in (new areas in) the risk assessment area.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Qu. 2.10. Estimate the overall likelihood of establishment in the risk assessment area under foreseeable climate change conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under foreseeable climate change conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of establishment (e.g. increase in average winter temperature, increase in drought periods)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely establishment within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

3 PROBABILITY OF SPREAD

Important instructions:

- Spread is defined as the expansion of the geographical distribution of an alien species within the risk assessment area.
- Repeated releases at separate locations do not represent continuous spread and should be considered in the probability of introduction and entry section (Qu. 1.7).

Qu. 3.1. How important is the expected spread of this organism within the risk assessment area by natural means? (List and comment on each of the mechanisms for natural spread.)

including the following elements:

- a list and description of the natural spread mechanisms of the species in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of spread discussed in relation to the species biology and the environmental conditions in the risk assessment area.

The description of spread patterns here refers to the CBD pathway category “Unaided (Natural Spread)”. It should include elements of the species life history and behavioural traits able to explain its ability to spread, including: reproduction or growth strategy, dispersal capacity, longevity, dietary requirements, environmental and climatic requirements, specialist or generalist characteristics.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Qu. 3.2a. List and describe relevant pathways of spread other than "unaided". For each pathway answer questions 3.3 to 3.9 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 3.3a, 3.4a, etc. and then 3.3b, 3.4b etc. for the next pathway.

including the following elements:

- a list and description of pathways of spread with an indication of their importance and associated risks (e.g. the likelihood of spread in the risk assessment area, based on these pathways; likelihood of survival, or reproduction, or increase during transport and storage; ability and likelihood of transfer from the pathway to a suitable habitat or host) in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of spread for each pathway discussed in relation to the species biology and the environmental conditions in the risk assessment area.
- All relevant pathways of spread (except “Unaided (Natural Spread)”, which is

assessed in Qu. 3.1) should be considered. The classification of pathways developed by the Convention of Biological Diversity shall be used (see Annex IV).

Pathway name:

Qu. 3.3a. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional	CONFIDENCE	low
	unintentional		medium
			high

Response:

Qu. 3.4a. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely	CONFIDENCE	low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response:

Qu. 3.5a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Qu. 3.6a. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Qu. 3.7a. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Qu. 3.8a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Qu. 3.9a. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area.(please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response:

End of pathway assessment, repeat Qu. 3.3 to 3.9. as necessary using separate identifiers.

Qu. 3.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?

RESPONSE	very easy easy with some difficulty difficult very difficult	CONFIDENCE	low medium high
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Response:

Qu. 3.11. Estimate the overall potential rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (indicate any key issues and provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in current conditions, providing insight in the risk of spread into (new areas in) the risk assessment area.

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response:

Qu. 3.12. Estimate the overall potential rate of spread in relevant biogeographical regions in foreseeable climate change conditions (provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if rates of spread are likely slowed down or accelerated.

RESPONSE		CONFIDENCE	
	very slowly		low
	slowly		medium
	moderately		high
	rapidly		
	very rapidly		

Response:

4 MAGNITUDE OF IMPACT

Important instructions:

- Questions 4.1-4.5 relate to biodiversity and ecosystem impacts, 4.6-4.8 to impacts on ecosystem services, 4.9-4.13 to economic impact, 4.14-4.15 to social and human health impact, and 4.16-4.18 to other impacts. These impacts can be interlinked, for example, a disease may cause impacts on biodiversity and/or ecosystem functioning that leads to impacts on ecosystem services and finally economic impacts. In such cases the assessor should try to note the different impacts where most appropriate, cross-referencing between questions when needed.
- Each set of questions starts with the impact elsewhere in the world, then considers impacts in the risk assessment area (=EU excluding outermost regions) separating known impacts to date (i.e. past and current impacts) from potential future impacts (including foreseeable climate change).
- Only negative impacts are considered in this section (socio-economic benefits are considered in Qu. A.7)
- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable).

Biodiversity and ecosystem impacts

Qu. 4.1. How important is the impact of the organism on biodiversity at all levels of organisation caused by the organism in its non-native range excluding the risk assessment area?

including the following elements:

- Biodiversity means the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems
- impacted chemical, physical or structural characteristics and functioning of ecosystems

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Qu. 4.2. How important is the current known impact of the organism on biodiversity at all levels of organisation (e.g. decline in native species, changes in native species communities, hybridisation) in the risk assessment area (include any past impact in your response)?

Discuss impacts that are currently occurring or are likely occurring or have occurred in the past in the risk assessment area. Where there is no direct evidence of impact in the risk assessment area (for example no studies have been conducted), evidence from outside of the risk assessment area can be used to infer impacts within the risk assessment area.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Qu. 4.3. How important is the potential future impact of the organism on biodiversity at all levels of organisation likely to be in the risk assessment area?

See comment above. The potential future impact shall be assessed only for the risk assessment area. A potential increase in the distribution range due to climate change does not *per se* justify a higher impact score.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Qu. 4.4. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism currently in the risk assessment area?

including the following elements:

- native species impacted, including red list species, endemic species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy

Framework Directive

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

<p>Qu. 4.5. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism likely to be in the future in the risk assessment area?</p> <ul style="list-style-type: none"> • See guidance to Qu. 4.3. and 4.4.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Ecosystem Services impacts

<p>Qu. 4.6. How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?</p> <ul style="list-style-type: none"> • For a list of services use the CICES classification V5.1 provided in Annex V. • Impacts on ecosystem services build on the observed impacts on biodiversity (habitat, species, genetic, functional) but focus exclusively on reflecting these changes in relation to their links with socio-economic well-being. • Quantitative data should be provided whenever available and references duly reported.
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RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Qu. 4.7. How important is the impact of the organism on provisioning, regulating, and cultural services currently in the different biogeographic regions or marine sub-regions where the species has established in the risk assessment area (include any past impact in your response)?

- See guidance to Qu. 4.6.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Qu. 4.8. How important is the impact of the organism on provisioning, regulating, and cultural services likely to be in the different biogeographic regions or marine sub-regions where the species can establish in the risk assessment area in the future?

- See guidance to Qu. 4.6.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Economic impacts

Qu. 4.9. How great is the overall economic cost caused by the organism within its current area of distribution (excluding the risk assessment area), including both costs of / loss due to damage and the cost of current management.

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the world these should be reported here. The assessment of the potential costs of / loss due to damage shall describe those costs quantitatively and/or qualitatively depending on what information is available. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage. As far as possible, it would be useful to separate costs of / loss due to the organism from costs of current management.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Qu. 4.10. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism currently in the risk assessment area (include any past costs in your response)?

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the EU these should be reported here. Assessment of the potential costs of damage on human health, safety, and the economy, including the cost of non-action. A full economic assessment at EU scale might not be possible, but qualitative data or different case studies from across the EU (or third countries if relevant) may provide useful information to inform decision making. In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable). Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Qu. 4.11. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.10.

RESPONSE	minimal minor moderate major	CONFIDENCE	low medium high
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	massive		
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Response:

Qu. 4.12. How great are the economic costs / losses associated with managing this organism currently in the risk assessment area (include any past costs in your response)?

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable).

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Qu. 4.13. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.12.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Social and human health impacts

Qu. 4.14. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism for the risk assessment area and for third countries, if relevant (e.g. with similar eco-climatic conditions).

The description of the known impact and the assessment of potential future impact on human

health, safety and the economy, shall, if relevant, include information on

- illnesses, allergies or other affections to humans that may derive directly or indirectly from a species;
- damages provoked directly or indirectly by a species with consequences for the safety of people, property or infrastructure;
- direct or indirect disruption of, or other consequences for, an economic or social activity due to the presence of a species.

Social and human health impacts can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Qu. 4.15. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism in the future for the risk assessment area.

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable).

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Other impacts

Qu. 4.16. How important is the organism in facilitating other damaging organisms (e.g. diseases) as food source, a host, a symbiont or a vector etc.?

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Qu. 4.17. How important might other impacts not already covered by previous questions be resulting from introduction of the organism?

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Qu. 4.18. How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area?

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Qu. 4.19. Estimate the overall impact in the risk assessment area under current climate conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, in current conditions.

RESPONSE	minimal minor	CONFIDENCE	low medium
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	moderate major massive		high
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Response:

Qu. 4.20. Estimate the overall impact in the risk assessment area in foreseeable climate change conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, under future conditions.

- See also guidance to Qu. 4.3.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

RISK SUMMARIES			
	RESPONSE	CONFIDENCE	COMMENT
Summarise Introduction and Entry*	very unlikely unlikely moderately likely likely very likely	low medium high	Provide a comprehensive short summary of your response to Questions 1.8-1.9.
Summarise Establishment*	very unlikely unlikely moderately likely likely very likely	low medium high	Provide a comprehensive short summary of your response to Questions 3.13-3.14.
Summarise Spread*	very slowly slowly moderately rapidly very rapidly	low medium high	Provide a comprehensive short summary of your response to Questions 4.11-4.12.
Summarise Impact*	minimal minor moderate major massive	low medium high	Provide a comprehensive short summary of your response to Question 5.19-5.20.
Conclusion of the risk assessment (overall risk)	low moderate high	low medium high	The combination of specific elements (scores) of a risk assessment into a final overall score is difficult. There is no accepted or agreed (or correct) formula or decision protocol for this final step. This risk assessment template uses many different sources of information to deliver assessment scores along the invasion continuum (introduction, entry, establishment, spread, impact), which are not necessarily equal. The conclusion of the risk assessment, however, needs to match the scores of the specific elements in a consistent and sensible way and requires justification of the overall risk.

*in current climate conditions and in foreseeable future climate conditions

REFERENCES

Distribution Summary

Please answer as follows:

- Yes if recorded, established or invasive
- if not recorded, established or invasive
- ? Unknown; data deficient

The columns refer to the answers to Questions A5 to A12 under Section A.

For data on marine species at the Member State level, delete Member States that have no marine borders. In all other cases, provide answers for all columns.

EU-Member States and the United Kingdom

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Austria					
Belgium					
Bulgaria					
Croatia					
Cyprus					
Czech Republic					
Denmark					
Estonia					
Finland					
France					
Germany					
Greece					
Hungary					
Ireland					
Italy					
Latvia					
Lithuania					
Luxembourg					
Malta					
Netherlands					
Poland					
Portugal					
Romania					
Slovakia					
Slovenia					
Spain					
Sweden					
United Kingdom					

Biogeographical regions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Alpine					
Atlantic					
Black Sea					
Boreal					
Continental					
Mediterranean					
Pannonian					
Steppic					

Marine regions and subregions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Baltic Sea					
Black Sea					
North-east Atlantic Ocean					
Bay of Biscay and the Iberian Coast					
Celtic Sea					
Greater North Sea					
Mediterranean Sea					
Adriatic Sea					
Aegean-Levantine Sea					
Ionian Sea and the Central Mediterranean Sea					
Western Mediterranean Sea					

ANNEX I Scoring of Likelihoods of Events

(taken from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Description	Frequency
Very unlikely	This sort of event is theoretically possible, but is never known to have occurred and is not expected to occur	1 in 10,000 years
Unlikely	This sort of event has occurred somewhere at least once in the last millenium	1 in 1,000 years
Moderately Likely	This sort of event has occurred somewhere at least once in the last century	1 in 100 years
Likely	This sort of event has happened on several occasions elsewhere, or on at least once in the last decade	1 in 10 years
Very likely	This sort of event happens continually and would be expected to occur	Once a year

ANNEX II Scoring of Magnitude of Impacts

(modified from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Biodiversity and ecosystem impact	Ecosystem Services impact	Economic impact (Monetary loss and response costs per year)	Social and human health impact, and other impacts
	<i>Question 5.1-5</i>	<i>Question 5.6-8</i>	<i>Question 5.9-13</i>	<i>Question 5.14-18</i>
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected ⁵	Up to 10,000 Euro	No social disruption. Local, mild, short-term reversible effects to individuals.
Minor	Some ecosystem impact, reversible changes, localised	Local and temporary, reversible effects to one or few services	10,000-100,000 Euro	Significant concern expressed at local level. Mild short-term reversible effects to identifiable groups, localised.
Moderate	Measureable long-term damage to populations and ecosystem, but reversible; little spread, no extinction	Measureable, temporary, local and reversible effects on one or several services	100,000-1,000,000 Euro	Temporary changes to normal activities at local level. Minor irreversible effects and/or larger numbers covered by reversible effects, localised.
Major	Long-term irreversible ecosystem change, spreading beyond local area	Local and irreversible or widespread and reversible effects on one / several services	1,000,000-10,000,000 Euro	Some permanent change of activity locally, concern expressed over wider area. Significant irreversible effects locally or reversible effects over large area.
Massive	Widespread, long-term population loss or extinction, affecting several species with serious ecosystem effects	Widespread and irreversible effects on one / several services	Above 10,000,000 Euro	Long-term social change, significant loss of employment, migration from affected area. Widespread, severe, long-term, irreversible health effects.

⁵ Not to be confused with “no impact”.

ANNEX III Scoring of Confidence Levels

(modified from Bacher et al. 2017)

Each answer provided in the risk assessment must include an assessment of the level of confidence attached to that answer, reflecting the possibility that information needed for the answer is not available or is insufficient or available but conflicting.

The responses in the risk assessment should clearly support the choice of the confidence level.

Confidence level	Description
Low	There is no direct observational evidence to support the assessment, e.g. only inferred data have been used as supporting evidence <i>and/or</i> Impacts are recorded at a spatial scale which is unlikely to be relevant to the assessment area <i>and/or</i> Evidence is poor and difficult to interpret, e.g. because it is strongly ambiguous <i>and/or</i> The information sources are considered to be of low quality or contain information that is unreliable.
Medium	There is some direct observational evidence to support the assessment, but some information is inferred <i>and/or</i> Impacts are recorded at a small spatial scale, but rescaling of the data to relevant scales of the assessment area is considered reliable, or to embrace little uncertainty <i>and/or</i> The interpretation of the data is to some extent ambiguous or contradictory.
High	There is direct relevant observational evidence to support the assessment (including causality) <i>and</i> Impacts are recorded at a comparable scale <i>and/or</i> There are reliable/good quality data sources on impacts of the taxa <i>and</i> The interpretation of data/information is straightforward <i>and/or</i> Data/information are not controversial or contradictory.

ANNEX IV CBD pathway categorisation scheme

Overview of CBD pathway categorisation scheme showing how the 44 pathways relate to the six main pathway categories. All of the pathways can be broadly classified into 1) those that involve intentional transport (blue), 2) those in which the taxa are unintentionally transported (green) and 3) those where taxa moved between regions without direct transportation by humans and/or via artificial corridors (orange and yellow). **Note that the pathways in the category “Escape from confinement” can be considered intentional for the introduction into the risk assessment area and unintentional for the entry into the environment.**



ANNEX V Ecosystem services classification (CICES V5.1, simplified) and examples

For the purposes of this risk assessment, please feel free to use what seems as the most appropriate category / level / combination of impact (Section – Division – Group), reflecting information available.

Section	Division	Group	Examples (i.e. relevant CICES “classes”)
Provisioning	Biomass	Cultivated <i>terrestrial</i> plants	Cultivated terrestrial plants (including fungi, algae) grown for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from cultivated plants, fungi, algae and bacteria for direct use or processing (excluding genetic materials); Cultivated plants (including fungi, algae) grown as a <u>source of energy</u> <i>Example: negative impacts of non-native organisms to crops, orchards, timber etc.</i>
		Cultivated <i>aquatic</i> plants	Plants cultivated by in- situ aquaculture grown for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from in-situ aquaculture for direct use or processing (excluding genetic materials); Plants cultivated by in- situ aquaculture grown as an <u>energy source</u> . <i>Example: negative impacts of non-native organisms to aquatic plants cultivated for nutrition, gardening etc. purposes.</i>
		Reared animals	Animals reared for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from reared animals for direct use or processing (excluding genetic materials); Animals reared to provide <u>energy</u> (including mechanical) <i>Example: negative impacts of non-native organisms to livestock</i>
		Reared <i>aquatic</i> animals	Animals reared by in-situ aquaculture for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from animals grown by in-situ aquaculture for direct use or processing (excluding genetic materials); Animals reared by in-situ aquaculture as an <u>energy source</u> <i>Example: negative impacts of non-native organisms to fish farming</i>
		Wild plants (terrestrial and aquatic)	Wild plants (terrestrial and aquatic, including fungi, algae) used for <u>nutrition</u> ; <u>Fibres and other materials</u> from wild plants for direct use or processing (excluding genetic materials); Wild plants (terrestrial and aquatic, including fungi, algae) used as a <u>source of energy</u> <i>Example: reduction in the availability of wild plants (e.g. wild berries, ornamentals) due to non-native organisms (competition, spread of disease etc.)</i>
	Wild animals (terrestrial and aquatic)	Wild animals (terrestrial and aquatic) used for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from wild animals for direct use or processing (excluding genetic materials); Wild animals (terrestrial and aquatic) used as a <u>source of energy</u> <i>Example: reduction in the availability of wild animals (e.g. fish stocks, game) due to non-native organisms (competition, predations, spread of disease etc.)</i>	
	Genetic material from all biota	Genetic material from plants, algae or fungi	<u>Seeds, spores and other plant materials</u> collected for maintaining or establishing a population; Higher and lower plants (whole organisms) used to <u>breed new strains or varieties</u> ; Individual genes extracted from higher and lower plants for the

			<p><u>design and construction of new biological entities</u></p> <p><i>Example: negative impacts of non-native organisms due to interbreeding</i></p>
		Genetic material from animals	<p>Animal material collected for the purposes of maintaining or establishing a population; Wild animals (whole organisms) used to breed new strains or varieties; Individual genes extracted from organisms for the design and construction of new biological entities</p> <p><i>Example: negative impacts of non-native organisms due to interbreeding</i></p>
	Water⁶	Surface water used for nutrition, materials or energy	<p>Surface water for <u>drinking</u>; Surface water used as a material (<u>non-drinking purposes</u>); Freshwater surface water, coastal and marine water used as an <u>energy source</u></p> <p><i>Example: loss of access to surface water due to spread of non-native organisms</i></p>
		Ground water for used for nutrition, materials or energy	<p>Ground (and subsurface) water for <u>drinking</u>; Ground water (and subsurface) used as a material (<u>non-drinking purposes</u>); Ground water (and subsurface) used as an <u>energy source</u></p> <p><i>Example: reduced availability of ground water due to spread of non-native organisms and associated increase of ground water consumption by vegetation.</i></p>
Regulation & Maintenance	Transformation of biochemical or physical inputs to ecosystems	Mediation of wastes or toxic substances of anthropogenic origin by living processes	<p><u>Bio-remediation</u> by micro-organisms, algae, plants, and animals; <u>Filtration/sequestration/storage/accumulation</u> by micro-organisms, algae, plants, and animals</p> <p><i>Example: changes caused by non-native organisms to ecosystem functioning and ability to filtrate etc. waste or toxics</i></p>
		Mediation of nuisances of anthropogenic origin	<p><u>Smell reduction; noise attenuation; visual screening</u> (e.g. by means of green infrastructure)</p> <p><i>Example: changes caused by non-native organisms to ecosystem structure, leading to reduced ability to mediate nuisances.</i></p>
	Regulation of physical, chemical, biological conditions	Baseline flows and extreme event regulation	<p>Control of <u>erosion</u> rates; Buffering and attenuation of <u>mass movement</u>; <u>Hydrological cycle and water flow regulation</u> (Including flood control, and coastal protection); <u>Wind</u> protection; <u>Fire</u> protection</p> <p><i>Example: changes caused by non-native organisms to ecosystem functioning or structure leading to, for example, destabilisation of soil, increased risk or intensity of wild fires etc.</i></p>
		Lifecycle maintenance, habitat and gene pool protection	<p><u>Pollination</u> (or 'gamete' dispersal in a marine context); <u>Seed dispersal</u>; Maintaining <u>nursery populations and habitats</u> (Including gene pool protection)</p> <p><i>Example: changes caused by non-native organisms to the abundance and/or distribution of wild pollinators; changes to the availability / quality of nursery habitats for fisheries</i></p>
	Pest and disease control	<p>Pest control; Disease control</p> <p><i>Example: changes caused by non-native organisms to the</i></p>	

⁶ Note: in the CICES classification provisioning of water is considered as an abiotic service whereas the rest of ecosystem services listed here are considered biotic.

			abundance and/or distribution of pests
		Soil quality regulation	<p><u>Weathering processes</u> and their effect on soil quality; <u>Decomposition and fixing processes</u> and their effect on soil quality</p> <p><i>Example: changes caused by non-native organisms to vegetation structure and/or soil fauna leading to reduced soil quality</i></p>
		Water conditions	<p>Regulation of the <u>chemical condition</u> of freshwaters by living processes; Regulation of the chemical condition of salt waters by living processes</p> <p><i>Example: changes caused by non-native organisms to buffer strips along water courses that remove nutrients in runoff and/or fish communities that regulate the resilience and resistance of water bodies to eutrophication</i></p>
		Atmospheric composition and conditions	<p>Regulation of <u>chemical composition</u> of atmosphere and oceans; Regulation of <u>temperature and humidity</u>, including ventilation and transpiration</p> <p><i>Example: changes caused by non-native organisms to ecosystems' ability to sequester carbon and/or evaporative cooling (e.g. by urban trees)</i></p>
Cultural	Direct, in-situ and outdoor interactions with living systems that depend on presence in the environmental setting	Physical and experiential interactions with natural environment	<p>Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through <u>active or immersive interactions</u>;</p> <p>Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through <u>passive or observational interactions</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that make it attractive for recreation, wild life watching etc.</i></p>
		Intellectual and representative interactions with natural environment	<p>Characteristics of living systems that enable <u>scientific investigation</u> or the creation of traditional ecological knowledge;</p> <p>Characteristics of living systems that enable <u>education and training</u>;</p> <p>Characteristics of living systems that are resonant in terms of <u>culture or heritage</u>;</p> <p>Characteristics of living systems that enable <u>aesthetic experiences</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have cultural importance</i></p>
	Indirect, remote, often indoor interactions with living systems that do not require presence in the environmental setting	Spiritual, symbolic and other interactions with natural environment	<p>Elements of living systems that have <u>symbolic meaning</u>;</p> <p>Elements of living systems that have <u>sacred or religious meaning</u>;</p> <p>Elements of living systems used for <u>entertainment or representation</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have sacred or religious meaning</i></p>
		Other biotic characteristics that have a non-use value	<p>Characteristics or features of living systems that have an <u>existence value</u>;</p> <p>Characteristics or features of living systems that have an <u>option or bequest value</u></p> <p><i>Example: changes caused by non-native organisms to ecosystems designated as wilderness areas, habitats of endangered species etc.</i></p>

ANNEX VI EU Biogeographic Regions and MSFD Subregions

See <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2> ,
http://ec.europa.eu/environment/nature/natura2000/biogeog_regions/

and

<https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions-1/technical-document/pdf>

ANNEX VII Delegated Regulation (EU) 2018/968 of 30 April 2018

see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>

Supplement Risk Management Notes Template

Annex with evidence on measures and their implementation cost and cost-effectiveness

Species (scientific name)	
Species (common name)	
Author(s)	
Reviewer	
Date Completed	

<ul style="list-style-type: none"> Summary¹ Highlight of measures that provide the most cost-effective options to prevent the introduction, achieve early detection, rapidly eradicate and manage the species, including significant gaps in information or knowledge to identify cost-effective measures.

Detailed assessment			
	Description of measures²	Assessment of implementation cost and cost-effectiveness (per measure)³	Level of confidence⁴
Methods to achieve prevention of intentional and unintentional introduction⁵			
Methods to achieve early detection⁶			
Methods to achieve eradication⁷			
Methods to achieve management⁸			

References

Guidelines for Completing the Annex

1 Provide a brief summary description of the most cost-effective methods drawing on the reviews in the detailed assessments

2 Provide a description of the potential method. This should be based on the available key scientific evidence which should be gathered from sources including articles and reviews in technical and scientific journals, internet searches, online databases, grey literature and relevant books and personal communications from scientists, stakeholders, conservation practitioners and governmental bodies. This information should include a full bibliographic list detailing the literature and sources considered.

3 Provide an assessment of the likely cost and effectiveness of the method. Where information is available, consider the following range of questions, accepting that not all questions will be appropriate in all circumstances.

- How effective has this approach proven to be in the past or in an analogous situation?
- How publically acceptable is the approach likely to be?
- Over what period of time would this approach need to be applied to be effective?
- What is the direct cost of implementing this approach?
- How likely are the methods used in the approach to be available?
- How likely is it that relevant licences or other approvals to undertake the approach would be difficult to obtain?
- How likely is it that health and safety issues would prevent the use of this approach?
- How significant is the environmental harm caused by this approach?
- How significant is the economic harm caused by this approach?
- How significant is the social harm caused by this approach?
- How likely is it that the approach will be criticised on welfare grounds?
- How likely is it that the approach will be acceptable to other stakeholders?

Where available, factual information on the costs of specialist equipment, or case studies of management costs from across the Union or third countries should be provided. When describing case studies, if the information is available then provide both total cost and the area over which control was undertaken so that a cost per unit area might be derived. Where such quantitative information is not available, then any qualitative information from the literature is acceptable to help guide decision making. It is accepted that in the majority of cases the information required to assess the potential total cost of management at a member state level is unlikely to be available. This would normally require information on the extent and abundance of the species which is beyond the scope of this assessment. Assessors are not expected to extrapolate the potential total costs of management at a member state level, only to report on the information provided within the literature.

4 Provide an overall assessment of the confidence that can be applied to the information provided for this method. This confidence should relate to the quality of the available information using the guidance below. It should NOT relate to the confidence in the effectiveness of the method

The confidence scores are:

- High: Information comes from published material, or current practices based on expert experience applied in one of the EU countries or third country with similar environmental, economic and social conditions.
- Medium: Information comes from published data or expert opinion, but it is not commonly applied, or it is applied in regions that may be too different from Europe (e.g. tropical regions) to guarantee that the results will be transposable.
- Low: data are not published in reliable information sources and methods are not commonly practiced or are based solely on opinion; This is for example the case of a novel situation where there is little evidence on which to base an assessment.

If there are further factors beyond these that have determined the chosen level of confidence, then provide a brief written description to support the choice of the level of confidence.

5 Measures for preventing the species being introduced intentionally or unintentionally into the territory of a Member State (cf. Articles 7(1)(a), 7(2), 13, 15), i.e. preventing a species entering by blocking its pathways, including pre-border prevention and prevention of escape or release into the environment and secondary spread whenever relevant. Measures for preventing the species reproducing while in containment should be included, if appropriate. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

6 Measures to run an effective surveillance system for achieving an early detection of a new occurrence (cf. Article 16). This section assumes that the species is not currently present in a Member State, or part of a Member State's territory.

7 Measures to achieve eradication. Preferably, but not exclusively, used at an early stage of invasion, after an early detection of a new occurrence (cf. Article 17). Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

8 Measures to achieve management of the species once it has become widely spread within a Member State territory (cf. Article 19). These measures can be aimed at eradication, population control or containment of a population of the species. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

The development and completion of this template forms part of the EU project No 07.0202/2020/834529/ETU/ENV.D.2 Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention.

ANNEXES TO THIS FINAL REPORT

**Risk assessment template developed under the "Study on Invasive Alien Species –
Development of risk assessments to tackle priority species and enhance prevention"
Contract No 07.0202/2020/834529/ETU/ENV.D.2¹**

Name of organism: *Bipalium kewense* Moseley, 1878

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Risk Assessment Area: The risk assessment area is the territory of the European Union 27 and the
United Kingdom, excluding the EU-outermost regions.

Peer review 1: Dr Richard Shaw, CABI, Egham, UK

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Date of completion: 15/10/2021

¹ This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA). A number of amendments have been introduced to ensure compliance with Regulation (EU) 1143/2014 on IAS and relevant legislation, including the Delegated Regulation (EU) 2018/968 of 30 April 2018, supplementing Regulation (EU) No 1143/2014 of the European Parliament and of the Council with regard to risk assessments in relation to invasive alien species (see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>).

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SECTION A – Organism Information and Screening

A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?

including the following elements:

- the taxonomic family, order and class to which the species belongs;
- the scientific name and author of the species, as well as a list of the most common synonym names;
- names used in commerce (if any)
- a list of the most common subspecies, lower taxa, varieties, breeds or hybrids

As a general rule, one risk assessment should be developed for a single species. However, there may be cases where it may be justified to develop one risk assessment covering more than one species (e.g. species belonging to the same genus with comparable or identical features and impact). It shall be clearly stated if the risk assessment covers more than one species, or if it excludes or only includes certain subspecies, lower taxa, hybrids, varieties or breeds (and if so, which subspecies, lower taxa, hybrids, varieties or breeds). Any such choice must be properly justified.

Response:

Bipalium kewense Moseley, 1868

Phylum Platyhelminthes, Class Rhabditophora, Order Tricladida, Sub-Order Continenticola, Family Geoplanidae, Subfamily Rhynchodeminae

Synonyms: *Bipalium kewense* Moseley var *viridis* Lehnert; *Placocephalus kewensis* (Moseley, 1878), *Placocephalus isabellinus* Geba, 1909; *Bipalium costaricensis* Hyman, 1939. A comprehensive synonymy is available (Winsor, 1983).

The organism is a single taxonomic entity. There are no known valid varieties, breeds, or hybrids. COI sequences for *Bipalium kewense* suggest that it is clonal, with identical molecular records from several continents (Justine *et al.*, 2018).

Common names: Kew flatworm, Shovel-headed Garden worm; Hammerhead flatworm; Hamerhoofdlandplatworm (NL); Hammerhaidwurm (DE).

A2. Provide information on the existence of other species that look very similar [that may be detected in the risk assessment area, either in the environment, in confinement or associated with a pathway of introduction]

Include both native and non-native species that could be confused with the species being assessed, including the following elements:

- other alien species with similar invasive characteristics, to be avoided as substitute species (in this case preparing a risk assessment for more than one species together may be considered);
- other alien species without similar invasive characteristics, potential substitute species;
- native species, potential misidentification and mis-targeting

Response:

Bipalium kewense is a long, thin flatworm, whose body shape is characterized by a typical “hammerhead” (see Figure 1, *Bipalium kewense* from Townsville, tropical north Queensland, Australia Life size). Living specimens may attain a length of up to 450 mm. The dorsal ground colour is usually a light ochre, with five black to grey-brown-coloured longitudinal stripes: a median, paired lateral, and paired marginal stripes which all begin at or near the base of the headplate where the latter joins the body at the “neck”. The headplate is usually the same colour as the body, or slightly darker, with recurved posterior margins. The median stripe is black, narrow, with sharp margins, extending caudally from below the neck over the entire body length, and is broadest over the pharyngeal area. Paired dark to pale brown-coloured lateral stripes with diffuse margins, constant over the entire body length, are separated from the median and marginal stripes by an equal width of ground colour. The paired black, fine, marginal stripes, with sharp margins, extend the entire body length. The paired lateral and marginal stripes unite just behind the neck to form an incomplete black transverse neck band, interrupted dorsally by a small median gap, and ventrally by the creeping sole. The headplate is a greyish colour with a light ochre margin. The ventral surface is a light ochre colour, with a distinct off-white creeping sole, delineated by paired, narrow, longitudinal diffuse grey-violet stripes beginning at the ventral termination of the collar, and extending the entire body length (Justine *et al.*, 2018). Variations observed in the external morphology of *B. kewense* have been described by Winsor (1983a). A specimen 450 mm long, and 7.6 mm wide weighed 1.5 g (Daly *et al.*, 1976). Juvenile specimens hatched from an egg cocoon have the same dark collar and paired dorsal medio-lateral stripes as the adults, some may exhibit a dark mid-dorsal stripe, but do not exhibit the paired marginal stripes. The headplate is lightly coloured, darker around the anterior margin; the mass of juveniles varied from 7.5 mg – 14.7 mg (Ducey *et al.*, 2006). The ground colour and darker longitudinal stripes of juveniles develop further with age.



The egg capsule or cocoon of *B. kewense* measures 5 – 6 mm diameter in two successive lays over a week (specimen from New Orleans, Louisiana, USA, Connella and Stern, 1969), and 9.5 mm diameter declining to 3 mm diameter in nine successive lays over 103 days from a single copulation, with cocoons 3 mm - 6 mm sterile (specimen from Charters Towers, Queensland, Australia, Winsor, 1984, unpubl.). The cocoon wall is stabilized by quinone tanning on exposure to air, usually within 24 hours changing in colour from lemon when freshly laid by the planarian through cherry-red, tan, dark brown then black, as has been described for species in all the subfamilies of the Geoplanidae (Winsor, 1998).

Morphologically, *B. kewense* is differentiated externally from similar striped species by the incomplete (or interrupted) black transverse band at the neck (the “collar”), the thin dorsal median longitudinal stripe that begins at or below the transverse neck band, the pattern and form of the dorsal and ventral stripes, and the relative position of body apertures. A detailed comparison with similar bipaliine taxa is provided elsewhere (Winsor 1983a; Justine *et al.*, 2018).

Bipalium kewense cannot be confused with any native European land flatworm species as none of the latter possess the characteristic bipaliine headplate (hammerhead). The species can be confused with *Diversibipalium multilineatum*, another five striped bipaliine species originally from Japan (Kawakatsu *et al.*, 2001), with similar characteristics, also recorded in Europe (Justine *et al.*, 2018). Corrêa (1947) considered that the juvenile *B. kewense* of Arndt (1934, Fig. 5a) collected from the Berlin Botanic Gardens to be a *Dolichoplana* (Rhynchodemini), because juvenile *B. kewense* have a headplate the same as an adult specimen and are not tapered anteriorly as in species of *Dolichoplana*. Following examination of the specimen it was later identified as *Dolichoplana feildeni* (syn *D. striata*), a non-bipaliine species alien in Europe (Winsor, 1983a).

A3. Does a relevant earlier risk assessment exist? Give details of any previous risk assessment, including the final scores and its validity in relation to the risk assessment area.

Response:

There are no qualitative or quantitative risk assessments available for this species.

A4. Where is the organism native?

including the following elements:

- an indication of the continent or part of a continent, climatic zone and habitat where the species is naturally occurring.
- if applicable, indicate whether the species could naturally spread into the risk assessment area

Response:

The natural range of *Bipalium kewense*, appears to extend from northern Vietnam south to Cambodia. There is some uncertainty in determining the likely natural range of *B. kewense*, and the co-occurrence of other Bipaliine species assists in differentiating areas of possible natural occurrences from areas where the species may have been introduced. Records of non-sexual specimens in Malaysia and Indonesia may represent southern extensions in this species natural range (Winsor, 1983a). Records from Thailand may represent the western extension of its range. All the records of *B. kewense* in Singapore appear to be from urban locations and are possibly introductions.

Based upon the occurrence of sexual and non-sexual specimens of *B. kewense* obtained from the central highlands of Vietnam at 1300 – 2000 m elevation, where they were found together with a suite

of species of Bipaliinae with similar occurrences, de Beauchamp (1939, 1961) considered that *B. kewense* originated from Vietnam. At that time, this area was relatively remote, with an average density of under 10 inhabitants per sq. km, areas of which were used for big game hunting, with a tea plantation at 1500 m, and coffee plantation at 600 m (Stuttard, 1943), and within the area of the natural occurrence of the Bipaliinae that extends throughout the Indomalayan zoogeographic realm. Non-sexual specimens have also been recorded in Ninh Binh, northern Vietnam.

The Central Highlands region in Vietnam is characterized by a Tropical Monsoon climate (“Am” in the Köppen-Geiger climate classification), and most months in the year are marked by significant rainfall, with a short dry season. The minimum temperatures range from 14.5°C (January) to 18.7°C (May), and maximum temperatures range from 23.5°C (November) to 27.1 °C (March), with precipitation ranging from 27 mm (February) to 297 mm (September), with about 2161 mm annually (data for Dalat at 1,486 m elevation from Climate-data.org 2020). Lower temperatures would be expected at the higher altitudes around 2000 m elevation. Seasonal evaporation in the highlands is dependent upon the relative humidity, cloudiness, and winds. The high plateaux of Vietnam that include the Biosphere Reserve Lang Bien, are characterized by local winds that differ in their excessive aridity from the general character of the monsoon (Stoddard, 1943).

Several forest systems are present in the area, the composition of which depends upon altitude, aspect, soil nutrients, and other factors. The vegetation of the area is characterized as lower montane forests (1000 m – 1800 m elevation) (Tran Van Do *et al.*, 2017). The plant family best represented in the upper canopy of these forests is the Fagaceae, together with the Magnoliaceae, Aceraceae, Podocarpaceae, Lauraceae, and Theaceae. The diversity of conifers is high in montane forests, with five genera present (*Podocarpus* sensu latu., *Calocedrus*, *Fokienia*, *Cephalotaxus*, and *Taxus*). Several important endemic species are present, including *Pinus dalatensis* and *P. krempfii*. Epiphytes form a notable part of the biodiversity of these montane forests. There is high diversity of the orchids in the upper canopy and ferns in the middle and lower canopy. *Pinus kesiya* extends over a broad range of montane areas of the Dalat Plateau and other uplands in southern Vietnam at elevations up to 1,800 m (World Wildlife Fund <https://www.worldwildlife.org/ecoregions/im0152>).

Unfortunately, detailed habitat data for the few records within the natural range are lacking. Of the two records from Thailand, one was from secondary rainforest, and the other unknown. The climate of both locations (Sihanoukville and Chumphon) is similar to those in Vietnam, characterized by a Tropical Monsoon climate (“Am” in the Köppen-Geiger climate classification), and most months in the year are marked by significant rainfall, with a short dry season. The sole location in Cambodia (Ream, Surat Thani) has a Tropical Savannah climate (“Aw” in the Köppen-Geiger climate classification), characterized a monthly mean temperature above 18°C in every month of the year and typically a pronounced dry season, with the driest month having precipitation less than 60mm. In essence, a tropical savanna climate tends to either see less rainfall than a tropical monsoon climate or have more pronounced dry seasons than a tropical monsoon climate. The Tropical Savannah climate are most commonly found in Africa, Asia and South America and is also prevalent in sections of Central America, northern Australia and North America, specifically in sections of Mexico and the state of Florida in the US (<https://www.weatherbase.com/weather/weather-summary.php3?s=66984&cityname=Siem+Reap,+Cambodia>) retrieved 24 June 2021 (*B. kewense*, as an alien species, is recorded outdoors in all these countries).

Bipalium kewense, like all land planarians, is sensitive to moisture and atmospheric humidity. Land planarians have practically no water-saving adaptations, being dependent upon moisture in their

microhabitat, yet are sensitive to too much water and thus generally avoid wet places. Flatworms are mostly strongly seasonal in occurrence, probably reflecting attempts to maintain themselves within a constant microclimate by vertical migration through the continuum of habitat niches, between one extreme with those of the litter and soil faunas, and the other extreme with the faunas of rocks and trees (Wallwork, 1970). When the moisture conditions are optimal or too wet, and cover is present, they will occupy the cryptozoic and litter microhabitats on the soil surface and may even venture up trees. When conditions are too dry for them, they retreat into the soil. In the absence of surface cover terrestrial flatworms can live entirely in soil as permanent members of the soil fauna and can be generally regarded as facultative soil animals. Most species appear not to burrow but make use of existing pore spaces to migrate within the soil (Winsor *et al.*, 1998). Land planarians naturally disperse by creeping on the substrate surface.

Like many species of land planarians, *B. kewense* climbs trees, and given that epiphytic orchids form a notable part in the biodiversity of the montane forests within its natural range (World Wildlife Fund <https://www.worldwildlife.org/ecoregions/im0152>), this could explain the view (Bell, 1886a, 1886b) that it was introduced to Europe by the orchid trade. It could not have spread naturally into the risk assessment area.

A5. What is the global non-native distribution of the organism outside the risk assessment area?

Response:

Bipalium kewense is undoubtedly the most widespread species of land flatworm worldwide. It was first found in Hong Kong in 1856, then in Australia in 1874, before the species was formally described in 1878 from a specimen found in the hothouse, Kew Gardens, London. It is now recorded in 67 countries outside the risk assessment area. In the United States, the early records were from hothouses on the east coast, and more recent records are from outdoor urban gardens and parks in centres further inland, suggesting acclimatization (Winsor, 1983) The colonizing success of *B. kewense* can largely be attributed to its ability to reproduce asexually by fragmentation (Hyman, 1943, 1951).

When the world distribution of the species was first mapped (Graff, 1899), *B. kewense* had been recorded in eight countries or territories (other than England, Ireland, Germany, and Austria which are in the risk assessment area), namely: Australia, Brazil, Hong Kong, Madeira, New Zealand, Samoa, South Africa, and the United States of America.

With the taxonomic revision of *B. kewense*, and mapping of its distribution worldwide and in the US (Winsor, 1983a), a further 32 countries or territories were added (but excluding four countries in the risk assessment area where it had been recorded): Argentina, Azores autonomous region of Portugal, Barbados, Bermuda, Cambodia, Canada, Cape Verde Islands, Colombia, Costa Rica, El Salvador, Fiji, French Polynesia (Tahiti, Tabuai Islands), Hawaii, India, Indonesia, Israel, Jamaica, Japan, La Reunion, Madagascar, Malaysia, Mauritius, Mexico, Peru, Puerto Rico, Ryukyus Islands, Saint Helena Island, Singapore, Taiwan, Tonga, Vietnam, and Zimbabwe.

Sporadic reports of occurrence of *B. kewense* in a further 30 countries or territories outside of the risk assessment area include: Kenya, China (mainland), the Natuna Islands, Ogasawara Islands of Japan,

Sri Lanka, Thailand, and Uruguay (summarized by Ogren *et al.*, 1992), Pakistan (Justine *et al.*, 2018), Pitcairn Island (Winsor, 1992 unpubl.); Egypt (Ali, 2008); Ecuador (Wizen, 2015); Cuba (Morffe, 2016); Sao Tome (Sluys *et al.*, 2017), Caribbean French Islands (Martinique, Guadeloupe, Saint Barthelemy, Saint Martin, and Monserrat), French Guiana, Monaco, and Pakistan (Justine *et al.*, 2018). Observations and mapped locations of *B. kewense* in iNaturalist <https://www.inaturalist.org/> (individually checked and verified by Winsor in 2021 specifically in preparation for this risk assessment) in new countries outside the risk area include Chile, Galapagos Islands, Nicaragua, and Paraguay (2018); Afghanistan, Guatemala, and Philippines (2020); and Panama (2021).

A6. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species been recorded and where is it established? The information needs be given separately for recorded (including casual or transient occurrences) and established occurrences.

“Established” means the process of an alien species successfully producing viable offspring with the likelihood of continued survival².

A6a. Recorded: List regions

A6b. Established: List regions

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

- Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea.

Comment on the sources of information on which the response is based and discuss any uncertainty in the response.

For delimitation of EU biogeographical regions please refer to <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2> (see also Annex VI).

For delimitation of EU marine regions and subregions consider the Marine Strategy Framework Directive areas; please refer to <https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions/technical-document/pdf> (see also Annex VI).

Response (6a): *Bipalium kewense* is recorded from the Alpine, Atlantic, Continental, and Mediterranean biogeographic regions (see A.8 for references).

Response (6b): *Bipalium kewense* is established outdoors in the Atlantic, Continental, and Mediterranean biogeographic regions (see A.8 for references).

² Convention on Biological Diversity, Decision VI/23

A7. In which biogeographic region(s) or marine subregion(s) in the risk assessment area could the species establish in the future under current climate and under foreseeable climate change? The information needs to be given separately for current climate and under foreseeable climate change conditions.

A7a. Current climate: List regions

A7b. Future climate: List regions

With regard to EU biogeographic and marine (sub)regions, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (7a):

Alpine, Atlantic, Black Sea, Continental, Mediterranean, Pannonian and Steppic in the risk assessment area; and in the Anatolian and Macaronesia biogeographic regions (refer to Figures 1 and 2, Annex VIII).

Response (7b):

Under Climate Change with timeframe 2070, and emission scenarios RCP 2.6 and RCP 4.5: Alpine, Atlantic, Black Sea, Continental, Mediterranean, Pannonian and Steppic in the risk assessment area; and in the Anatolian and Macaronesia biogeographic regions (Figure 1 Annex VIII). There are increases in the projected suitability for the establishment of *B. kewense* generally north and northeastwards under both RCP 2.6 and RCP 4.5 in the Atlantic, Black Sea, Continental, Pannonian and Steppic biogeographical regions in the risk assessment area, and in the Anatolian biogeographic region (Figures 3 and 4 Annex VIII). The lower to mid-levels of the Alpine region appear to become increasingly suitable for the establishment of the species under the RCP 2.6 and 4.5 scenarios. For the Mediterranean region, there is little change between the current projected suitability and those under RCP 2.6 and RCP 4.5.

In the Atlantic biogeographical region, Belgium, Denmark, Ireland, the Netherlands, and the United Kingdom contain some of the largest conglomerations of people in Europe, many of whom live near the coast (https://www.eea.europa.eu/publications/report_2002_0524_154909/biogeographical-

[regions-in-europe/the_atlantic_region.pdf/view](#) retrieved 25 June 2021). In the Continental region countries such as Austria, Czech Republic, Germany, Poland, and Slovakia, the urban areas are among the largest and most extensive in Europe. A large proportion of the population lives in the vicinity of forests. Some new afforestation occurs around big cities for recreational purposes (https://www.eea.europa.eu/publications/report_2002_0524_154909/biogeographical-regions-in-europe/continental_biogeographical_region.pdf/view retrieved 25 June 2021).

In these countries within both the Atlantic and Continental biogeographical regions, *B. kewense* is currently present largely in “protected” environments such as hothouses. Under the climate warming scenarios, there would probably no longer be the need for hothouses in these countries, and there could be a loss of containment of *B. kewense* from “protected” environments into outdoor urban gardens and parks where it could establish in the warmer conditions.

A8. In which EU Member States has the species been recorded and in which EU Member States has it established? List them with an indication of the timeline of observations. The information needs be given separately for recorded and established occurrences.

A8a. Recorded: List Member States

A8b. Established: List Member States

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, and the United Kingdom

The description of the invasion history of the species shall include information on countries invaded and an indication of the timeline of the first observations, establishment and spread.

Response (8a):

Bipalium kewense has been recorded in managed facilities (hothouses) in the following Member States:

Austria (Graff, 1893), Belgium (Woestinje, 1907), Czech Republic (Mrazek, 1902), Denmark (Kirkegaard, 1971), Finland (Söderstrom, 1936), Germany (Graff, 1899), Ireland (Bell, 1892), Netherlands (De Waart, 2016), Poland (Paxa, 1921), Slovakia (Košel, 2002), and the United Kingdom (Moseley, 1878).

Bipalium kewense has been recorded in urban gardens and urban environments in the following Member States:

France, including Corsica (Justine *et al.*, 2018), Italy, including Sardinia (Gremigni, 2003), Portugal (Ogren *et al.*, 1992, Silva, 2020), Malta (T. Cassar and D. Mifsud, 2021 unpubl.), Spain (Filella-Subirà, 1983), United Kingdom Glasgow Botanic Gardens and gardens in Sussex and Liverpool (Boag *et al.*, 2010).

Bipalium kewense was first recorded in 1877 in the United Kingdom, in the hothouse in Kew Gardens, London (Moseley, 1878). Subsequently in 1866 in Germany, from the orchid house, Botanic Gardens, Berlin (Graff, 1899); Ireland, in the hothouse at Stafford House, Kildare, 1892 (Bell, 1892); Austria, 1893 (Graff, 1893), in a hothouse in Graz; Poland, 1898, in a hothouse, Wroclaw (Paxa, 1921); Czech Republic, 1902, in a greenhouse in Prague (Mrazek, 1902); Belgium 1907 in a hothouse (Woestijnje, 1907) Netherlands 1912, in a hothouse (De Waart, 2016), Finland 1935, in a hothouse (Söderstrom, 1936), Denmark, in a hothouse (Kirkegaard, 1971), Spain 1982, in private gardens (Filella-Subirà, 1983), France 1999, in private gardens (Justine *et al.* 2018); Portugal, urban outdoors (Ogren *et al.*, 1992, Silva, 2020); Slovakia, in a hothouse (Košel, 2002), Italy 2021, urban outdoors (Bello *et al.*, 1995, Gremigni, 2003, Mori *et al.* 2021); Malta 2021, in an urban garden (T. Cassar and D. Mifsud, 2021, unpubl.).

Response (8b):

Of the foregoing countries in which *B. kewense* has been recorded, it is present in the following outdoor locations: in a private garden in Malta in 2021 (T. Cassar and D. Mifsud, 2021, unpubl.) in Portugal in private gardens in Mealharda circa 1990 and Gemunde 24 Aug 2020, (Silva, 2021, 2020), numerous outdoor occurrences in France (Justine *et al.*, 2018), in outdoor urban sites and private gardens in Italy, Mori *et al.* (2021), and outdoor locations in Spain, all from private gardens, are taken, in chronological order: Caldes d'Estrac (Barcelona) 1982, Girona (Girona) 1994, Barcelona, 1995, and Bètera (València), 1999 (Álverz-Presas *et al.* (2014).

The most comprehensive recent study of the distribution *B. kewense* is from France (Justine *et al.*, 2018). From metropolitan France, including Corsica, 36 records were obtained, with *B. kewense* established in nine administrative departments; 34 of these records were from outdoor sites, with more than half (16) from the department Pyrénées-Atlantiques. The dates of records ranged from 1999-2017, with the oldest record in the Department of Pyrénées-Atlantiques: “In France, the outdoors occurrence of *B. kewense* was reported in Orthez and Bayonne in 2005 (Vivant 2005), Urcuit in 1999, in Arthez de Béarn, Hasparren, Villefranque, Urt (all in 2014), near Jurançon (2016), Nay (2016) and Saint Jean de Luz (2016), Ustaritz (2017) and in Bayonne and Orthez again (2014). Specimens records were from Saint-Pée-sur-Nivelle (2013), Ustaritz (2014), Bassussary (2014) and Orthez (2014). All these localities are in the Department of Pyrenées-Atlantiques, plus three records from the Department of Landes, north of Pyrenées-Atlantiques, along the Atlantic coast. The remark by Vivant that the animal was collected “five times in the last 20 years”, the record from 1999, and the recent record and specimens in the same locality (Orthez) in 2014 strongly suggests that the species is now established in the open in Orthez and in several localities of the Department of Pyrenées-Atlantiques (Justine *et al.*, 2018). All these records are from gardens, and it is not known whether the species has colonized semi-natural habitats.

Additional unpublished results (2018 - 2021) confirm that the species is well established in France, mainly in the Department of the Pyrénées-Atlantiques (Justine, unpubl, records of *B. kewense* in iNaturalist https://www.inaturalist.org/observations?place_id=any&subview=map&taxon_id=64221).

A9. In which EU Member States could the species establish in the future under current climate and under foreseeable climate change? The information needs to be given separately for current climate and under foreseeable climate change conditions.

A9a. Current climate: List Member States

A9b. Future climate: List Member States

With regard to EU Member States, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (9a): Under present climate conditions, *B. kewense* could establish in the following European countries. Figures in brackets are the central estimate of the percentage of the country projected to be suitable in the species distribution model – please note uncertainties around these estimates (Table 3, Annex VIII): Austria (3%), Belgium (70%), Bulgaria (60%), Croatia (76%), Cyprus (100%), France (78%), central Germany (14%), Greece (92%), Hungary (43%), Italy (82%), Luxembourg (60%), Malta (not modelled but deemed suitable), Netherlands (41%), Portugal (100%), southern Romania (18%), Slovakia (2%), Slovenia (28%), Spain (89%), and the United Kingdom (but not northern Scotland) (18%) (refer also to Figures 2 and 5, Annex VIII).

Bipalium kewense is established outdoors in the Atlantic and Mediterranean biogeographic regions. In the Atlantic region the area most at risk is the Department of Pyrénées-Atlantiques, France. However, all current records are from gardens, and it is not known whether the species has colonised semi-natural habitats.

In order to model the establishment of *B. kewense* under current climate and under predicted climate change conditions in the 2070s under low and medium emissions scenarios (RCP2.6 and RCP4.5), climate variables based upon the biology of *B. kewense* were selected, and an ensemble model constructed (Annex VIII). The model suggested that the suitability of particular location for the establishment of *B. kewense* was most strongly determined by the minimum temperature of the coldest month in that region, followed by annual precipitation, then the presence of human-disturbed habitats, then the mean temperature of the warmest quarter, followed last by the Climatic Moisture Index for that area. The seasonality of precipitation did not impact on the suitability of an area for the establishment of *B. kewense*.

Response (9b):

Under predicted future warmer climate conditions in the 2070s, *B. kewense* could establish in the following European countries. Figures in brackets are the central estimates of the percentage of each country projected to be suitable in the species distribution model for low (RCP2.6) and medium (RCP4.5) emissions scenarios. Please note uncertainties around these estimates (Table 3, Annex VIII): Austria (30-39%), Belgium (97-98%), Bulgaria (89-94%), Croatia (95-99%), Cyprus (88-50%), Czech Republic (41-81%), southern Denmark (4-7%), France (90-92%), Germany (86-95%), Greece (95-85%), Hungary 100%), Ireland (1%), Italy 88-89%), Luxembourg (100%), Malta (not modelled, but deemed suitable), Netherlands 74-83%), western and central Poland (22-49%), Portugal (100%), Romania (59-75%), Slovakia (45-62%), Slovenia (72-82%), Spain (95-89%), southern coastal Sweden (1%), and the United Kingdom excluding Scotland (38-42%).

Refer to Figures 7(a) Annex VIII illustrating the projected suitability for *Bipalium kewense* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP2.6, and Figure 8(a) Annex VIII showing the projected suitability for *Bipalium kewense* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP4.5.

A10. Is the organism known to be invasive (i.e. to threaten or adversely impact upon biodiversity and related ecosystem services) anywhere outside the risk assessment area?

Response:

There is no evidence that the species is invasive (i.e. threatens or adversely impacts upon biodiversity and related ecosystem services) anywhere outside the risk assessment area. However, no studies have been conducted that would support or refute this statement. The data for this species is deficient.

Bipalium kewense is a minor pest in a few urban earthworm farms (vermiculture of non-native earthworm species) in New South Wales and in Queensland, Australia (Winsor, 1998) where it is readily removed by handpicking or flooding the beds.

A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea,

Aegean-Levantine Sea

Response:

There is presently no evidence that the species is invasive in the risk assessment areas of the EU (i.e. threatens or adversely impacts upon biodiversity and related ecosystem services). However, no studies have been conducted that would support or refute this statement. The data for this species is deficient.

A12. In which EU Member States has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, and the United Kingdom

Response:

There is presently no evidence that the species is invasive in the risk assessment areas of the EU (i.e. threatens or adversely impacts upon biodiversity and related ecosystem services). However, no studies have been conducted that would support or refute this statement. The data for this species is deficient.

A13. Describe any known socio-economic benefits of the organism.

including the following elements:

- Description of known uses for the species, including a list and description of known uses in the risk assessment area and third countries, if relevant.
- Description of social and economic benefits deriving from those uses, including a description of the environmental, social and economic relevance of each of those uses and an indication of associated beneficiaries, quantitatively and/or qualitatively depending on what information is available.

If the information available is not sufficient to provide a description of those benefits for the entire risk assessment area, qualitative data or different case studies from across the risk assessment area or third countries shall be used, if available.

Response:

There is evidence that other species alien flatworms have been inadvertently disseminated in potted plants from infected garden centres and botanic gardens to the public or other botanic gardens. This is non-intentional transfer and if known it can adversely affect the reputation of the business or botanic garden concerned (Boag and Neilson, 2014).

Bipalium kewense has no known uses outside the research field. It is undoubtedly the most studied species of land planarian and acts as an experimental animal and as an example of a terrestrial flatworm, largely because of its widespread occurrence, size, and availability. There has been, and continues to be, economic benefits from funds directed to research on this organism.

B. kewense has been used as the subject of various anatomical and histological studies (Bergendal, 1892; Graff, 1899; Pfitzner, 1958; Hauser, 1966; Silveira, 1969, 1970, 1971, 1972, 1978; Storch and Abraham, 1972; Sun *et al.*, 1975, 1979; Storch and Welsch, 1977), behavioural studies (Lehnert, 1891; Kew, 1900; Barnwell *et al.*, 1964; Barnwell *et al.*, 1965; Barnwell, 1966; Morton and Kleinginna, 1971), biochemical studies (Campbell, 1960, 1965, 1970, 1973; Campbell and Lee, 1963; Reddy and Campbell, 1970; Tramell and Campbell, 1971; Scheid and Awapara, 1972; Phillips and Dresden, 1973; Dresden and Landsperger, 1977), biology (Lehnert, 1891; Johri, 1952; Dundee and Dundee, 1963; Froelich, 1956; Connella and Stern, 1969; Olewine, 1972; Chandler, 1974; Ducey *et al.*, 2006), genomic studies (Gastineau *et al.*, 2019), laboratory maintenance (Barnwell, 1967; Neck, 1987; Olewine and Morton, 1971), physiological studies (Daly and Matthews, 1975, 1982; Daly *et al.*, 1977), pseudoparasitism (Walton and Yokogawa, 1972; Daly *et al.*, 1976, 1977; Winsor, 1980, 1983b), regeneration (Morgan, 1900; Brøndsted, 1969), and toxicology (Arndt, 1925; Stokes *et al.*, 2014).

Understanding the processes of cell proliferation, differentiation and pattern formation in regenerative organisms can help find ways to enhance the poor regenerative abilities shown by many other animals, including humans. Studying regeneration in a relatively simple creature, like a planarian whose neoblasts act as stem cells, can hold important clues into how human cells can be coaxed to behave similarly and help humans regenerate injured or missing tissues. Some of the earliest studies on planarian regeneration used *B. kewense* as the experimental model (Morgan, 1900; Brøndsted, 1969). Progress towards understanding the mechanics of regeneration was hampered by an incomplete understanding of basic cell biology and genetics, in addition to a lack of tools for experimentation, and only recently have significant advances in molecular biology (for example Gastineau *et al.*, 2019 using *B. kewense*), genetics, and sequencing technologies reignited interest in planarians as an attractive model in which to study regeneration (Elliot and Alvarado, 2013).

The social benefits of the knowledge generated by these studies of *B. kewense* are intangible and difficult to quantify, let alone place a monetary value upon them. As such, in economic terms, these studies and their outcomes are regarded as externalities - the overall cost and benefit to society being defined as „the sum of the imputed monetary value of benefits and costs to all parties involved“ (Laffont, 2008). Research and development, and education are examples of such positive externalities ("[Externalities - Definition and examples](#)"). *Conceptually*. Retrieved 21 June 2021).

SECTION B – Detailed assessment

Important instructions:

- In the case of lack of information the assessors are requested to use a standardized answer: “No information has been found.”
- With regard to the scoring of the likelihood of events or the magnitude of impacts see Annexes I and II.
- With regard to the confidence levels, see Annex III.
- Highlight the selected response score and confidence level in **bold** but keep the other scores in normal text (so that the selected score is evident in the final document).

1 PROBABILITY OF INTRODUCTION AND ENTRY

Important instructions:

- **Introduction** is the movement of the species into the risk assessment area (it may be either in captive conditions and/or in the environment, depending on the relevant pathways).
- **Entry** is the release/escape/arrival in the environment, i.e. occurrence in the wild
- Introduction and entry may coincide for species entering through pathways such as “corridor” or “unaided”, but it also may differ. If different, please consider all relevant pathways, both for the introduction into the risk assessment area and the entry in the environment.
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used (see Annex IV). For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document³ and the provided key to pathways⁴.
- For organisms which are already present (recorded or established) in the risk assessment area, the likelihood of introduction and entry should be scored as “very likely” by default.
- Repeated (independent) introductions and entries at separate locations in the risk assessment area should be considered here (see Qu. 1.7).

Qu. 1.1. List relevant pathways through which the organism could be introduced into the risk assessment area and/or enter into the environment. Where possible give details about the specific origins and end points of the pathways as well as a description of any associated commodities.

For each pathway answer questions 1.2 to 1.7 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 1.2a, 1.3a, etc. and then 1.2b, 1.3b etc. for the next pathway.

In this context a pathway is the route or mechanism of introduction and/or entry of the species.

The description of commodities with which the introduction of the species is generally associated shall include a list and description of commodities with an indication of associated risks (e.g. the

³ <https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf>

⁴ <https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf>

volume of trade; the likelihood of a commodity being contaminated or acting as vector).

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 1.2-1.9.

Response:

The main driver of historic biological invasions and the passive dispersal of terrestrial flatworms was probably horticulturalists of the 19th Century using the then recently invented Wardian cases that are wooden hermetically sealed glazed cases, the forerunner of the modern terrarium, named after their inventor Dr Nathaniel Ward designed to safely transport back by sea to the hothouses and gardens of Europe rare plants (Keogh, 2020), together with soil containing cryptic exotic animal species (Winsor *et al.*, 2004, Keogh, 2020). Therefore, over 30 non-native species of land planarians, including *B. kewense*, have established in various countries outside their native range (Winsor *et al.*, 2004). In human-modified habitats, flatworms and their cocoons continue to be associated with rooted and potted plants, rhizomes, and certain types of fresh vegetable produce (Alford *et al.*, 1996). Subsequent secondary dispersal of these invasive flatworm species occurs through the exchange and purchase of plants from nurseries, botanical gardens, garden centres and gardeners (Alford *et al.*, 1996) especially infested nurseries and garden centres (Boag *et al.*, 1994; Moore *et al.*, 1998), and active inadvertent dispersal through social traditions of exchanging plants and recycling topsoil (Christensen and Mather 1998).

The pathways considered in this risk assessment for *B. kewense* are “contaminant nursery material” (unintentional), and “Botanical Garden / Zoos & Aquaria (excluding domestic aquaria)” (unintentional). These two pathways are similar and represent unintentional introduction via trade and movement of living plants.

Pathway name: Contaminant nursery material

Qu. 1.2a. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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This pathway is unintentional. Trade in exotic plants is increasing and landscaping, the process of making a garden or other piece of land more attractive by altering the existing design, adding ornamental features, often requiring potted trees and shrubs, poses a particular risk. Terrestrial flatworms have been intercepted in the UK on plant material, particularly tree ferns (*Dicksonia* spp.), from Australia and New Zealand (Cannon and Baker, 2007; Matthews, 2005). Data from other EU countries on interceptions of terrestrial flatworms on imported products at ports are sparse. *Bipalium kewense* was detected in potted flowering plants (*Agapanthus*) imported from South Africa to France in August 2019 by the authorities of Paris Charles de Gaulle airport. The single specimen was barcoded for molecular confirmation of identification (Justine, 2019 unpubl.)

Qu. 1.3a. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication.
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

With greater awareness of the problems caused by alien flatworms and more stringent EU plant health legislation under the ‘Plant Health Law’ (Regulation (EU) 2016/2031), the likelihood of entry of alien flatworms should be lessened. However, this is set against increasing international trade in plants (Migliorini *et al.*, 2015), with the value of imported plants for planting increasing by 60% over the past fifteen years (Eschen *et al.*, 2015). The key factor here is the level of importation of potted plants from affected regions. The largest risk to the risk assessment area would be from regions like China (via Hong Kong), Indonesia, Thailand, and Florida in the US, which are producers of potted plants (van Uffelen and de Groot, 2005; EPPO, 2012).

There are no data on *B. kewense* interceptions during plant trade except anecdotal records in France and U.K. only (see Q 1.2a), so an estimate of the propagule pressure is difficult. Experience with other alien flatworms would suggest that the number of individuals would be low and sporadic, with one or two individuals in a contaminated shipment.

Land planarians are hermaphrodite. *Bipalium kewense* can reproduce asexually by fragmentation. The colonizing success of *B. kewense* can largely be attributed to this asexual mode of reproduction (Hyman, 1943, 1951), in which an individual specimen can eventually give rise to a colony through reproduction by fission, as opposed to sexual reproduction that will require another sexually mature individual, and the time and energy expended in searching for this sexual partner.

In the fission process (Barnwell, 1967; Connella and Stern, 1969) one or two portions of the tail section, 1 - 4 cm long (zooids), are shed a few days after the adult fed and are able to crawl. Although fragile, there is the possibility that these zooids, especially given their small size, could pass

undetected and be transported together with potted plants. After several days or weeks, the dropped tail sections (zooids) regenerate so that they grow to become small versions of adult specimens. A regenerating *B. kewense* will not attack and eat earthworms until the head region has completely regenerated into the adult shape. However, the ability of a regenerated *B. kewense* to eat depends primarily upon a regenerated pharynx than a regenerated head (Barnwell, 1966). Adult planarians raised in laboratory terraria shed an average of one or two fragments per month (Connell and Stern, 1969). This average rate of fragment production under laboratory conditions may not be an accurate estimate of fragment production in outdoor environments. Field observations of a *B. kewense* population in Tennessee US revealed few sightings of fragments over a year, with the ratio of fragments to adult specimens was greatest during the cold months (Chandler, 1976).

The likelihood of the introduction of an egg cocoon of *B. kewense* is considered unlikely.

Qu. 1.4a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Within a potted plant or enclosed in a plant’s root ball, *B. kewense* would be in a “protected” environment. The conditions necessary to keep the plant healthy, would presumably match those of the source location and therefore be amenable to *B. kewense*. Cold storage may have an impact on *B. kewense* survival as it is largely a tropical species, albeit originally from the cool central highlands in Vietnam. There are no specific data looking at this, but Barnwell (1969) noted that when the temperature dropped to -6.7°C or -9.4°C, planarians were not found in their usual places, but a few days later when conditions were warmer, they reappeared. He also stated that they could survive temperatures near -17°C, but after temperatures fell below this for several nights in 1966 in Athens, Georgia, US, no *B. kewense* were seen for over two years. The likelihood of survival of adults, egg cocoons and juveniles and fragments will vary with shipment journey time and storage conditions, which will be dependent on the plant product transported.

Qu. 1.5a. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely	CONFIDENCE	low medium high
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	likely very likely		
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Response:

Plant health inspection and phytosanitation are the most practical means of preventing invasion by cryptic flatworm species. Legislation in the EU now requires that plants for planting from third countries meet strict criteria. In particular, the growing media must be free from soil or subjected to suitable treatment to ensure freedom from pests (EU Commission Implementing Regulation (EU) 2019/2072). However, legislation is dependent on plant health certification by exporting producers and only a small proportion of the plant trade can be directly inspected by the importing country.

However, as of the 14th December 2019, Professional Sellers, Importers / Exporters of plants, and Online / Offline sellers of plants who may sell plants on online platforms such as eBay, Etsy, Facebook, a private website, Instagram or any other venue, will need to be able to issue plant passports in accord with the EU Plant Passport Regulation (Implementing Regulation (EU) 2017/2313), enacted to protect the environment, and trade, from plant pests and diseases (<https://www.bb-automation.com/pflanzenpass?lang=en#:~:text=The%20plant%20passport%20is%20used,producer%20to%20the%20retail%20trade>). Inspections, if thorough, and other requirements of the EU Plant Passporting may go some way to limiting the inadvertent importation of flatworms.

Aside from plant health inspection, and the EU Plant Passport Regulation, there are no existing management practices implemented specifically against invasive flatworms. Hot water treatment has been considered for flatworm management for imported potted plants (Justine *et al.*, 2014; Murchie and Moore, 1998).

As the flatworms are sheltering within the soil or in the plant container, other pest management practices along the pathway are unlikely to affect them. For example, treatment with insecticides to control foliar pests is unlikely to penetrate the root ball or if systemic affect the flatworms as they are not feeding on the plant.

Qu. 1.6a. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

If *B. kewense* is embedded in the growing medium or root ball, detection is difficult without destruction of the potted plant. Adult flatworms are comparatively large and distinctive, so once

uncovered would be visible easily with the naked eye. Egg capsules (cocoon) are 3-9.5 mm in diameter and would be much harder to detect and to differentiate from other detritus.

As *B. kewense* is cryptic, soil-dwelling, and nocturnal, it is most likely detected in the environment under refuges, such as plant pots or other items in close contact with the soil, when these are moved. Whilst the flatworms are likely to be noticed by gardeners in these cases, it is worthwhile bearing in mind the comments of Justine *et al.* (2018) with respect to *Bipalium* spp., *Diversibipalium* spp. in France, where the authors expressed their amazement that these highly noticeable non-native flatworms had escaped attention of scientists and officials for 20 years.

Qu. 1.7a. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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Response:

Importation of potted plants into the EU occurs throughout Europe. The pathway would encompass ports to wholesale suppliers to local dissemination via garden centres, landscapers, and supermarkets. There is substantial internal trade in the EU. Increasing online trade may make dissemination points more dispersed as wholesalers may freight directly to the public.

As the flatworms would be enclosed in potted plants, opportunities for entry into the environment would also be widespread. In warm areas of Europe, ornamental plants that could house *B. kewense* may be placed directly outdoors allowing direct dissemination of the flatworm into the local environment. In northern areas of Europe such plants may be housed indoors, with less opportunity for transfer. However, during the summer months tropical plants may be housed in conservatories or glasshouses from which the flatworm could escape into the wild.

Qu. 1.8a. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Bipalium kewense has already been introduced and established in the risk assessment area through this pathway. The risk of further introductions into the EU depends on the extent of trade with regions maintaining populations of *B. kewense*, and the effectiveness of measures by the trading partners to prevent the introduction, achieve early detection, rapidly eradicate, and manage the species.

End of pathway assessment, repeat Qu. 1.3 to 1.8 as necessary using separate identifier.

Pathway name: Botanical Garden / Zoos and Aquaria

Qu. 1.2b. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?			
RESPONSE	intentional unintentional	CONFIDENCE	Low medium high

Response:

Botanic gardens are a main pathway for the introduction / entry of *Bipalium kewense*, evidenced by the following records that include some additional records from Germany to those in earlier responses (8a and 8b): *Bipalium kewense* was first recorded in the risk assessment region in the hothouse in Kew Gardens, London, United Kingdom, 1877 (Moseley, 1878). Subsequently it was found in the orchid house, old Botanic Gardens, Berlin, Germany 1886 (Graff, 1899), and within Germany a hothouse in Frankfurt am Main, 1887, hothouses in Dresden and Leipzig in 1891, hothouse in Heidelberg, 1896, Botanic Gardens and Zoological Gardens, Hamburg, 1901 (associated with plants from Brazil), orchid house, Bellevue Palace, Berlin, 1912, palm house, Dahlem Botanic Gardens, 1923, fern house and hothouse Botanic Gardens Breslau, 1930, hothouse, Bonn, 1933, and a hothouse, Göttingen (no date) (locations summarized by Arndt, 1934) ; the hothouse at Stafford House, Kildare, Ireland, 1892 (Bell, 1892); a hothouse in Graz, Austria, 1893 (Graff, 1893); in a hothouse, Wrocław, Poland, 1898 (Paxa, 1921); a greenhouse in Prague, Czech Republic, 1902 (Mrazek, 1902); hothouse Belgium 1907 (Woestinje, 1907); hothouse in the Netherlands 1912 (De Waart, 2016), and a hothouse in Finland 1935 (Söderstrom, 1936).

Although these records are historical, there remains a high possibility that these protected locations continue to harbour populations of *B. kewense*, and may act as reservoirs of “infection”, especially if the garden sells plants to the public. For example, in Kew Gardens, *B. kewense* was found in a hothouse in 1966, and again in May 1980 by the Keeper in the tropical fern house in which the original specimen of *B. kewense* was found in 1877 (Winsor, 1983a), the implication being that the species has continued to survive in this location for 103 years (whether other people have seen specimens over the 103 years is not known; there were no museum records from this location). However, many exotic plants are transferred to hothouses, often historic in nature, and once the plants are in place they are not disturbed further. There is a possibility that Botanic Gardens will continue to be an entry point from outside the EU.

There is a single report of *B. kewense* found in the Glasgow Botanic Gardens close to but outside the hothouses (Boag et al., 2010). It was found in summer *B. kewense* outdoors in Glasgow botanic gardens but within a couple of metres of a heated greenhouse where it was established. There was no suggestion it was able to live under Scottish climatic conditions, but it did show it had the capability

to migrate when conditions were right, in other words it could have moved from one greenhouse to another when weather conditions were favourable but the general climate for the region was not (Boag 2021, unpublished).

Qu. 1.3b. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	Low medium high
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Response:

The introduction of alien flatworms via importation of exotic plants to botanic gardens has occurred with *B. kewense* (Winsor, 1983). However, most of these introductions happened in the past when biosecurity and quarantine protocols were less stringent.

The mechanism of contamination of botanical specimen plants and propagule pressure is similar to that of nursery plants (above 1.3a). Land planarians are hermaphrodite. *Bipalium kewense* can reproduce asexually by fragmentation. The colonizing success of *B. kewense* can largely be attributed to this asexual mode of reproduction (Hyman, 1943, 1951), in which an individual specimen can eventually give rise to a colony through reproduction by fission, as opposed to sexual reproduction that will require another sexually mature individual, and the time and energy expended in searching for this sexual partner.

In the fission process (Barnwell, 1967; Connella and Stern, 1969) one or two portions of the tail section, 1 - 4 cm long, are shed a few days after the adult fed and are able to crawl. After several days or weeks, the dropped tail sections regenerate so that they grow to become small versions of adult specimens. Adult planarians raised in laboratory terraria shed an average of one or two fragments per month (Connella and Stern, 1969). This average rate of fragment production under laboratory conditions may not be an accurate estimate of fragment production in outdoor or managed environments. Field observations of a *B. kewense* population in Tennessee US revealed few sightings

of fragments over a year, with the ratio of fragments to adult specimens was greatest during the cold months (Chandler, 1976).

Botanic gardens may seek more exotic plants collected from the wild, which may increase the likelihood of contamination with flatworms compared to contamination by flatworms of locally reared plants. On the other hand, the quantities imported will be smaller and awareness of biosecurity risk and mitigation procedures are likely to be greater in botanic gardens, which justifies a reduced likelihood compared to the pathway “contaminant nursery material”. In addition, in temperate countries at least, exotic plants collected from the tropics will probably be kept in hothouses as is already the case with many locations from which *B. kewense* has been recorded. Such arrangements are likely to limit the flatworm’s entry to the human-modified environment outside the hothouse or managed facility. Estimates of the propagule pressure along this pathway are difficult to generate because of unknown variables, for example the availability of food, temperatures at which the facility is run, introduction of new plant stock, and plant hygiene practices in the facility.

Qu. 1.4b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

As with the nursery plant pathway (above, 1.4a), *B. kewense* is likely to survive well in transported and stored exotic plants, assuming that the conditions necessary for the plants’ survival matches that of the flatworms. Without more details of the conditions of transport of contaminated material, it is difficult to go beyond generalisations, but *B. kewense* has certainly been transported successfully around the world with plant material.

Qu. 1.5b. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

The response to this question is similar to that for nursery plants (above, 1.5a). There are no management measures widely specifically implemented against *B. kewense*. One would expect that high value or rare exotic plants in transit to botanic gardens would be subject to high biosecurity assessments with intense inspection and scrutiny for pests and diseases. Biosecurity risk and

mitigation procedures are likely to be greater in botanic gardens. However, flatworms sheltering in root balls can be difficult to detect without damaging the plant, so the confidence score is low.

Qu. 1.6b. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Bipalium kewense would shelter in soil surrounding root balls and is unlikely to be detected without destructive sampling (see response above, 1.6a). For high value exotic plants, it may be possible to wash the roots and re-pot, which would expose the flatworms. However, small individuals or egg capsules may still go undetected.

As botanic gardens have dedicated botanists and gardeners and are open to the public, it could be argued that detection outside the hothouse or managed facility may be more likely as they may be trained in the recognition of pest and exotic species, than for nursery stock. However, the focus of phytosanitary and biosecurity measures, and education of staff in botanic gardens appear to be exclusively concerned with plant pests, especially microbial pathogens, insects, and invasive plant species (Hulme, 2011, Heyward with Sharrock, 2013, Wondafrash et al., 2021), rather than on non-plant pests such as alien land planarians that may be unintentionally brought into the gardens as contaminant on plants.

Qu. 1.7b. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	Isolated widespread ubiquitous	CONFIDENCE	Low medium high
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Response:

Most cities in Europe have botanic gardens and similar establishments. Typically, these would comprise both “protected” environments such as glasshouses, hothouses) and landscaped gardens.

Qu. 1.8b. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely	CONFIDENCE	low
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	unlikely moderately likely likely very likely		medium high
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Response:

There are many botanic gardens in Europe often dating from the 19th century. Most have civic, educational, conservation or academic functions. Yet, botanic gardens have been criticised as sources of invasive alien species and encouraged to adopt and implement codes of practice to prevent the spread of unwanted species (Hulme, 2011). Botanic Gardens Conservation International, the body representing some 600 institutional members in 100 countries, specifically acknowledges the threats posed by invasive alien species and the role of botanic gardens in their dissemination but also detection (Hayden, 2020). BGCI advocates careful planning, preparation and management of plant material being exchanged, together with good record keeping and robust procedures to ensure that „neither the plant itself nor any associated pests or diseases will affect the collections of the botanic garden or the wider environment“ (<https://www.bgci.org/our-work/plant-conservation/plant-health-and-biosecurity/>).

Bipalium kewense has certainly been introduced many times into new regions via botanic gardens, and the recent finding of *Platydemus manokwari* in a botanic garden hothouse in France (Justine *et al.*, 2018) demonstrates that this remains a viable and active pathway. As with nursery plants, the extent of the risk depends on the quantities and types of plants transported from regions with *B. kewense* to Europe by botanic gardens.

End of pathway assessment, repeat Qu. 1.2 to 1.8 as necessary using separate identifier.

Qu. 1.9. Estimate the overall likelihood of introduction into the risk assessment area or entry into the environment based on all pathways and specify if different in relevant biogeographical regions in current conditions.

Provide a thorough assessment of the risk of introduction in relevant biogeographical regions in current conditions: providing insight into the risk of introduction into the risk assessment area.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Bipalium kewense has been introduced into the risk assessment area by the botanical garden and contaminated nursery material pathways in the past as evidenced by the widespread findings of this flatworm in botanical gardens and urban hothouses. The most likely pathway for introduction of *B. kewense* is with living plants, either through botanical gardens or the contaminated nursery material pathways, which appear to be still active today. Major risk factors include (a) the extent to which

botanical gardens act as reservoirs of *B. kewense*, (b) propagule pressure arising from the asexual mode of reproduction of *B. kewense* in which fragments are shed that will subsequently regenerate into mature flatworms, (c) the efficacy of phytosanitary and biosecurity measures employed by botanical gardens and nurseries to plants, (d) trade and exchange of plants between botanical gardens within and between Member States, and between Member States and other countries, and the phytosanitary and biosecurity measures taken in these countries (e) and the extent of education of botanical garden and nursery staff, and the public, about alien flatworms.

Bipalium kewense is established outdoors in the Atlantic and Mediterranean biogeographic regions. In the Atlantic region the area most at risk is the Department of Pyrénées-Atlantiques, France. However, all records are from gardens, and it is not known whether the species has colonised semi-natural habitats.

Qu. 1.10. Estimate the overall likelihood of introduction into the risk assessment area or entry into the environment based on all pathways in foreseeable climate change conditions?

Thorough assessment of the risk of introduction in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of introduction (e.g. change in trade or user preferences)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely introduction within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Bipalium kewense has already been introduced to the risk assessment area through the botanical garden, and contaminated nursery material pathways. The pathways themselves (transport of exotic plants) will be little affected directly by climate change. However, as the climate warms, there are two

issues that will increase the risk of *B. kewense* establishment in the wild, and possible further introductions and entry into the wild.

The first is that the flatworm is likely to extend its range outside the risk assessment area. The current predicted areas for establishment of *B. kewense* includes substantial regions of Asia, south-eastern USA, north-western North America – Alaska, central and South America, and central east Africa (SDM Annex VIII Figure 6). With greater colonisation of these regions, the risk of inadvertent transport with ornamental or exotic plants to the risk assessment area increases. This factor will likely apply equally to the biogeographical regions suitable for establishment (e.g., mainly the Atlantic and Mediterranean regions), although as this movement is dependent on human agency, the risk will be dependent on human population density, and also the success of phytosanitary and plant passport controls. Second, as the EU climate warms, the range of garden plants likely to be grown outdoors will alter. This may include exotic plants from flatworm-infected regions and therefore increase the risk of entry of *B. kewense* into the surrounding environment. This risk will be greater in the Atlantic biogeographical region as it extends further north, and probably presents a relatively higher risk than import from external countries.

2 PROBABILITY OF ESTABLISHMENT

Important instructions:

- For organisms which are already established in parts of the risk assessment area or have previously been eradicated, the likelihood of establishment should be scored as “very likely” by default.
- Discuss the risk also for those parts of the risk assessment area, where the species is not yet established.

Qu. 2.1. How likely is it that the organism will be able to establish in the risk assessment area based on similarity of climatic and abiotic conditions in its distribution elsewhere in the world?

RESPONSE		CONFIDENCE	
	very unlikely		low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response:

Bipalium kewense is already established outdoors in the risk assessment area in the low altitude region of the Pyrénées-Atlantiques along the ocean that has an Atlantic climate, suggesting that the region is particularly suitable for land planarians (Justine *et al.*, 2018). It has also established outdoors in Malta in 2021 (T. Cassar and D. Mifsud, 2021, unpubl.) in Portugal in Mealharda and Gemunde (Silva, 2021, 2020), in Italy, especially western Italy, Mori *et al.* (2021), and outdoor locations in Spain, Caldes d’Estrac (Barcelona), Girona (Girona), Barcelona, and Bètera (València), (Álverz-Presas *et al.* (2014). Therefore, the confidence is that establishment in the risk assessment area is very likely, and confidence is high.

Countries within the natural range of *Bipalium kewense* have Köppen Climate Classification Type A Tropical climates (Tropical Rainforest, Tropical Monsoon, and Tropical Savannah climates) that do not occur in the risk assessment area, and Köppen classification Type C Temperate climates (Humid sub-tropical, and Monsoon-influenced humid subtropical climate) that are present in the risk assessment area.

The locations within the risk assessment area where *B. kewense* has established outdoors all have Köppen Climate Classification Type C climates as follows:

The low altitude region of the Pyrénées-Atlantiques in France (numerous records), the northern and eastern Italy (sparse records), and Girona, Spain have Humid sub-tropical climates characterized by the coldest month averaging above 0 °C, at least one month's average temperature above 22 °C, and at least four months averaging above 10 °C. There is no significant precipitation difference between seasons, and no dry months in the summer. This climate type extends to the UK, and the Atlantic coast of western Europe.

Most of central and western Italy (numerous records), Malta, Barcelona, and Spain have Hot summer Mediterranean climates characterized by the coldest month averaging above 0 °C, at least one month's average temperature above 22 °C, and at least four months averaging above 10 °C. There is at least three times as much precipitation in the wettest month of winter as in the driest month of summer, and driest month of summer receives less than 30 mm. This climate type extends to the Mediterranean coast of France and Greece.

Mealharda and Gemunde in Portugal, and Valencia in Spain have Cold summer Mediterranean climates characterized by the coldest month averaging above 0 °C and 1–3 months averaging above 10 °C. There is at least three times as much precipitation in the wettest month of winter as in the driest month of summer, and driest month of summer receives less than 30 mm.

Laboratory work suggests that *B. kewense* will tolerate a wide range of temperatures, with the optimum temperature for growth and reproduction being between 15.6°C and 32°C; the species will survive near freezing temperatures, but they do not regenerate or grow as rapidly at these low temperatures (Barnwell, 1967). Soil temperatures will generally be higher than air temperatures, depending upon the thermal conductivity of the substratum. In another study (Chandler, 1976) the numbers of specimens of *B. kewense* that were sighted declined when the soil temperature reached a minimum of 3.9°C (minimum air temperature in the same location was -6.6°C); sightings of *B. kewense* increased with a rise in soil temperature. “In Billère (Pyrénées-Atlantiques, France), repeated records of *B. kewense* from the same garden were obtained between September and December 2017 and January 2018, live and outdoors, even in winter; they were also found at various depths under the soil surface in January, clearly a way for the species to survive the cold season.”(Justine *et al*, 2018).

However, *B. kewense* has only been found in hothouses and not outdoors in Austria, Belgium, Czech Republic, Denmark, Finland, Germany, Netherlands, Poland, and Slovakia. Except for the Netherlands and the UK (in proximity to a hothouse), the other remaining countries all have Köppen Climate Classification Type D Continental climates, characterized by having at least one month averaging below 0 °C and at least one month averaging above 10 °C. Most of these countries have Hot summer humid continental climates, with the coldest month averaging below 0 °C, at least one month's average temperature above 22 °C, and at least four months averaging above 10 °C, with no significant precipitation difference between seasons.

As would be expected, the establishment of *B. kewense* outdoors (in countries and regions with Type C climates with the minimum temperature of the coldest month averaging above 0 °C), and the factors limiting this, chiefly minimum temperature of the coldest month (countries with Type D climates with the minimum temperature of the coldest month averaging below 0 °C) broadly accord with the ensemble model (Annex VIII). The model suggested that the suitability of particular location for the establishment of *B. kewense* was most strongly determined by the minimum temperature of the coldest month in that region, followed by annual precipitation, (then the presence of human-disturbed habitats), then the mean temperature of the warmest quarter, followed last by the Climatic Moisture Index for that area. The seasonality of precipitation did not impact on the suitability of an area for the establishment of *B. kewense*.

(Climate data for the Köppen Climate Classifications previously discussed were derived from https://en.wikipedia.org/wiki/K%C3%B6ppen_climate_classification retrieved 25 June 2021).

Qu. 2.2. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area? Consider if the organism specifically requires another species to complete its life cycle.

RESPONSE	very isolated isolated moderately widespread widespread ubiquitous	CONFIDENCE	low medium high
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Response:

Habitats, and prey suitable for the survival of *Bipalium kewense*, are widespread in the risk assessment area.

The habitat of the natural range of *Bipalium kewense* includes tropical montane forests and rainforests, absent in the Risk Assessment area. The preferred microhabitats of land planarians including *B. kewense* are those of the litter fauna, cryptofauna, and soil fauna (Winsor, 1998). The niches occupied by these flatworms are continuous between one extreme with those of the litter and soil faunas, and the other extreme with the faunas of rocks and trees (Wallwork, 1970). The prey of *Bipalium kewense* in its natural range is unknown. Nothing is known about the preferences of soil type by *B. kewense*, other than a requirement for a humid microhabitat.

Outside its natural range, the outdoor occurrence of *B. kewense* appears to be almost exclusively restricted to public and private gardens and parks. Unlike some other species of alien Bipaliinae, for example *Bipalium adventitium* in the USA (Ducey *et al.*, 2005), there is no evidence to date of *B. kewense* surviving, developing, and reproducing in native forests or other natural habitats in countries with Type C Temperate climates (Köppen Climate Classifications – see Qu 2.1).

In a four-year experimental study of *Bipalium kewense* it was found that of the prey proffered to the planarians, *B. kewense* very rarely predated upon slugs, but preferred live earthworms (Lehnert, 1891). This preference for earthworms by *B. kewense* has been confirmed by subsequent studies and observations (Johri, 1952; Wallen, 1954; Froehlich, 1956; Dundee and Dundee, 1963; Barnwell, 1967; Connella and Stern, 1969; Olewine, 1972; Chandler, 1974; Winsor, 1985). There are no quantitative studies on the impacts of *B. kewense* upon native or alien populations of earthworms.

Based on evidence from countries outside the natural range of *B. kewense*, and outside of the Risk Assessment area, the most common habitats of this flatworm are public and private urban parks and gardens. To these could be added private and public (communal) allotments used to grow produce for private consumption. Under the EUNIS Habitat Classification, these habitats would be classified as “Regularly or recently cultivated agricultural, horticultural and domestic habitats”, defined as “Habitats maintained solely by frequent tilling or arising from recent abandonment of previously tilled ground such as arable land and gardens. Includes tilled ground subject to inundation. Excludes lawns and sports fields (E2.6), shrub orchards (FB), tree nurseries (G5.7) and tree-crop plantations (G3.F etc.)” (European Nature Information System EUNIS <https://eunis.eea.europa.eu/habitats/352> retrieved 26 June 2021).

In these sites there are undoubtedly refuges on the soil surface for sheltering during the day and in adverse weather conditions, such as mulch, leaf litter, masonry, timber, plant pots, and especially within the soil of potted plants. In addition, there is a strong likelihood that such urban habitats will be well watered, and they are also likely to be excellent habitats for species of earthworms, thus providing prey upon which *B. kewense* feeds.

Bipalium kewense is already established outdoors in private urban gardens in France (Justine *et al.*, 2018), Italy (Mori *et al.*, 2021), Malta (T. Cassar and D. Mifsud, 2021 unpubl.), Portugal (Silva, 2020), Spain (Filella-Subirà, 1983; Alvarez-Presas *et al.* 2014), and United Kingdom (Boag *et al.*, 2010).

Qu. 2.3. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

No direct information has been found concerning competition in the establishment of *B. kewense* from existing species in any area. However, *B. kewense* is established in the risk assessment area despite potential predators so the response is very likely with high confidence. It has been postulated that invasive flatworms in Europe may be exploiting an underdeveloped predatory niche, which comes from the abundance of prey species combined with the paucity of the native flatworm fauna in Europe compared to Asia, South America, and Australasia (Boag and Yeates, 2001; Boag *et al.*, 2010).

Qu. 2.4. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Land planarians are apex predators in their microhabitat (Sluys, 2016). Although few of the studies on the natural enemies of flatworms specifically include *B. kewense*, the results can be reasonably extrapolated to land planarians in general because of their shared characteristics such as diet, dermal secretions, and habitat. Some species of land planarians prey on others (Boll *et al.*, 2015), including *O. nungara* (Boll and Leal-Zanchet, 2016). Amongst potential vertebrate predators, cats and amphibians find planarians distasteful, though a shrew ate a specimen of *A. triangulatus* under laboratory conditions (Cannon *et al.*, 1999) and there is anecdotal evidence that some birds in the risk assessment area may take planarians (Cannon *et al.*, 1999). The larvae and adults of staphylinid and carabid beetles have devoured *A. triangulatus* under laboratory and field conditions (Gibson *et al.*, 1997), and the larvae of the mycetophilid fly, *Planivora insignis* parasitizes some species of Tasmanian land planarians (Hickman, 1964). These largely isolated observations provide no indication of the overall impact on land planarian populations and their establishment. Gregarine parasites, common in the seminal vesicles of earthworms, are frequently found infesting the gut of flatworms (Graff, 1899), and where the planarians testes are involved, may cause sterility (Winsor *et al.*, 2004). The parasitic burden of a flatworm may be significant under conditions of physiological stress such as partial desiccation. Laboratory cultures of *B. kewense* are vulnerable to bacterial and fungal infections (Barnwell, 1969, Rodrigues, 1972). Our knowledge of pathogens and parasites of land planarians is extremely limited.

Qu. 2.5. How likely is the organism to establish despite existing management practices in the risk assessment area? Explain if existing management practices could facilitate establishment.

RESPONSE	CONFIDENCE
very unlikely unlikely moderately likely likely very likely	low medium high

Response:

There are no specific management practices routinely applied for *B. kewense* in the risk assessment area. Nevertheless, there are risk management practices in the applied against other pests that may have an untargeted impact on *B. kewense*. However, there is no evidence of pesticide effects on *B. kewense*, and terrestrial flatworms are difficult to target as they are normally sheltered under refuges or in the soil.

Cultivation of the soil is likely to affect *B. kewense* as they are soft-bodied organisms and susceptible to physical damage, though the species successfully reproduces by fission, and damaged specimens will readily regenerate if the conditions are conducive to this.

Bipalium kewense is associated with disturbed and man-modified habitats, so agricultural practices and forestry that disturb habitats and create refuges on the soil surface may benefit flatworms. These would include practices such as logging, baled silage and use of plastic membrane weed suppressants.

Prolonged, or intensive drought will dry topsoil and could destroy populations of land planarians, though in an urban environment these effects may be ameliorated by people watering their gardens. However, in the event of accompanying water restrictions the resulting reduction in moisture deficit in the soil could adversely affect planarian populations (Winsor, 2021, unpubl).

Qu. 2.6. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Bipalium kewense is a cryptic soil dwelling species. Once established in the wild it would be difficult to eradicate from anything other than a small, localised area. Timing of any eradication campaign would be important, as flatworms are seasonal. Under adverse dry or cold weather conditions, or removal of surface litter to expose and dry the soil surface and remove surface microhabitat, land planarians, including *B. kewense*, are likely move deeper into the soil where they are still likely to find prey in the soil fauna. Egg cocoons are small (4mm – 9.5 mm diameter), black and inconspicuous, and would be readily mixed within disturbed soil, and could easily be overlooked, though sexual reproduction is rare and unlikely to occur in the risk assessment area. Hot water treatment may be used to cleanse plants of terrestrial flatworms, either by immersion (Sugiura, 2008; Murchie and Moore, 1998) or as a drench (Justine *et al.*, 2014), and these methods could be effective for *B. kewense*.

Similarly, small regenerating body fragments shed by asexual means of reproduction by adult *B. kewense* are easily overlooked and are able to hide in refugia such as tiny cracks and holes in available microhabitats such as timber and rocks. Shed fragments are vulnerable to desiccation and they cannot feed until the head and pharynx are regenerated (Barnwell, 1967).

Planarians are able to live for long periods without feeding and remain intact even after severe starvation has reduced their body size significantly (Brøndsted, 1969), though there are no experimental data for the survival and regeneration of land planarians following starvation. Depletion of potential food sources may be unsuccessful strategy in eradicating flatworms.

Cultivation of the soil is likely to affect *B. kewense* as they are soft-bodied organisms and susceptible to physical damage and dehydration. However, as the species successfully reproduces by fission, damaged specimens will readily regenerate provided the conditions are conducive for this. *Bipalium kewense* is well adapted to survive most eradication campaigns at anything other than a very small scale so the response is very likely with a medium confidence.

Qu. 2.7. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?

including the following elements:

- a list and description of the reproduction mechanisms of the species in relation to the environmental conditions in the risk assessment area
- an indication of the propagule pressure of the species (e.g. number of gametes, seeds, eggs or propagules, number of reproductive cycles per year) of each of those reproduction mechanisms in relation to the environmental conditions in the risk assessment area.
- If relevant, comment on the likelihood of establishment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in establishment whereas for others high propagule pressure (many thousands of individuals) may not.
- If relevant, comment on the adaptability of the organism to facilitate its establishment and if low genetic diversity in the founder population would have an influence on establishment.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Bipalium kewense, in common with many species of land planarians, has biological characteristics that would facilitate its establishment in the risk assessment area. Chief of these are its modes of reproduction.

Land planarians are hermaphrodite. *Bipalium kewense* can reproduce asexually by fragmentation, a more efficient method than sexual reproduction for converting food energy to offspring, favoured in ecological circumstances over sexual reproduction when food is limiting (Calow *et al.*, 1979). The colonizing success of *B. kewense* can largely be attributed to this asexual mode of reproduction (Hyman, 1943, 1951), in which an individual specimen can give rise to a colony through reproduction by fission, as opposed to sexual reproduction that will require another sexually mature individual, and the time and energy expended in searching for this sexual partner.

In the fission process (Barnwell, 1967; Connella and Stern, 1969) one or two portions of the tail section, 1 - 4 cm long that are able to crawl, are shed a few days after the adult has fed. After several days or weeks, the dropped tail sections will regenerate so that they grow to become small versions of adult specimens. A regenerating *B. kewense* will not attack and eat earthworms until the head region has completely regenerated into the adult shape. However, the ability of a regenerated *B. kewense* to eat depends primarily upon a regenerated pharynx than a regenerated head (Barnwell, 1966). Adult planarians raised in laboratory terraria shed an average of one or two fragments per month (Connella and Stern, 1969). Field observations of a *B. kewense* population in Tennessee US revealed few sightings of fragments over a year, with the ratio of fragments to adult specimens greatest during the cold months (Chandler, 1976). Anterior fission has also been observed in damaged and diseased individuals of *B. kewense* (Ducey *et al.*, 2005).

Bipalium kewense also reproduces sexually, and mature specimens, indicated by the presence of a gonopore, have been found in eleven countries with tropical or subtropical climates, none of which are in the risk assessment area (Winsor, 1983a). Sexual reproduction by *B. kewense* is an uncommon or underreported event (Ducey *et al.*, 2006). Sexual maturity is seasonal. In the USA (Louisiana), adult *B. kewense* were found to be sexually mature over a five-month period from October to February, and non-sexual from March to September (Connella and Stern, 1969).

Following copulation, the fertilized eggs of the planarian are enclosed within a cocoon that is then expelled via the gonopore and attached with mucus to the substratum. The cocoon wall is stabilized by quinone tanning on exposure to air, usually within 24 hours changing in colour from lemon when freshly laid by the planarian through cherry-red, tan, dark brown then black, as has been described for species in all the subfamilies of the Geoplanidae (Winsor, 1998). The cocoon protects the developing young from desiccation.

A single specimen from New Orleans, Louisiana US (Connella and Stern 1969), laid three cocoons, 8 and 21 days apart, with one hatching after 21 days containing 3 juveniles. Another specimen from Cypress, Texas US laid a single cocoon from which emerged seven juveniles after 21 days after laying (Ducey *et al* 2006). The adult flatworm was reproducing by fission 10 days prior to, and five days following laying the cocoon. Cocoons laid by a specimen of *B. kewense* from Queensland, Australia (Charters Towers) measured 9.5 mm diameter declining to cocoons 3 mm diameter from nine successive occasions over 103 days from a single copulation, resulting in a total of six hatchlings (2 juveniles present in each of two cocoons, and one juvenile in each of two single cocoons, with one cocoon damaged); the four cocoons 3 mm - 6 mm were sterile (Winsor, 1984, unpubl.).

In Murfreesboro, Tennessee US (Type C Humid subtropical climate in the Köppen climate classification.) field observations of *B. kewense* revealed the most sightings occurred when the mean air temperature was 18.3°C, and mean soil temperature at 25 mm depth was 18.9°C. Less sightings occurred when the mean air temperature was 5°C and mean soil temperature 10.6°C, and most monthly sightings occurred when the mean air temperature was 22.8°C, and mean soil temperature 19.5°C (Chandler, 1976). Soil moisture was usually above 90% for the entire period of observations. Barnwell (1969) noted that when the temperature dropped to -6.7°C or -9.4°C, planarians were not found in their usual places, but a few days later when conditions were warmer, they reappeared. He also stated that they could survive temperatures near -17°C, but after temperatures fell below this for several nights in 1966 in Athens, Georgia, US, no *B. kewense* were seen for over two years; regions where *B. kewense* could survive outdoors would be limited by the minimum temperature of the coldest month (refer to Qu 2.1).

Like the subtropical humid climates in Tennessee and Georgia in the US, locations within the risk assessment area where *B. kewense* has established outdoors also have Köppen Climate Classification Type C climates (see details in Qu 2.1). Citizen Science observations in France report that specimens of *B. kewense* bury themselves in soil to a depth of up to 20 mm during winter (Justine, 2018, unpubl.).

Bipalium kewense, like all land planarians, is sensitive to moisture and atmospheric humidity. “Land planarians have practically no water-saving adaptations, being dependent upon moisture in their microhabitat, yet are sensitive to too much water and thus generally avoid wet places. Flatworms are mostly strongly seasonal in occurrence, probably reflecting attempts to maintain themselves within a constant microclimate by vertical migration through the continuum of habitat niches. When the

moisture conditions are optimal or too wet, and cover is present, they will occupy the cryptozoic and litter microhabitats on the soil surface and may even venture up trees. When conditions are too dry for them, they retreat into the soil. In the absence of surface cover terrestrial flatworms can live entirely in soil as permanent members of the soil fauna and can be generally regarded as facultative soil animals. Most species appear not to burrow but make use of existing pore spaces to migrate within the soil” (Winsor *et al.*, 1998)

The limit of desiccation without fatal effects, determined for a *Diversibipalium* sp. that can generally be applied to other land planarians, is 40%-50% of body weight (Kawaguchi, 1932). The rate of evaporation from a planarian is proportional to its surface area (Kawaguchi, 1932). *Bipalium kewense* is one of the particularly elongate species of land planarians and consequently has a relatively large surface area and is potentially more vulnerable to desiccation. This may also explain the apparent relatively low vagility of *B. kewense*, as there are costs in energy and moisture loss in moving its particularly elongate body over the substratum. This is especially evident in specimens found crawling over cement pathways in which the latter readily absorb moisture, and on asphalt pathways where the specimens may still be found at sunrise, and subsequently desiccate in the heat (Fletcher, 1887). The proportional rate of evaporation for smaller specimens is greater than for larger ones (Kawaguchi, 1932), and this would put regenerating fission propagules at risk in dry conditions. Prolonged, or intensive drought will dry topsoil and could destroy populations of land planarians, though in an urban environment these effects may be ameliorated by people watering their gardens. However, in the increasingly frequent event of accompanying water restrictions the resulting reduction in moisture deficit in the soil could adversely affect planarian populations (Winsor, 2021, unpubl). Under summer conditions in north Queensland, Australia, living specimens of *Bipalium kewense* were found coiled between an impervious plastic plant box and the hard dry soil beneath; the planarians were entirely covered with a thick coat of mucus that was more viscous than is normally encountered (Winsor, 2021, unpubl). This behaviour would protect the planarian from desiccation, but for what duration is unknown.

Low propagule pressure through asexual reproduction could result in the establishment of the species if climatic conditions were favourable. The rarity of sexual reproduction in the species, and the relatively low fecundity of this means of reproduction, suggests that propagules generated through sexual reproduction are insignificant compared to those produced by asexual reproduction. It is not known whether low genetic diversity in the founder population would have an influence on establishment of the species. However, genetic analyses suggests that many introduced populations are clonal.

Qu. 2.8. If the organism does not establish, then how likely is it that casual populations will continue to occur?

Consider, for example, a species which cannot reproduce in the risk assessment area, because of unsuitable climatic conditions or host plants, but is present because of recurring introduction, entry and release events. This may also apply for long-living organisms.

RESPONSE	N/A very unlikely	CONFIDENCE	low medium
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	unlikely moderately likely likely very likely		high
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Response:

Even if specimens of *B. kewense* do not establish in an area, there is a possibility that they could be re-introduced via potted plants.

Bipalium kewense is already established outdoors in some countries within the risk assessment area. However, continuing trade in plants from regions in which the species is established will continue to pose a risk of introduction to uninfected areas. If such an introduction is to a “protected environment” (e.g. a hothouse) in an uninfected region, a flatworm population will probably survive and likely establish within these limited confines. In the wild, if conditions are not suitable for establishment, then it is unlikely that a transient population would survive. It would either establish or die-off.

Bipalium kewense is one of the particularly elongate species of land planarians and consequently has a relatively large surface area and is potentially more vulnerable to desiccation. This may also explain the apparent relatively low vagility of *B. kewense*, as there are costs in energy and moisture loss in moving its particularly elongate body over the substratum. The rate of evaporation for smaller specimens is greater than for larger ones (Kawaguchi, 1932), and this would put regenerating fission propagules at risk in dry conditions. Under summer conditions in north Queensland, Australia, living specimens of *Bipalium kewense* were found coiled between an impervious plastic plant box and the hard dry soil beneath; the planarians were entirely covered with a thick coat of mucus that was more viscous than is normally encountered (Winsor, 2021, unpubl). This behaviour would protect the planarian from desiccation, but for what duration is unknown. Prolonged, or intensive drought will dry topsoil and could destroy populations of land planarians, though in an urban environment these effects may be ameliorated by people watering their gardens. However, in the event of accompanying water restrictions the resulting reduction in moisture deficit in the soil could adversely affect planarian populations (Winsor, 2021, unpubl.). When drought conditions ease, re-introduction of *B. kewense* could occur if people re-establish their gardens using contaminated nursery material.

Qu. 2.9. Estimate the overall likelihood of establishment in the risk assessment area under current climatic conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under current climatic conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in current conditions: providing insight in the risk of establishment in (new areas in) the risk assessment area.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Bipalium kewense is a predominantly a tropical and subtropical species, and at present it is established in urban gardens outdoors in the risk assessment area. Therefore the score is very likely with high confidence.

In the Atlantic biogeographical region, *B. kewense* is established outdoors in France in the department Pyrénées-Atlantiques, the climate of which appears particularly conducive to flatworms, and food and habitat are readily available in the urban gardens that thrive in this climate (Justine *et al.* 2018). In the United Kingdom, *B. kewense* has been recorded from “protected” environments (e.g. hothouses), just outside hothouses of the Glasgow Botanic Gardens, and urban gardens in Liverpool and Sussex (Boag *et al.*, 2010). Australian and New Zealand land planarians have been recorded outdoors from the warmer regions in the U.K. such as the Isles of Scilly or Cornwall, Continental (Jones, 2005), but to date not *B. kewense*.

In the Mediterranean biogeographic region, the species is established in Italy (Mori *et al.* 2021), Malta (T. Cassar and D. Mifsud, 2021 unpubl.), Portugal (Silva, 2020), and Spain (Filella-Subirà, 1983; Alvarez-Presas *et al.* 2014). In urban gardens the availability of food (earthworms) is unlikely to be a limiting factor. If the urban habitat is conducive to land planarians, the species could establish in Cyprus, Greece and Turkey. For example the Australian flatworm *Caenoplana bicolor* is established in western Crete, in the urban environment of Vryses square with Plane trees and the Vryisianos stream (Vardinoyannis and Alexandrakis, 2019). This type of habitat would probably also suit *B. kewense*.

In the Continental biogeographical region, *B. kewense* has only been recorded in “protected” environments (e.g. hothouses) in Austria, Belgium, Czech Republic, Denmark, Finland, Germany, Netherlands, Poland, Slovakia, and the United Kingdom. The main factor constraining the establishment of *B. kewense* outdoors in this biogeographic region is chiefly the minimum temperature of the coldest month (See Qu 2.1).

Qu. 2.10. Estimate the overall likelihood of establishment in the risk assessment area under foreseeable climate change conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under foreseeable climate change conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of establishment (e.g. increase in average winter temperature, increase in drought periods)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely establishment within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided.

However, if new, original models are executed for this risk assessment, the following RCP pathways

shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

With warmer temperatures and increased rainfall, climate change is highly likely to make biogeographical regions more amenable for the expanded establishment of *B. kewense* so the score remains very likely with high confidence.

Under Climate Change with timeframe 2070, and emission scenarios RCP 2.6 and RCP 4.5: Alpine, Atlantic, Black Sea, Continental, Mediterranean, Pannonian and Steppic in the risk assessment area; and in the Anatolian and Macaronesia biogeographic regions (Refer to Figure 1 Annex VIII). There are increases in the projected suitability for the establishment of *B. kewense* generally north and northeastwards under both RCP 2.6 and RCP 4.5 in the Atlantic, Black Sea, Continental, Pannonian and Steppic biogeographical regions in the risk assessment area, and in the Anatolian biogeographic region (Refer to Figures 3 and 4 Annex VIII). The lower to mid-levels of the Alpine region appear to become increasing suitable for the establishment of the species under the RCP 2.6 and 4.5 scenarios. For the Mediterranean region, there is little change between the current projected suitability and those under RCP 2.6 and RCP 4.5. However, in the Macaronesia region, the projected suitability for the establishment of *B. kewense* is the same for current and RCP 2.6 scenarios, but a decline in suitability under the RCP 4.5 scenario as region will exceed the mean temperature of the warmest quarter.

3 PROBABILITY OF SPREAD

Important instructions:

- Spread is defined as the expansion of the geographical distribution of an alien species within the risk assessment area.
- Repeated releases at separate locations do not represent continuous spread and should be considered in the probability of introduction and entry section (Qu. 1.7).

Qu. 3.1. How important is the expected spread of this organism within the risk assessment area by natural means? (List and comment on each of the mechanisms for natural spread.)

including the following elements:

- a list and description of the natural spread mechanisms of the species in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of spread discussed in relation to the species biology and the environmental conditions in the risk assessment area.

The description of spread patterns here refers to the CBD pathway category “Unaided (Natural Spread)”. It should include elements of the species life history and behavioural traits able to explain its ability to spread, including: reproduction or growth strategy, dispersal capacity, longevity, dietary requirements, environmental and climatic requirements, specialist or generalist characteristics.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Terrestrial flatworms move by creeping on the soil surface. In general, human-aided dispersal is long distance with natural dispersal local. Flatworms will gradually move out from the initial foci to colonise favourable surrounding areas. *Bipalium kewense* appears to have low vagility (Winsor, 2021 unpubl.). Natural spread within the risk assessment area will be dependent on climatic conditions. It may be that *B. kewense* is restricted to particular microhabitats and is unable to move naturally between these.

The life stages of *B. kewense* (mainly fission, with adult and regenerating propagules, and very rarely egg capsule and resulting juveniles) are all soil-litter bound and there is no specific dispersal phase. Some species of terrestrial flatworms can survive long periods of submersion in water, and it is possible that they can be dispersed by rafting on various objects such as logs in floodwater, or by sea, although this has not been confirmed by direct observation in the field. The occurrence of *B. kewense* on the second terrace of the Guadeloupe River in the Guadeloupe River State Park, Kendall County Texas US, was attributed to flood-borne specimens from an upstream urban area (Neck, 1987). There

is no evidence to support dispersal of *B. kewense* by birds or other animals, though other flatworm species have been reported on pet and livestock fur (Moore et al., 1998).

Bipalium kewense feeds exclusively upon earthworms and there is no evidence of dispersal to follow prey populations; rather, the flatworms will move locally to hunt individuals.

Qu. 3.2a. List and describe relevant pathways of spread other than "unaided". For each pathway answer questions 3.3 to 3.9 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 3.3a, 3.4a, etc. and then 3.3b, 3.4b etc. for the next pathway.

including the following elements:

- a list and description of pathways of spread with an indication of their importance and associated risks (e.g. the likelihood of spread in the risk assessment area, based on these pathways; likelihood of survival, or reproduction, or increase during transport and storage; ability and likelihood of transfer from the pathway to a suitable habitat or host) in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of spread for each pathway discussed in relation to the species biology and the environmental conditions in the risk assessment area.
- All relevant pathways of spread (except “Unaided (Natural Spread)”, which is assessed in Qu. 3.1) should be considered. The classification of pathways developed by the Convention of Biological Diversity shall be used (see Annex IV).

Response:

Invasive alien terrestrial flatworms are cryptic soil-dwelling organisms that are mostly spread through human activities. As with ‘introduction into the risk assessment area’, the most likely pathway for spread of *B. kewense* within the risk assessment area, at least initially, involves potted plants.

Bipalium kewense was described from a specimen found in a hothouse at Kew Gardens, and subsequently in gardens with direct relations with Kew, but around this period the species was also found in hothouses that had no connection with Kew; the consensus of opinion at that time was that both Kew and other hothouses had been stocked from an unidentified common source, and all were agreed that it had come to them in connection with orchids from Burma (Bell, 1886a, 1886b) during the Victorian craze for orchids that lasted up to the first World War (Hansen 2001). There is no evidence that this pathway “Botanical Garden / zoo /aquaria” remains active for *B. kewense* and will not be discussed further (See also Q 1.2b). There is a report (Boag *et al.*, 2010), of a specimen of *B. kewense* found in the Glasgow Botanic Gardens, close to but outside the glasshouses. It is not known whether the specimen escaped from the glasshouses, or came to that spot via some other pathway such as contaminate nursery material (unintentional), or transportation of habitat material (unintentional).

Potted plants can be bought by the public and disseminated from garden centres, nurseries, DIY stores and supermarkets and these have all been classed under the ‘Contaminant nursery material’ pathway (unintentional). The second pathway considered is ‘Transportation of habitat material’ (unintentional). This refers to the movement of soil and compost, which may contain *B. kewense*. A third pathway is ‘Machinery/equipment’. There is some overlap with the movement of soil, as flatworms may be carried in soil adhering to machinery or equipment. A fourth pathway is Contaminant on plants

(except parasites, species transported by host/vector” in which there is personal transfer of potentially contaminant plants between gardeners .

For another flatworm species (*Arthurdendyus triangulatus*) a range of miscellaneous pathways for spread have been documented, including being caught on pet and livestock fur, stuck to plastic silage bale wrapping and broadcast with farmyard manure (Boag and Yeates, 2001; Moore *et al.*, 1998; Boag *et al.*, 1999). However there is no evidence of these pathways being active for *B. kewense*.

For all pathways, contamination with *B. kewense* is likely to be sporadic and random. The potential propagule pressure along these pathways is likely to be individually low; however, a single adult flatworm reproducing asexually could eventually give rise to a population under suitable conditions. Given the rarity and climatic constraints on sexual reproduction in *B. kewense*, the potential for this to happen from a single fertilized egg capsule is extremely low in the risk assessment area.

Pathway name: **Contaminant nursery material**

Qu. 3.3a. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response:

There is evidence that other alien flatworms have been unintentionally disseminated in potted plants from infected garden centres to the public (key reference Alford *et al.*, 1996). There is no direct evidence of *B. kewense* being disseminated in this way within the risk assessment area, or outside it; it is assumed that like other alien flatworms for which there is evidence, this is pathway also applies to *B. kewense*.

Qu. 3.4a. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication.
- if appropriate, indicate the rate of spread along this pathway.
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Unintentional transport of *B. kewense* via contaminant nursery material is considered the most likely means for spread of *B. kewense*. Usually, such spread is anecdotal and undocumented. However, Fletcher (1887) gives the historic example of specimens of *B. kewense* found in Hyde Park, Sydney, that had evidently strayed from the enclosure around Captain Cook's statue, which had been stocked with plants from the Botanic Gardens nursery.

Low propagule pressure through asexual reproduction (refer to Qu 2.7) could still result in the establishment of the species if climatic conditions were favourable. Similarly the species may be re-introduced via the same pathway following eradication (refer to Qu 2.6).

There are no data or estimates on the number or volume of specimens passing along this pathway in the course of a year, but the trade pathway is large with more than 22 billion euros of plant and flowers produced in the EU in 2019. (https://ec.europa.eu/info/sites/default/files/food-farming-fisheries/plants_and_plant_products/documents/flowers-ornamental-plants-statistics_en.pdf).

Bipalium kewense is one of the particularly elongate species of land planarians and consequently has a relatively large surface area and is potentially more vulnerable to desiccation than for example the smaller alien species *Obama nungara*. This may explain the apparent relatively low vagility of *B. kewense*, as there are costs in energy and moisture loss in moving its particularly elongate body over the substratum. This is especially evident in specimens found crawling over cement pathways in which the latter readily absorb moisture, and on asphalt pathways where the specimens may still be found at sunrise, and subsequently desiccate in the heat (Fletcher, 1887).

Generally, *B. kewense* is only found in the open at night when the humidity is relatively high, and on wet overcast days, possibly seeking earthworm prey that is also often in the open under these conditions. The distance the flatworm travels appears to be only within a few metres from its normal place of refuge but may vary depending upon the availability of prey and alternative refugia (Winsor, 2021, unpubl.).

Qu. 3.5a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely	CONFIDENCE	low medium high
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	very likely		
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Response:

If *B. kewense* is transferred with potted plants containing soil, then providing these plants are not exposed to temperature extremes, and sufficient moisture is retained, it is likely that flatworms will survive. Reproduction by fragmentation may be possible in transit and during storage in the garden centres or nurseries depending upon the availability of earthworms, as asexual reproduction generally follows feeding on prey.

Qu. 3.6a. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

National phytosanitary measures within Member States may be implemented at nurseries / garden centers to control flatworms, if a consignment is suspected to be contaminated, but are rarely executed in practice. In such a case, use of hot-water treatment shows promise for flatworm management (Murchie and Moore, 1998; Sugiura, 2008a; Justine *et al.*, 2014). However, we know of no instances where any routine management is practiced against invasive flatworms.

Qu. 3.7a. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Bipalium kewense is a cryptic, nocturnal soil and litter-dwelling species. It can shelter within plant pots, root balls and within plant material making it difficult to detect unless the plants are uprooted and checked by experts, and the growing medium and root balls examined directly. *Bipalium kewense* has already spread along this pathway, and has been largely undetected, as documented in France (Justine *et al.*, 2018). It is very likely that its spread would be undetected and confidence is high.

Qu. 3.8a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

If local conditions are favourable, it is highly probably that *B. kewense* would transfer from plant root balls or plant pots) to an urban garden or other site where there was earthworm prey. This is essentially direct transfer to an urban environment.

Qu. 3.9a. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response:

If *B. kewense* established itself in garden centres or nurseries in the risk assessment area, then there is great capacity for secondary spread through this means, especially in countries with temperate climates (Type C climates with the minimum temperature of the coldest month averaging above 0 °C. see Qu 2.1) where the species is presently established outdoors in urban gardens.

However, *B. kewense* will have limited distribution within the risk assessment area in countries with the minimum temperature of the coldest month averaging below 0 °C (Type D climates – see Qu 2.1) where the species is only found in “protected facilities” (e.g. hothouses).

The extent of spread is difficult to predict as although most garden centres will operate locally, some may trade on the internet, whilst customers may purchase plants on holiday or travelling. Such spread is therefore likely to be mostly localised but with some random long-distance transfers.

As transfer of plants is human-mediated and likely to be via cars and vans, environmental conditions are unlikely to have an impact on this means of transfer *per se*. *Bipalium kewense* has already established in suitable climates within the risk assessment area. However, spread has not been

particularly fast compared to species such as *Obama nungara*. Justine *et al.* (2018) admitted surprise that *B. kewense* has probably been undetected in some regions of France for over 20 years, which also suggests that spread had not been extensive or rapid.

There is no quantitative data available that would facilitate estimates for the potential rate of spread for *B. kewense* or other flatworm species.

End of pathway assessment, repeat Qu. 3.3 to 3.9. as necessary using separate identifier.

Pathway name: Transportation of habitat material

Qu. 3.3b. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional unintentional	CONFIDENCE	Low medium high
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Response:

Terrestrial flatworms may be spread with contaminated soil or material left on the soil surface beneath which they have sheltered. Such transport is unintentional.

Qu. 3.4b. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication.
- if appropriate, indicate the rate of spread along this pathway.
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	Low medium high
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Response:

Movement of topsoil, mulch, and compost have spread terrestrial flatworms, such as *A. triangulatus* (Justine *et al.*, 2014; Cannon *et al.*, 1999; Christensen and Mather, 1998; Moore *et al.*, 1998). Given that *B. kewense* shelters under soil refuges in the same way as *A. triangulatus*, it is reasonable to assume that this flatworm species could be spread in a similar manner. As with potted plants, much will depend on the ability of *B. kewense* to survive outdoors within the risk assessment area. Such

spread is largely random and unpredictable. There are no specific data for *B. kewense*. The volume of topsoil and compost movement is such that the starter populations will be larger than with individual potted plants making the chance of successful establishment higher.

Qu. 3.5b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	Low medium high
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Response:

Soil, mulch, and compost can provide microhabitats that retain moisture and buffer temperature fluctuations. Depending on the environmental conditions, *B. kewense* would likely survive transport and storage in soil especially if the volume is large. Large fragile elongate specimens may get damaged in the process of loading and unloading, possibly resulting in additional propagules via reproductive fragmentation.

Qu. 3.6b. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	Low medium high
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Response:

Management practices vary depending on the substrate moved. There are currently no management practices that would prevent transport of *B. kewense* if carried in large quantities of soil, mulch, or compost. Subsequent cultivation of the soil would be detrimental to *B. kewense* as the flatworm could be physically damaged (bearing in mind comments about regeneration of fragments).

Qu. 3.7b. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely	CONFIDENCE	Low medium high
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	very likely		
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Response:

Detecting *B. kewense*, a cryptic and a brown-ochre coloured flatworm, in large quantities of soil, mulch, or compost would be exceedingly difficult. Most often terrestrial flatworms are found in the location afterwards, and retrospective association made with a recent delivery of materials.

Qu. 3.8b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	Low medium high
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Response:

Topsoil, mulch, or compost used in garden and landscaping would directly transfer *B. kewense* into a suitable habitat assuming that the minimum temperature of the coldest month in the area averaged above 0 °C.

Qu. 3.9b. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	Low medium high
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Response:

As with movements of ornamental and garden plants, most topsoil, mulch, and compost will be used in the local vicinity. It is difficult to quantify the potential for spread *B. kewense* as there is a deficiency of relevant data. As with potted plants, much will depend on the ability of the flatworm and any fragments resulting from asexual reproduction of *B. kewense*, to survive outdoors provided that the minimum temperature of the coldest month in the area averaged above 0 °C.

If the topsoil, mulch, and compost are heaped and the flatworm can burrow into and below the mass, then it is expected that specimens would survive in this “semi-protected” environment. However if the topsoil, mulch, or compost is spread, then there is a high likelihood that the flatworms would desiccate.

End of pathway assessment, repeat Qu. 3.3 to 3.9. as necessary using separate identifier.

Pathway name: Machinery/equipment (transport stowaway)

Qu. 3.3c. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional unintentional	CONFIDENCE	Low medium high
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Response:

Transfer along this pathway is unintentional. Terrestrial flatworms can be carried in soil remnants left on machinery or equipment. In addition, flatworms are covered in mucus and may adhere directly to machinery and equipment, and in particular wooden and plastic pallets that have remained in-situ for some period of time.

Qu. 3.4c. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication.
- if appropriate, indicate the rate of spread along this pathway.
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	Low medium high
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Response:

There are no records of *B. kewense* being transported inadvertently in soil on boots or machinery. However, it is possible that flatworms will stick directly to soil-working machinery or equipment, especially wooden and plastic pallets in contact with the soil, and particularly if it is left to stand in one place for a prolonged period prior to relocation and thus allowing flatworms time to settle underneath. This is random and sporadic spread. There are no data available to estimate the propagule pressure. The rate of spread along this pathway will be determined by the movement of machines or equipment including pallets. As these are likely to horticultural or agricultural, most movement will be local. The likelihood of transport in this manner and the confidence level is dependent on distance. The longer the distance travelled the less likely flatworms will survive as they will be subject to mechanical damage and desiccation (please see below, Qu 3.5c).

Qu. 3.5c. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Adult *B. kewense* are unlikely to survive well when transported stuck to machinery or equipment, including wooden and plastic pallets. They will be prone to desiccation, exposed to temperature extremes and physical damage. Flatworms are only likely to survive short-range transport along this pathway. Egg cocoons may be more resilient than adults and being smaller and immobile, may be carried embedded within small quantities of soil stuck to machinery, but there is no evidence of this occurring for *B. kewense*.

Qu. 3.6c. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Sometimes agricultural equipment and machinery may be power washed and / or sprayed with disinfectant between sites for biosecurity purposes. The effects of disinfectants on *B. kewense* are not

known but the power washing process may dislodge them. Working machinery is likely to be detrimental to *B. kewense* survival as moving parts will increase the risk of physical damage but bearing in mind the ability of this flatworm to regrow from fragments.

Qu. 3.7c. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Other terrestrial flatworm species have been seen in transit on equipment or machinery, but this is mostly happenstance. In Scotland and Northern Ireland, *A. triangulatus* has been seen on hay and silage bales and the equipment used to move these (Boag *et al.*, 1999; Moore *et al.*, 1998). There is no evidence of spread of *Bipalium kewense* via this, or the food contaminant pathways.

Qu. 3.8c. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Soil-working machinery or equipment could transfer *B. kewense* directly to a new soil habitat. However, terrestrial flatworms are susceptible to physical damage, especially elongate specimens such as *B. kewense*, and may not survive intensive cultivation practices. Furthermore, the absence of refuges and greater movement of air (and hence lower relative humidity) that occur near the centre of fields, may explain why terrestrial flatworms have difficulty in becoming established in arable land, yet can readily be found in nurseries, gardens centres and urban gardens (Yeates *et al.*, 1998). Movement of more static equipment, especially wooden and plastic pallets resting on the soil surface, to garden and semi-natural sites poses the greatest risk for this pathway.

Qu. 3.9c. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response:

Agricultural and horticultural machinery and equipment are predominantly used locally, although sometimes they can be moved long distances between sites. There are no quantitative data on the rate of spread of *B. kewense* or other terrestrial flatworms along this pathway. As with the other pathways above, it is likely to be random and sporadic.

End of pathway assessment, repeat Qu. 3.3 to 3.9. as necessary using separate identifier.

Pathway name: “**Contaminant on plants (except parasites, species transported by host/vector)**”
Unintentional.

Qu. 3.3d. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response:

The pathway “Contaminant on plants” concerns the unintentional transfer of potentially contaminated plants between gardeners. There is a single report of this type of occurrence in London, Ontario, Canada (Judd, 1957), where a resident obtained two plants of *Amaryllis* that had been potted and kept in a greenhouse for the previous six months; on receipt the plants were placed in a sink and thoroughly watered with liquid fertilizer; a few minutes later a specimen of *B. kewense* emerged from one of the pots.

Qu. 3.4d. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or

frequency of passage through pathway), including the likelihood of reinvasion after eradication.

- if appropriate, indicate the rate of spread along this pathway.
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

There are no data or estimates on the number or volume of specimens passing along this pathway in the course of a year. Exchange of plants and seeds (plant swapping) is a widespread social practice amongst gardeners.

Low propagule pressure through asexual reproduction of *B. kewense* (refer to Qu 2.7) could still result in the establishment of the species if conditions were favourable in the area to which the contaminated potted plant was taken (refer to Qu 2.1).

Similarly the species may be re-introduced via the same pathway following eradication (refer to Qu 2.6).

Qu. 3.5d. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

If *B. kewense* is transferred with potted plants containing soil, then providing these plants are not exposed to temperature extremes, and sufficient moisture is retained, it is likely that flatworms will survive. Reproduction by fragmentation may be possible in transit and during storage in the garden centres or nurseries depending upon the availability of earthworms, as asexual reproduction generally follows feeding on prey.

Qu. 3.6d. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

National phytosanitary measures within Member States may be implemented at nurseries / garden centers to control flatworms but such measures are highly unlikely to be implemented by private individuals socially exchanging plants.

Qu. 3.7d. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Bipalium kewense is a cryptic, nocturnal soil and litter-dwelling species. It can shelter within plant pots, root balls and within plant material making it difficult to detect unless the plants are uprooted and checked by experts, and the growing medium and root balls examined directly. The state of knowledge by the average home gardener about alien flatworms throughout the risk assessment area is not known, but from the lack of public awareness of terrestrial flatworms in France (Justine *et al.*, 2018) it is probably poor, possibly because the planarians are generally cryptic and concealed. However, many urban gardeners have joined the citizen science network organized in France for collecting information about land planarians (Justine *et al.*, 2018), and as a result the awareness of the public about land planarians has improved. *Bipalium kewense* has already spread along this pathway in Canada (Judd, 1957), but is not documented in the risk assessment area.

Qu. 3.8d. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely	CONFIDENCE	low medium
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	moderately likely likely very likely		high
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Response:

If climatic conditions are favourable (see Qu 2.1), it is highly probable that *B. kewense* would transfer from plant pots arising from a social plant exchange to an urban garden where there was earthworm prey. Also the plants may well be planted out into the garden after being transported in pots.

Qu. 3.9d. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response:

If *B. kewense* established itself in urban gardens in the risk assessment area, then there is great capacity for spread through this pathway, especially in countries with temperate climates (Type C climates with the minimum temperature of the coldest month averaging above 0 °C. see Qu 2.1) where the species is presently established outdoors in urban gardens.

However, *B. kewense* will have limited potential for spread via social plant exchanges within the risk assessment area in countries with the minimum temperature of the coldest month averaging below 0 °C (Type D climates – see Qu 2.1) where the species has only been found in “protected facilities” (e.g. hothouses).

The extent of spread is difficult to predict as social plant exchanges are likely to be local, such as within families and social networks. Such spread is therefore likely to be mostly localised but with some random long-distance transfers.

As transfer of plants is human-mediated and likely to be via cars and vans, environmental conditions are unlikely to have an impact on this means of transfer *per se*. *Bipalium kewense* has already established in suitable climates within the risk assessment area. Justine *et al.* (2018) admitted surprise that *B. kewense* has probably been undetected in some regions of France for over 20 years, which also suggests that spread had not been extensive or rapid.

There is no quantitative data available that would facilitate estimates for the potential rate of spread for *B. kewense* or other flatworm species via this pathway.

End of pathway assessment, repeat Qu. 3.3 to 3.9. as necessary using separate identifier.

Qu. 3.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?

RESPONSE	very easy easy with some difficulty difficult very difficult	CONFIDENCE	low medium high
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Response:

Bipalium kewense is a cryptic soil-dwelling flatworm that is already present in the risk assessment area. It can be spread by potted plants, soil, and agricultural/horticultural equipment. The potential individual pathways within this context are numerous and exceedingly difficult to manage. Other than direct and potentially destructive inspection, *B. kewense* would be difficult to detect and there are no universal control measures that could be applied to bulk quantities of either plants, soil, mulch, or compost.

Qu. 3.11. Estimate the overall potential rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (indicate any key issues and provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in current conditions, providing insight in the risk of spread into (new areas in) the risk assessment area.

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response:

Bipalium kewense is a predominantly a tropical and subtropical species, and at present it is established in urban gardens outdoors in the risk assessment area.

Wherever it is established *B. kewense* will most likely continue to be spread via various pathways given that the species has been in the risk assessment area mainly within “protected” environments such as hothouses since the late 19th Century, and outdoors in more recent times. Potted plants kept in the same locality would be the initial reservoir for spread within the risk assessment area; spread via contaminant nursery material, and contaminant on plants via the horticultural trade or between amateur gardeners would be difficult to detect and manage.

Local spread would possibly occur via the machinery / equipment pathway through transport of soil or equipment and materials in contact with the soil surface. Given the right conditions, *B. kewense* might also gradually colonise surrounding areas through natural dispersal.

In the Atlantic biogeographical region, *B. kewense* is established outdoors in France in the department Pyrénées-Atlantiques, the climate of which appears particularly conducive to flatworms, and food and habitat are readily available in the urban gardens that thrive in this climate (Justine *et al.* 2018). In the U.K., *B. kewense* has only been recorded from “protected” environments (e.g. hothouses). Australian and New Zealand land planarians have been recorded outdoors from the warmer regions in the U.K. such as the Isles of Scilly or Cornwall, Continental (Jones, 2005), but to date not *B. kewense*.

In the Mediterranean biogeographic region, the species is established in Italy (Mori *et al.* 2021), Malta (T. Cassar and D. Mifsud, 2021 unpubl.), Portugal (Silva, 2020), and Spain (Filella-Subirà, 1983; Alvarez-Presas *et al.* 2014). In urban gardens the availability of food (earthworms) is unlikely to be a limiting factor. If the urban habitat is conducive to land planarians, the species could establish in Cyprus, Greece and Turkey. For example the Australian flatworm *Caenoplana bicolor* is established in western Crete, in the urban environment of Vryses square with Plane trees and the Vrysianos stream (Vardinoyannis and Alexandrakis, 2019). This type of habitat would probably also suit *B. kewense*.

In the Continental biogeographical region, *B. kewense* has only been recorded in “protected” environments (e.g. hothouses) in Austria, Belgium, Czech Republic, Denmark, Finland, Germany, Netherlands, Poland, Slovakia, and the United Kingdom. The main factor constraining the establishment of *B. kewense* outdoors in this biogeographic region is chiefly the minimum temperature of the coldest month (See Qu 2.1).

The rate of spread of *B. kewense* in the biogeographical regions in the risk assessment area under current conditions can be crudely estimated as follows: based upon literature records, the first occurrence of *Bipalium kewense* in countries and territories in the risk assessment area (RAA) can be divided into two distinct periods between 1877-1971 and 1982-2021.

The first period concerns the occurrence of the species in managed facilities such as hothouses largely in the Alpine, Atlantic, and Continental biogeographic regions, from 1877 – 1912, with a rate of spread of one country every 4.4 years, slowing between 1913 and 2002 with an overall rate (1877 – 2002) of one country every 11.4 years. These reports concern land planarians such as *B. kewense*, *Dolichoplana striata*, and some South American species brought to Europe from overseas via Wardian Cases largely during the period of Empires. The three later hothouse records in 1935, 1971 and 2002 may have been as a result of distribution from existing sources within the risk assessment area.

The second period concerns the occurrence of the species outdoors in urban situations largely in the southern Atlantic and Mediterranean biogeographic regions from 1982 - 2002, with a rate of one country every 4 years, starting to slow between 2002 – 2021 with only a single report, and an overall rate (1982-2021) of one country every 6.5 years. These reports possibly concern “new arrivals” via the plant trade rather than redistribution from managed facilities and may have been spread together with other alien land planarians such as recently reported outdoor occurrences of species of *Caenoplana*, *Bipalium*, *Diversibipalium* and *Obama* that have not generally been recorded in managed facilities.

Both periods are characterised by a rapid series of species occurrence reports, followed by a slowing of reports trending towards a plateauing of occurrences. Given these approximately parallel trends,

under current conditions, it is expected that the rate of spread (taken as the rate of publication of occurrences in new countries within the risk assessment area) should slow. However, the rate of spread within countries where *B. kewense* is present outdoors could increase. A more accurate study might be based on identified museum specimens, and / or dated photographs confirmed as of *B. kewense*, as in GBIF and iNaturalist. Also, new reports in all countries should be considered.

An additional indicator for the rate of spread of *B. kewense* that could be used to predict possible areas of likely establishment and measure spread rate is new urban developments within the Atlantic and Mediterranean biogeographic regions. In Australia, through enquiries to State museums and posts on iNaturalist Australia and Questagame, the interstate spread of a suite of native and alien land planarians via contaminant nursery material (unintentional) and transportation of habitat material (unintentional) is being casually monitored and suggests that the spread of the flatworms has some relationship to new housing developments in outer suburbs where owners plant gardens and landscape their properties (Winsor, 2021 unpubl.).

Qu. 3.12. Estimate the overall potential rate of spread in relevant biogeographical regions in foreseeable climate change conditions (provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if rates of spread are likely slowed down or accelerated.

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response:

Bipalium kewense is mostly found in tropical and subtropical regions, albeit at altitude within these areas (Justine *et al.*, 2014). The optimum temperature for rearing *B. kewense* is between 15.6°C and 32°C and the flatworm is dependent on high humidity and soil moisture (Barnwell, 1968, as see Qu 2.7). Increased temperature and rainfall brought upon climate change could increase the rate of natural spread of *B. kewense*. For the human-dependent pathways, it is more difficult to suggest an effect of climate change. Changes in consumer preference for ornamental plants and the possibility of growing more exotic species may lead to greater south-to-north European plant trade, which would facilitate spread of *B. kewense*. The Atlantic biogeographical region would be most affected by this scenario as it extends further north.

4 MAGNITUDE OF IMPACT

Important instructions:

- Questions 4.1-4.5 relate to biodiversity and ecosystem impacts, 4.6-4.8 to impacts on ecosystem services, 4.9-4.13 to economic impact, 4.14-4.15 to social and human health impact, and 4.16-4.18 to other impacts. These impacts can be interlinked, for example, a disease may cause impacts on biodiversity and/or ecosystem functioning that leads to impacts on ecosystem services and finally economic impacts. In such cases the assessor should try to note the different impacts where most appropriate, cross-referencing between questions when needed.
- Each set of questions starts with the impact elsewhere in the world, then considers impacts in the risk assessment area (=EU excluding outermost regions) separating known impacts to date (i.e. past and current impacts) from potential future impacts (including foreseeable climate change).
- Only negative impacts are considered in this section (socio-economic benefits are considered in Qu. A.7)
- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

Biodiversity and ecosystem impacts

Qu. 4.1. How important is the impact of the organism on biodiversity at all levels of organisation caused by the organism in its non-native range excluding the risk assessment area?

including the following elements:

- Biodiversity means the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems.
- impacted chemical, physical or structural characteristics and functioning of ecosystems

RESPONSE		CONFIDENCE	
	minimal		low
	minor		medium
	moderate		high
	major		
	massive		

Response:

No specific information has been found on this issue. Full evaluation of the impacts of *B. kewense* is hampered by the absence of ecological studies.

The prey of *Bipalium kewense* in its natural range is unknown.

However, in a four-year experimental study of two species of land planarians *Rhynchodemus sylvaticus*, a molluscivorous species, and *Bipalium kewense* (Lehnert, 1891), it was found that of the prey proffered to the planarians, *B. kewense* very rarely predated upon slugs, but preferred live

earthworms. This prey preference, confirmed by subsequent studies and observations (Johri, 1952; Wallen, 1954, Froehlich, 1956, Dundee and Dundee, 1963; Barnwell, 1967; Connella and Stern, 1969; Olewine, 1972; Chandler, 1974, Winsor, 1985), is reinforced to the extent that the collagenase present in this species is most active against earthworm (invertebrate-type) collagen (Dresden and Landsperger, 1977).

There are no quantitative studies on the impacts of *B. kewense* upon native or alien populations of earthworms.

Bipalium kewense is practicably solely a predator of earthworms, yet in New South Wales and Queensland, Australia it is regarded as an occasional minor pest in some urban domestic and commercial vermiculture farms that cultivate alien species of earthworms, where it is readily controlled by hand picking following water-flooding the beds (Winsor, 1998). Distribution data worldwide suggests that *B. kewense* is largely restricted to urban gardens, parks, and human-modified habitats. In Australia, the common earthworms in south-eastern urban gardens are mostly introductions from Europe with the Grey worm, *Aporrectodea caliginosa*, an alien species of earthworm originally from and common in the UK, to be most common species in urban habitats and orchards, with native species rarer in disturbed habitats (Baker *et al.*, 1997); consequently, these introduced earthworms that predominate in urban habitats (public and private gardens and parks) are the most likely prey for *B. kewense* in south-eastern Australia.

In one study under laboratory conditions (Connella and Stern, 1969), *B. kewense* was fed the native North American earthworm species *Diplocardia singularis* raised specifically for that purpose.

Therefore despite *B. kewense* being recorded in many countries around the world, only isolated accounts of predation of “earthworms”, largely under experimental conditions, have been reported (Lehnert, 1891, Johri, 1952, Dundee and Dundee, 1963, Connella and Stern, 1969, Olewine, 1972; Chandler, 1974, Winsor *et al.*, 2004, Ducey *et al.*, 2006).

Data on predatory behaviour and reproduction are available for two invasive bipaliines in North America *Bipalium adventitium* (key recent reference Ducey *et al.*, 2005), and *Bipalium* sp. cf *vagum* (Ducey *et al.*, 2007), though unlike *B. kewense* both these species are relatively small (5-10 cm long, and 3.5 cm long respectively), and propagate principally by sexual rather than asexual reproduction. Given the different principal reproductive strategies, and relative fecundity, the ecological impacts of *B. adventitium* may not necessarily extrapolate to become those of *B. kewense*.

Whilst these studies confirm that *B. kewense* feeds upon earthworms, the actual impact of the planarian on earthworm populations and possibly flow-on effects on biodiversity are unknown.

Qu. 4.2. How important is the current known impact of the organism on biodiversity at all levels of organisation (e.g. decline in native species, changes in native species communities, hybridisation) in the risk assessment area (include any past impact in your response)?

Discuss impacts that are currently occurring or are likely occurring or have occurred in the past in the risk assessment area. Where there is no direct evidence of impact in the risk assessment area (for example no studies have been conducted), evidence from outside of the risk assessment area can be used to infer impacts within the risk assessment area.

RESPONSE	minimal minor	CONFIDENCE	low medium
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	moderate major massive		high
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Response:

No information has been found on this issue. *Bipalium kewense* does not appear to be established outside urban and agricultural land, or in wild habitats in the risk assessment area.

However, following treatment of pasture land via contaminated “transportation of habitat material”, the species *Bipalium kewense* established in grassland in the warmer far north of the North Island of New Zealand. European earthworms have been deliberately introduced into pasture in New Zealand to increase their yields and *B. kewense* might have a negative impact if they were to significantly reduce the numbers of the European earthworms (Boag 2021, unpublished).

No studies have been conducted on the ecological impact of the *B. kewense* on earthworm populations, nor on species preyed upon by the flatworm, either within the risk assessment area, or in other countries outside the risk assessment area where the species is established. Whilst not conclusive, the lack of studies on these topics suggest that concerns have not been raised nor obvious impacts reported.

Qu. 4.3. How important is the potential future impact of the organism on biodiversity at all levels of organisation likely to be in the risk assessment area?

See comment above. The potential future impact shall be assessed only for the risk assessment area.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Unlike *Arthurdendyus triangulatus*, *Bipalium kewense* does not appear to have invaded large areas of agricultural land and there are no records from pasture in the Risk Assessment Area; *Bipalium kewense* established in grassland in the warmer far north of New Zealand; whether there is any impact on earthworm populations in this area is unknown. At the present time, *B. kewense* is mostly regarded as a species of urban habitats in Europe. Data from the species distribution models (Appendix VIII) suggest that *B. kewense* will spread under climate change scenarios and will likely invade natural habitats. However, the impact of *B. kewense* on earthworms seems to be less than other invasive alien flatworms (e.g. *A. triangulatus*) and it does not appear to achieve the high population densities seen with *O. nungara*.

Qu. 4.4. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism currently in the risk assessment area?

including the following elements:

- native species impacted, including red list species, endemic species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

No specific information has been found on the issue. However, there is no evidence that *Bipalium kewense* is established in natural habitats in the risk assessment areas.

Qu. 4.5. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.4.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

No information has been found on the issue. The impact on habitats and sites is difficult to determine at this stage, as the earthworm species preyed upon by *B. kewense* in outdoor locations, currently all urban, have not been identified.

Ecosystem Services impacts

Qu. 4.6. How important is the impact of the organism on provisioning, regulating, and cultural

services in its non-native range excluding the risk assessment area?

- For a list of services use the CICES classification V5.1 provided in Annex V.
- Impacts on ecosystem services build on the observed impacts on biodiversity (habitat, species, genetic, functional) but focus exclusively on reflecting these changes in relation to their links with socio-economic well-being.
- Quantitative data should be provided whenever available and references duly reported.

RESPONSE	N/A	CONFIDENCE	low
	minimal		medium
	minor		high
	moderate		
	major		
	massive		

Response:

No information has been found on the issue. The impact on habitats and sites is difficult to determine at this stage, as the earthworm species preyed upon by *B. kewense* in outdoor locations, currently all urban, have not been identified.

Qu. 4.7. How important is the impact of the organism on provisioning, regulating, and cultural services currently in the different biogeographic regions or marine sub-regions where the species has established in the risk assessment area (include any past impact in your response)?

- See guidance to Qu. 4.6.

RESPONSE	N/A	CONFIDENCE	low
	minimal		medium
	minor		high
	moderate		
	major		
	massive		

Response:

No information has been found on the issue. The impact on habitats and sites is difficult to determine at this stage, as the earthworm species preyed upon by *B. kewense* in outdoor locations, currently all urban, have not been identified.

Qu. 4.8. How important is the impact of the organism on provisioning, regulating, and cultural services likely to be in the different biogeographic regions or marine sub-regions where the

species can establish in the risk assessment area in the future?

- See guidance to Qu. 4.6.

RESPONSE	N/A minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

No information has been found on this issue that specifically relates to *Bipalium kewense*. Although *Bipalium kewense* is an earthworm predator it does not seem to occur in the high population densities seen in other invasive earthworm predators, *A. triangulatus* and *O. nungara*. It has not been recorded from fields and agricultural land, nor natural habitats in the Risk Assessment Area, and has only been recorded in urban habitats such as gardens and parks.

Economic impacts

Qu. 4.9. How great is the overall economic cost caused by the organism within its current area of distribution (excluding the risk assessment area), including both costs of / loss due to damage and the cost of current management.

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the world these should be reported here. The assessment of the potential costs of / loss due to damage shall describe those costs quantitatively and/or qualitatively depending on what information is available. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage. As far as possible, it would be useful to separate costs of / loss due to the organism from costs of current management.

RESPONSE	N/A minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

No information has been found on this issue that specifically relates to *Bipalium kewense* in the Risk Assessment Area.

Bipalium kewense has been recorded as a minimal pest of urban earthworm farming cultivating alien earthworms species in Australia and is readily controlled by occasional hand picking from water-flooded beds as required. (The native Australian flatworm species, *Dolichoplana* sp., was more damaging for commercial earthworm farmers (Winsor, 1998) as the earthworm stock provided to these franchisee farmers was found to be already contaminated by the flatworm, and as a consequence at least one grower lost his business (Winsor, 2021 unpubl.)).

Bipalium kewense is established in grassland in the warmer far north of the North Island of New Zealand. New Zealand depends upon agricultural exports, mainly from sheep and cattle, for their livelihood. European earthworms have been deliberately introduced into pasture in New Zealand to increase their yields and *B. kewense* might have a negative impact if they were to significantly reduce the numbers of the European earthworms (Boag 2021, unpublished).

In the absence of data on this question from the RAA, the question is regarded as inapplicable to the species.

Qu. 4.10. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism currently in the risk assessment area (include any past costs in your response)?

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the EU these should be reported here. Assessment of the potential costs of damage on human health, safety, and the economy, including the cost of non-action. A full economic assessment at EU scale might not be possible, but qualitative data or different case studies from across the EU (or third countries if relevant) may provide useful information to inform decision making. In absence of specific studies or other direct evidence this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	N/A	CONFIDENCE	low medium high
	minimal minor moderate major massive		

Response:

No information has been found on the issue specifically relating to *Bipalium kewense*, and the question considered Not Applicable to the species.

There is evidence that other species alien flatworms have been inadvertently disseminated in potted plants from infected garden centres and botanic gardens to the public or other botanic gardens. This is non-intentional transfer and if known it can adversely affect the reputation of the business or botanic garden concerned (Boag and Neilson, 2014).

Qu. 4.11. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.10.

RESPONSE	N/A minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

No information has been found on the issue.

There is no evidence of *B. kewense* causing economic losses in any of the countries that it has become established. Following treatment of pasture land via contaminated “transportation of habitat material”, the species *Bipalium kewense* established in grassland in the warmer far north of the North Island of New Zealand. European earthworms have been deliberately introduced into pasture in New Zealand to increase their yields and *B. kewense* might have a negative impact if they were to significantly reduce the numbers of the European earthworms (Boag 2021, unpublished).

In addition, unlike *A. triangulatus*, and apart from the single example in New Zealand (above) it has not established widely in agricultural pasture, where predation on earthworms causes economic losses to grass production (Murchie, 2018). There is potential that *B. kewense* could migrate from garden locations to surrounding habitats but so far it does not appear to have done so, despite being established outdoors in France for c. 30 years.

Qu. 4.12. How great are the economic costs / losses associated with managing this organism currently in the risk assessment area (include any past costs in your response)?

- In absence of specific studies or other direct evidence this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	N/A minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

No information has been found on the issue, hence rated as Not Applicable

Bipalium kewense has not established in natural or agricultural habitats in the risk assessment area but rather in “protected” sites such as hothouses, and in urban gardens. Management practices are therefore limited to these locations and are not thought to be costly. Management of the flatworm in private urban gardens would presumably be up to landowners to implement, if indeed they were concerned. Management practices for other invasive alien flatworms in Europe, e.g. *A. triangulatus* are mainly physical control measure applied by horticultural producers, garden centres and nurseries (EPPO, 2001b; MAFF, 1996). The extent of implementation of these and the associated costs are unknown.

Qu. 4.13. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.12.

RESPONSE	N/A minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

No information has been found on the issue, hence rated as Not Applicable

At the present time, there are no published economic costs associated with managing this flatworm.

In the future management options applied to other more damaging flatworm species, would equally affect *B. kewense* but would not necessarily be justified for this species alone unless problems become more apparent than have been currently observed.

Social and Human health impacts

Qu. 4.14. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism for the risk assessment area and for third countries, if relevant (e.g. with similar eco-climatic conditions).

The description of the known impact and the assessment of potential future impact on human health, safety and the economy, shall, if relevant, include information on

- illnesses, allergies or other affections to humans that may derive directly or indirectly from a species;
- damages provoked directly or indirectly by a species with consequences for the safety of people, property or infrastructure;

- direct or indirect disruption of, or other consequences for, an economic or social activity due to the presence of a species.

Social and human health impacts can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Bipalium kewense is not known to have any important social or human health impacts.

However, there is evidence that other species alien flatworms have been inadvertently disseminated in potted plants from infected garden centres and botanic gardens to the public or other botanic gardens. This non-intentional transfer if known, can adversely affect the reputation of the business or botanic garden concerned (Boag and Neilson, 2014).

The presence of invasive alien flatworms can be distressing to some gardeners as they regard these species to be ‘slimy’ and harmful.

Bipalium kewense has been implicated in cases of gastrointestinal pseudoparasitism (where there is no harm to humans, native or domestic animals), based upon circumstantial evidence, of humans (Walton and Yokogawa, 1972; Daly *et al.*, 1977).

Three toxins have been reported from *B. kewense*: a cardiotoxin, related in its effects but not necessarily in its chemistry, to cardiac glucosides, that is localized in dermal mucus, and a haemolytic toxin distributed throughout the planarian body (Arndt, 1925). More recently tetrodotoxin (TTX) was detected in *B. kewense*, largely concentrated in the head region, used to subdue large earthworm prey by reducing it to a partial paralytic state to facilitate feeding (Stokes *et al.*, 2014). The presence of TTX may also serve for defence by *B. kewense* against predators, and the species may accumulate TTX in the egg cocoon as does *Bipalium adventitium* (Stokes *et al.*, 2014). There is no evidence that these toxins result in any injury to humans through normal handling.

On the basis of this evidence, the impacts are considered minimal (only relating to possible loss of reputation of nurseries selling contaminated stock) with high confidence.

Qu. 4.15. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism in the future for the risk assessment area.

- In absence of specific studies or other direct evidence this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	N/A minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

No information has been found on this issue, hence rated as Not Applicable.

It is expected that the importance of social, health or other impacts caused by the organism (as in Qu 4.14) would change little in the future.

Other impacts

Qu. 4.16. How important is the organism in facilitating other damaging organisms (e.g. diseases) as food source, a host, a symbiont or a vector etc.?

RESPONSE	N/A minor moderate major massive	CONFIDENCE	low medium high
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Response:

Unlike species of the Rhynchodemid planarians, species of the Bipaliinae that includes *Bipalium kewense* have not yet been implicated as paratenic or carrier hosts of the Rat Lung worm, the nematode *Angiostrongylus cantonensis* that is responsible for eosinophilic meningitis, especially in the Pacific Region, and other species of *Angiostrongylus*. *Bipalium kewense* predate solely upon earthworms and does not predate upon land molluscs.

On the basis of the biological characteristics of *B. kewense*, this question is Not Applicable to the species.

Qu. 4.17. How important might other impacts not already covered by previous questions be resulting from introduction of the organism?

RESPONSE	N/A	CONFIDENCE	low
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	minimal minor moderate major massive		medium high
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Response:

No information has been found on the issue, hence rated as Not Applicable.

Bipalium kewense has been present in the risk assessment area in “protected” environment sites, such as hothouses etc., for some 144 years, and possibly in outdoor locations for at least 30 years.

The absence of ecological studies on this species hampers an informed assessment of possible impacts. However, there are no reports of *B. kewense* impacting on specific members of the soil fauna, such as earthworm species identified in studies within the risk assessment area (Rutgers et al., 2016).

Boag *et al.* (2010) argue that there is a readily available niche for land planarians from the southern hemisphere to exploit in Europe and North America, and this seems to be supported by recent accounts of alien land planarians in France (Justine *et al.*, 2018) and in Italy (Mori *et al.*, 2021), and the spread of Hammerhead flatworms in North America, perhaps facilitated by *Lumbricus terrestris*, itself a problematic alien species in North America (Murchie and Gordon, 2013).

Bipalium kewense has been implicated in cases of gastrointestinal pseudoparasitism, based upon circumstantial evidence, of humans (Walton and Yokogawa, 1972; Daly *et al.*, 1977), a dog and a cat (Daly *et al.*, 1976). On the basis of experimental physiological studies of *B. kewense* (Daly *et al.* 1977) demonstrated the failure to establish even short-term passage of the flatworm in the digestive tract of dogs, lethality of 37°C body temperature, and sensitivity to faeces makes gastrointestinal pseudoparasitism unlikely in these organisms. A more reasonable explanation for the phenomenon is the accidental attachment, in an outdoor environment, of highly sticky specimens of *B. kewense* to dogs, cats and humans. This phenomenon has observed in interactions between other species of land planarians and native and domestic animals (Winsor, 1980).

The presence of toxins in *B. kewense*, together with repugnatorial dermal secretions, may explain the three reports of vomiting of specimens of *B. kewense* by cats (Winsor, 1983b). These toxins are significant only in the flatworm’s natural environment in obtaining prey, and in defense against potential predators.

In the absence of information on additional potential impacts resulting from the introduction of *B. kewense*, the response is that the question is not applicable to this species.

Qu. 4.18. How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area?

RESPONSE	N/A minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

No information has been found on the issue, and the question regarded as Not Applicable to the species.

The impact on *B. kewense* by predators, parasites (nematodes) and pathogens is expected to be low (Winsor 2021, unpublished), and the importance of the expected impacts of *B. kewense*, essentially be unchanged.

Qu. 4.19. Estimate the overall impact in the risk assessment area under current climate conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, in current conditions.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Bipalium kewense is exclusively a predator of earthworms. It has been present in the risk assessment area for some 144 years, initially in botanic garden and urban hothouses, and in outdoor urban environments for at least 30 years in south-eastern France. Despite numerous studies in which *B. kewense* was used as a representative of a free-living triclad flatworm, there is a deficiency of ecological studies for this species. There are no quantitative studies on the impacts of populations of *B. kewense* upon earthworms.

However, world-wide, *B. kewense* has not been implicated in the observable decline of earthworm populations, nor implicated in concerns about the demise of earthworms causing changes to pastureland in the risk assessment area (U.K.) as is the situation for the New Zealand flatworm *A. triangulatus*. Nor has it exhibited invasiveness of outdoor natural habitats as has *B. adventitium* in the US. *Bipalium kewense* has had no discernable impacts on ecological services, the economy, or adversely impacted aspects of social and human health. There is presently no evidence to suggest that it will cease being benign.

The response to this question is that the impacts are considered minimal (relating to possible loss of reputation of nurseries selling contaminated stock, and despite the deficiency of ecological studies and absence of quantitative studies on the impacts of the species earthworm populations) with medium confidence based on comparison with other alien terrestrial flatworms and ratings elsewhere in this section.

Qu. 4.20. Estimate the overall impact in the risk assessment area in foreseeable climate change conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, under future conditions.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Under Climate Change with timeframe 2070, and emission scenarios RCP 2.6 and RCP 4.5 there are increases in the projected suitability for the establishment of *B. kewense* generally north and northeastwards under both RCP 2.6 and RCP 4.5 in the Atlantic, Black Sea, Continental, Pannonian and Steppic biogeographical regions in the risk assessment area, and in the Anatolian biogeographic region (refer to Figures 3 and 4 Annex VIII). The lower to mid-levels of the Alpine region appear to become increasing suitable for the establishment of the species under the RCP 2.6 and 4.5 scenarios.

For the Mediterranean region, there is little change between the current projected suitability and those under RCP 2.6 and RCP 4.5. Arid and desert conditions are increasing, and water will become more and more scarce (https://www.eea.europa.eu/publications/report_2002_0524_154909/biogeographical-regions-in-europe/mediterranean_biogeographical_region.pdf/view retrieved 25 June 2021), making this region inimical for the establishment of *B. kewense*.

These predicted changes to the climate in the risk assessment area could result in the establishment and spread of *B. kewense* as the Continental, Alpine and Boreal biogeographical regions warm and become more temperate.

In the Atlantic biogeographical region Belgium, Denmark, Ireland, the Netherlands, and the United Kingdom contain some of the largest conglomeration of people in Europe, any of whom live near the coast (https://www.eea.europa.eu/publications/report_2002_0524_154909/biogeographical-regions-in-europe/the_atlantic_region.pdf/view retrieved 25 June 2021). In the Continental region countries such as Austria, Czech Republic, Germany, Poland, and Slovakia, the urban areas are among the largest and most extensive in Europe. A large proportion of the population lives in the vicinity of

forests. Some new afforestation occurs around big cities for recreational purposes (https://www.eea.europa.eu/publications/report_2002_0524_154909/biogeographical-regions-in-europe/continental_biogeografical_region.pdf/view retrieved 25 June 2021).

In these countries within both the Atlantic and Continental biogeographical regions, *B. kewense* is currently present only in “protected” environments such as hothouses. Under the climate warming scenarios, there would probably no longer be the need for hothouses in these countries, and there could be a loss of containment of *B. kewense* from “protected” environments into outdoor urban gardens and parks where it could establish in the warmer conditions. This would bring *B. kewense* into contact with a broader range of potential prey species, but the impact would likely remain localised and reversible. ‘Low’ confidence reflects a lack of current information on the ecological impact of *B. kewense* under current conditions but especially given potential additional prey species.

Any impacts on biodiversity, ecosystem services and human health are likely to increase under climate change and mostly occur in the same biogeographic regions.

RISK SUMMARIES

	RESPONSE	CONFIDENCE	COMMENT
Summarise Introduction and Entry*	very unlikely unlikely moderately likely likely very likely	low medium high	<i>Bipalium kewense</i> has already been unintentionally introduced via the live plant trade, especially potted plants for gardens and via the botanical garden pathway. The species has been present in “protected” environments (e.g. hothouses) in Europe for 144 years, and in urban outdoor habitats for possibly 30+ years. Climate change will probably see the movement of the flatworm from the “protected” facilities into outdoor urban gardens and parks.
Summarise Establishment*	very unlikely unlikely moderately likely likely very likely	low medium high	The species is already established outdoors in urban gardens in several Member States: France, Italy, Portugal, Spain, and Malta. It is expected that the outdoor occurrence of <i>B. kewense</i> will follow the increasing urbanization in the risk assessment area. Climate change will enable the increased spread northwards from the present biographical regions where <i>B. kewense</i> occurs.
Summarise Spread*	very slowly slowly moderately rapidly very rapidly	low medium high	<i>Bipalium kewense</i> was originally spread by human-assisted transport over long distances followed subsequently by slow and local natural dispersal within urban habitats. Flatworms can be readily spread via ‘Contaminant nursery material’ pathway that includes potted plants and plants for planting; the ‘Transportation of habitat material’ (unintentional) pathway movement of soil and compost, which may contain <i>B. kewense</i> . The ‘Machinery / equipment’ pathway with which there is some overlap with the movement of soil, as flatworms may be carried in soil adhering to machinery or equipment. The “Contaminant on plants” pathway concerns the personal exchange of potentially contaminant plants between gardeners. This will probably lead to moderately rapid spread but confidence in this score is low because of lack of monitoring data.

Summarise Impact*	minimal minor moderate major massive	low medium high	There is lack of information on the ecological, economic, and social impact of <i>B. kewense</i> , despite the flatworm having colonized urban habitats in many regions worldwide and having been the subject of numerous scientific studies. It is likely that there would only be local impacts on ecosystems and only localised control costs and public concern. If future data becomes available revealing biodiversity impacts the PRA would need to be revised.
Conclusion of the risk assessment (overall risk)	low moderate high	low medium high	<i>Bipalium kewense</i> is the most widely dispersed flatworm species in the world. It is established in many EU Member States and it is likely that it will continue to spread, at least in urban situations. While the risk assessment area has been colonized by many other species of terrestrial flatworms that have had clear deleterious consequences, no information to this effect was found for <i>B. kewense</i> . However, deleterious impacts on soil invertebrates are not as readily observed as above-ground impacts and it remains feasible that <i>B. kewense</i> predation could have unforeseen effects on soil fauna. The overall assessment is therefore ‘moderate’ based on expert opinion regarding the ability of the flatworm to establish and spread, and that it predated upon earthworms; in the absence of detailed studies on the impact of <i>B. kewense</i> , the confidence however is low.

*in current climate conditions and in foreseeable future climate conditions

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Distribution Summary

Please answer as follows:

- Yes if recorded, established or invasive
- if not recorded, established or invasive
- ? Unknown; data deficient

The columns refer to the answers to Questions A5 to A12 under Section A.

For data on marine species at the Member State level, delete Member States that have no marine borders. In all other cases, provide answers for all columns.

Member States, and the United Kingdom

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Austria	Yes	-	Yes	Yes	
Belgium	Yes	?	Yes	Yes	
Bulgaria	?	-	Yes	Yes	
Croatia	?	-	Yes	Yes	
Cyprus	?	-	Yes	Yes	
Czech Republic	Yes	?		Yes	
Denmark	Yes	?		Yes	
Estonia	?	-			
Finland	Yes	?			
France	Yes	Yes	Yes	Yes	Yes
Germany	Yes	?	Yes	Yes	
Greece	?	-	Yes	Yes	
Hungary	?	-	Yes	Yes	
Ireland	Yes	?		?	
Italy	Yes	Yes	Yes	Yes	Yes
Latvia	?	-			
Lithuania	?	-			
Luxembourg	?	-	Yes	Yes	
Malta	Yes	Yes	Yes	Yes	?
Netherlands	Yes	-	Yes	Yes	
Poland	Yes	?		Yes	
Portugal	Yes	Yes	Yes	Yes	Yes
Romania	?	-	Yes	Yes	
Slovakia	?#	-	Yes	Yes	
Slovenia	?	-	Yes	Yes	
Spain	Yes	Yes	Yes	Yes	Yes
Sweden	?	-		?	
United Kingdom	Yes	-	Yes	Yes	Yes

Biogeographical regions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Alpine	yes	yes	yes	yes	yes
Atlantic	yes	yes	yes	yes	yes
Black Sea			yes	yes	
Boreal					
Continental	yes	yes	yes	yes	yes
Mediterranean	yes	yes	yes	yes	yes
Pannonian			yes	yes	
Steppic			yes	yes	

ANNEX I Scoring of Likelihoods of Events

(taken from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Description	Frequency
Very unlikely	This sort of event is theoretically possible, but is never known to have occurred and is not expected to occur	1 in 10,000 years
Unlikely	This sort of event has occurred somewhere at least once in the last millennium	1 in 1,000 years
Moderately likely	This sort of event has occurred somewhere at least once in the last century	1 in 100 years
Likely	This sort of event has happened on several occasions elsewhere, or on at least once in the last decade	1 in 10 years
Very likely	This sort of event happens continually and would be expected to occur	Once a year

ANNEX II Scoring of Magnitude of Impacts

(modified from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Biodiversity and ecosystem impact	Ecosystem Services impact	Economic impact (Monetary loss and response costs per year)	Social and human health impact, and other impacts
	<i>Question 5.1-5</i>	<i>Question 5.6-8</i>	<i>Question 5.9-13</i>	<i>Question 5.14-18</i>
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected ⁵	Up to 10,000 Euro	No social disruption. Local, mild, short-term reversible effects to individuals.
Minor	Some ecosystem impact, reversible changes, localised	Local and temporary, reversible effects to one or few services	10,000-100,000 Euro	Significant concern expressed at local level. Mild short-term reversible effects to identifiable groups, localised.
Moderate	Measureable long-term damage to populations and ecosystem, but reversible; little spread, no extinction	Measureable, temporary, local and reversible effects on one or several services	100,000-1,000,000 Euro	Temporary changes to normal activities at local level. Minor irreversible effects and/or larger numbers covered by reversible effects, localised.
Major	Long-term irreversible ecosystem change, spreading beyond local area	Local and irreversible or widespread and reversible effects on one / several services	1,000,000-10,000,000 Euro	Some permanent change of activity locally, concern expressed over wider area. Significant irreversible effects locally or reversible effects over large area.
Massive	Widespread, long-term population loss or extinction, affecting several species with serious ecosystem effects	Widespread and irreversible effects on one / several services	Above 10,000,000 Euro	Long-term social change, significant loss of employment, migration from affected area. Widespread, severe, long-term, irreversible health effects.

⁵ Not to be confused with “no impact”.

ANNEX III Scoring of Confidence Levels

(modified from Bacher *et al.* 2017)

Each answer provided in the risk assessment must include an assessment of the level of confidence attached to that answer, reflecting the possibility that information needed for the answer is not available or is insufficient or available but conflicting.

The responses in the risk assessment should clearly support the choice of the confidence level.

Confidence level	Description
Low	There is no direct observational evidence to support the assessment, e.g. only inferred data have been used as supporting evidence <i>and/or</i> Impacts are recorded at a spatial scale which is unlikely to be relevant to the assessment area <i>and/or</i> Evidence is poor and difficult to interpret, e.g. because it is strongly ambiguous <i>and/or</i> The information sources are considered to be of low quality or contain information that is unreliable.
Medium	There is some direct observational evidence to support the assessment, but some information is inferred <i>and/or</i> Impacts are recorded at a small spatial scale, but rescaling of the data to relevant scales of the assessment area is considered reliable, or to embrace little uncertainty <i>and/or</i> The interpretation of the data is to some extent ambiguous or contradictory.
High	There is direct relevant observational evidence to support the assessment (including causality) <i>and</i> Impacts are recorded at a comparable scale <i>and/or</i> There are reliable/good quality data sources on impacts of the taxa <i>and</i> The interpretation of data/information is straightforward <i>and/or</i> Data/information are not controversial or contradictory.

ANNEX IV CBD pathway categorisation scheme

Overview of CBD pathway categorisation scheme showing how the 44 pathways relate to the six main pathway categories. All of the pathways can be broadly classified into 1) those that involve intentional transport (blue), 2) those in which the taxa are unintentionally transported (green) and 3) those where taxa moved between regions without direct transportation by humans and/or via artificial corridors (orange and yellow). **Note that the pathways in the category “Escape from confinement” can be considered intentional for the introduction into the risk assessment area and unintentional for the entry into the environment.**



ANNEX V Ecosystem services classification (CICES V5.1, simplified) and examples

For the purposes of this risk assessment, please feel free to use what seems as the most appropriate category / level / combination of impact (Section – Division – Group), reflecting information available.

Section	Division	Group	Examples (i.e. relevant CICES “classes”)
Provisioning	Biomass	Cultivated <i>terrestrial</i> plants	Cultivated terrestrial plants (including fungi, algae) grown for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from cultivated plants, fungi, algae and bacteria for direct use or processing (excluding genetic materials); Cultivated plants (including fungi, algae) grown as a <u>source of energy</u> <i>Example: negative impacts of non-native organisms to crops, orchards, timber etc.</i>
		Cultivated <i>aquatic</i> plants	Plants cultivated by in- situ aquaculture grown for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from in-situ aquaculture for direct use or processing (excluding genetic materials); Plants cultivated by in- situ aquaculture grown as an <u>energy source</u> . <i>Example: negative impacts of non-native organisms to aquatic plants cultivated for nutrition, gardening etc. purposes.</i>
		Reared animals	Animals reared for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from reared animals for direct use or processing (excluding genetic materials); Animals reared to provide <u>energy</u> (including mechanical) <i>Example: negative impacts of non-native organisms to livestock</i>
		Reared <i>aquatic</i> animals	Animals reared by in-situ aquaculture for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from animals grown by in-situ aquaculture for direct use or processing (excluding genetic materials); Animals reared by in-situ aquaculture as an <u>energy source</u> <i>Example: negative impacts of non-native organisms to fish farming</i>
		Wild plants (terrestrial and aquatic)	Wild plants (terrestrial and aquatic, including fungi, algae) used for <u>nutrition</u> ; <u>Fibres and other materials</u> from wild plants for direct use or processing (excluding genetic materials); Wild plants (terrestrial and aquatic, including fungi, algae) used as a <u>source of energy</u> <i>Example: reduction in the availability of wild plants (e.g. wild berries, ornamentals) due to non-native organisms (competition, spread of disease etc.)</i>
		Wild animals (terrestrial and aquatic)	Wild animals (terrestrial and aquatic) used for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from wild animals for direct use or processing (excluding genetic materials); Wild animals (terrestrial and aquatic) used as a <u>source of energy</u> <i>Example: reduction in the availability of wild animals (e.g. fish</i>

			stocks, game) due to non-native organisms (competition, predations, spread of disease etc.)
	Genetic material from all biota	Genetic material from plants, algae or fungi	<u>Seeds, spores and other plant materials</u> collected for maintaining or establishing a population; Higher and lower plants (whole organisms) used to <u>breed new strains or varieties</u> ; Individual genes extracted from higher and lower plants for the <u>design and construction of new biological entities</u> <i>Example: negative impacts of non-native organisms due to interbreeding</i>
		Genetic material from animals	Animal material collected for the purposes of maintaining or establishing a population; Wild animals (whole organisms) used to breed new strains or varieties; Individual genes extracted from organisms for the design and construction of new biological entities <i>Example: negative impacts of non-native organisms due to interbreeding</i>
	Water ⁶	Surface water used for nutrition, materials or energy	Surface water for <u>drinking</u> ; Surface water used as a material (<u>non-drinking purposes</u>); Freshwater surface water, coastal and marine water used as an <u>energy source</u> <i>Example: loss of access to surface water due to spread of non-native organisms</i>
		Ground water for used for nutrition, materials or energy	Ground (and subsurface) water for <u>drinking</u> ; Ground water (and subsurface) used as a material (<u>non-drinking purposes</u>); Ground water (and subsurface) used as an <u>energy source</u> <i>Example: reduced availability of ground water due to spread of non-native organisms and associated increase of ground water consumption by vegetation.</i>
Regulation & Maintenance	Transformation of biochemical or physical inputs to ecosystems	Mediation of wastes or toxic substances of anthropogenic origin by living processes	<u>Bio-remediation</u> by micro-organisms, algae, plants, and animals; <u>Filtration/sequestration/storage/accumulation</u> by micro-organisms, algae, plants, and animals <i>Example: changes caused by non-native organisms to ecosystem functioning and ability to filtrate etc. waste or toxics</i>
		Mediation of nuisances of anthropogenic origin	<u>Smell reduction</u> ; <u>noise attenuation</u> ; <u>visual screening</u> (e.g. by means of green infrastructure) <i>Example: changes caused by non-native organisms to ecosystem structure, leading to reduced ability to mediate nuisances.</i>
	Regulation of physical, chemical, biological conditions	Baseline flows and extreme event regulation	Control of <u>erosion</u> rates; Buffering and attenuation of <u>mass movement</u> ; <u>Hydrological cycle and water flow regulation</u> (Including flood control, and coastal protection); <u>Wind</u> protection; <u>Fire</u> protection <i>Example: changes caused by non-native organisms to ecosystem functioning or structure leading to, for example, destabilisation of soil, increased risk or intensity of wild fires etc.</i>

⁶ Note: in the CICES classification provisioning of water is considered as an abiotic service whereas the rest of ecosystem services listed here are considered biotic.

		<p>Lifecycle maintenance, habitat and gene pool protection</p>	<p><u>Pollination</u> (or 'gamete' dispersal in a marine context); <u>Seed dispersal</u>; Maintaining <u>nursery populations and habitats</u> (Including gene pool protection)</p> <p><i>Example: changes caused by non-native organisms to the abundance and/or distribution of wild pollinators; changes to the availability / quality of nursery habitats for fisheries</i></p>
		<p>Pest and disease control</p>	<p>Pest control; Disease control</p> <p><i>Example: changes caused by non-native organisms to the abundance and/or distribution of pests</i></p>
		<p>Soil quality regulation</p>	<p><u>Weathering processes</u> and their effect on soil quality; <u>Decomposition and fixing processes</u> and their effect on soil quality</p> <p><i>Example: changes caused by non-native organisms to vegetation structure and/or soil fauna leading to reduced soil quality</i></p>
		<p>Water conditions</p>	<p>Regulation of the <u>chemical condition</u> of freshwaters by living processes; Regulation of the chemical condition of salt waters by living processes</p> <p><i>Example: changes caused by non-native organisms to buffer strips along water courses that remove nutrients in runoff and/or fish communities that regulate the resilience and resistance of water bodies to eutrophication</i></p>
		<p>Atmospheric composition and conditions</p>	<p>Regulation of <u>chemical composition</u> of atmosphere and oceans; Regulation of <u>temperature and humidity</u>, including ventilation and transpiration</p> <p><i>Example: changes caused by non-native organisms to ecosystems' ability to sequester carbon and/or evaporative cooling (e.g. by urban trees)</i></p>
Cultural	Direct, in-situ and outdoor interactions with living systems that depend on presence in the environmental setting	<p>Physical and experiential interactions with natural environment</p>	<p>Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through <u>active or immersive interactions</u>;</p> <p>Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through <u>passive or observational interactions</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that make it attractive for recreation, wild life watching etc.</i></p>
		<p>Intellectual and representative interactions with natural environment</p>	<p>Characteristics of living systems that enable <u>scientific investigation</u> or the creation of traditional ecological knowledge;</p> <p>Characteristics of living systems that enable <u>education and training</u>;</p> <p>Characteristics of living systems that are resonant in terms of <u>culture or heritage</u>;</p> <p>Characteristics of living systems that enable <u>aesthetic experiences</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have cultural importance</i></p>

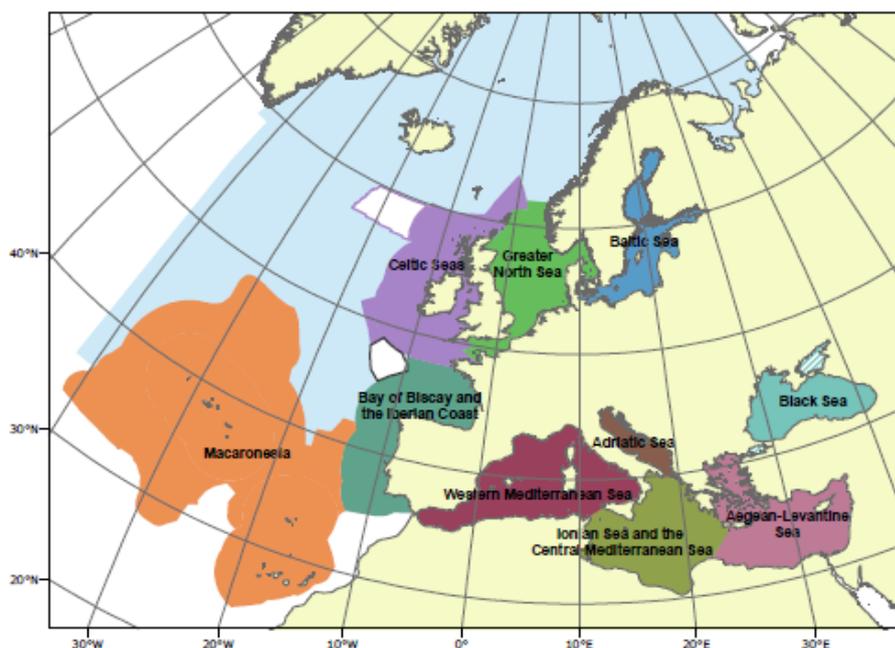
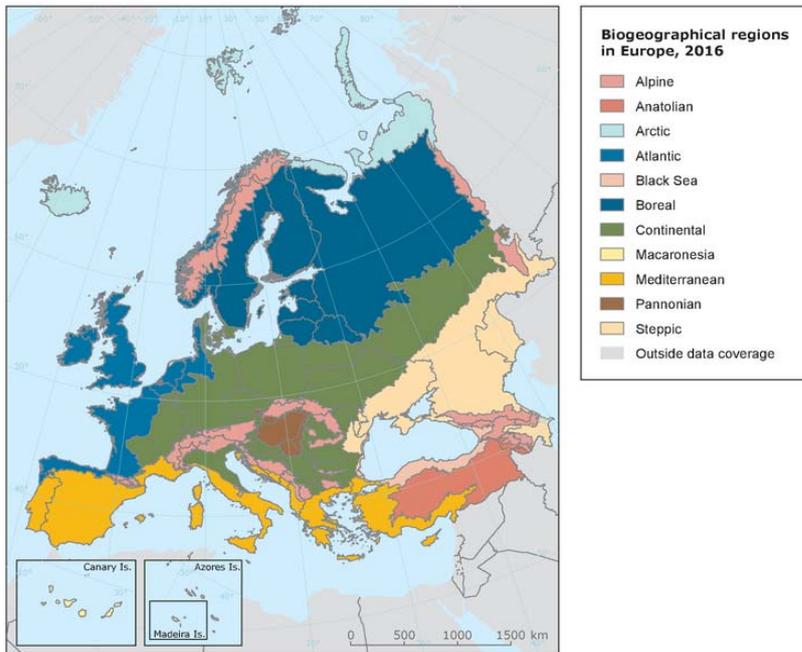
	Indirect, remote, often indoor interactions with living systems that do not require presence in the environmental setting	Spiritual, symbolic and other interactions with natural environment	Elements of living systems that have <u>symbolic meaning</u> ; Elements of living systems that have <u>sacred or religious meaning</u> ; Elements of living systems used for <u>entertainment or representation</u> <i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have sacred or religious meaning</i>
		Other biotic characteristics that have a non-use value	Characteristics or features of living systems that have an <u>existence value</u> ; Characteristics or features of living systems that have an <u>option or bequest value</u> <i>Example: changes caused by non-native organisms to ecosystems designated as wilderness areas, habitats of endangered species etc.</i>

ANNEX VI EU Biogeographic Regions and MSFD Subregions

See <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2> ,
http://ec.europa.eu/environment/nature/natura2000/biogeog_regions/

and

<https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions-1/technical-document/pdf>



ANNEX VII Delegated Regulation (EU) 2018/968 of 30 April 2018

see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>

ANNEX VIII Species Distribution Model

Projection of environmental suitability for *Bipalium kewense* establishment in Europe

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19 June 2021

Aim

To project the suitability for potential establishment of *Bipalium kewense* in Europe, under current and predicted future climatic conditions.

Data for modelling

Species occurrence data were obtained from iNaturalist (1776 records), the Global Biodiversity Information Facility (GBIF) (1001 records), the Biodiversity Information Serving Our Nation database (BISON) (145 records), the Atlas of Living Australia (9 records), and additional records from the risk assessment team. We scrutinised occurrence records from regions where the species is not known to be established and removed any dubious records or where the georeferencing was too imprecise (e.g. records referenced to a country or island centroid) or outside of the coverage of the predictor layers (e.g. small island or coastal occurrences). The remaining records were gridded at a 0.25 x 0.25 degree resolution for modelling, yielding 688 grid cells with occurrences (Figure 1a). As a proxy for recording effort, the density of Platyhelminthes records held by GBIF was also compiled on the same grid (Figure 1b).

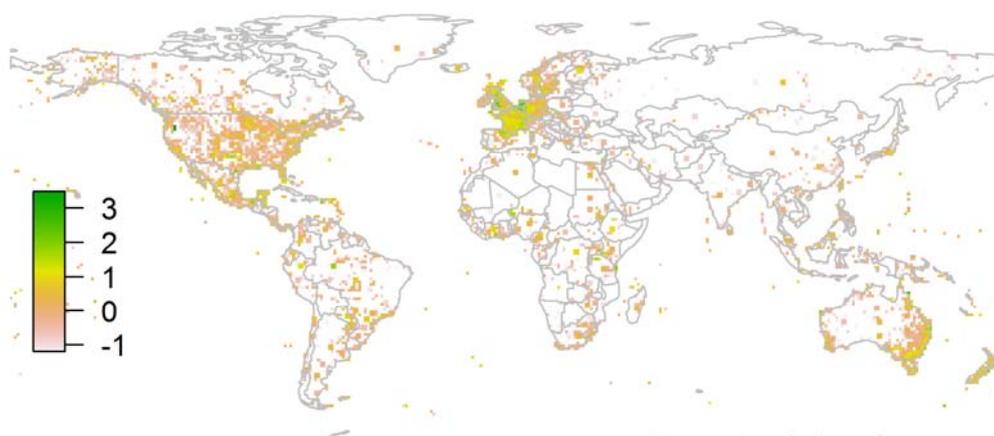
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Figure 1. (a) Occurrence records obtained for *Bipalium kewense* and used in the modelling, showing native and invaded distributions. (b) The recording density of Platyhelminthes on GBIF, which was used as a proxy for recording effort.

(a) Species distribution used in modelling



(b) Estimated recording effort (log₁₀-scaled)



Climate data were selected from the 'Bioclim' variables contained within the WorldClim database (Hijmans et al., 2005), originally at 5 arcminute resolution (0.083 x 0.083 degrees of longitude/latitude) and aggregated to a 0.25 x 0.25 degree grid for use in the model.

Based on the biology of *Bipalium kewense*, the following climate variables were used in the modelling:

- Minimum temperature of the coldest month (Bio6)
- Mean temperature of the warmest quarter (Bio10)
- Annual precipitation (Bio12)
- Precipitation seasonality (Bio15)
- Climatic moisture index (CMI): ratio of mean annual precipitation to potential evapotranspiration, log+1 transformed. For its calculation, monthly potential evapotranspirations were estimated from the WorldClim monthly temperature data and solar radiation using the simple method of Zomer et al. (2008) which is based on the Hargreaves evapotranspiration equation (Hargreaves, 1994).

To estimate the effect of climate change on the potential distribution, equivalent modelled future climate conditions for the 2070s under the Representative Concentration Pathways (RCP) 2.6 and

4.5 were also obtained. These represent low and medium emissions scenarios, respectively. The above variables were obtained as averages of outputs of eight Global Climate Models (BCC-CSM1-1, CCSM4, GISS-E2-R, HadGEM2-AO, IPSL-CM5A-LR, MIROC-ESM, MRI-CGCM3, NorESM1-M), downscaled and calibrated against the WorldClim baseline (see http://www.worldclim.org/cmip5_5m).

The following habitat layers were also used:

- Human influence index (HII): As many non-native invasive species associate with anthropogenically disturbed habitats. We used the Global Human Influence Index Dataset of the Last of the Wild Project (Wildlife Conservation Society - WCS & Center for International Earth Science Information Network - CIESIN - Columbia University, 2005), which is developed from nine global data layers covering human population pressure (population density), human land use and infrastructure (built-up areas, nighttime lights, land use/land cover) and human access (coastlines, roads, railroads, navigable rivers). The index ranges between 0 and 1 and was $\ln+1$ transformed for the modelling to improve normality.

Species distribution model

A presence-background (presence-only) ensemble modelling strategy was employed using the BIOMOD2 R package version 3.4.6 (Thuiller et al., 2020, Thuiller et al., 2009). These models contrast the environment at the species' occurrence locations against a random sample of the global background environmental conditions (often termed 'pseudo-absences') in order to characterise and project suitability for occurrence. This approach has been developed for distributions that are in equilibrium with the environment. Because invasive species' distributions are not at equilibrium and subject to dispersal constraints at a global scale, we took care to minimise the inclusion of locations suitable for the species but where it has not been able to disperse to (Chapman et al. 2019). Therefore the background sampling region included:

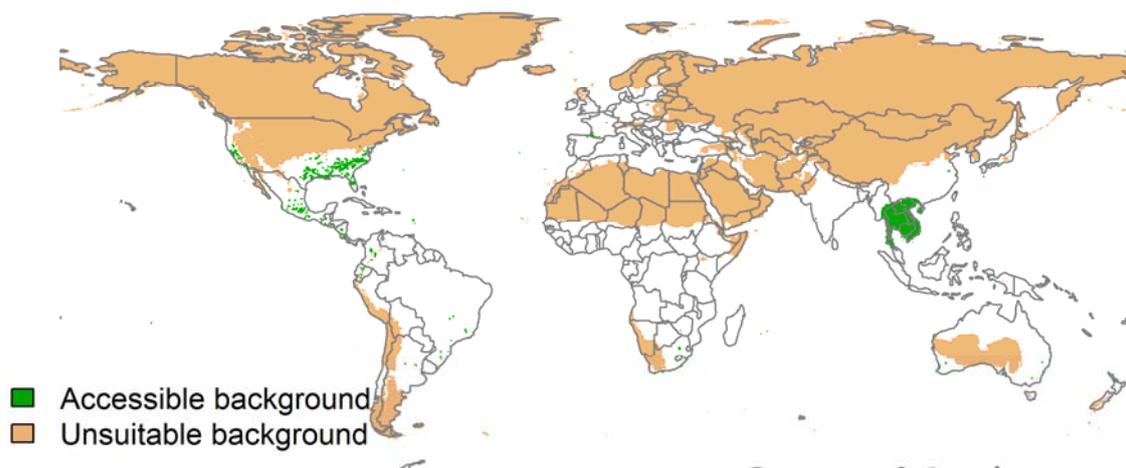
- The area accessible by native *Bipalium kewense* populations, in which the species is likely to have had sufficient time to disperse to all locations. Based on presumed maximum dispersal distances, the accessible region was defined as a 300km buffer around the native range occurrences; AND
- A 30km buffer around the non-native occurrences, encompassing regions likely to have had high propagule pressure for introduction by humans and/or dispersal of the species; AND
- Regions where we have an *a priori* expectation of high unsuitability for the species so that absence is assumed irrespective of dispersal constraints (see Figure 2). The following rules were applied to define a region expected to be highly unsuitable for *Bipalium kewense* at the spatial scale of the model:
 - Minimum temperature of the coldest month (Bio6) < -7°C
 - Mean temperature of the warmest quarter (Bio10) < 13°C
 - Annual precipitation (Bio12) < 270mm

Altogether, 1.2% of occurrence grid cells were located in the unsuitable background region.

Within the unsuitable background region, 10 samples of 5000 randomly sampled grid cells were obtained. In the accessible background (comprising the accessible areas around native

and non-native occurrences as detailed above), the same number of pseudo-absence samples were drawn as there were presence records (688), weighting the sampling by a proxy for recording effort (Figure 2).

Figure 2. The background from which pseudo-absence samples were taken in the modelling of *Bipalium kewense*. Samples were taken from a 300km buffer around the native range and a 30km buffer around non-native occurrences (together forming the accessible background), and from areas expected to be highly unsuitable for the species (the unsuitable background region). Samples from the accessible background were weighted by a proxy for recording effort (Figure 1(b)).



Each dataset (i.e. combination of the presences and the individual background samples) was randomly split into 80% for model training and 20% for model evaluation. With each training dataset, seven statistical algorithms were fitted with the default BIOMOD2 settings and rescaled using logistic regression, except where specified below:

- Generalised linear model (GLM)
- Generalised boosting model (GBM)
- Generalised additive model (GAM) with a maximum of four degrees of freedom per smoothing spline
- Artificial neural network (ANN)
- Multivariate adaptive regression splines (MARS)
- Random forest (RF)
- Maxent

Since the total background sample was larger than the number of occurrences, prevalence fitting weights were applied to give equal overall importance to the occurrences and the background. Normalised variable importance was assessed and variable response functions were produced using BIOMOD2's default procedure.

Model predictive performance was assessed by the following three measures:

- AUC, the area under the receiver operating characteristic curve (Fielding & Bell 1997). Predictions of presence-absence models can be compared with a subset of records set aside for model evaluation (here 20%) by constructing a confusion matrix with the number of true positive, false positive, false negative and true negative cases. For models generating non-dichotomous scores (as here) a threshold can be applied to transform the scores into a dichotomous set of presence-absence predictions. Two measures that can be derived from the confusion matrix are sensitivity (the proportion of observed presences that are predicted as such, quantifying omission errors), and specificity (the proportion of observed absences that are predicted as such, quantifying commission errors). A receiver operating characteristic (ROC) curve can be constructed by using all possible thresholds to classify the scores into confusion matrices, obtaining sensitivity and specificity for each matrix, and plotting sensitivity against the corresponding proportion of false positives (equal to 1 - specificity). The use of all possible thresholds avoids the need for a selection of a single threshold, which is often arbitrary, and allows appreciation of the trade-off between sensitivity and specificity. The area under the ROC curve (AUC) is often used as a single threshold-independent measure for model performance (Manel, Williams & Ormerod 2001). AUC is the probability that a randomly selected presence has a higher model-predicted suitability than a randomly selected absence (Allouche et al. 2006).
- Cohen's Kappa (Cohen 1960). This measure corrects the overall accuracy of model predictions (ratio of the sum of true presences plus true absences to the total number of records) by the accuracy expected to occur by chance. The kappa statistic ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random. Advantages of kappa are its simplicity, the fact that both commission and omission errors are accounted for in one parameter, and its relative tolerance to zero values in the confusion matrix (Manel, Williams & Ormerod 2001). However, Kappa has been criticised for being sensitive to prevalence (the proportion of sites in which the species was recorded as present) and may therefore be inappropriate for comparisons of model accuracy between species or regions (McPherson, Jetz & Rogers 2004, Allouche et al. 2006).
- TSS, the true skill statistic (Allouche et al. 2006). TSS is defined as sensitivity + specificity - 1, and corrects for Kappa's dependency on prevalence. TSS compares the number of correct forecasts, minus those attributable to random guessing, to that of a hypothetical set of perfect forecasts. Like kappa, TSS takes into account both omission and commission errors, and success as a result of random guessing, and ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random (Allouche et al. 2006).

An ensemble model was created by first rejecting poorly performing algorithms with relatively extreme low AUC values and then averaging the predictions of the remaining algorithms, weighted by their AUC. To identify poorly performing algorithms, AUC values were converted into modified z-scores based on their difference to the median and the median absolute deviation across all algorithms (Iglewicz & Hoaglin 1993). Algorithms with $z < -2$ were rejected. In this way, ensemble projections were made for each dataset and then averaged to give an overall suitability, as well as its standard deviation.

Projections were classified into suitable and unsuitable regions using a "lowest presence threshold" (Pearson et al. 2007), setting the cut-off as the lowest value at which 98% of all presence records are classified correctly under the current climate (here 0.59). In order to express the sensitivity of classifications to the choice of this threshold, thresholds at which 95% and 99% of records are classified correctly (here 0.71 and 0.45 respectively) were used in the calculation of error bars in Figs. 9 and 10 below in addition to taking account of uncertainty in the projections themselves.

We also produced a limiting factor map for Europe following Elith et al. (2010). For this, projections were made separately with each individual variable fixed at a near-optimal value. These were chosen as the median values at the occurrence grid cells. Then, the most strongly limiting factors were identified as the ones resulting in the highest increase in suitability in each grid cell.

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Results

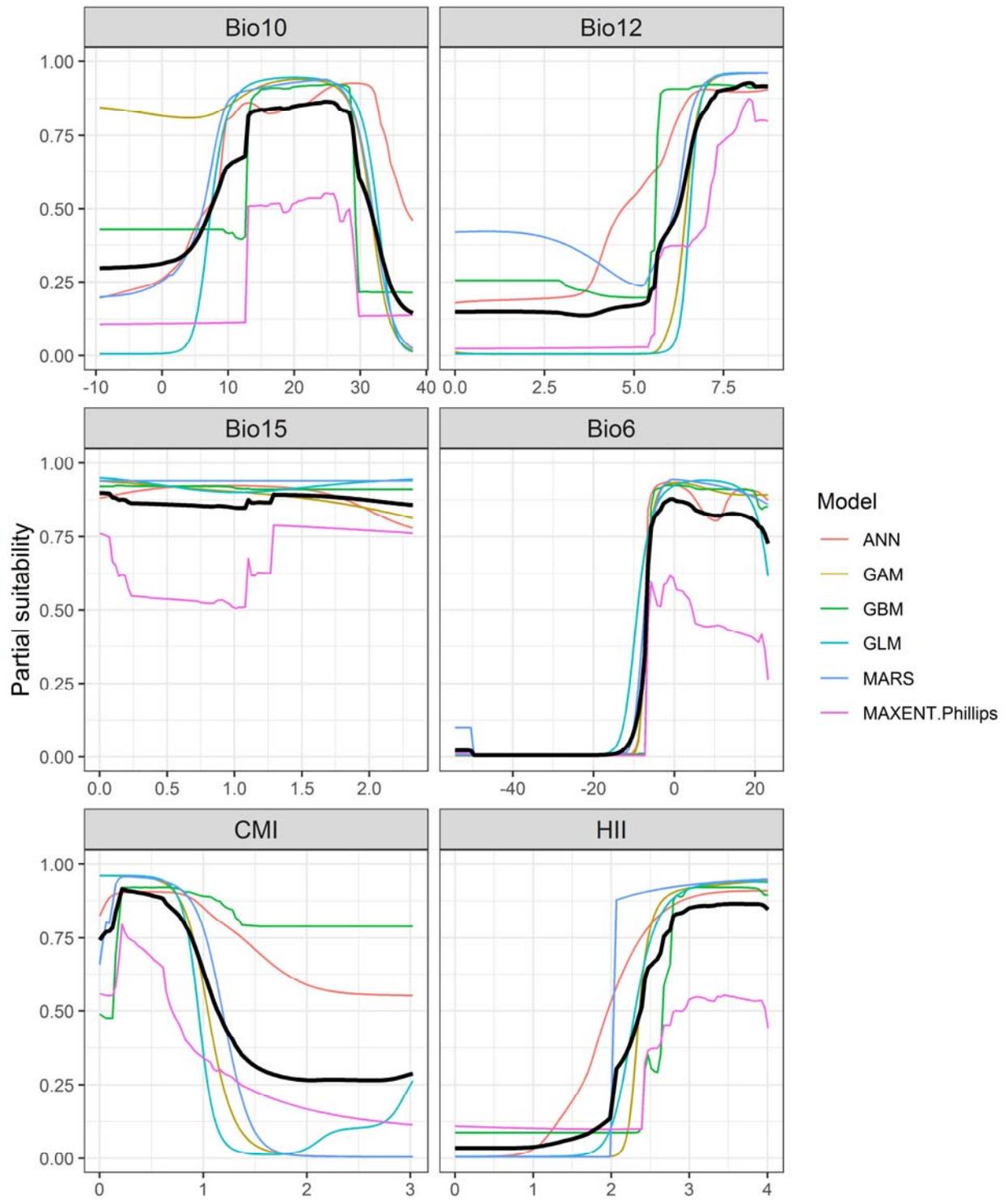
The ensemble model suggested that suitability for *Bipalium kewense* was most strongly determined by Minimum temperature of the coldest month (Bio6), accounting for 37.5% of variation explained, followed by Annual precipitation (Bio12) (23.5%), Human influence index (HII) (22.5%), Mean temperature of the warmest quarter (Bio10) (9.7%), Climatic moisture index (CMI) (6.4%) and Precipitation seasonality (Bio15) (0.4%) (Table 1, Figure 3).

Table 1. Summary of the cross-validation predictive performance (AUC, Kappa, TSS) and variable importance of the fitted model algorithms and the ensemble (AUC-weighted average of the best performing algorithms). Results are the average from models fitted to 10 different background samples of the data.

Algorithm	AUC	Kappa	TSS	Used in the ensemble	Variable importance (%)					
					Minimum temperature of the coldest month (Bio6)	Annual precipitation (Bio12)	Human influence index (HII)	Mean temperature of the warmest quarter (Bio10)	Climatic moisture index (CMI)	Precipitation seasonality (Bio15)
GLM	0.944	0.623	0.873	yes	24	37	21	8	10	0
GAM	0.951	0.621	0.875	yes	30	34	25	3	7	0
GBM	0.944	0.618	0.875	yes	47	10	28	12	3	0
ANN	0.953	0.627	0.871	yes	48	15	19	14	4	0
MARS	0.949	0.619	0.876	yes	37	22	25	8	8	0
RF	0.920	0.595	0.865	no	45	21	13	11	5	5
Maxent	0.945	0.617	0.872	yes	40	22	17	13	7	1
Ensemble	0.949	0.623	0.876		37	23	23	10	6	0

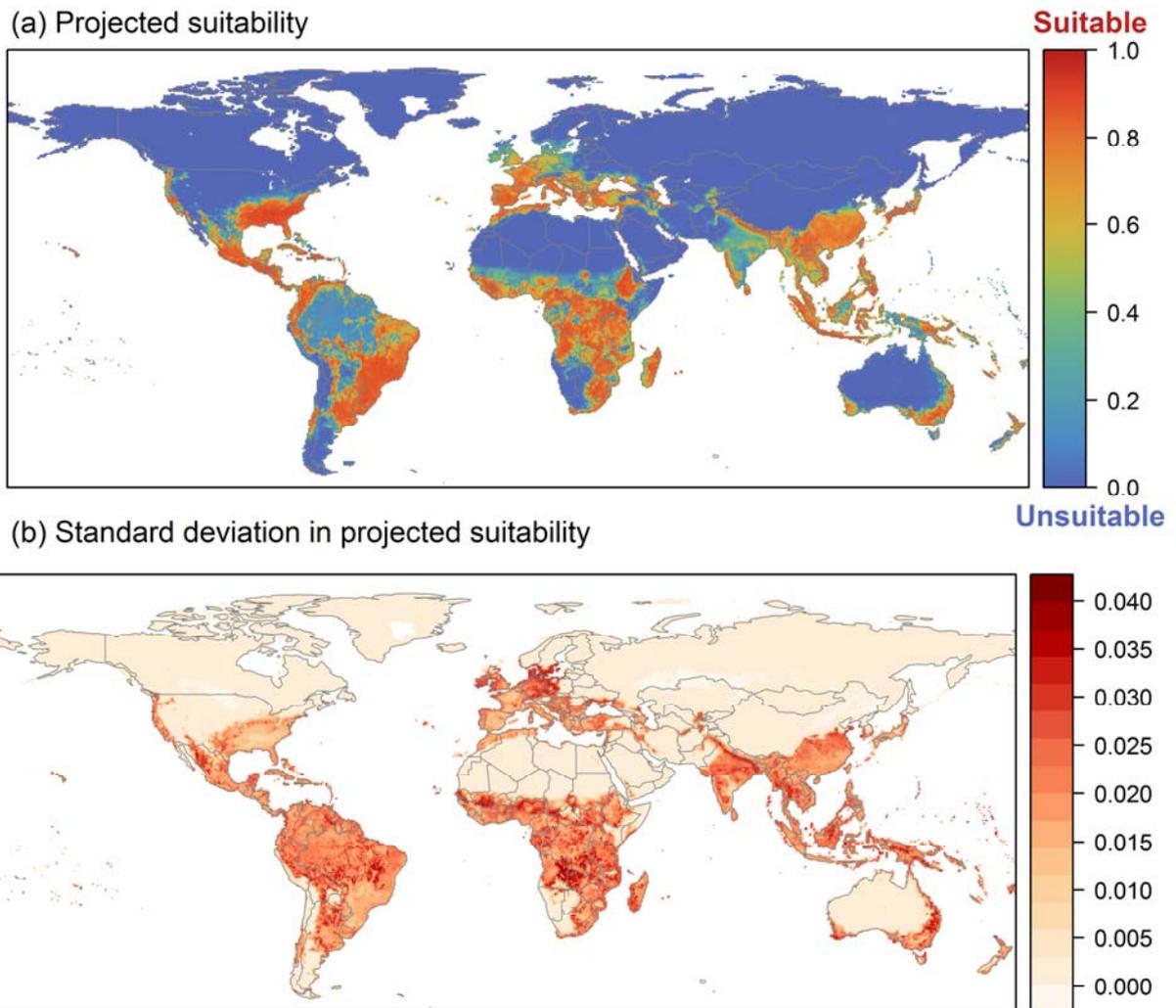
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Figure 3. Partial response plots from the fitted models. Thin coloured lines show responses from the algorithms in the ensemble, while the thick black line is their ensemble. In each plot, other model variables are held at their median value in the training data. Some of the divergence among algorithms is because of their different treatment of interactions among variables.



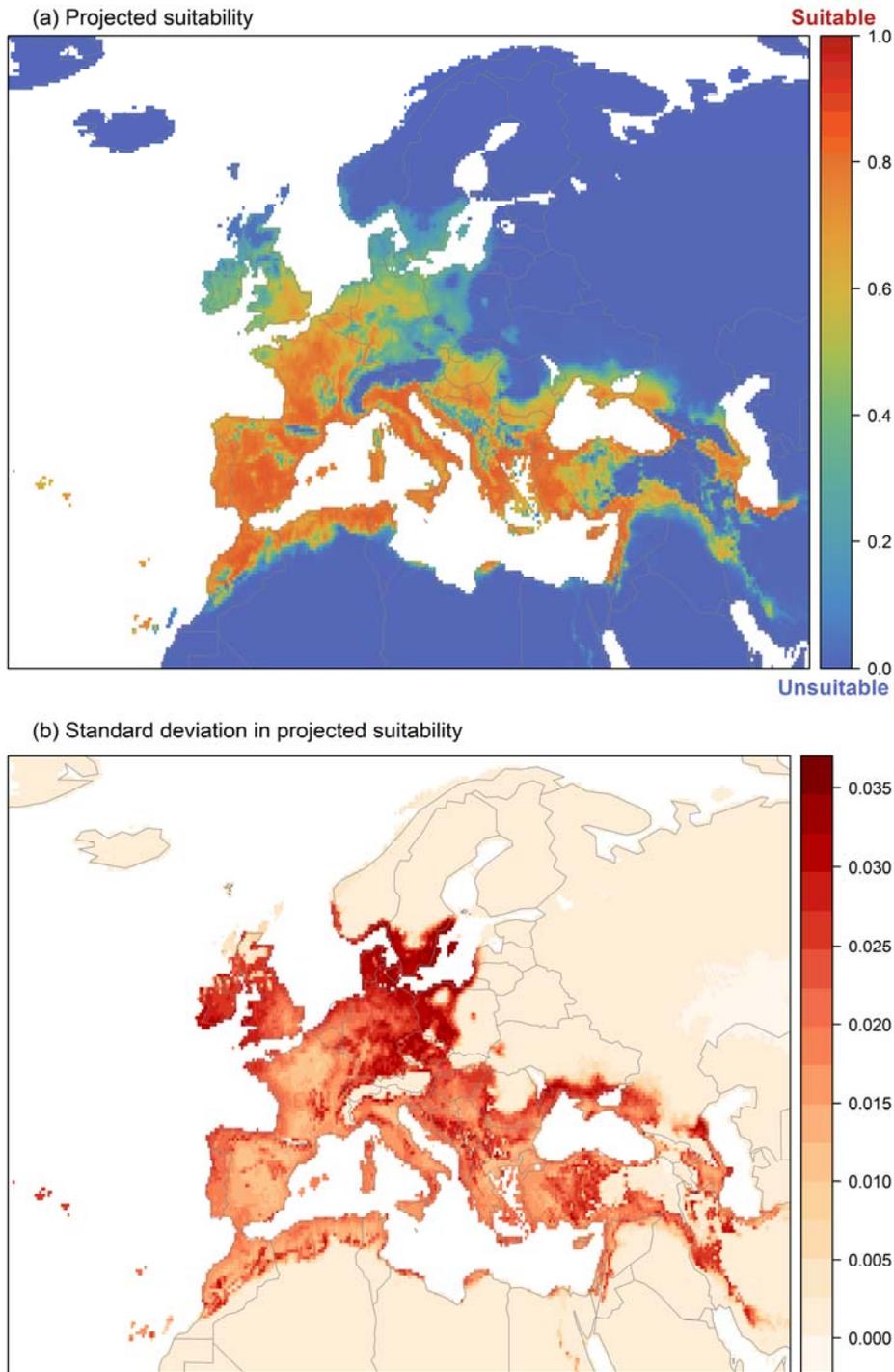
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Figure 4. (a) Projected global suitability for *Bipalium kewense* establishment in the current climate. For visualisation, the projection has been aggregated to a 0.5 x 0.5 degree resolution, by taking the maximum suitability of constituent higher resolution grid cells. Values > 0.59 are suitable for the species, with 98% of global presence records above this threshold. Values below 0.59 indicate lower relative suitability. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.



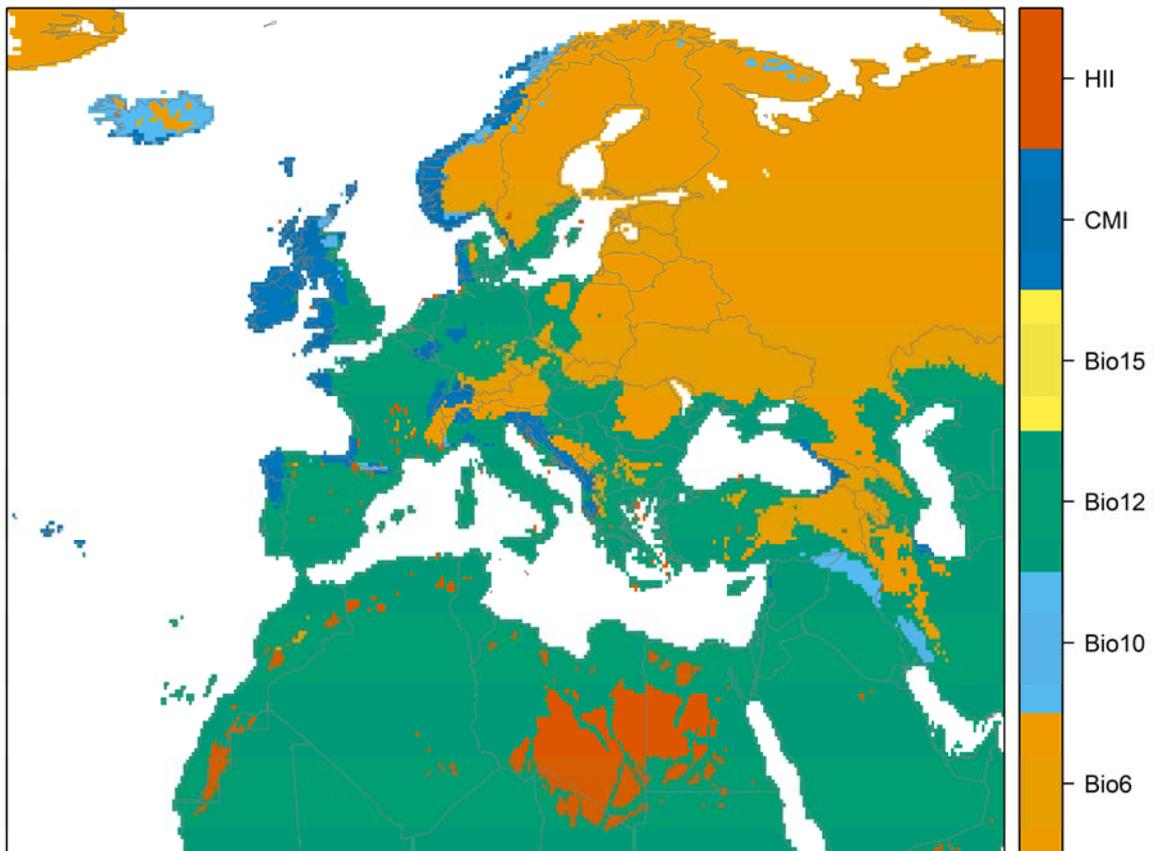
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Figure 5. (a) Projected current suitability for *Bipalium kewense* establishment in Europe and the Mediterranean region. Values > 0.59 are suitable for the species, with 98% of global presence records above this threshold. Values below 0.59 indicate lower relative suitability. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.



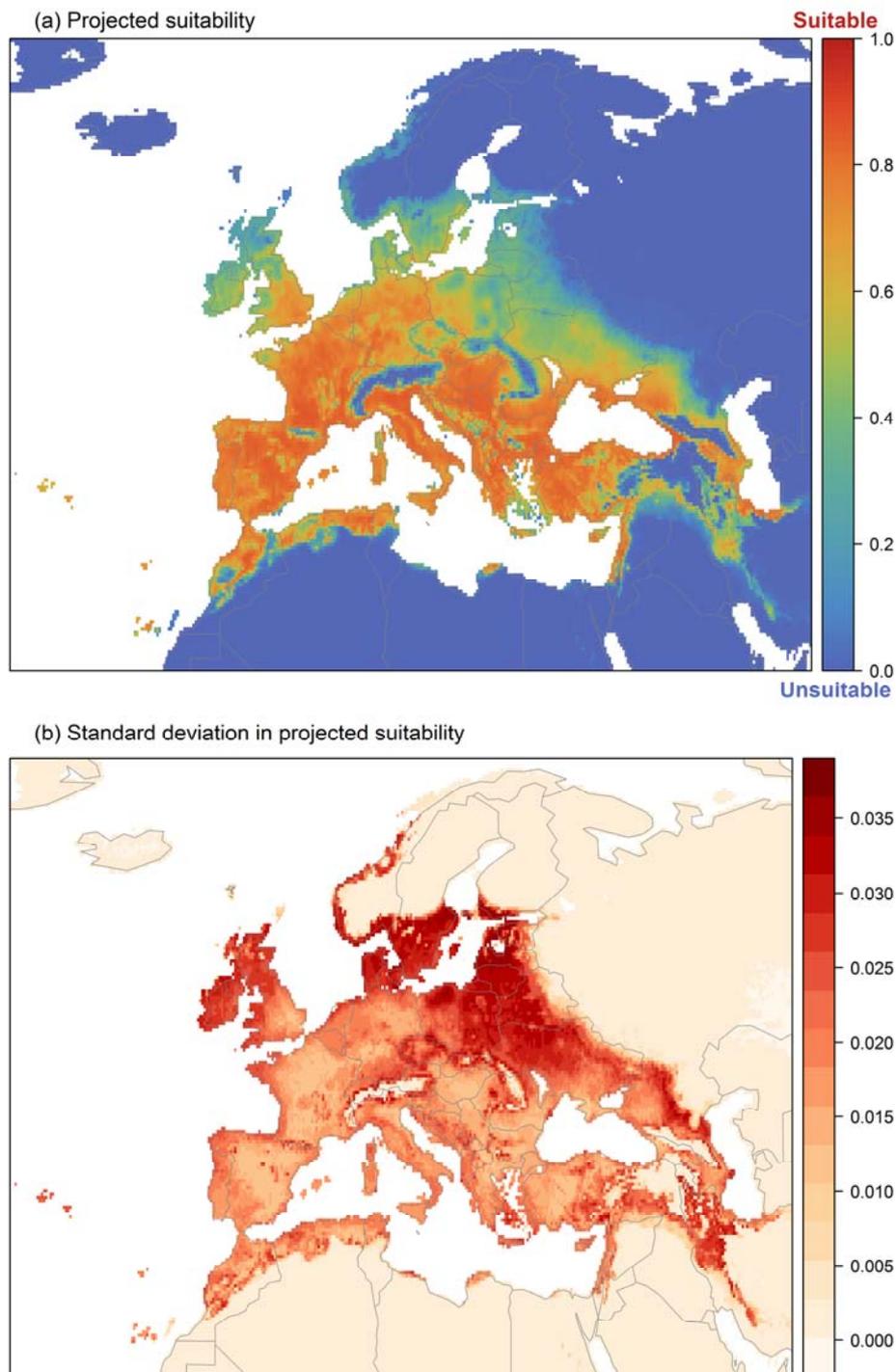
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Figure 6. The most strongly limiting factors for *Bipalium kewense* establishment estimated by the model in Europe and the Mediterranean region in current climatic conditions.



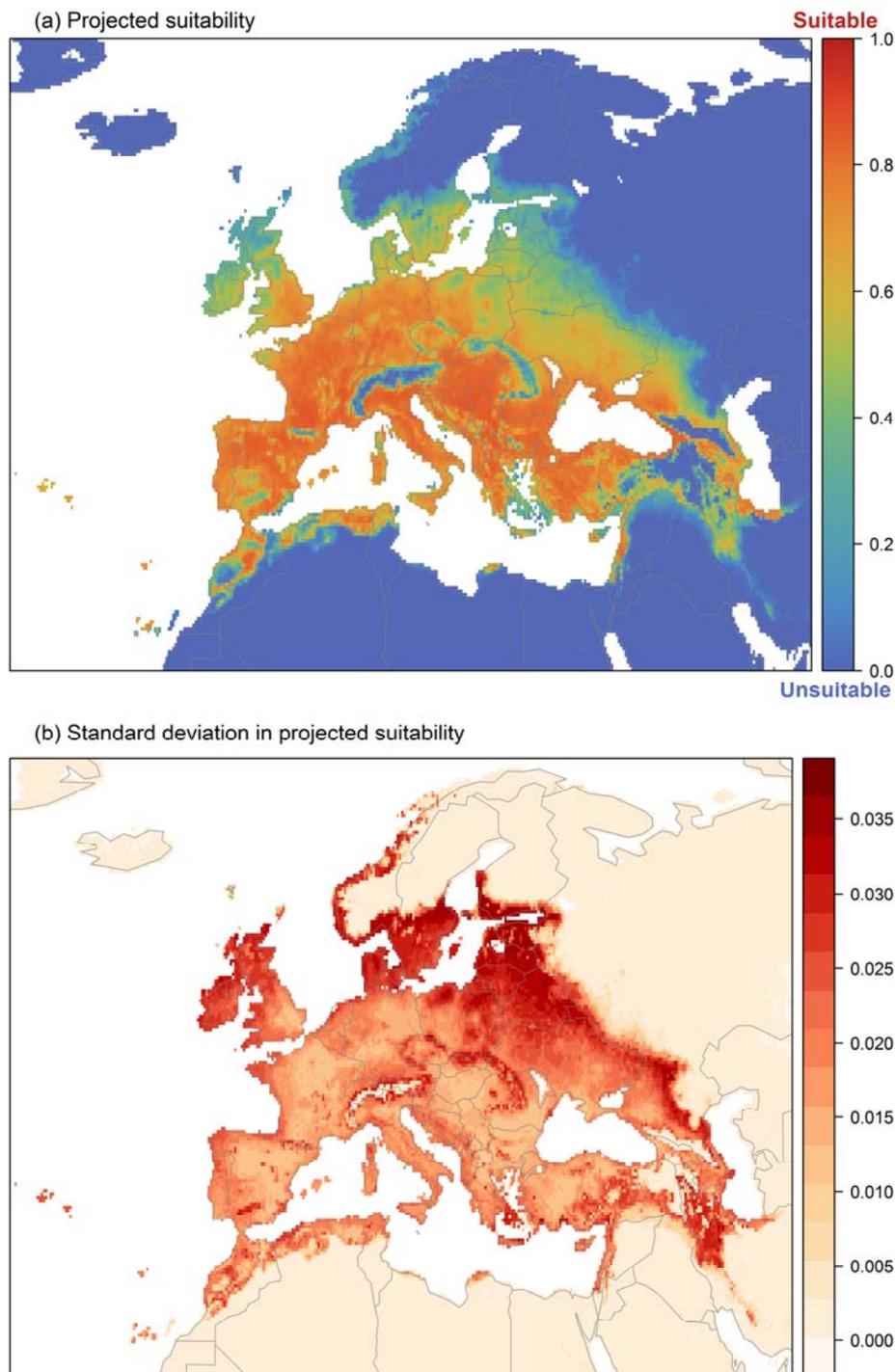
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Figure 7. (a) Projected suitability for *Bipalium kewense* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP2.6. Values > 0.59 are suitable for the species, with 98% of global presence records above this threshold under current climate. Values below 0.59 indicate lower relative suitability. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.



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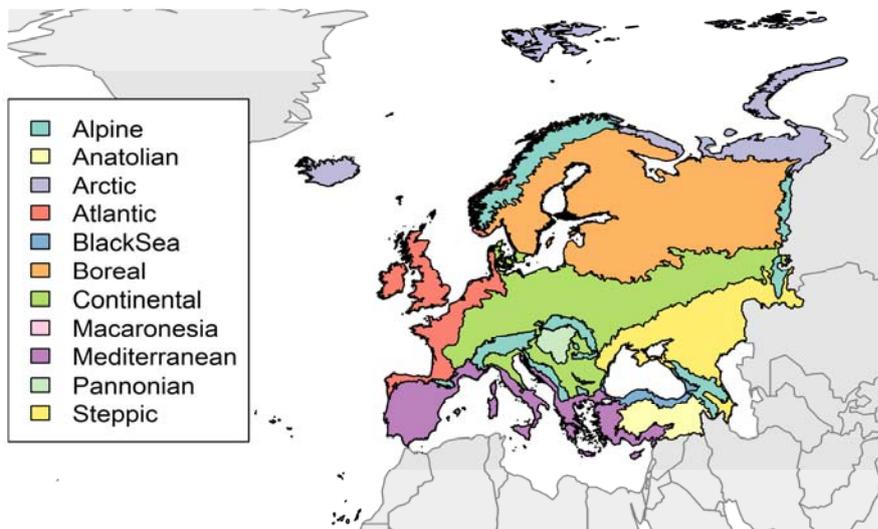
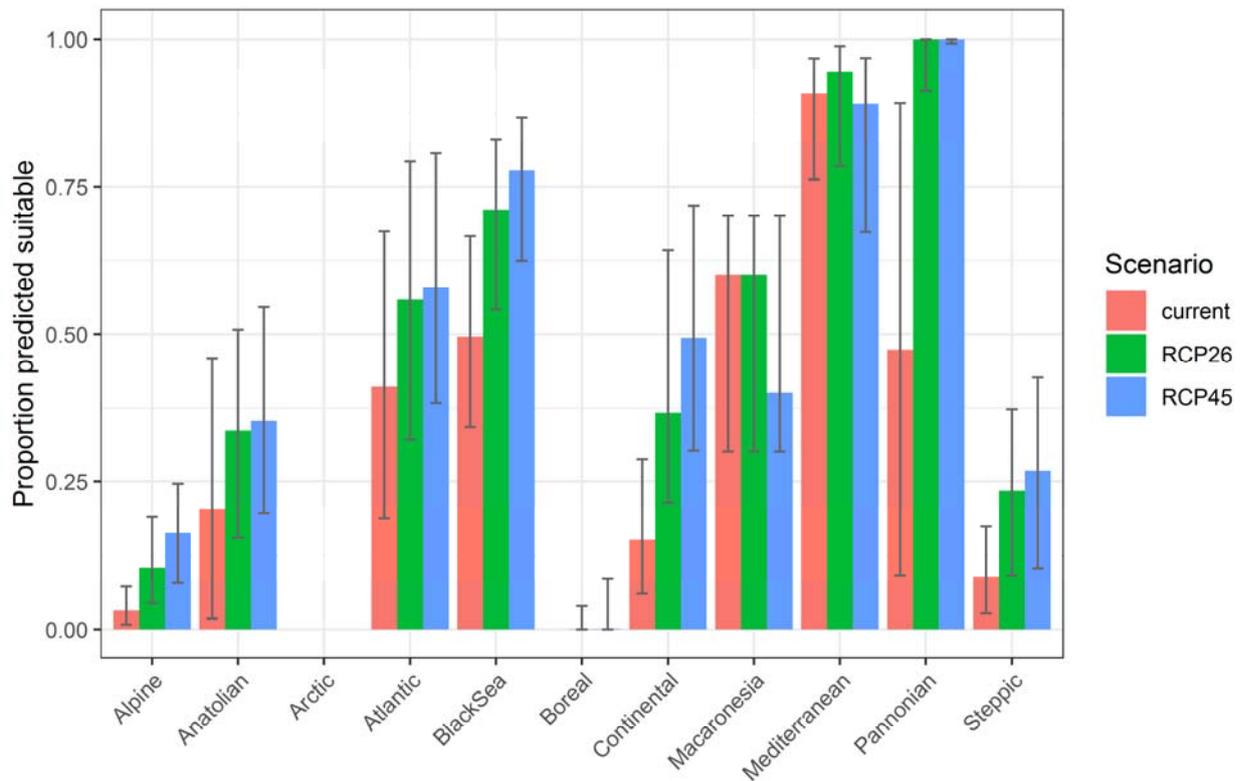
Figure 8. (a) Projected suitability for *Bipalium kewense* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP4.5. Values > 0.59 are suitable for the species, with 98% of global presence records above this threshold under current climate. Values below 0.59 indicate lower relative suitability. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.



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Figure 9. Variation in projected suitability for *Bipalium kewense* establishment among Biogeographical Regions of Europe (<https://www.eea.europa.eu/data-and-maps/data/biogeographical-regions-europe-3>). The bar plots show the proportion of grid cells in each region classified as suitable (with values > 0.59) in the current climate and projected climate for the 2070s under two RCP emissions scenarios. Error bars indicate uncertainty due to both the choice of classification threshold (cf. p.6) and uncertainty in the projections themselves (cf. part (b) of Figs.

5,7,8). The location of each region is also shown. The Arctic and Macaronesian regions are not part of the study area, but are included for completeness.



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Table 2. Variation in projected suitability for *Bipalium kewense* establishment among Biogeographical regions of Europe (numerical values of Figure 9 above). The numbers are the proportion of grid cells in each region classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. The Arctic and Macaronesian biogeographical regions are not part of the study area, but are included for completeness.

	current climate	2070s RCP2.6	2070s RCP4.5
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	lower	central estimate	upper	lower	central estimate	upper	lower	central estimate	upper
Alpine	0.01	0.03	0.07	0.04	0.10	0.19	0.08	0.16	0.25
Anatolian	0.02	0.20	0.46	0.15	0.34	0.51	0.20	0.35	0.54
Arctic	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Atlantic	0.19	0.41	0.67	0.32	0.56	0.79	0.38	0.58	0.81
Black Sea	0.34	0.50	0.67	0.54	0.71	0.83	0.62	0.78	0.87
Boreal	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.00	0.09
Continental	0.06	0.15	0.29	0.21	0.37	0.64	0.30	0.49	0.72
Macaronesia	0.30	0.60	0.70	0.30	0.60	0.70	0.30	0.40	0.70
Mediterranean	0.76	0.91	0.97	0.78	0.94	0.99	0.67	0.89	0.97
Pannonian	0.09	0.47	0.89	0.91	1.00	1.00	0.99	1.00	1.00
Steppic	0.03	0.09	0.17	0.09	0.23	0.37	0.10	0.27	0.43

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Figure 10. Variation in projected suitability for *Bipalium kewense* establishment among European Union countries and the UK. The bar plots show the proportion of grid cells in each country classified as suitable (with values > 0.59) in the current climate and projected climate for the 2070s under two RCP emissions scenarios. Error bars indicate uncertainty due to both the choice of classification threshold (cf. p.6) and uncertainty in the projections themselves (cf. part (b) of Figs. 5,7,8). Malta has been excluded because the Human Influence Index dataset lacks coverage for Malta.

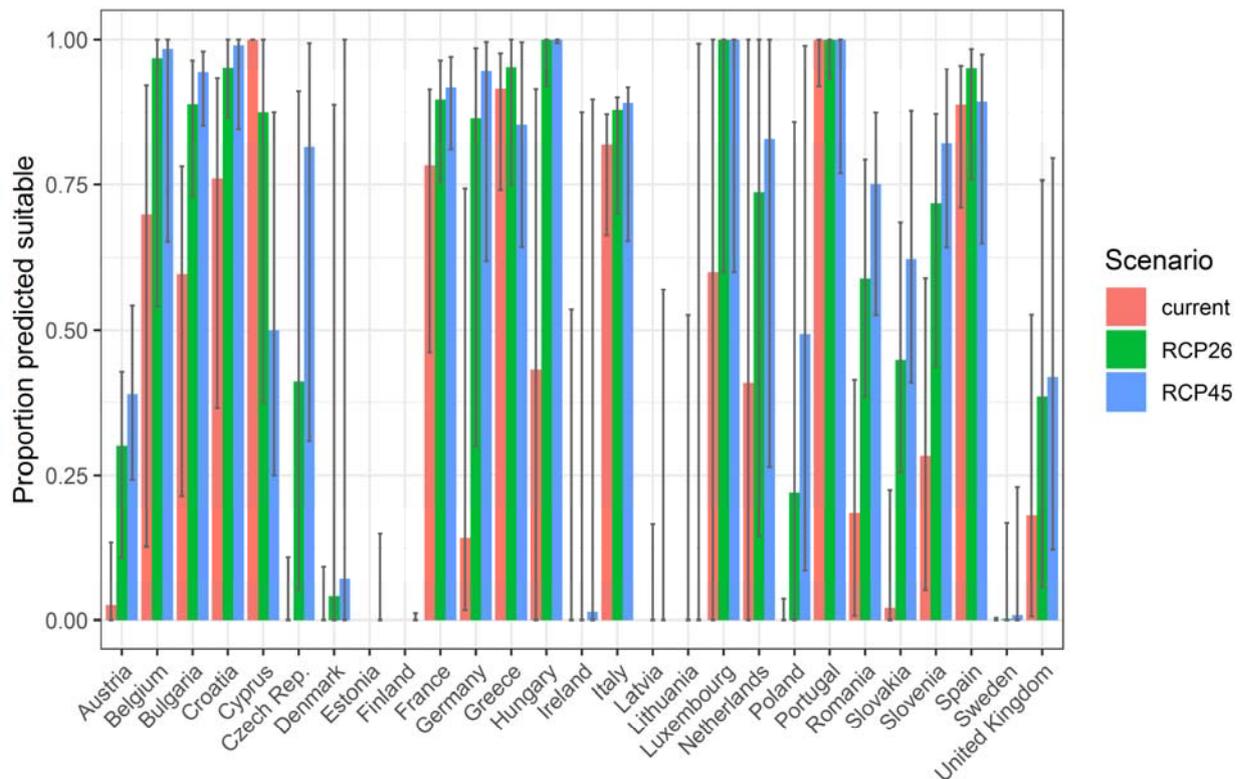


Table 3. Variation in projected suitability for *Bipalium kewense* establishment among European Union countries and the UK (numerical values of Figure 10 above). The numbers are the proportion of grid cells in each country classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios.

	current climate			2070s RCP2.6			2070s RCP4.5		
	lower	central estimate	upper	lower	central estimate	upper	lower	central estimate	upper
Austria	0.00	0.03	0.13	0.11	0.30	0.43	0.24	0.39	0.54
Belgium	0.13	0.70	0.92	0.54	0.97	1.00	0.65	0.98	1.00
Bulgaria	0.21	0.60	0.78	0.73	0.89	0.96	0.85	0.94	0.98
Croatia	0.37	0.76	0.93	0.87	0.95	1.00	0.85	0.99	1.00
Cyprus	1.00	1.00	1.00	0.38	0.88	1.00	0.25	0.50	0.88
Czech Rep.	0.00	0.00	0.11	0.05	0.41	0.91	0.31	0.81	0.99
Denmark	0.00	0.00	0.09	0.00	0.04	0.89	0.00	0.07	1.00
Estonia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15
Finland	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
France	0.46	0.78	0.91	0.75	0.90	0.96	0.81	0.92	0.97
Germany	0.02	0.14	0.74	0.30	0.86	0.99	0.62	0.95	1.00
Greece	0.74	0.92	0.98	0.75	0.95	1.00	0.64	0.85	1.00
Hungary	0.00	0.43	0.91	0.92	1.00	1.00	0.99	1.00	1.00
Ireland	0.00	0.00	0.53	0.00	0.00	0.88	0.00	0.01	0.90
Italy	0.66	0.82	0.87	0.70	0.88	0.90	0.65	0.89	0.92
Latvia	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.57
Lithuania	0.00	0.00	0.00	0.00	0.00	0.52	0.00	0.00	0.99
Luxembourg	0.00	0.60	1.00	0.60	1.00	1.00	0.60	1.00	1.00
Netherlands	0.00	0.41	1.00	0.14	0.74	1.00	0.26	0.83	1.00
Poland	0.00	0.00	0.04	0.00	0.22	0.86	0.09	0.49	0.99
Portugal	0.92	1.00	1.00	0.93	1.00	1.00	0.77	1.00	1.00
Romania	0.01	0.18	0.41	0.38	0.59	0.79	0.53	0.75	0.87
Slovakia	0.00	0.02	0.22	0.26	0.45	0.68	0.41	0.62	0.88
Slovenia	0.05	0.28	0.59	0.44	0.72	0.87	0.64	0.82	0.95
Spain	0.71	0.89	0.95	0.76	0.95	0.98	0.65	0.89	0.97
Sweden	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.01	0.23
UK	0.01	0.18	0.53	0.06	0.38	0.76	0.12	0.42	0.79

Caveats to the modelling

To remove spatial recording biases, the selection of the background sample from the accessible background was weighted by the density of Platyhelminthes records on the Global Biodiversity Information Facility (GBIF). While this is preferable to not accounting for recording bias at all, it may not provide the perfect measure of recording bias.

There was substantial variation among modelling algorithms in the partial response plots (Figure 3). In part this will reflect their different treatment of interactions among variables. Since partial plots are made with other variables held at their median, there may be values of a particular variable at which this does not provide a realistic combination of variables to predict from.

Other variables potentially affecting the distribution of the species, such as types of land cover were not included in the model.

Page Break

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Annex with evidence on measures and their implementation cost and cost-effectiveness

Species (scientific name)	<i>Bipalium kewense</i> Moseley, 1878
Species (common name)	Kew flatworm
Author(s)	Archie K. Murchie, Leigh Winsor, Jean-Lou Justine
Reviewer	Brian Boag, Peter Robertson, Richard Shaw
Date completed	15/10/2021

Summary¹

Highlight of measures that provide the most cost-effective options to prevent the introduction, achieve early detection, rapidly eradicate and manage the species, including significant gaps in information or knowledge to identify cost-effective measures.

Bipalium kewense is an alien terrestrial flatworm, originally from South East Asia. It is the most widespread species of terrestrial flatworm worldwide. In the risk assessment area, it has been recorded from many European countries, albeit mainly in hothouses (the species name relates to Royal Botanic Gardens, Kew, London, UK). Nevertheless, *B. kewense* has established outdoors in France, Italy, Malta, Portugal and Spain. *Bipalium kewense* is a potentially problematic species because it is a predator of earthworms. If established in a region, eradication and management options are very limited. Prevention of introduction, prevention of establishment and prevention of spread are by far the best options.

Unintentional introduction: Legislative control and phytosanitary practices at exporting plant producers would be the first step to prevent introduction of *B. kewense*. If *B. kewense* is found, detention notices should be enforced to prevent further stock movement. Plants from the same consignment should be traced and thoroughly examined for flatworms.

Detection: An education and awareness programme should be implemented to raise the profile of *B. kewense* and the potential risk it poses to the environment within the industry. Importing nurseries, botanic gardens or garden centres should inspect their stock regularly for non-indigenous flatworms.

Eradication: Eradication is only likely to be feasible in very small, controlled areas before the species has entered the wild. If on concrete, biocides could be used, but on a soil-base, removal and scorching would be necessary. The flatworm can reproduce by fragmentation, so may regenerate if physically damaged. If *B. kewense* spreads to the agricultural land and the wild, eradication will be virtually impossible.

Long-term management: Management options are limited. There are no chemical or classical biological control methods currently available for invasive alien flatworms. Spread may be limited by good biosecurity measures, cleaning machinery and equipment before moving between sites. Soil, compost and rooted plants should not be moved from infested gardens or other sites.

Detailed assessment			
	Description of measures ²	Assessment of implementation cost and cost-effectiveness (per measure) ³	Level of confidence ⁴
<p>Methods to achieve prevention of intentional and unintentional introduction⁵</p>	<p>Plant health inspection Terrestrial flatworms are mostly introduced via inadvertent transport by the plant trade, particularly nursery and potted plants. Regular plant health inspections and surveillance are necessary to prevent establishment. Current EU Commission Implementing Regulation (EU) 2019/2072 stipulates the special requirements for growing medium attached to plants to sustain them and this largely prohibits soil but includes plants imported bare root or maintained on an artificial media. If implemented correctly, this should reduce the likelihood of <i>B. kewense</i> being carried in plant containers. Legislation to guarantee that exported plants are free from infestation by invasive flatworms would be helpful.</p> <p>EPPO (2001) guidelines for another alien flatworm <i>Arthurdendyus triangulatus</i> specify that: 1) Plants are grown on slatted benches</p>	<p>Plant health inspection Plant health inspections and surveillance are important universal controls for crop pests and pathogens, as well as preventing incursion of invasive species. They are widely accepted and expected across the horticulture sector. It is likely that surveillance for <i>B. kewense</i> would be incorporated into routine surveillance. However, examination for soil-borne or cryptic pests such as <i>B. kewense</i> is likely to be very time-consuming and expensive. Plant inspections at source would be more effective than portal inspections. Environmental DNA technologies could aid in detection but have not been tested as yet. There would be an incremental cost from increasing the level of plant health inspection at ports of entry as well as the cost of training inspectors in new monitoring and control measures, which could be passed on to the industry.</p>	<p>Medium (Some information from published sources on terrestrial flatworms but not applied to <i>B. kewense</i>)</p>

	<p>2) The consignment must come from a place of production found free from flatworms</p> <p>3) The consignment must have been subjected to a disinfestation treatment to eliminate flatworms.</p>		
	<p>Hot-water treatment Hot water treatment may be used to cleanse plants of terrestrial flatworms, either by immersion (Sugiura, 2008; Murchie and Moore, 1998) or as a drench (Justine <i>et al.</i>, 2014). The temperature will depend on the flatworm species. For <i>A. triangulatus</i>, immersion in water at 34°C, resulted in death after 5 minutes (Murchie and Moore, 1998). No data are available for <i>B. kewense</i>. Treatment at source would be preferable, as this would meet the requirement that the consignment has been subject to disinfestation treatment (as above). The guidelines above can be stipulated by importers and National Plant Protection Organisations.</p>	<p>Hot-water treatment Hot water treatment is used for nematode control and could be effective for <i>B. kewense</i>. The method is likely to be acceptable to the public for <i>B. kewense</i> management as these measures have been agreed for other invasive flatworms and hot water is the recommended method of euthanising them when found in domestic settings. However, on a commercial scale it requires specialist equipment and risks damaging the plants. There may be some health and safety implications of hot water treatment such as scalding but these are likely to be routine. Justine <i>et al.</i> (2014) considered that drenching may be more practical in a commercial setting. There is little information on whether hot-water treatment has been used for flatworm control at any nurseries or producers. Placing compost in glasshouses to raise the temperature has been occasionally used against <i>A. triangulatus</i> but its effectiveness against <i>B. kewense</i>, a sub-tropical species, would be reduced. There are no environmental implications for heat-treatment control as measures will take place in a managed</p>	<p>Medium (Some information from published sources on terrestrial flatworms but not applied to <i>B. kewense</i>)</p>

		environment at a local scale. Eradication at site would need to be implemented as rapidly as possible.	
Methods to achieve early detection⁶	<p>Awareness and education (detection by industry and public)</p> <p>UK and Irish guidelines (MAFF, 1996; Kelly, 2012) for nurseries and garden centres suggest the following methods to detect terrestrial flatworms:</p> <ol style="list-style-type: none"> 1) Check regularly under matting or pots standing directly on the ground for flatworms or their egg cocoons. 2) Lift plants from their pots frequently to check for the presence of flatworms or their egg cocoons. Standing potted plants in water overnight has been practised by some gardeners as a means of detecting <i>A. triangulatus</i> (B. Boag 2005, pers. comm.). 3) Set traps (e.g. gravel or sand filled polythene bags) along the edges of the holding especially where it adjoins private gardens. Regularly check these for the presence of flatworms or their egg cocoons. 4) Inspect all outgoing consignments of plants carefully whether for export or not. <p>For <i>A. triangulatus</i>, pictorial identification guides have been produced and it is</p>	<p>Awareness and education (detection by industry and public)</p> <p>As <i>B. kewense</i> is likely to be spread from nurseries, garden centres and botanic gardens, detection at these sites would be most cost-effective. Raising awareness of invasive flatworms and their potential impact to industry and consumers, would be one of the most effective ways to detect this species. The methods proposed are low-cost and easily implemented as part of normal nursery practice. Awareness and vigilance are key. If a nursery or garden centre were found to disseminate <i>B. kewense</i> in their plants, this would cause significant reputational damage with consequent loss in consumer confidence. There is therefore a clear commercial incentive for producers to be involved.</p> <p>Citizen science approaches have been used to map the spread of invasive flatworms, including <i>B. kewense</i> (Moore <i>et al.</i>, 1998; Justine <i>et al.</i>, 2020; Justine <i>et al.</i>, 2015; Justine <i>et al.</i>, 2018). This is a valuable approach that has become increasingly effective with the development of mobile phone cameras and online recording applications.</p> <p>Large adult flatworms are distinctive from other soil organisms such as earthworms and insect larvae. However, for species identification</p>	<p>High</p> <p>(Professionally produced guidelines for invasive flatworm detection published and available on several websites)</p>

	<p>recommended that similar be done for <i>B. kewense</i>.</p> <p>Environmental DNA (eDNA) technologies could be used for detection by, for example, analysing water samples passed through growing media held in plant pots.</p>	<p>specialist training would be required. A citizen science approach would cost tens of thousands of euros, but this would comprise many hours of individual unpaid activity, rather than direct costs to government or industry. There are environmental benefits of engaging the public in this process, as it aids in the overall awareness of invasive species and other issues. The health and safety and welfare issues are minimal.</p> <p>Detection is likely if industry and the public can be proactively engaged. As <i>B. kewense</i> has not established in all its potential European range, ongoing vigilance is required, and refresher messages should be disseminated to promote continued engagement.</p>	
<p>Methods to achieve eradication⁷</p>	<p>Eradication has only been attempted in infested earthworm farms Australia where <i>B. kewense</i> can be a minor pest, by simple hand picking or by water-flooding the beds (Winsor, 1998). Eradication is likely only to be possible if detected very early (within weeks) in a small, controlled area, following intensive treatments, such as physical removal, soil removal, soil scorching, soil flooding and treatments with biocides. Fumigation may be a possibility for eradicating <i>B. kewense</i> in hothouses, providing phytotoxic effects were kept to a minimum; however, this has not been assessed.</p>	<p>Hand picking and water flooding</p> <p>This technique has been successful in removing <i>B. kewense</i> from earthworm beds in Australia. There are no environmental or ethical constraints to this method. However, this is a labour-intensive approach and limited to an enclosed area.</p> <p>Scorching / soil removal</p> <p>Murchie (2017) considered that soil removal and scorching could eradicate invasive flatworms from a small area. However, such techniques have not been tested against flatworms and may not be effective if flatworms hide deep in the soil. Furthermore, any removed soil would also have to</p>	<p>Low</p> <p>Hand-picking and water-flooding have been successful in earthworm beds but only in a small area. Flatworms may re-invade over time.</p>

	<p>Once established in the wider environment, eradication of <i>B. kewense</i> is unlikely. Removal trapping was attempted for <i>A. triangulatus</i> as a means of eradication/management but was not successful, as shelter traps did not capture a sufficiently high proportion of the population and also it was very labour intensive (Cannon <i>et al.</i>, 1999; Blackshaw <i>et al.</i>, 1996).</p>	<p>be treated to eliminate flatworms therein, most likely with a form of heat treatment or freezing. Scorching and / or soil removal is only effective for very small areas (10s of m²) and at the very early stages of detection. Even so, the likely costs for small-scale eradication at a suspected nursery / garden site would be in the tens of thousands of euros if soil needed to be removed and monitoring implemented. Scorching and soil removal would have health and safety implications and need to be carried out by professional operators. The environmental impact would be highly localised. Stakeholders are generally likely to be supportive but may call for financial support to fund the eradication process or seek compensation for damaged produce and / or facilities. Eradication will be rapid if techniques are successful, but these are untested.</p> <p>Fumigation</p> <p>Fumigation is carried out in commercial glasshouses to eliminate outbreaks of phytophagous pests. There is therefore potential that this method could be used for eradication of <i>B. kewense</i> in hothouses. However, this has not been tested and it is questionable whether the fumigant used would be effective against terrestrial flatworms and whether it would penetrate the soil to target flatworms therein. Use</p>	
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		of fumigants is subject to strict procedures and must be carried out by licensed personnel.	
Methods to achieve management⁸	<p>Management options</p> <p>In general, prevention of spread through the measures detailed in the ‘prevention of introduction’ section (above) would be valuable for management, particularly education and awareness, coupled with good biosecurity/phytosanitary measures and limitations on the movement of infested soil or plants.</p> <p>Techniques developed for eradication could be extended where appropriate to a control/management scenario. For example, water-flooding and hand picking of <i>B. kewense</i> as used in earthworm beds (Winsor, 1998), may be classed more as management than eradication if it has to be continually applied to prevent re-infestation.</p> <p>If native species are threatened by the presence of flatworms, species conservation measures may be required. For earthworms, this may entail methods to enhance their populations such as minimum-till cultivation and application of farmyard manure.</p> <p>Chemical control has not been used against terrestrial flatworms. No pesticides are approved for control and it is difficult</p>	<p>Management options</p> <p>Management options for <i>B. kewense</i> are extremely limited and only hand removal has been used in the field (as described under the eradication section) (Winsor, 1998). Hand collection of flatworms may be possible within a limited area (e.g. earthworm beds, small gardens) but is very labour-intensive and therefore costly. As an indicator, a retired gardener collected and removed <i>A. triangulatus</i> from shelter traps on a weekly basis, taking c. 45 minutes each time (A.K. Murchie 2021, unpubl.). If this was extrapolated to a larger area, the costs in terms of person-hours would be substantial.</p> <p>Chemical control will depend on identifying a suitable active ingredient and applying it in a way that minimises any effects on earthworms and other soil fauna. Even if this could be done, widespread use of pesticides in natural environments is likely to be met with public scrutiny and could have other environmental consequences (e.g. non-target impacts, water pollution, etc.).</p> <p>It is probable that native predatory beetles and some birds will feed upon <i>B. kewense</i> in Europe and their activity should be encouraged through land management practices and judicious use of pesticides. However, terrestrial flatworms are</p>	<p>Medium</p> <p>(Little evidence of successful management techniques described in the literature)</p>

	<p>to target flatworms in the soil, whilst avoiding impacts on non-target organisms. B. Boag quoted in Murchie (2017) reported that slug pellets (metaldehyde) had an impact on <i>A. triangulatus</i> sheltering under refuges on the soil surface. Veterinary anti-helminthics could potentially target invasive alien flatworms but this has not been tested and there would be significant regulatory hurdles to widespread environmental use.</p> <p>No specific predators, parasitoids or pathogens have been documented as potential biocontrol agents for <i>B. kewense</i>.</p>	<p>generally distasteful and native predation would be insufficient to limit populations. Protection and enhancement of earthworms through conservation measures may mitigate some flatworm damage. This could be a long-term and costly commitment, although it would be a beneficial approach despite the flatworm threat. The large-scale management of such a cryptic and inaccessible species is impractical and unproven and would probably be limited to preventing further local spread. Unfortunately, there are no examples of terrestrial flatworm management programmes on which to base cost estimates for the management of <i>B. kewense</i>.</p>	
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Guidelines for Completing the Annex

1 Provide a brief summary description of the most cost-effective methods drawing on the reviews in the detailed assessments

2 Provide a description of the potential method. This should be based on the available key scientific evidence which should be gathered from sources including articles and reviews in technical and scientific journals, internet searches, online databases, grey literature and relevant books and personal communications from scientists, stakeholders, conservation practitioners and governmental bodies. This information should include a full bibliographic list detailing the literature and sources considered.

3 Provide an assessment of the likely cost and effectiveness of the method. Where information is available, consider the following range of questions, accepting that not all questions will be appropriate in all circumstances.

- How effective has this approach proven to be in the past or in an analogous situation?
- How publically acceptable is the approach likely to be?
- Over what period of time would this approach need to be applied to be effective?
- What is the direct cost of implementing this approach?
- How likely are the methods used in the approach to be available?
- How likely is it that relevant licences or other approvals to undertake the approach would be difficult to obtain?
- How likely is it that health and safety issues would prevent the use of this approach?
- How significant is the environmental harm caused by this approach?
- How significant is the economic harm caused by this approach?
- How significant is the social harm caused by this approach?
- How likely is it that the approach will be criticised on welfare grounds?
- How likely is it that the approach will be acceptable to other stakeholders?

Where available, factual information on the costs of specialist equipment, or case studies of management costs from across the Union or third countries should be provided. When describing case studies, if the information is available then provide both total cost and the area over which control was undertaken so that a cost per unit area might be derived. Where such quantitative information is not available, then any qualitative information from the literature is acceptable to help guide decision making. It is accepted that in the majority of cases the information required to assess the potential total cost of management at a member state level is unlikely to be available. This would normally require information on the extent and abundance of the species which is beyond the scope of this assessment. Assessors are not expected to extrapolate the potential total costs of management at a member state level, only to report on the information provided within the literature.

4 Provide an overall assessment of the confidence that can be applied to the information provided for this method. This confidence should relate to the quality of the available information using the guidance below. It should NOT relate to the confidence in the effectiveness of the method

The confidence scores are:

- **High:** Information comes from published material, or current practices based on expert experience applied in one of the EU countries or third country with similar environmental, economic and social conditions.
- **Medium:** Information comes from published data or expert opinion, but it is not commonly applied, or it is applied in regions that may be too different from Europe (e.g. tropical regions) to guarantee that the results will be transposable.
- **Low:** data are not published in reliable information sources and methods are not commonly practiced or are based solely on opinion; This is for example the case of a novel situation where there is little evidence on which to base an assessment.

If there are further factors beyond these that have determined the chosen level of confidence, then provide a brief written description to support the choice of the level of confidence.

5 Measures for preventing the species being introduced intentionally or unintentionally into the territory of a Member State (cf. Articles 7(1)(a), 7(2), 13, 15), i.e. preventing a species entering by blocking its pathways, including pre-border prevention and prevention of escape or release into the environment and secondary spread whenever relevant. Measures for preventing the species reproducing while in containment should be included, if appropriate. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

6 Measures to run an effective surveillance system for achieving an early detection of a new occurrence (cf. Article 16). This section assumes that the species is not currently present in a Member State, or part of a Member State's territory.

7 Measures to achieve eradication. Preferably, but not exclusively, used at an early stage of invasion, after an early detection of a new occurrence (cf. Article 17). Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

8 Measures to achieve management of the species once it has become widely spread within a Member State territory (cf. Article 19). These measures can be aimed at eradication, population control or containment of a population of the species. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

The development and completion of this template forms part of the EU project **No 07.0202/2020/834529/ETU/ENV.D.2** Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention.

**Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention"
Contract No 07.0202/2020/834529/ETU/ENV.D.2¹**

Name of organism: Sika deer (*Cervus nippon*)

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Risk Assessment Area: The risk assessment area is the territory of the European Union 27 and the United Kingdom, excluding the EU-outermost regions

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Date of completion: 15/10/2021

¹ This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA). A number of amendments have been introduced to ensure compliance with Regulation (EU) 1143/2014 on IAS and relevant legislation, including the Delegated Regulation (EU) 2018/968 of 30 April 2018, supplementing Regulation (EU) No 1143/2014 of the European Parliament and of the Council with regard to risk assessments in relation to invasive alien species (see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>).

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SECTION A – Organism Information and Screening

A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?

including the following elements:

- the taxonomic family, order and class to which the species belongs;
- the scientific name and author of the species, as well as a list of the most common synonym names;
- names used in commerce (if any)
- a list of the most common subspecies, lower taxa, varieties, breeds or hybrids

As a general rule, one risk assessment should be developed for a single species. However, there may be cases where it may be justified to develop one risk assessment covering more than one species (e.g. species belonging to the same genus with comparable or identical features and impact). It shall be clearly stated if the risk assessment covers more than one species, or if it excludes or only includes certain subspecies, lower taxa, hybrids, varieties or breeds (and if so, which subspecies, lower taxa, hybrids, varieties or breeds). Any such choice must be properly justified.

This risk assessment covers one species, the sika deer *Cervus nippon* Temminck, 1838, also known as Japanese Sika (deer) (Class: Mammalia, Order: Artiodactyla, Family: Cervidae, Subfamily: Cervinae, Genus: *Cervus*).

Synonym(s): Main synonyms are *Cervus sika* and *Cervus japonicus* (for additional information see Feldhamer 1980).

Sika are native to Japan, Taiwan and Eastern Asia (namely China, Korea, Russia, Vietnam) and two main lineages are recognised, a northern and a southern one, with several subspecies, ranging from 10 to 16 (see, for example, Wilson and Reeder 2005, Wilson and Mittermeier 2011) whose validity is questionable. The study of the species taxonomy is also affected by the fact that many source populations have uncertain or mixed origin due to several introductions and translocations that occurred in the past (Feldhamer 1980, Wilson and Reeder 2005, Wilson and Mittermeier 2011, Harris 2015, CABI 2021). Some subspecies are possibly extinct in the wild (Wilson and Mittermeier 2011).

For the purpose of the present risk assessment, it is important to point out that sika deer represent a monophyletic lineage that is endemic to Asia and has speciated long ago away from *C. elaphus* (Cook et al. 1999). It is also of interest to remark that introduced populations are mostly from animals originating from Japan, but also from other parts of Asia (as in the case of the Czech Republic, Linnell and Zachos 2011). However, the exact geographical origin of sika deer introduced in Europe cannot be clearly ascertained due to the lack of documented evidences, and the fact that different subspecies had been mixed and intercrossed (Bartoš 2009). In particular, animals introduced in Europe are deemed to originate mainly from captive Asian populations, which were probably subject to human manipulation through farming, artificial selection and translocations (Baiwy et al. 2013).

Sika may hybridise and produce fertile offspring with the only congeneric species in the risk assessment area, the native red deer *Cervus elaphus* (Wilson and Reeder 2005). Introgression may also occur, as the fertile hybrids usually backcross with either parent species, and this leads to increased phenotypic similarity between the two species, which makes further hybridization more likely (Zachos and Hartl 2011). Hybrids occur when sika are introduced outside their natural range, however, sika and red deer also hybridize naturally where they overlap in Far East Russia (Bartoš 2009). Since sika and sika-red deer hybrids are present in Europe both in

captivity and in the wild, and since many free ranging populations of sika include to some extent sika-hybrids (Baiwy et al. 2013), the present risk assessment applies both to pure sika deer and its hybrids.

A2. Provide information on the existence of other species that look very similar [that may be detected in the risk assessment area, either in the environment, in confinement or associated with a pathway of introduction]

Include both native and non-native species that could be confused with the species being assessed, including the following elements:

- other alien species with similar invasive characteristics, to be avoided as substitute species (in this case preparing a risk assessment for more than one species together may be considered);
- other alien species without similar invasive characteristics, potential substitute species;
- native species, potential misidentification and mis-targeting

Sika are small to medium-sized deer, and are extremely variable in size and body weight both between and within subspecies. Females are about 60-95 cm at shoulder height, and males 65-115 cm. Their weight ranges from 20-90 kg in females and 30-140 kg in males (Wilson and Mittermeier 2011). Sexual dimorphism is very pronounced, as body measurements of adult males averaged 8.7% more than those of females (Feldhamer 1980), while males are 40-70% heavier than females (Wilson and Mittermeier 2011). The species shows extensive variation in size and colours within and between populations (Baiwy et al. 2013). Pelage can vary from chestnut to yellow in summer, and grey to almost black in winter, depending on the subspecies. Similarly, the distinct white spots can be present throughout the year, or only in summer, depending on the subspecies. The rump (caudal) patch, which is the most characteristic feature, is white with a black outline on top, and is crossed by the tail, which is white with a median black stripe of variable thickness. Also the head has some distinctive features, with dark lines above the eyes and a short stubby muzzle which give sika deer a typical “frowning” expression (Booy et al. 2015). The pale U-shaped band between the eyes is also distinctive. A good distinguishing character is the prominent white scent gland often visible on the lower hind legs. Stags have relatively simple antlers, but can grow up to 98 cm long. At maturity, they usually develop up to four points on each side (CABI 2021, Feldhamer 1980, Wilson and Mittermeier 2011).

Sika can be easily mistaken for red deer and fallow deer (*Dama dama*). In general, adult sika are similar in size to adult fallow deer but smaller than red deer (CABI 2021). Adult sika can be confused with adult fallow deer for the spotted coat and the tail that look very similar. The rump can help distinguish the three species, as most fallow deer have a peculiar dark border, which is absent in the red deer, and which is only partially developed in the sika. The antlers may look similar to those of the red deer, but in sika, the brow tines of the antlers are always at an acute angle to the main beam of the antler while in red deer, they present an obtuse angle (CABI 2021). Moreover, the antlers of the sika are not palmate as in the fallow deer. While adult red deer and sika are usually easier to distinguish because of the lack of the spotted coat and of dark stripes in tail and rump patch, calves can be similar.

Hybrids can be difficult to distinguish from red deer or pure sika (CABI 2021). First generation (F1) hybrids show traits of both species, and introgressed hybrids are difficult to identify in the field (MacDevitt et al. 2009, Lammertsma et al. 2012). Not surprisingly, the presence of sika

hybrids may go undetected for a long time, and may be disbelieved even by hunters until demonstrated by DNA analysis (Lammertsma et al. 2012). Some authors stressed that species determination of shot specimen is difficult even for experienced gamekeepers and zoologists (Macháček et al. 2014).

A3. Does a relevant earlier risk assessment exist? Give details of any previous risk assessment, including the final scores and its validity in relation to the risk assessment area.

Sika deer was assessed at the EU level in 2014, following a workshop (Roy et al. 2015) aimed at assessing and integrating the information from a previous risk assessment made at the GB level which resulted in a “high” risk with “low” uncertainty (GB Non-Native Species Secretariat 2011). The participants to the workshop confirmed the compliance of the GB risk assessment with the EU Regulation No 1143/2014 and therefore its conclusions were upscaled at the EU level. However, the species was not retained for being listed among those of Union concern at that time.

A risk assessment for the sika deer exists for Poland (Solarz et al. 2018). The species is considered very invasive based on its impact on the environment, and the resulting statement in relation to the risk for the country is that the sika is a “*High risk species, occurring in the wild, with isolated populations (black list)*” (Solarz et al. 2018). It is one of the top six priority species for management actions and, possibly, eradication according to the level of spread, impacts and manageability. In relation to the risk assessment area, this result may be considered valid particularly for the Continental biogeographic region.

A risk analysis of the sika deer was carried out in Belgium (Baiwy et al. 2013), and as reported in the summary of impact “*It is very likely that the establishment of sika deer in Belgium will result in an increase of the biomass and density of deer per hectare and will produce strong negative impacts on native vegetation through overgrazing, especially on acidic soils. It could also cause the local decline of native deer species, favour disease transmission to native ungulates and induce extensive hybridization with the red deer. During irruptive events, a degradation of the conservation status of ecosystems is also expected to occur locally (alteration of vegetation, inhibition of tree regeneration, soil trampling, etc.)*.” Also in this case, in relation to the risk assessment area, such conclusions may be considered valid for the Continental biogeographic region.

In the Netherlands, the risk assessment of the sika deer (Lammertsma et al. 2012) resulted the species being classified as a category A “black list species” and since the sika is not yet naturalized in the country, is also classified as an “Alert list species” (according to the ISEIA score Branquart et al. 2007). Otherwise, the threat category in the Dutch risk assessment using Bomford (2003) is “extreme”. The main concerns in the country are damage to forestry (silviculture, timber production) and agriculture, Natura 2000 areas, competition with native ungulates and hybridization and introgression with native red deer.

In Ireland, the sika was considered among the 15 high risk impact terrestrial vertebrates (O’Flynn et al. 2014).

In Italy, the sika deer is the second one in the ranked lists of priority species for management actions and, possibly, eradication according to the predicted likelihood of spread, potential impacts on biodiversity and well-being and manageability (Bertolino et al. 2020).

In Germany, sika deer has been assessed with the GABLIS-protocol (Rabitsch & Nehring 2015). The result of the assessment was “potentially invasive – Watch List”, based on the – at this time – still rare hybridization with red deer.

A global assessment was also carried out to rank alien ungulates based on their risks of causing environmental impacts using the EICAT classification: the highest impact score reached by the sika is "Major" (Volery et al. 2021, IUCN 2020a, 2020b). The species was previously assessed also by Nentwig et al. (2018) according to whom out of 486 alien species established in Europe from a wide range of taxonomic groups, the sika deer ranked 31, among those with the highest environmental and socioeconomic impact (following the generic impact scoring system GISS, as calculated by Nentwig et al. 2010).

A4. Where is the organism native?

including the following elements:

- an indication of the continent or part of a continent, climatic zone and habitat where the species is naturally occurring
- if applicable, indicate whether the species could naturally spread into the risk assessment area

The sika is native to Japan, Taiwan and east Asia, from eastern Russia to China (where it is now rare and localized). In Taiwan it was reintroduced; it occurred also in North and South Korea, and Vietnam, where it is now probably extinct (see Wilson and Reader 2005, Harris 2015, CABI 2021).

In its native range the sika deer inhabits a wide range of habitats in low-elevation plains and surrounding hills, and a wide range of forest types, from the tropical jungles of Vietnam to broad-leaved deciduous forests of Far East Russia and northern Japan, with a preference for early succession stages over mature woodlands (Baiwy et al. 2013). Low temperatures in winter and the depth of snow cover are the main limiting factors. In a study carried out in Japan, Kaji et al. (2004) suggest that there is strong density dependence in population and the availability of food is a limiting factor in concordance with climatic factors such as variation in snow depth and/or duration of deep snow (see also Kaji et al. 2009, Takatsuki 1992). In particular, more than 40 cm of snow is considered limiting by Wilson and Mittermeier (2011) while according to Genovesi and Putman (2009) sika deer is favored by warm climates (12° to 46° N) and selects areas where snowfall does not exceed 10-20 cm and where snow-free sites are available. In Japan, sika deer are mostly concentrated in areas with snow depths less than 50 cm, with some populations living in the 'habitable area' of 50-100 cm of snow, but they rarely live in the 'inhabitable area' with snow depths higher than 100 cm (Takatsuki 1992).

The species appears highly adaptable and is found on a wide range of forest habitats with dense undergrowth and adjacent open grounds, from the sea level up to 3000 meters a.s.l. according to Wilson and Mittermeier (2011) (but only up to 1800 meters according to Feldhamer 1980). In Europe, managed and natural forests such as coniferous or mixed forests (on acidic soils with dense undergrowth), are the preferred habitats, but since sika are an opportunistic species and forage in open, grassy areas, they are also found in open mid-forest spaces, heathlands, meadows near the forest border, plantations and orchards, grasslands (grazing systems), freshwater marshes and riverbanks, moorlands and estuarine reed beds, saltmarshes provided some woodland cover is available (Lammertsma et al. 2012, Baiwy et al. 2013, Harris 2015, Solarz et al. 2018, CABI 2021). Overall, sika deer has a very broad ecological amplitude. Its

distribution and habitat selection are also affected by landscape configuration and human disturbance (Uzal et al. 2013).

With respect to feeding ecology, sika may act both as a browser and a grazer, and feed on a wide variety of plant species (Harris 2015, Takatsuki 2009b). Food composition is highly variable and depends on genetic groups, resource availability, vegetation type and climatic conditions (Baiwy et al. 2013, Takatsuki 2009b). Like other ungulates sika deer feed on a variety of grasses, herbs, young shoots of trees and shrubs, and even fruit (Solarz et al. 2018). Their diet is most often constituted of graminoids, forbs, shrubs, twigs, tree foliage, fruits, fungi and agricultural crops, but also pine needles, bark, gorse, with no significant reliance on any particular food sources (CABI 2021, Baiwy et al. 2013, Harris 2015). In fact, also plants that are usually considered unpalatable to deer are eaten (Feldhamer 1980). In total, more than a hundred species of plant are known to be part of the sika deer diet. Feldhamer and Demarais (2008) reported details on plant species found in the diet of sika in the US.

Both habitat and diet requirements are described and discussed extensively in the monograph by McCullough et al. (2009).

A5. What is the global non-native distribution of the organism outside the risk assessment area?

Outside the risk assessment area, the sika deer was successfully introduced in the following countries: Armenia, Azerbaijan, New Zealand, Philippines (Solo Island), United States, and also small islands off Japan (Kerama Islands, Ryukyu Islands). For details, see Long (2003), Lever (1985), Wilson and Reader (2005), Harris et al. (2015), CABI (2021).

The species is also present in Europe outside the risk assessment area, e.g. in Switzerland, Ukraine, western Russia (including in the Kaliningrad District of Russian Federation), and possibly Moldova (Bartoš 2009, Biedrzycka et al. 2012, CABI 2021).

Historic introductions of sika deer occurred also in Australia, South Africa, and possibly Morocco and Madagascar, but no established populations are confirmed (Long 2003, Lever 1985). Nevertheless, in Australia, the species is still being kept in captivity, for example in deer farms in Victoria (Forsyth et al. 2015).

A6. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species been recorded and where is it established? The information needs be given separately for recorded (including casual or transient occurrences) and established occurrences. “Established” means the process of an alien species successfully producing viable offspring with the likelihood of continued survival².

A6a. Recorded: List regions

A6b. Established: List regions

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

² Convention on Biological Diversity, Decision VI/23

Marine subregions:

- Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea.

Comment on the sources of information on which the response is based and discuss any uncertainty in the response.

For delimitation of EU biogeographical regions please refer to <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2> (see also Annex VI).

For delimitation of EU marine regions and subregions consider the Marine Strategy Framework Directive areas; please refer to <https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions/technical-document/pdf> (see also Annex VI).

Response (6a): Alpine, Atlantic, Boreal, Continental, Mediterranean, Pannonian.

Response (6b): Alpine, Atlantic, Boreal, Continental, Mediterranean, Pannonian.

The source of information on which the response is based can be found in Qu. A8.

A7. In which biogeographic region(s) or marine subregion(s) in the risk assessment area could the species establish in the future under current climate and under foreseeable climate change? The information needs to be given separately for current climate and under foreseeable climate change conditions.

A7a. Current climate: List regions

A7b. Future climate: List regions

With regard to EU biogeographic and marine (sub)regions, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (7a):

Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic (see details in Annex VIII).

Response (7b):

Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic (see details in Annex VIII).

Note: establishment and invasiveness in Boreal and Alpine biogeographic regions is uncertain because of species sensitivity to deep snow cover as described above (Kaji et al. 2004). Moreover, it is uncertain how sika will respond to the gradual desertification of the Mediterranean region (Alastair I Ward, pers. comm. 2021).

A8. In which EU Member States has the species been recorded and in which EU Member States has it established? List them with an indication of the timeline of observations. The information needs be given separately for recorded and established occurrences.

A8a. Recorded: List Member States

A8b. Established: List Member States

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden

The description of the invasion history of the species shall include information on countries invaded and an indication of the timeline of the first observations, establishment and spread.

Response (8a): Austria, Belgium, Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, Lithuania, Luxembourg, Netherlands, Poland; and the United Kingdom.

Response (8b): Austria, Czech Republic, Denmark, France, Germany, Hungary, Ireland, Lithuania, Poland; and the United Kingdom.

The first introductions of sika deer in the risk assessment area date back to 1860 in Ireland (McDevitt et al. 2009). In the same year the first introductions were carried out also in the UK (GB Non-Native Species Secretariat 2011).

In France, sika were successively introduced at least since 1966 (Pascal et al. 2006, Bartoš 2009, Lever 1985). In the Czech Republic sika were introduced in 1891 (Bartoš 2009, Lever 1985), in Germany in 1893 (Bartoš 2009) with the first releases into the wild in 1930 (Niethammer 1963). In Denmark, the first introductions of sika deer occurred in 1900 (Asferg and Madsen 2007) while in Austria the species was first introduced in 1907 (Bartoš 2009). In Hungary the sika was introduced in 1910 (Bartoš 2009), while in Poland the species was introduced in 1985 with first releases into the wild in 1910 (Bartoš 2009).

In Lithuania the sika deer was released into the wild in 1954 (Pūraitė and Paulauskas 2016, Baleišis et al. 1987), where there is documented evidence of hybridization with red deer where both species are known to occur (Biedrzycka et al. 2012). A study by Pūraitė and Paulauskas (2016) suggests hybridization of the two species in the wild, as there were wild living specimens, which are attributed to red deer according to their phenotype, but are closer to sika deer according to their genotype.

There are records of the species also in Estonia where sika deer were introduced in 1956, but the population is probably extinct at present, although single immigrants from neighboring areas may occur (Bartoš 2009).

Also in Luxembourg the species was first reported in 2012-2013, when some hunted animals were identified as sika. The presence of the species in the area was explained with the escape of sika deer from an enclosure in Gemünd, Germany, near the Luxembourg border, between 2011 and 2012 (Cellina and Schley 2014). It is not clear whether the species is currently established in Luxembourg.

In Belgium casual observations of sika deer were reported from different localities during recent years (Baiwy et al. 2013). In particular, two individuals were seen in Les Marionville (Saint-Ghislain) in November 2011 (Lammertsma et al. 2012) and a single male was shot in 2011 in the Famenne, near Houyet (Cellina and Schley 2014). In Flanders, sika deer have been present in Limburg province (Zonhoven) since 2012 and reproduce since at least 2018 (e.g. <https://waarnemingen.be/observation/160293818/>).

In the Netherlands sika deer were reported near 's-Graveland since 2005 on several occasions, but also here no established population is considered present (Lammertsma et al. 2012).

The species and its hybrids were recently reported also for Italy (Raganella Pelliccioni et al. 2013) in the Modena and Bolzano provinces, where animals have apparently been present since the early 2000s (Ferri et al. 2014, Ferri et al. 2016).

According to Bartoš (2009) there are internet advertisements for hunting sika deer in countries such as Finland, Bulgaria, and Slovenia, suggesting the existence of the species there as well, but it was not possible to confirm the status of the species. The only additional information retrieved for Finland is that sika deer is listed in Section 5 of the Hunting Act among the 34 mammal game species (see <https://mmm.fi/en/wildlife-and-game/hunting-and-game-management/finnish-game-resources>). An advertisement for hunting sika deer was also found for Slovakia (i.e. <http://www.shootingenterprise.com/japanese-sika-deer-hunting-and-stalking>). However, Moravčíková et al. (2017) did not confirm the species occurrence in the country. Moreover, no sign of hybridisation of red deer was found in Slovakia based on genetic studies (Moravčíková et al. 2017).

A9. In which EU Member States could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A9a. Current climate: List Member States

A9b. Future climate: List Member States

With regard to EU Member States, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global

warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (9a):

Austria, Belgium, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden; and the United Kingdom (see Annex VIII for details).

The result also shows that the high level of uncertainty in the case of Cyprus does not allow to assess the actual suitability of the country for the species, while no data is available for Malta.

Response (9b):

Austria, Belgium, Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden; and the United Kingdom (see Annex VIII for details).

The result also shows that the high level of uncertainty in the case of Cyprus does not allow to assess the actual suitability of the country for the species, while no data is available for Malta.

A10. Is the organism known to be invasive (i.e. to threaten or adversely impact upon biodiversity and related ecosystem services) anywhere outside the risk assessment area?

The species is considered invasive in many countries outside its native range, for example in the US (Feldhamer and Demarais 2008) and New Zealand (Davidson 1973).

As detailed in the section below, sika is known to pose a threat to native species through competition with other deer species and through hybridisation with native red deer. They can also have an impact on vegetation and habitats, which in turn can affect ecosystem services, as well as human health, crops and the economy. The effects of sika deer on vegetation in its native range Japan was reviewed by Takatsuki (2009a). In Japan, roadkills and rail accidents are also reported (Kaji et al. 2010).

Further details and refs. see answer to Qu 4.1 below.

A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea

Response:

Alpine, Atlantic, Boreal, Continental, Mediterranean, Pannonian.

A12. In which EU Member States has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden

Response: Czech Republic, Denmark, France, Ireland, Poland; and the United Kingdom. The list of Member States could be extended to other countries where the presence of hybrids has been recorded (although the establishment of the species is not confirmed yet, e.g. Italy and Lithuania) because if sika hybrids are within a population of red deer, then hybrids must be established and therefore sika are invading and invasive even if pure sika (if any of those exist anywhere) are not present (Alastair I Ward, pers. comm. 2021).

A13. Describe any known socio-economic benefits of the organism.

including the following elements:

- Description of known uses for the species, including a list and description of known uses in the risk assessment area and third countries, if relevant.
- Description of social and economic benefits deriving from those uses, including a description of the environmental, social and economic relevance of each of those uses and an indication of associated beneficiaries, quantitatively and/or qualitatively depending on what information is available.

If the information available is not sufficient to provide a description of those benefits for the entire risk assessment area, qualitative data or different case studies from across the risk assessment area or third countries shall be used, if available.

As summarised by Roy et al. (2014) and CABI (2021) the sika deer is a game species frequently introduced outside Asia as an additional asset for hunting, although there is little or no quantitative information available on the economic value of this.

As noted by the GB Non-Native Species Secretariat (2011) for UK, sika stags have increasingly become a valuable sporting asset (Pérez-Espona et al. 2009), and the species seems still in high demand for hunting purposes.

In Germany the International Sika Deer Society forced policy to step back from eradication attempts in NRW (<http://sikawild.org/ausrottung>). The number of animals culled per year is low in most European countries (a few hundred individuals) with an associated hunting value of several hundred to more than 2000 euro per individual (e.g. [12](http://www.globus-</p></div><div data-bbox=)

[jagdreisen.de/de/jagdlaender/europa/schottland/jagd-auf-sikahirsche/](http://www.premium-jagdreisen.de/de/jagdlaender/europa/schottland/jagd-auf-sikahirsche/) , http://www.premium-jagdreisen.de/product_info.php?products_id=29 (Solarz & Okarma, 2014).

Sika are also widely kept on farms for production of venison and, at least within their native range, antler velvet – in China, Japan and Russia. Economic values vary with demand, and although sika are farmed for meat in numbers significantly lower than other cervids, may be an important constituent of local economies. For example, in Poland, the commercial value of farmed sika deer was roughly estimated at 215,000 to 251,000 € (Solarz & Okarma, 2014).

In the US, the primary goal of deer farms is the production of brood stock, venison, and by-products such as antler velvet and hides, but some enterprises also offer opportunities for visitor viewing and photography (Feldhamer and Demarais 2008).

The species is kept in zoological gardens and for ornamental purposes by private owners. The ISIS database roughly estimates that 885 individuals are kept across 48 European institutions (ISIS, 2014). Sika deer are also perceived as a local attraction for nature lovers and the general public (Solarz & Okarma, 2014).

SECTION B – Detailed assessment

Important instructions:

- In the case of lack of information the assessors are requested to use a standardized answer: “No information has been found.” In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable).
- With regard to the scoring of the likelihood of events or the magnitude of impacts see Annexes I and II.
- With regard to the confidence levels, see Annex III.
- Highlight the selected response score and confidence level in **bold** but keep the other scores in normal text (so that the selected score is evident in the final document).

1 PROBABILITY OF INTRODUCTION AND ENTRY

Important instructions:

- **Introduction** is the movement of the species into the risk assessment area (it may be either in captive conditions and/or in the environment, depending on the relevant pathways).
- **Entry** is the release/escape/arrival in the environment, i.e. occurrence in the wild
- Introduction and entry may coincide for species entering through pathways such as “corridor” or “unaided”, but it also may differ. If different, please consider all relevant pathways, both for the introduction into the risk assessment area and the entry in the environment.
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used (see Annex IV). For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document³ and the provided key to pathways⁴.
- For organisms which are already present (recorded or established) in the risk assessment area, the likelihood of introduction and entry should be scored as “very likely” by default.
- Repeated (independent) introductions and entries at separate locations in the risk assessment area should be considered here (see Qu. 1.7).

Qu. 1.1. List relevant pathways through which the organism could be introduced into the risk assessment area and/or enter into the environment. Where possible give details about the specific origins and end points of the pathways as well as a description of any associated commodities.

For each pathway answer questions 1.2 to 1.8 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you

³ <https://circabc.europa.eu/sd/a/7e5f0bd4-34e8-4719-a2f7-c0cd7ec6a86e/2020-CBD-pathways-interpretation.pdf>

⁴ <https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf>

consider more than one pathway, e.g. 1.2a, 1.3a, etc. and then 1.2b, 1.3b etc. for the next pathway.

In this context a pathway is the route or mechanism of introduction and/or entry of the species.

The description of commodities with which the introduction of the species is generally associated shall include a list and description of commodities with an indication of associated risks (e.g. the volume of trade; the likelihood of a commodity being contaminated or acting as vector).

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 1.2-1.9.

The following active pathways of introduction have been identified in the risk assessment area:

- a) Hunting (Release in nature)
- b) Farmed animals (including animals left under limited control) (Escape from confinement)
- c) Botanical garden/zoo/aquaria (excluding domestic aquaria) (Escape from confinement)
- d) Natural dispersal across borders of invasive alien species that have been introduced through pathways 1 to 5.

For some of the introductions carried out in Europe the ultimate purpose of the releases is not explicitly reported, therefore it is not possible to establish with certainty which is the relevant pathway. For example, in Lithuania fenced sika deer herds are numerous and possibly formed hybrids with red deer after escape (Baleišis et al. 1987, Andersone-Lilley et al. 2010), but details on the purpose of the enclosures, e.g. whether for hunting, farming or ornamental purposes are not provided. The same applies in the case of Austria where sika escaped from the enclosures when they fell into disrepair or were destroyed at the end of World War II (Bartoš 2009). However, the three pathways a), b), and c) above are considered active in the EU and as such are described in detail in the next questions below. Here follows a short summary of the main evidence regarding the relevant introductions in the EU (with examples also from other countries, if appropriate).

- a) There is documented evidence that the species was introduced into the EU through for **hunting purposes**, and it is possible to expect further introductions of this species through the same pathway, although data on relevant frequency and volumes of animals are not available. In France, for example, deliberate introductions into the wild of sika as a game species were carried out during the twentieth century (Pascal et al. 2006). Also in Denmark the species was introduced as a game species for hunting purposes (Asferg and Madsen 2007). The species was bred for game and used for sport hunting also in the European part of the former USSR (Bartoš 2009), as well as in other countries outside Europe, e.g. in the US (Feldhamer and Demarais 2008). For some of the introductions carried out in Europe the ultimate purpose of the releases is not explicitly reported, therefore it is not possible to establish with certainty whether there was any link with the hunting sector. For example, in Poland no information about cases of intentional introduction of species for hunting purposes was retrieved for at least several decades, if not for a century (Wojciech Solarz, pers. comm. 2021), however according to Solarz et al. (2018) there is some risk of introducing this species in new areas because

some hunters are interested in introducing sika deer in locations not suitable for the red deer (although so far no relocations from the two existing Polish populations took place). Moreover, in Europe, as reported by Bartoš (2009), various breeders intentionally crossed red and sika deer for trophy improvement, although this was rarely documented, which led to the risk of deer translocations being carried out with animals of unknown status. In his review Bartoš (2009) pointed out how “European hunters’ interests may be the main factor leading to ignoring the threat of sika deer presence”.

- b) **Deer farming** for sika has been growing consistently in Europe (with the notable exception of Denmark where there are some legal restrictions) and escapes from farms are reported in the risk assessment area (Bartoš 2009). For example, in Poland the species is kept in deer farms and agrotourism farms (the number of farm deer may exceed 1,000 individuals) where cases of escapes are known (Solarz et al. 2018). In Lithuania, about 1,000 sika deer are farmed in the Kaunas and Klaipeda districts (Bartoš 2009). Also in the Netherlands a possible pathway is escape or deliberate release from deer farms, which according to Lammertsma et al. (2012) was the origin of all of the present European populations. In Italy, deer farming is also present, and the escape of 3-4 sika deer from a farm was reported in Emilia Romagna in 1999 (Ferri et al. 2014). According to Bartoš (2009) there is an unknown number of farms keeping sika deer in continental Europe, however numbers and locations continually change over time, and only fragmentary information is available, hence it is difficult to get any reliable data and/or trend estimates. In Germany, according to Bartoš (2009), frequent escapes of sika deer from an enclosure near Neuhaus, Möhnesee, occurred. Conversely, in Belgium, deer farming is not considered as a major pathway for introduction in the country (Baiwy et al. 2013). In Denmark the species was frequently kept in deer farms (Asferg and Madsen 2007). In the UK, escape of sika as well as of sika-red hybrids from deer farms is considered a main pathway (GB Non-Native Species Secretariat 2011). In fact, escape from farms is a well-known risk also in regions other than the EU, e.g. in the US (Feldhamer and Demarais 2008) and in Asia, particularly in China, where sika are widely kept in captivity for farming of venison or antler velvet (Harris 2015, CABI 2021). Also, the translocation of sika from the Russian far east to Ukraine and other areas of the former Soviet Union, e.g. around 1933, was aimed at meeting the increasing demand for medical products from antlers (Bartoš 2009).
- c) Escapes of sika deer from **deer parks** are well documented in the risk assessment area (Bartoš 2009). For example, in France, an increasing number of small free-living sika deer populations have been reported during the last decades, mostly as a result of escapes from deer parks (Baiwy et al. 2013). In this country the reported origin of the sika deer in the Forêt de la Harth, is the Mulhouse Zoo and the Huttenheim enclosure near Strasbourg (Bartoš 2009). In Poland the species is kept in zoological gardens, as well as minizoo where cases of escapes are known (Solarz et al. 2018). According to Bartoš (2009), in Czech Republic, sika deer were frequently imported in the late nineteenth and early twentieth century for deer parks, from where they escaped (see also Dvořák and Palyzová 2016). Also, in Denmark, sika were introduced in deer parks and zoos from where they managed to escape and disperse over long distances, but their numbers have declined since the 1970s (Asferg and Madsen 2007, Bartoš 2009). In Germany, the largest sika population (in Möhnesee) apparently originated from Hagenbeck and from the Hellabrun Zoo (Munich), while the second largest population located at Hochrhein originated from Basel Zoo (Switzerland), along with St. Peter Game Park in Freiburg, and Hagenbeck (Hamburg) (Bartoš 2009). The most probable pathway of sika deer entry in Belgium, where observations of isolated specimens escaped from captivity have been reported in recent years, is the accidental/deliberated

releases of individuals from deer parks (Baiwy et al. 2013). Sika deer destined to hobby parks as ornamental species have been imported to Belgium several times from Ireland and Germany (Baiwy et al. 2013). Similarly, a possible pathway in the Netherlands is escape or deliberate release from enclosed parks, which was the origin of all of the present European populations (Lammertsma et al. 2012). In this country, sika are kept in several parks, and the few observed sika deer reported in the wild near ‘s Graveland are deemed likely to originate from captivity (Lammertsma et al. 2012). In Ireland, sika were introduced in deer parks, some of which are not enclosed (Carden et al. 2011). Also, in the UK, escape of sika as well as of sika-red hybrids from enclosed parks is considered a main pathway from the late 19th century onwards (GB Non-Native Species Secretariat 2011).

- d) The “Natural dispersal across borders of invasive alien species that have been introduced through pathways 1 to 5” is the fourth pathway still active in the EU, see details below.

Another pathway known for the sika is “Landscape / flora / fauna “improvement” in the wild (release in nature)”. This pathway is only known in regions other than Europe, for example in New Zealand, e.g. in relation to introductions carried out by acclimatisation societies which were active in the 19th century (Nugent and Speedy 2021, Banwell, 2009). Sika deer were also translocated from the Russian far east to Ukraine and other areas of the former Soviet Union to supplement sika deer populations that had declined during the second half of the nineteenth century (Bartoš 2009). Such translocations can be assigned to the pathway “Introduction for conservation purposes or wildlife management”. However, as no escapes are reported in Europe from the two pathways above, they are not considered active in the risk assessment area, and not considered further in this risk assessment.

a) Pathway name: Hunting (release in nature)

Qu. 1.2a. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional	CONFIDENCE	low
	unintentional		medium
			high

This pathway refers to animals introduced into the risk assessment area to be hunted for venison and/or to provide recreational hunting opportunities (including collection of hunting trophies).

Sika were widely introduced to many countries to hunting parks from which they have subsequently escaped or have been released. Only fragmentary information is available on numbers and locations where sika were introduced for hunting, hence it is difficult to get any reliable data and/or trend estimates (Bartoš 2009).

Both the introduction and the entry in the risk assessment area through this pathway is intentional (except in the case of misidentification, in which case the entry (and possibly the introduction as well) would be unintentional. See further details below.

Qu. 1.3a. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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As discussed under Qu. 1.1, there is documented evidence that the species was introduced into the EU through for hunting purposes, although it is unlikely that large numbers of animals (e.g. hundreds) are introduced for hunting within the course of one year. Depending on the objectives of the release, it would be expected, however, to be a number large enough to establish viable wild populations, considering that a very small number of hinds and a few stags are deemed sufficient to found a new population. In New Zealand, for example, it is documented that a herd descended from only 4-5 deer, of which one male only (Nugent and Speedy 2021). Also in Japan, a population introduced in a small island descended from one male and two females only (Kaji et al. 2009). Also in the EU, there is evidence of populations founded by few individuals, i.e. in Poland, where one of the two populations went through narrow bottlenecks down to 5 individuals, from which it recovered successively (Solarz 2011, and <https://www.iop.krakow.pl/gatunkiobce/default4e83.html?nazwa=opis&id=108&je=pl>).

The capacity to hybridise with red deer and produce offspring with intermediate morphological features carries the inherent risk of introducing sika hybrids mislabelled as red deer, as postulated for Italy (Ferri et al. 2014, Ferri et al. 2016).

Qu. 1.4a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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The species is able to survive during passage along the pathway, as demonstrated by the fact that it has been successfully introduced in several countries in the EU and that secondary translocations occurred too. Hence, it is very likely that the animals survive during transport and storage along this pathway, which has an interest in ensuring animal welfare standards,

although overall sika appear more prone to and suffer higher levels of mortality as result of stress from handling and transport than red and fallow deer (GB Non-Native Species Secretariat 2011). The species is unlikely to reproduce or increase during transport, but this might happen during extended periods of storage.

Qu. 1.5a. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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The likelihood of the sika deer to survive existing management practices in a released area will vary depending on the type of hunting regime applied to ungulate in general and the extent of disturbance in the area (e.g. agricultural practices). In principle it might be high, provided that the species requirements are duly considered and ensured. According to the GB Non-Native Species Secretariat (2011), sika “is very adaptable and has established and persisted under current agricultural and forestry practices and commercial exploitation by shooting”.

Qu. 1.6a. How likely is the organism to be introduced into the risk assessment area or enter into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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In principle, the intentional introduction for hunting purposes cannot go undetected (but see below about the risk of misidentification and/or mislabelling, particularly of hybrids).

This is a medium sized deer which may be easily detected by hunters (as well as naturalists, and farmers, provided that access in hunting estates is allowed) but only in areas where there are no other similar deer species or hybrids (e.g. sika are mistaken by some for fallow or red deer, see section A.2). In fact, as pointed out by the GB Non-Native Species Secretariat (2011), the co-occurrence of other deer species would allow the presence and spread of sika and sika hybrids to go undetected for a long time not only by landowners and the general public (which may not be fully familiar with deer species) but even by hunters, until demonstrated by DNA analysis (Bartoš 2009, Biedrzycka et al. 2012, Lammertsma et al. 2012).

The preliminary results of the surveys carried out in Italy show that the species is present in the country and was likely imported as a generic “deer” (hence was likely considered to be red deer *C. elaphus*) or simply the documentation of the animals imported for deer farming were not

checked. This led to the hypothesis that such deer could originate from animals introduced from Scotland (where hybrids are known to occur) which have escaped in the area where sika and their hybrids were now detected (Ferri et al. 2014, Ferri et al. 2016). It required a large awareness campaign among hunters to start detecting more sika individuals in the wild, and there is concern that the species may be more widespread than is currently known (Ferri et al. 2014, Ferri et al. 2016).

As a result, sika deer is moderately likely to be introduced in the risk assessment area undetected (although this is valid for authorised releases only, as any illegal introduction would very likely go undetected).

Qu. 1.7a. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated	CONFIDENCE	low
	widespread		medium
	ubiquitous		high

Possible points of introduction and subsequent entry are widespread. This is supported by the fact that the sika deer has already escaped (or been deliberately released) several times from various locations in the risk assessment area.

Qu. 1.8a. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely	CONFIDENCE	low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

The species was already introduced to the risk assessment area in the past along this pathway, although this was explicitly documented in only a limited number of occasions.

**b) Pathway name: Farmed animals (including animals left under limited control)
(Escape from confinement)**

Qu. 1.2b. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional	CONFIDENCE	low
	unintentional		medium
			high

This pathway refers to animals that have been introduced for farming into deer farms, where they were kept with the primary purpose to provide food (it does not include animals held in zoos, deer parks and the likes, which are treated below in chapters 1.2c to 1.7c).

Sika were widely introduced to many countries to deer farms from which they have subsequently escaped or have been released. However, the number of sika deer farms present in the EU is unknown, and no information is available about the numbers of sika deer kept in such facilities.

The introduction in the risk assessment area through this pathway is intentional. However, the entry into the environment is either intentional or unintentional, depending on whether it is the result of deliberate releases or accidental escapes.

Qu. 1.3b. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?
including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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As discussed under Qu. 1.1, there is documented evidence that the species was introduced in the EU through this pathway, and it is possible to expect further introductions of sika deer motivated by farming purposes, although quantitative data are lacking.

It is not very likely that large numbers of animals are introduced for farming within one year. Introduction is not expected to take place with large quantities (e.g. hundreds) of animals at one time. It would be expected, however, to be a number large enough to establish viable wild populations, considering that a very small number of hinds and a few stags are deemed sufficient to found a new population. This also leads to the conclusions that in the event of an escape or a deliberate release, a few individuals may fund a new population. In New Zealand, for example, it is documented that a herd descended from only 4-5 deer, of which one male only (Nugent and Speedy 2021). Also in Japan, a population introduced in a small island descended from

Qu. 1.4b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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The species is able to survive during passage along the pathway, as demonstrated by the fact that it has been frequently kept in captive facilities and from there successfully introduced in the past in several countries in the EU. Hence, it is very likely that the animals survive during transport and storage along the pathway, provided appropriate animal welfare standards are ensured (as reported by the GB Non-Native Species Secretariat (2011) overall sika appear more prone to and suffer higher levels of mortality as result of stress from handling and transport than is the case for red and fallow deer). Also, the likelihood of the sika deer to survive, reproduce, or increase in a fenced area is high, provided that the species requirements are duly considered and ensured. The species is unlikely to reproduce or increase during such transport.

Qu. 1.5b. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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The likelihood of the sika deer to survive existing management practices in a fenced area will vary depending on the type of deer management and extent of disturbance in the area. In principle it might be very likely, provided that the species requirements are duly considered and ensured. According to GB Non-Native Species Secretariat (2011), sika “is very adaptable and has established and persisted under current agri-forestry practices and commercial exploitation by shooting”.

See answer to Qu. 1.5a.

Qu. 1.6b. How likely is the organism to be introduced into the risk assessment area or enter into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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In principle, the intentional introduction for farming purposes cannot go undetected (but see below about the risk of misidentification and/or mislabelling, particularly of hybrids). However, things may be different for escaped animals.

See answer to Qu. 1.6a.

Qu. 1.7b. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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Possible points of introduction and subsequent entry are widespread. This is supported by the fact that the sika deer has already escaped (or been deliberately released) several times from various locations as documented in the risk assessment area (see also Qu 1.3b).

Qu. 1.8b. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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The species has already entered the wild through this pathway (as escapee from a deer farm), and the risk for such events to happen again is very high as long as animals are kept in such facilities. Despite the lack of precise information about the distribution of deer farms in Europe, the quality of the deer farm facilities, the numbers of animals they hold and their security appliances and contingency plans (if available), sika deer are deemed to be abundant in deer farms and hence likelihood of further introductions or transport of animals between existing facilities (from outside the EU into the risk assessment area) and/or entry into the environment is very likely.

**c) Pathway name: Botanical garden/zoo/aquaria (excluding domestic aquaria)
(Escape from confinement)**

Qu. 1.2c. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Sika deer are known to be kept in deer parks, zoological gardens, minizoo's, hobby zoo's, wildlife parks and similar captive facilities for ornamental reasons.

The introduction in the risk assessment area through this pathway is intentional. However, the entry into the environment is either intentional or unintentional, depending on whether it is the result of deliberate releases or accidental escapes.

Qu. 1.3c. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?
including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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According to Bartoš (2009) there is an unknown number of deer parks and zoological gardens containing sika deer in continental Europe. Numbers and locations continually change over time, and only fragmentary information is available, hence it is difficult to get any reliable data and/or trend estimates.

As discussed under Qu. 1.1, there is documented evidence that sika deer was introduced into the EU for deer farming, and it is possible to expect further introductions of this species for the purposes of keeping animals in deer parks and zoological facilities. It is considered unlikely that large numbers of animals escape from zoos or deer parks within the same year yet few founders are needed to establish new populations in the environment. It would be expected, however, to be a number large enough to establish viable wild populations, considering that a very small number of hinds and a few stags are deemed sufficient to found a new population (see also Qu. 1.3b).

Qu. 1.4c. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely	CONFIDENCE	Low medium
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	moderately likely likely very likely		high
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The species is able to survive during passage along the pathway, as demonstrated by the fact that it has been frequently kept in captive facilities and from there successfully introduced in the past in several countries in the EU. Hence, it is very likely that the animals survive during transport and storage along the pathway, provided appropriate animal welfare standards are ensured (as reported by the GB Non-Native Species Secretariat (2011) overall sika appear more prone to and suffer higher levels of mortality as result of stress from man-handling and transport than is the case for red and fallow deer). Also, the likelihood of the sika deer to survive, reproduce, or increase in a fenced area is high, provided that the species requirements are duly considered and ensured. The species is unlikely to reproduce or increase during such transport, but this can definitely happen once released in the wild.

See Qu 1.4b.

Qu. 1.5c. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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The likelihood of the sika deer to survive existing management practices in a fenced area will vary depending on the type of deer management and extent of disturbance in the area. In principle it might be high, provided that the species requirements are duly considered and ensured. According to GB Non-Native Species Secretariat (2011), sika “is very adaptable and has established and persisted under current agri-forestry practices and commercial exploitation by shooting”.

See Qu 1.5b.

Qu. 1.6c. How likely is the organism to be introduced into the risk assessment area or enter into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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In principle, the intentional introduction in deer parks, zoological gardens, and similar captive facilities cannot go undetected (but see below about the risk of misidentification and/or mislabelling, particularly of hybrids). However, things may be different for escaped animals.

See Qu. 1.6a.

Qu. 1.7c. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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Possible points of introduction and subsequent entry are widespread. This is supported by the fact that the sika deer has already escaped (or been deliberately released) several times from various locations as documented in the risk assessment area.

Qu. 1.8c. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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The species has already entered the wild through this pathway (as escapee from a zoo or deer park), and the risk for such events to happen again is high as long as animals are kept in such facilities. Despite the lack of precise information about the distribution of zoos and deer parks in Europe where the species is held and their biosecurity, sika deer are known to be abundant in zoos and deer parks and hence likelihood of further introductions or transport of animals between existing facilities (from outside the EU into the risk assessment area) is likely.

d) Pathway name: Natural dispersal across borders of invasive alien species that have been introduced through pathways 1 to 5.

Qu. 1.2d. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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This pathway refers to animals that spread to new regions by natural dispersal, without action or assistance by humans, from regions in which they are alien and were introduced by one of the other introduction pathways.

This pathway is unintentional, as it depends on the dispersal capacities of the species. It is facilitated by the habitat conditions present in the area (including, for instance, the forest management regime and the recreational hunting practices, the extent of suitable ecological corridors etc.).

Qu. 1.3d. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?
including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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According to Solarz et al (2018) the dispersal capacity of the species would allow the immigration of individuals from outside the risk assessment area, e.g. from Kaliningrad Oblast (Russia), to Poland. The same authors report that records from northern Poland suggest that there could have been immigration in the past, when single individuals could have dispersed from the Kaliningrad Oblast to the area of the Kadyń population. Also the occurrence of the species in Estonia may be due to animals dispersing from Russia, for example there is evidence of single individuals dispersed to Alutaguse from the population in neighboring areas of Russia (Bartoš 2009).

Further introductions of this species due to natural dispersal from areas outside the risk assessment area, particularly from Russia, are likely to occur. It is considered possible that large numbers of animals can disperse naturally across countries within one year.

Qu. 1.4d. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely	CONFIDENCE	low medium
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	moderately likely likely very likely		high
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The likelihood of the animals to survive, reproduce, or increase during natural dispersal will vary on the extent of deer management and disturbance in the area (for examples in relation to land use practices, hunting, and other pressures). According to GB Non-Native Species Secretariat (2011), sika “is very adaptable and has established and persisted under current agri-forestry practices and commercial exploitation by shooting”.

Qu. 1.5d. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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See Qu. 1.4d.

Qu. 1.6d. How likely is the organism to be introduced into the risk assessment area or enter into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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The unintentional introduction due to natural dispersal may go undetected, at least in early stage of colonisation, until it gets the attention of hunters, naturalists, and farmers (but also in this case only in areas where there are no other similar deer species or hybrids, because sika can be mistaken for fallow or red deer, see section A.2). In fact, as pointed out by the GB Non-Native Species Secretariat (2011), the co-occurrence of other deer species would allow the presence and spread of sika and sika hybrids to go undetected for a long time not only by landowners and the general public not fully familiar with deer species, but even by hunters until demonstrated by DNA analysis (Bartoš 2009, Biedrzycka et al. 2012, Lammertsma et al. 2012).

See answer to Qu. 1.6a.

Qu. 1.7d. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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Possible points of entry are possibly isolated, i.e. mostly localised in the European Russia, i.e. in the Kaliningrad District of Russian Federation (Biedrzycka et al. 2012), Switzerland, Ukraine, and possibly Moldova (Bartoš 2009, CABI 2021).

Qu. 1.8d. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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The species was already introduced into the risk assessment area in the past along this pathway (see Qu. 1.3d and the case of single individuals deemed to immigrate into the Polish population from Russia), and the risk for such events to happen again is high as long as animals are present in the wild in EU neighbouring countries. Hence likelihood of natural spread of animals from outside the EU into the risk assessment area is likely.

Qu. 1.9. Estimate the overall likelihood of introduction into the risk assessment area or entry into the environment based on all pathways and specify if different in relevant biogeographical regions in current conditions.
Provide a thorough assessment of the risk of introduction in relevant biogeographical regions in current conditions: providing insight in to the risk of introduction into the risk assessment area.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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The most likely pathway of sika deer entry into the wild within the EU, as documented in the risk assessment area already, is the escape of individuals from deer parks and deer farms and, less likely, the deliberate release for hunting. Natural dispersal across EU-borders is another important pathway.

This seems to contradict CABI (2021), according to which further introductions seem unlikely because most countries/states now do not allow deliberate introductions or releases of exotic ungulates. CABI (2021) also argued that there is now a greater awareness of risks associated with such introductions through damage to agriculture and forestry, competition with native wildlife species and the risk of hybridization with red deer. However, as documented above, as long as sika populations exist in the wild in neighbouring regions, along with deer parks and deer farms within EU Member States and neighboring countries, the risk of further escapes/releases or dispersal cannot be ruled out. Therefore the overall risk is deemed very likely.

Qu. 1.10. Estimate the overall likelihood of introduction into the risk assessment area or entry into the environment based on all pathways in foreseeable climate change conditions?

Thorough assessment of the risk of introduction in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of introduction (e.g. change in trade or user preferences)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely introduction within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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There is no evidence that climate change will have any effect on the likelihood of introduction or entry into the environment via hunting, farming or keeping animals in zoological facilities. The degree to which natural dispersal from regions outside the risk assessment area could be affected by climate change, is unknown.

2 PROBABILITY OF ESTABLISHMENT

Important instructions:

- For organisms which are already established in parts of the risk assessment area or have previously been eradicated, the likelihood of establishment should be scored as “very likely” by default.
- Discuss the risk also for those parts of the risk assessment area, where the species is not yet established.

Qu. 2.1. How likely is it that the organism will be able to establish in the risk assessment area based on similarity of climatic and abiotic conditions in its distribution elsewhere in the world?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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The species is already established in the risk assessment area, where the environmental conditions are in general similar to those of the native and alien range of the species. Sika are well adapted across a wide range of regions characterized by a diversity of climatic and abiotic conditions (see descriptions in A.4) which are likely to favour the establishment of the species in the risk assessment area.

For example, according to GB Non-Native Species Secretariat (2011), sika have adapted well to the mild climatic conditions of southern Britain and the more extreme and cooler conditions in northern Scotland. Such considerations apply to many other European countries as well.

In Belgium, according to Baiwy et al. (2013) the sika deer is likely to establish in most parts of the country on the basis of climatic conditions.

Qu. 2.2. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area? Consider if the organism specifically requires another species to complete its life cycle.

RESPONSE	very isolated isolated moderately widespread widespread ubiquitous	CONFIDENCE	low medium high
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Sika deer seem characterised by a high degree of flexibility and are well adapted to a wide variety of natural and semi-natural habitats and food, according to availability (see descriptions in A.4). Habitats and food necessary for its survival, development and reproduction are widespread throughout the risk assessment area, as demonstrated by the successful establishment of the species in several locations in the risk assessment area.

As noted by Lammertsma et al. (2012) for the Netherlands, it is plausible that sika and sika-hybrids can occupy the same habitats as red deer, which is a widespread species in the risk assessment area.

No other organism is required for sika deer to complete its life cycle.

Qu. 2.3. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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There is potential for competition with the native red deer and roe deer (*Capreolus capreolus*), as well as other ungulates in the risk assessment area, but as noted for other introduced deer species such competition is unlikely to prevent establishment (sika may in fact outcompete native ungulates). See Qu. 4.2 for details.

As pointed out by Baiwy et al. (2013) invasion histories of sika deer in habitats already occupied by other ungulate species in the UK, Germany and North America show that competition is unlikely to prevent its establishment or even slow down population expansion. Similarly, Lammertsma et al. (2012) noted that sika deer are sympatric with other deer species in Europe, but competition with these species did not prevent establishment in Germany and the Czech Republic, as well as the UK (GB Non-Native Species Secretariat 2011).

Qu. 2.4. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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The risk assessment area is certainly characterised by the presence of potential predators, parasites or pathogens of sika deer - the same that would be considered for other ungulates, like the wolf (*Canis lupus*), which is a main predator (Wilson and Mittermeier 2011), but also lynx (*Lynx lynx*), red fox (*Vulpes vulpes*) and raptors. However, there are several species of native and alien deer already occurring in the risk assessment area, and predators do not seem to represent a limiting factor for their populations (Feldhamer 1980). In any case, such predators are not widespread in large parts of Europe. For Belgium, the only predator mentioned in the national risk assessment for sika (Baiwy et al. 2013) is the red fox, which may play a role in limiting population growth rate through predation on fawns. This risk assessment however predated the arrival and establishment of the wolf in Belgium. Similarly, as noted by the GB Non-Native Species Secretariat (2011) the only abundant natural enemy in the UK is the red fox,

which may predate on neonates, and potentially some raptors (e.g. the golden eagle *Aquila chrysaetos*) which could also take sika fawns, but have been rarely reported to do so.

In Europe, sika deer can be infected by numerous endemic wildlife diseases and parasites (see Baiwy et al. 2013, Böhm et al. 2007) but they do not seem to suffer very much from them and usually show a high resistance to infection (although several of those diseases often represent lethal infections among farmed deer).

Qu. 2.5. How likely is the organism to establish despite existing management practices in the risk assessment area? Explain if existing management practices could facilitate establishment.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Deer in Europe are usually subject to hunting and culling, which are regulated by law (see Bartoš 2009, Apollonio et al. 2010). However, the management of the sika or its commercial exploitation by shooting did not seem to prevent its establishment (which in any case would not be the goal of this kind of management at all).

On the other hand, the species persisted under current agricultural and forestry practices, e.g. in the UK, where the GB Non-Native Species Secretariat (2011) also noted that “Continued increases in afforestation in general would produce further suitable habitat, aiding the spread and establishment of sika, whereas current policies to establish fewer conifer thickets (one of their preferred habitats) in preference for more deciduous planting may possibly negate this to some extent”.

Poaching and overhunting are also factors which may lead to the extinction of introduced populations, but this did not seem to represent a limiting factor for their populations in the risk assessment area. It can be expected that many populations introduced for hunting are subject to sustainable harvesting as for red deer.

Qu. 2.6. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Sika deer are medium sized animals and there are effective eradication methods available. However, one particular concern associated with the effective eradication of sika deer is hybridisation with native red deer. The presence of hybrids complicates the practicalities of eradication programmes, as it would require the selective culling of animals with hybrid characteristics which are not always easily identifiable in the field. As pointed out by the GB Non-Native Species Secretariat (2011) regarding the situation in the UK. However, it should

be noted that several sika populations are likely to be represented by sika-hybrid, which would entail the need to remove also those red deer population showing a certain level of introgression, which may be considered unlikely given the many caveats and constraints of a similar operation.

Qu. 2.7. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?

including the following elements:

- a list and description of the reproduction mechanisms of the species in relation to the environmental conditions in the risk assessment area
- an indication of the propagule pressure of the species (e.g. number of gametes, seeds, eggs or propagules, number of reproductive cycles per year) of each of those reproduction mechanisms in relation to the environmental conditions in the risk assessment area.
- If relevant, comment on the likelihood of establishment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in establishment whereas for others high propagule pressure (many thousands of individuals) may not.
- If relevant, comment on the adaptability of the organism to facilitate its establishment and if low genetic diversity in the founder population would have an influence on establishment.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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The sika deer presents a suite of life-history traits typical of successful invasive deer species such as a large native range with high local abundance, a gregarious behaviour, a wide habitat and diet breadth, a long life span, an early sexual maturity (Baiwy et al. 2013). Moreover sika may be able to overcome severe environmental conditions sometimes better than the local species (Bartoš 2009). The resilience of sika towards changes in its environment is also considered greater than for red deer (Ferretti and Lovari 2014). The diverse dietary requirements and the ecological flexibility which characterise the sika deer, along with the species’ adaptability may facilitate the establishment of the species. Low numbers of founding individuals were not associated with a significant reduction in genetic diversity (founder effect) as shown in a study in Germany and Austria (Pitra and Lutz 2005). Another trait that is deemed to be correlated with greater establishment success is the interspecific variation in adult body size (overall or within sex): according to González-Suárez et al (2015) sika deer was predicted to establish a population in about 88% of introductions of 10 individuals (observed success was 82%, nine out of 11 attempts, with a median of seven released individuals)

According to Harris (2015) the species is crepuscular (i.e. active primarily during the twilight period), but sometimes active by day and night, and forages solitarily or in small herds, with dominant males with harems during the period of rut. Potentially sika reach reproductive maturity at one year and can live up to 15-16 years, and may reach 25 years in captivity (Wilson and Mittermeier 2011). However, the mean life span reported in a study in Japan is 2.1–3.1 years for males and 3.6–3.9 years for females (Kaji et al. 2010). In Europe, sika deer breed in September-November (exceptionally until mid-February), hence the rutting season may overlap

with that of red deer, with which they hybridise (Bartoš 2009, Baiwy et al. 2013, Biedrzycka et al. 2012, Harris 2015, CABI 2021). Sika are polygamous and the breeding group of a successful territorial male may number as many as 12 females (Feldhamer 1980). Mating strategy within sika may be much more flexible (Endo 2009), with stags adopting a number of different strategies depending on circumstance, and hinds reaching puberty at 16-18 months, and thereafter breeding each year (Wilson and Mittermeier 2011, CABI 2021). Sika usually give birth between April and June to a single fawn, occasionally twins, after a 30 weeks gestation period (Feldhamer 1980). The reproductive rate is high, with a conception rate of 80-90% and an adult pregnancy rate of 85-100% (hence higher than for red deer). No signs of density-dependent reduction in fecundity among sika were reported, not even at population densities up to 35 animals per km², but juvenile mortality can be high, with about 50% of fawns surviving their first winter in Ireland and Japan (Feldhamer 1980, CABI 2021). Apparently, as discussed in the sections above (see for example point 1.3a), it seems that a very small number of hinds and a few stags are sufficient to establish a new population. In New Zealand it is documented that a herd descended from only 4-5 deer, with one male only (Nugent and Speedy 2021). Also, in Japan, a population introduced to a small island descended from one male and two females only (Kaji et al. 2009). Increase in population size can be very high following introduction or colonization of new areas and density can exceed 50-100 individuals per km². An irruptive population in Japan increased from 54 animals in 1986 to 592 animals in 1998, then after a decline to 177 animals in 1999, it reached a second peak of 626 animals in 2003, decreased to 341 deer in 2004, and eventually reached a third peak of 603 in 2005 (Kaji et al. 2004, Kaji et al. 2009). As summarized by CABI (2021) sika are less social than other deer species, living in small groups or solitarily (sexes are strongly segregated) with group size depending on the type of habitat (Wilson and Mittermeier 2011).

Qu. 2.8. If the organism does not establish, then how likely is it that casual populations will continue to occur?

Consider, for example, a species which cannot reproduce in the risk assessment area, because of unsuitable climatic conditions or host plants, but is present because of recurring introduction, entry and release events. This may also apply for long-living organisms.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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It is likely that high numbers of individuals are still kept and bred in captivity in the risk assessment area, which leads to a constant risk of some being intentionally released or accidentally escaping in the wild, building up casual occurrences.

The risk that casual population will occur seems likely, but no sufficient data are available to support any statement on this regard.

Qu. 2.9. Estimate the overall likelihood of establishment in the risk assessment area under current climatic conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under current climatic conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in current conditions: providing insight in the risk of establishment in (new areas in) the risk assessment area.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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The species is already established in the risk assessment area and it is very likely that this will continue in current climatic conditions.

Qu. 2.10. Estimate the overall likelihood of establishment in the risk assessment area under foreseeable climate change conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under foreseeable climate change conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of establishment (e.g. increase in average winter temperature, increase in drought periods)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely establishment within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Under foreseeable climate change conditions, the projected suitability for sika establishment is relatively stable in both the Atlantic and Continental biogeographic regions, while it is deemed to increase in the Alpine and Boreal biogeographic regions. Conversely, in the Mediterranean biogeographic region the suitability is expected to decrease in future climate change (see Annex VIII for details).

In contrast to the results of the SDM in Annex VIII, Polaina et al. (2021) predict suitability only in Western Europe (Ireland, UK, France, Belgium, Netherlands, Germany, Denmark), and a decline (contraction to the north-west) with climate change. While the modelling approach of the study in general seems valid, it may suffer from three flaws for this species: 1) The model uses 18 predictors, which is a large number, especially given the low number of presence points used (368). 2) The predictors are the same as for all the other species studied (mammals, birds, a reptile and an amphibian), suggesting little species-specific selection of predictors. The two most important predictors in the model (temperature seasonality and precipitation seasonality) would not a priori be considered particularly important for the species, while others that should be considered important, such as snow cover, are missing. 3) While the definition of the area for drawing pseudo-absences is based on a global climate model for the species, the projection of suitability is based on presences only in some European countries, and excludes presences in Ukraine and Russia as well as occurrences in the native range and North America. Taken together, these three issues seem to result in far too specific a fit of the model to the Western European presence points of the species, and poor ability to extrapolate to other areas and climates. In a large meta-analysis of invasive alien species distribution models, Liu et al. (2020) make exactly these points – models are most likely to have poor transferability between regions and climates if they are based on few presence points, many predictors, and little species-specific expert input.

As described by Ward (2005), sika have expanded their range in the UK at an annual rate of 5.3% in recent years and are predicted to be capable of spreading throughout the majority of Great Britain (Acevedo et al. 2010). Each of the few significant population declines throughout the 100-year history of the species presence in Poland was contributed by severe winter conditions, particularly by the deep snow cover (Solarz & Okarma 2014). The species favours warm climates, naturally ranging throughout the subtropics of Japan and China. Warmer, wetter conditions and milder winters in the risk assessment area, as predicted by climate change models, are therefore likely to favour the establishment of sika in Europe.

3 PROBABILITY OF SPREAD

Important instructions:

- Spread is defined as the expansion of the geographical distribution of an alien species within the risk assessment area.
- Repeated releases at separate locations do not represent continuous spread and should be considered in the probability of introduction and entry section (Qu. 1.7).

Qu. 3.1. How important is the expected spread of this organism within the risk assessment area by natural means? (List and comment on each of the mechanisms for natural spread.)

including the following elements:

- a list and description of the natural spread mechanisms of the species in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of spread discussed in relation to the species biology and the environmental conditions in the risk assessment area.

The description of spread patterns here refers to the CBD pathway category “Unaided (Natural Spread)”. It should include elements of the species life history and behavioural traits able to explain its ability to spread, including: reproduction or growth strategy, dispersal capacity, longevity, dietary requirements, environmental and climatic requirements, specialist or generalist characteristics.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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The potential of sika deer to spread within the risk assessment area by natural means is likely to be major. Sika have the capacity to migrate long distances, especially in mountainous areas, although characterized as sedentary (Bartoš 2009, Wilson and Mittermeier 2011). Overall, patterns of dispersal and range expansion seem to be depending on local population densities, sex and age of animals (young males are likely to disperse longer distances), presence of barriers such as railways and canals, types of habitats and water availability, presence of woodland corridors, extent of deer management and disturbance (Ward 2005, GB Non-Native Species Secretariat 2011, CABI 2021, Harris 2015). Although the information on dispersal patterns is often anecdotal, showing the need to fill in a number of gaps in knowledge through dedicated research (CABI 2021), there are several data which may help understand the spread potentialities of the species, as described below.

In New Zealand sika deer dispersed rapidly at a speed reaching 1.6 km per year (Davidson 1973, Nugent et al. 2001), while in the UK the range expansion was estimated at 3-5 km per year (Ward 2005). In Poland for some reason, both populations have been remaining virtually within the same small areas since their introduction in 1910 (Wojciech Solarz, pers. comm. 2021) However, travel distances up to 80-160 km/year are also reported, e.g. in Poland (Bartoš, 2009), although this is an estimate of possible migration of single individuals that appeared in areas where there were no sika, and whose appearance could as well be a result of an escape from some local farm (Wojciech Solarz, pers. comm. 2021). This is in line with data of seasonal (altitudinal) migration distances reported in Japan, where sika are known to cover mean distances of 35 km between winter and summer ranges (Wilson and Mittermeier 2011). Also,

a study on female sika deer in their native range in Japan, showed that the distance of their seasonal movements ranged between 7.2 and 101.7 km (Igota et al. 2004). Sika deer are excellent swimmers and can travel 12 km by swimming in the sea (Feldhamer 1980).

In Ireland, between 1978-2008 the range increased by 353%, with an annual rate of 5%, while range expansion in the UK varied from 5.3% to 7.3% yearly, depending on the studies, mostly as a consequence of both slow natural expansion and accidental escapes from parks and farms (Ward 2005, Pérez-Espona et al. 2009; Carden et al. 2011). Also in the Czech Republic, in the period 2003 – 2010, sika population occupied on average 55 thousand ha of new territories every year (Dvořák and Palyzová 2016).

It is worth to consider that in the UK, where natural spread is considered a main pathway, the establishment of new populations may be supported not only by the recruitment of further animals through the concurrent releases from enclosures, but also by the possibility of dispersing animals hybridising with red deer, if failing to encounter members of their own species (GB Non-Native Species Secretariat 2011). This may increase the risk of the animals to pass unnoticed, and the population of sika-hybrids to further spread. However, there are populations in the UK which showed a very slow expansion of their range, probably because of the presence of barriers, such as roads, railways and urban settlements (GB Non-Native Species Secretariat 2011, Carden et al. 2011). In Poland, although sika deer have been established for over 100 years, there was no significant expansion of their range, maybe due to limiting factors such as low temperatures in winter and the depth of snow cover (Solarz et al. 2018, Kopij 2017). No natural spread was reported in Austria and Denmark either (Baiwy et al. 2013, Asferg and Madsen 2007).

Qu. 3.2a. List and describe relevant pathways of spread other than "unaided". For each pathway answer questions 3.3 to 3.9 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 3.3a, 3.4a, etc. and then 3.3b, 3.4b etc. for the next pathway.

including the following elements:

- a list and description of pathways of spread with an indication of their importance and associated risks (e.g. the likelihood of spread in the risk assessment area, based on these pathways; likelihood of survival, or reproduction, or increase during transport and storage; ability and likelihood of transfer from the pathway to a suitable habitat or host) in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of spread for each pathway discussed in relation to the species biology and the environmental conditions in the risk assessment area.
- All relevant pathways of spread (except "Unaided (Natural Spread)", which is assessed in Qu. 3.1) should be considered. The classification of pathways developed by the Convention of Biological Diversity shall be used (see Annex IV).

No specific pathways other than the natural spread was identified, other than those already discussed under Qu. 1.1.

Qu. 3.3a. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: N/A

Qu. 3.4a. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: N/A

Qu. 3.5a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: N/A

Qu. 3.6a. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely	CONFIDENCE	low medium
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	moderately likely likely very likely		high
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Response: N/A

Qu. 3.7a. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: N/A

Qu. 3.8a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: N/A

Qu. 3.9a. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: N/A

Qu. 3.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?

RESPONSE	very easy easy with some difficulty difficult very difficult	CONFIDENCE	low medium high
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Effective containment measures to prevent the spread of sika deer through the pathway above are the same as those to control/eradicate the species (see for example discussion on **Qu. 2.6.**), hence their applicability is context dependent, and depends on the size of the population and the invasion stage.

Like for many other invasive alien species of vertebrates, eradication is likely achievable only in parts of the risk assessment area where recent introductions have occurred or sub-populations remain quite localized, and in any case nationwide containment is likely to be prohibitively costly, as pointed out by the GB Non-Native Species Secretariat (2011) regarding the situation in the UK. However, it should be noted that several sika populations are likely to be represented by sika-hybrid, which would entail the need to remove also those red deer population showing a certain level of introgression, which may be considered unlikely given the many caveats and constraints of a similar operation.

In the UK according to the GB Non-Native Species Secretariat (2011) *“the past level of culling has mostly failed to prevent range expansion in many areas, in large part likely due to lack of coordination of culling across sub-population ranges, with some landowners carrying out little or no control of numbers. However, in some areas such as the New Forest, where Sika have been established for many years, regular culling has succeeded in containing numbers, with limited range expansion observed over the past 30 years”*.

In Ireland there were several outlying records which may be a result of illegal translocations (Carden et al. 2011, Murphy et al. 2013), but the purpose of such introductions is not reported. It is not always easy to deal with illegal movement of species, as it requires the enforcement of a sound legislation and the implementation of effective communication campaigns.

Qu. 3.11. Estimate the overall potential rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (indicate any key issues and provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in current conditions, providing insight in the risk of spread into (new areas in) the risk assessment area.

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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There is documented evidence that the species spread in some EU countries by natural means, and it is possible to expect this to happen also in the future. For example, a possible pathway in the Netherlands is dispersal from seven established populations in Germany, especially from the Möhnesee area, which lies approximately 80 km from the Dutch border although this is considered not likely to occur given the current hunting management of the species in Germany (Lammertsma et al. 2012). On the other hand, sika were recorded to migrate 80-160 km in Poland (although there is no certainty, as discussed in Qu. 3.1), and were often seen roaming together with red deer (Bartoš 2009). Also in Luxembourg the presence of the species in the area was explained with the escape of sika deer from an enclosure in Gemünd, Germany, near the Luxembourg border (Cellina and Schley 2014). Similarly, a possible pathway of sika deer entry in Belgium is the natural spread of individuals from neighboring countries (Baiwy et al.

2013). It is worth to point out that also the sika population in Switzerland originated from animals dispersed from the population in Germany (Bartoš 2009).

Therefore, in the light of the evidence of the species spread by natural means within the EU, and the data discussed in Qu. 3.1, the rate of spread is considered moderate, as the species may suddenly appear in any Member State with sika populations (or their hybrids) already occurring in neighbouring countries.

Qu. 3.12. Estimate the overall potential rate of spread in relevant biogeographical regions in foreseeable climate change conditions (provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if rates of spread are likely slowed down or accelerated.

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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The rate of spread is expected to be the same whether in current conditions or future climate change conditions, because there are no specific elements of the species biology (i.e. in relation to dispersal patterns) which seem affected by the climate, except for the area which may be suitable, which may increase as shown in Annex VIII (i.e. in Northern Europe, in relation to a potential decrease of snow cover).

4 MAGNITUDE OF IMPACT

Important instructions:

- Questions 4.1-4.5 relate to biodiversity and ecosystem impacts, 4.6-4.8 to impacts on ecosystem services, 4.9-4.13 to economic impact, 4.14-4.15 to social and human health impact, and 4.16-4.18 to other impacts. These impacts can be interlinked, for example, a disease may cause impacts on biodiversity and/or ecosystem functioning that leads to impacts on ecosystem services and finally economic impacts. In such cases the assessor should try to note the different impacts where most appropriate, cross-referencing between questions when needed.
- Each set of questions starts with the impact elsewhere in the world, then considers impacts in the risk assessment area (=EU excluding outermost regions) separating known impacts to date (i.e. past and current impacts) from potential future impacts (including foreseeable climate change).
- Only negative impacts are considered in this section (socio-economic benefits are considered in Qu. A.7)
- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable).

Biodiversity and ecosystem impacts

Qu. 4.1. How important is the impact of the organism on biodiversity at all levels of organisation caused by the organism in its non-native range excluding the risk assessment area?

including the following elements:

- Biodiversity means the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems
- impacted chemical, physical or structural characteristics and functioning of ecosystems

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Sika is known to pose a threat to native species through competition with other deer species and through hybridisation with native red deer. They can also have an impact on vegetation and habitats, which in turn can affect ecosystem services, as well as on health, crops and the economy.

According to Bartoš (2009) sika appear to be successful competitors with local deer species due to anatomical and behavioral features. This is particularly the case with the red deer, e.g. in Russia and New Zealand, or white-tailed deer (*Odocoileus virginianus*) in North America (Feldhamer and Demarais 2008, CABI 2021, Lever 1994).

In New Zealand, competition between sika deer and red deer is reported. In areas where the vegetation had been heavily browsed by red deer, sika replaced them demonstrating greater adaptability and further degrading the plant communities (Lever 1994). In the United States, sika are displacing white-tailed deer because of their wider range of diet (Feldhamer and Demarais 2008, Lever 1994). In this country, the occurrence of parasites in sika is also reported, although no specific information on transmission is provided (Mason 1994). However, this shows the potential for the spread of pathogens to native wildlife.

There are studies carried out in the species native range, which are reported here to provide examples on additional possible impact of the species in its introduced range. In Japan, particularly in places where sika population density is high, the species demonstrates strong negative effects on vegetation in both agricultural and forested habitats, including on overall regeneration in many natural forests and conifer plantation (Takatsuki 2009a, Takatsuki and Ito 2009, Akashi et al. 2011). Deer grazing and browsing may cause significant changes in vegetation structure and species composition as well as significant soil erosion and changes in water flow (Diaz et al. 2005, Takatsuki 2009a, Lammertsma et al. 2012) which overall are deemed to exceed the impacts of comparable deer species (Baiwy et al. 2013). A recent study on dung beetles (Scarabaeinae) carried out in Japan, showed a larval resource shift from carnivore feces to deer feces associated with an increase in deer density (despite a number of caveats), which may affect ecosystem functions such as soil nutrient cycling and seed dispersal (Yama et al. 2019). By modifying the composition and physical structure of habitats deer may exert cascading effects on other animals (Reimoser and Putman 2011). For example, in a site in Japan, it was demonstrated that foraging opportunities and population densities of raccoon dogs (*Nyctereutes procyonoides*) and Japanese badgers (*Meles anakuma*) increased in response to the increased abundance of earthworms and insects caused by the habitat modification of high deer density (Seki and Koganezawa 2013, Seki et al. 2014). Other indirect effects of sika deer, although not well studied, seem to be the reduction of understory bamboo cover, which in turn improved the survival of tree seedlings and caused the decline of wood mice *Apodemus* spp. (Takatsuki 2009a). In Japan, high population densities of sika led to irreversible changes on the vegetation, with some plants locally decreasing to near extinction (Kaji et al. 2009, Baiwy et al. 2013). For example, a study carried out in a subalpine area at Mt. Kita showed that the impact of sika deer was more severe in the *Betula* forests than in grasslands, where more plant species are available for browsing. Specifically, browsing on less frequently occurring plants (including red listed species) led to deterioration of the vegetation, the disappearance of common species and consequently the homogenization of vegetation (Nagaite 2012).

Qu. 4.2. How important is the current known impact of the organism on biodiversity at all levels of organisation (e.g. decline in native species, changes in native species communities, hybridisation) in the risk assessment area (include any past impact in your response)?
 Discuss impacts that are currently occurring or are likely occurring or have occurred in the past in the risk assessment area. Where there is no direct evidence of impact in the risk assessment area (for example no studies have been conducted), evidence from outside of the risk assessment area can be used to infer impacts within the risk assessment area.

RESPONSE	minimal	CONFIDENCE	low
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	minor moderate major massive		medium high
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Competition with native ungulates

Sika deer competes with native ungulate species like roe deer, red deer, and wild boar, for both food and space (but also through mate competition with red deer), apparently reducing the fitness of other species (for example by outcompeting roe deer or disturbing red deer rutting) because of their ecological flexibility, as well as their anatomical and behavioural features (Bartoš 2009, Lammertsma et al. 2012, Macháček et al. 2014). However, there are very few definitive studies of the effects of sika on other deer species (Ferretti and Lovari 2014).

As reported by Solarz et al. (2018) sika deer have a strong effect on herbaceous vegetation, thus limiting the feeding base of European bison (*Bison bonasus*), although the lack of dedicated research makes it difficult to clearly state to what extent the food niche of these two species would overlap.

In general, outcomes of competition dynamics between sika and other ungulates cannot be predicted and differs between areas (Bartoš 2009). The lack of evidence in the scientific literature does not allow to demonstrate unequivocally the effect of competition between sika and other deer, also because most deer populations are under a management regime (CABI 2021, Ferretti and Lovari 2014, Solarz et al. 2018). Anecdotal observations, correlative studies and spatial segregation suggest that native deer species as well as other introduced ungulates (e.g. fallow deer) may decline in areas invaded by sika deer due to competition (Baiwy et al. 2013). On the other hand, there are studies which lead to the conclusion that sika are weak competitors and are not expected to displace native species (e.g. Acevedo et al. 2010 for the UK), but recognize that additional risks may occur, such as spread of diseases and genetic introgression). Overall, direct competition for resources between red deer and sika is not well documented in UK (GB Non-Native Species Secretariat 2011), despite the documented overlap of diet (Ferretti and Lovari 2014). Similarly, some overlap of habitat use was noted between roe deer and sika, but this did not seem to have any direct competitive effects in terms of population dynamics (Ferretti and Lovari 2014).

Hybridisation with red deer

A peculiar type of interspecific interaction is mate competition of sika deer with red deer. In particular, red deer stags (especially young) may be attacked by sika males, which are extremely aggressive in the rutting season and may mate with red deer hinds, with the inherent risk of hybridization and introgression between the two species (Zachos and Hartl 2011, Baiwy et al. 2013, Pérez-Espona et al. 2009). Because red deer harems are dispersed over large areas, even a low number of successful sika stags may trigger the hybridization process by simultaneously siring multiple hybrids (Zachos and Hartl 2011). The impact of hybridisation between sika and red deer is one of the greatest threats associated to the spread of this invasive alien species (see Linnell and Zachos 2011, Zachos and Hartl 2011, Bartoš 2009).

So far, hybridization between the two species has been documented in Ireland, Germany, Czech Republic, Lithuania, Austria and the United Kingdom (Pérez-Espona et al. 2009, Ferretti and Lovari 2014, Linnell and Zachos 2011, Bartoš 2009) as well as France (Pascal et al. 2006), Italy (Ferri et al. 2014, Ferri et al. 2016), Poland, Kaliningrad District (Russia), and Lithuania (Biedrzycka et al. 2012). It is likely that this list is not exhaustive, as shown by the unexpectedly

documented cases in countries where the species was not even known to occur, like in Italy (Ferri et al. 2014, Ferri et al. 2016). Some authors have also noted the development of a "hybrid swarm", e.g. in Poland (Biedrzycka et al. 2012), in the UK (Scotland, Senn and Pemberton 2009), although evidence is not always straightforward, e.g. see the case in Ireland (MacDevitt et al. 2009).

According to Bartoš (2009) in many areas where sika coexist with red deer, it is still believed by many hunters that no interbreeding has occurred, despite evidence to the contrary, including modern genetic techniques. Disregard of hybridization has resulted in introgression of sika and red deer genes in many areas (Bartoš 2009). Interbreeding of sika with red deer seems to be a rare event in general (Pérez-Espona et al. 2009) and there are sites where no hybrids are reported, however, the presence of isolated sika males in areas (harems) with red deer hinds may increase the likelihood of cross breeding (Pérez-Espona et al. 2009, Bartoš 2009). Overall, the impact may be locally significant, for example the level of introgression found in a study carried out in 5 regions in Poland, the Kaliningrad District (Russia), and Lithuania, is 15.5% in all regions studied (Biedrzycka et al. 2012), but reached up to 47% in some extremely strongly hybridised populations, i.e. in Ireland (Smith et al. 2014). Ireland is also known to have reported the first hybrids between sika deer and red deer in Europe (Powerscourt 1884), although red deer populations are alien too in this country (Smith et al. 2014).

As pointed out by Linnell and Zachos (2011) hybridisation in the wild between sika and red deer is not as common as previously feared but it nonetheless does occur, at least locally, in frequencies high enough to threaten the genetic integrity of red deer populations. For example, Goodman et al (1999) demonstrated how hybridisation is generally rarer than expected across Scotland (UK), but is quite pronounced at some locations. Future range shifts of both species may create new conditions of sympatry, which may increase the opportunities for further hybridization (Zachos and Hartl 2011).

Impact on vegetation and ecosystems

In Europe, sika deer can reach higher local densities than red deer and can induce strong vegetation damage due to overgrazing, as shown in a long term study in Ireland (Perrin et al. 2006, 2011). The impact seems especially evident on acidic soils (Baiwy et al. 2013), and may favour the spread of alien plants (Ferretti and Lovari 2014).

In the UK, sika have caused detrimental impacts on the biodiversity of ground vegetation in semi-natural heathland and wetland areas when present at high density (GB Non-Native Species Secretariat 2011). Severe and permanent changes in the structure and composition of vegetation in forests, heaths and wetlands are reported. In particular, the ecological impacts of sika deer on saltmarshes in the UK are described in detail (Diaz et al. 2005, Hannaford et al. 2006). Here, excessive browsing caused local decrease in number of several plant species, such as spartina (*Spartina anglica*) in saltmarshes. However, the studies also highlight that depending on the intensity of grazing, the effect of sika may be beneficial in terms of conservation management.

In forest habitats in Ireland sika deer suppressed the natural regeneration of most tree species (but canopy condition was another factor affecting regeneration), with the exception of the few plants not eaten, such as European beech *Fagus sylvatica* (Perrin et al. 2006, 2011).

The sika deer has a great potential for negative impacts on ecosystems (Ferretti and Lovari 2014). As noted by Baiwy et al. (2013) during irruptive events, a degradation of the conservation status of ecosystems is also expected to occur locally (alteration of vegetation, inhibition of tree regeneration, soil trampling, etc.).

No specific impacts on ecosystems is documented in France (Pascal et al. 2006).

By modifying the structure of forest ecosystems and the natural dynamics of vegetation through excessive browsing and trampling, sika may also cause soil erosion with cascading effects and this may also affect forest animal species, particularly soil invertebrates, birds nesting on the ground and in shrubs, small rodents and their predators (Reimoser and Putman 2011, Diaz et al. 2005, Baiwy et al. 2013, Solarz et al. 2018).

In conclusion, as pointed out by Lammertsma et al. (2012), effects of sika will depend on factors like population dynamics and density, habitat use, diet, available vegetation, interactions with other ungulates and site management.

Qu. 4.3. How important is the potential future impact of the organism on biodiversity at all levels of organisation likely to be in the risk assessment area?

See comment above. The potential future impact shall be assessed only for the risk assessment area. A potential increase in the distribution range due to climate change does not *per se* justify a higher impact score.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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In the event of substantial spread and increase in numbers of sika deer to new parts of the risk assessment area, the impact may be expected to increase in scale. The risk of introgression with native red deer populations is irreversible. Because there is evidence that this has already occurred and is still occurring, the confidence is high.

Qu. 4.4. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism currently in the risk assessment area?

including the following elements:

- native species impacted, including red list species, endemic species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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The sika deer represents a potential threat for a series of species and habitats protected by the Birds and Habitats directives, as well as a number of IUCN red-listed species in the risk assessment area. According to the IUCN Red list, because of the risk of hybridisation sika deer represent a main threat for the red deer, although the species is considered of Low concern at the global level (Lovari et al. 2018). The level of threat however may increase should the sika be further introduced and spread, and may be locally relevant in case of distinct isolated populations of red deer, such as the one in the Mesola wood (Po Delta Park, Italy). As mentioned above, the sika may also represent a threat to other ungulates, including *Cervus elaphus corsicanus*, whose populations in Corsica (France) and Sardinia (Italy) are protected by the Habitats directive (although their occurrence in the Tyrrhenian islands is due to ancient introductions). In Poland it is expected that, as a result of competition with native ungulates, the further spread of sika deer could cause decreases in the population size of native species, including those of special concern like the European bison *B. bonasus* (Solarz et al. 2018). While it cannot be excluded that interspecific interactions with sika deer may lead to the local decline of native deer species, they seem unlikely to cause large-scale species displacement (Baiwy et al. 2013).

Sika impact woodland habitats and saltmarshes by browsing on plants and trampling ground flora (Diaz et al. 2005, Hannaford et al. 2006, Perrin et al. 2006, 2011). In Ireland, the potential negative impact on woodland habitats protected under the European Union’s Habitats Directive is stressed by Carden et al. (2011). Besides the fact that several plants may be susceptible to sika deer impact, it is necessary to consider the cascading effects that sika deer populations may exert on other animals through the modification of vegetation structure and composition (Baiwy et al. 2013).

Qu. 4.5. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.3. and 4.4.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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In the event of substantial spread and increase in numbers of sika deer to new parts of the risk assessment area, the impact may be expected to increase accordingly. While there is no documented evidence of the species being able to cause the extinction of native species, the long-term irreversible risk of introgression on the European populations of red deer may be relevant.

Ecosystem Services impacts

Qu. 4.6. How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?

- For a list of services use the CICES classification V5.1 provided in Annex V.

- Impacts on ecosystem services build on the observed impacts on biodiversity (habitat, species, genetic, functional) but focus exclusively on reflecting these changes in relation to their links with socio-economic well-being.
- Quantitative data should be provided whenever available and references duly reported.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Sika deer is known to affect several ecosystem services, not only through the impacts on biodiversity (through competition on other ungulates, hybridisation with red deer, and damages to vegetation and ecosystems), but also due to the impacts documented on agricultural crops through browsing and bark stripping.

The erosion caused by the trampling behaviour associated with the death of trees caused by the habit to wipe their antlers on the barks, may result in destabilisation of stream banks, changes in stream flow and increased erosion. In New Zealand soil erosion was accelerated by sika through thinning of understory caused by excessive trampling (Lever 1994).

The potential effect on the nitrogen cycle has been suggested as documented for ungulates in general (Hobbs 1996, Solarz 2018). The role of sika deer in the transmission of diseases is a possible threat to both wildlife and livestock, and to humans (Solarz et al. 2018). On the basis of the impacts described above, the following list of potential impacts on ecosystem services was compiled:

Provisioning (Biomass)

- Cultivated terrestrial plants
- Reared animals
- Wild plants (terrestrial and aquatic)
- Wild animals (terrestrial and aquatic)
- Genetic material from animals

Regulation & Maintenance (Regulation of physical, chemical, biological conditions)

- Regulation of baseline flows and extreme event
- Lifecycle maintenance, habitat and gene pool protection
- Pest and disease control
- Regulation of soil quality

Cultural (Direct, in-situ and outdoor interactions with living systems that depend on presence in the environmental setting)

- Physical and experiential interactions with natural environment
- Intellectual and representative interactions with natural environment

Qu. 4.7. How important is the impact of the organism on provisioning, regulating, and cultural services currently in the different biogeographic regions or marine sub-regions where the species has established in the risk assessment area (include any past impact in your response)?

- See guidance to Qu. 4.6.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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The impacts on ecosystem services for the risk assessment area are the same as described in Qu. 4.6.

Specific references for the risk assessment area, are the following. Damages through fraying and bark stripping are reported in Ireland (Lever 1994). In the UK, intensive trampling was documented to trigger soil erosion processes on heaths and saltmarshes (Diaz et al. 2005).

Despite the lack of quantitative data, some impacts (particularly on provisioning services) is well documented in the risk assessment area, hence the medium confidence score.

Qu. 4.8. How important is the impact of the organism on provisioning, regulating, and cultural services likely to be in the different biogeographic regions or marine sub-regions where the species can establish in the risk assessment area in the future?

- See guidance to Qu. 4.6.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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In the event of substantial spread and increase in numbers of sika deer to new parts of the risk assessment area, the impact may be expected to increase accordingly. There is no evidence, however, that the level of impact will increase in the future. The level of confidence is medium because despite the lack of quantitative data, some impacts (particularly on provisioning services) are well documented in the risk assessment area.

Economic impacts

Qu. 4.9. How great is the overall economic cost caused by the organism within its current area of distribution (excluding the risk assessment area), including both costs of / loss due to damage and the cost of current management.

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the world these should be reported here. The assessment of the potential costs of / loss due to damage shall describe those costs quantitatively and/or qualitatively depending on what information is available. Cost of / loss due to damage within different

economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage. As far as possible, it would be useful to separate costs of / loss due to the organism from costs of current management.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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According to Harris (2015) in its native range in Japan, the sika deer is considered an agricultural and forest-plantation pest (see also Kaji et al. 2010).

On the island of Hokkaido, damage to forestry and agriculture increased to nearly 2 billion Japanese yen (JPY) (corresponding to 18 million USD) by 1990 and to over 5 billion JPY (corresponding to 45 million USD) by 1996 (Kaji 1999, Kaji et al. 2010). Damage to crops in Japan were significant in 2010 and were estimated at around 4.1 million USD, and the damage caused was demonstrated in 36.1% of 341 farms (Tsukada et al. 2013). In a dairy farm in central Japan, sika deer consumed an estimated 20% of the total herbage production with an economic loss estimated to exceed USD 0.13 million per year (Tsukada 2013). The level of herbage damage caused by sika deer, however, was different among different meadows (Tsukada et al. 2013). According to Kawata (2011) cost of agricultural/forestry damage caused by sika deer in Hokkaido, Japan, are slightly different, as they equal 0.35, 2.1 and 24 million USD, in 1970, 1980, and 1990 respectively; the damage reached its peak in 1996 with a cost of 60 million USD, and eventually the total cost of damage stabilized at around 36 million USD after 2000.

Qu. 4.10. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism currently in the risk assessment area (include any past costs in your response)?

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the EU these should be reported here. Assessment of the potential costs of damage on human health, safety, and the economy, including the cost of non-action. A full economic assessment at EU scale might not be possible, but qualitative data or different case studies from across the EU (or third countries if relevant) may provide useful information to inform decision making. In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable). Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Commercial forest plantations are used by sika deer for both food and cover and may suffer extensive damage due to bark stripping, browsing and antler abrasions (Murphy et al. 2013). In Ireland, where deer are generally perceived as a problem for the damage they cause to woodland, the types of impacts and damage in plantation forests and crops were reviewed by Murphy et al. (2013). The same authors also stressed that calculating the potential economic cost of damage is difficult since relatively few studies have been carried out in the country, and national baseline quantitative data are lacking. However, in Ireland, the potential loss of income caused by sika deer was estimated at approximately €1,200 per ha (Murphy et al. 2013).

In the UK, extensive damage by sika is reported to commercial forestry, especially at high density in Scotland, both through browsing of young trees and shoots, as well as bark-stripping, bole scoring and fraying (Gill et al. 2000, see also GB Non-Native Species Secretariat 2011). Damage to forestry is also considered significant in a number of countries to which sika have been introduced, once populations reach sufficient density (CABI 2021). In particular, damage levels to regenerating woodland are considered broadly tolerable if densities do not exceed 4 deer per 100 ha (Reimoser and Putman 2011). However, this threshold refers primarily to impacts of red and sika deer in commercial forests in Scotland, hence any generalisation would be too speculative in the absence of a detailed characterisation of other (potentially) affected plantations. Also Gill et al. (2000) in their review on the economic implications of deer damage in Scotland pointed out that no estimates are available specifically for sika deer, although it is likely that in certain habitat types the damage inflicted by this species is higher than that by the native red and roe deer.

On the other hand, agricultural crop damage has not been reported as of widespread economic significance in the UK. Economic losses to agriculture have not been studied in detail specifically for sika, but even at high density tend to be of mostly localised importance without causing a significant economic problem on a regional or a national scale (GB Non-Native Species Secretariat 2011, Putman et al. 2011, Pérez-Espona et al. 2009). In England, the economic losses to agriculture attributable to sika may be about £100,000, which is about 2% of the costs due to all deer species (Wilson 2003).

Similar conclusions arise from experience with sika in other European countries, where damage to arable and horticultural crops currently appears to be of minor significance (Pérez-Espona et al. 2009). These populations, however, are controlled at low densities by hunting. If sika deer were not controlled, it is likely that significant damage would occur to agricultural crops (Lammertsma et al. 2012). On the other hand, as pointed out by Solarz et al. (2018) there are no published studies on the effect of the species on the condition or yield of crops by changing the properties of the agroecosystem, including the circulation of nutrients, hydrology, physical properties, and trophic networks.

As reported by the GB Non-Native Species Secretariat (2011) for UK, it is important to note that much of the potential habitat available for the sika in the risk assessment area already has one or more other ungulates present (and often under management), hence economic impacts may not necessarily be additive or increase, unless the overall numbers of deer increase. Also, as noted for the UK, the presence of sika hybrids may compromise the genetic integrity of native red deer stocks, potentially reducing their trophy value, hence leading into net changes to income (e.g. through stalking and trophy shooting) and other associated economic losses which are however difficult to predict (GB Non-Native Species Secretariat 2011).

Qu. 4.11. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.10.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Overabundant deer are known to inflict major economic losses in forestry and, although to a lesser extent (hence with a reduced level of impact), can also cause damages in agriculture, and transportation and contribute to the transmission of several animal and human diseases. In the event of substantial spread and increase in numbers of sika deer to new parts of the risk assessment area, the impact (which in fact is calculated in euro/ha) may be expected to increase accordingly.

Qu. 4.12. How great are the economic costs / losses associated with managing this organism currently in the risk assessment area (include any past costs in your response)?

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable).

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Economic costs associated with managing the sika deer in the risk assessment area would depend on the objective and the status of the species.

In Ireland, a study carried out on a sample site demonstrated that the cost and maintenance of fencing reduced returns by €1,971.59 per ha, which was higher than the loss of €459.83 per ha were no fencing was used (Murphy et al. 2013). The losses without fencing were approximately one quarter of the overall costs of erecting and maintaining fencing, but the authors recognize that the cost benefit of fencing may differ in different types of plantations. In any case, it is worth mentioning that fencing may also have side effects, since the complete exclusion of grazing animals may be beneficial in terms of natural regeneration for certain species of woodlands, as shown on a long term study in Ireland, but may be undesirable if the management objectives are to achieve high levels of ground flora diversity (Perrin et al. 2006, 2011) and allowing for native deer species migrations.

In general, as noted by the GB Non-Native Species Secretariat (2011) for UK, within forest plantations greater effort tends to be required per sika culled as compared to red deer.

Qu. 4.13. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.12.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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In the event of substantial spread and increase in numbers of sika deer to new parts of the risk assessment area, the costs may be expected to increase accordingly. If the species spreads and is to be managed, some costs are bound to be incurred, even if there is no information on what these costs currently are. The spread of sika may cause extensive indirect costs for the reduction of damages to ecosystems or crops, or for roadside protections, due to the need for high net fences or culling by professional hunters and government agencies (GB Non-Native Species Secretariat 2011, Baiwy et al. 2013). However, it is not possible to estimate the exact monetary value, as it depends on deer management systems and policies involved, which vary considerably across the different countries of Europe depending on species present, legislation, cultural tradition and the status of deer as *res nullius* or *res communis*.

In the UK, according to a review on the economic implications of deer damage in Scotland (Gill et al. 2000) culling appears to be a far more cost effective option than fencing (which could cost in the region 10-30% of yield for Sitka spruce) in view of the fact that much of the cost of deer control can be offset against revenue from venison.

Social and human health impacts

Qu. 4.14. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism for the risk assessment area and for third countries, if relevant (e.g. with similar eco-climatic conditions).

The description of the known impact and the assessment of potential future impact on human health, safety and the economy, shall, if relevant, include information on

- illnesses, allergies or other affections to humans that may derive directly or indirectly from a species;
- damages provoked directly or indirectly by a species with consequences for the safety of people, property or infrastructure;
- direct or indirect disruption of, or other consequences for, an economic or social activity due to the presence of a species.

Social and human health impacts can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Overall, as pointed out for alien mammals in general (Capizzi et al. 2018), sika deer can act as vectors of both alien and native pathogens, and as host of either native or alien parasites (which in turn can be acting as vectors of either native or alien pathogens). In this way sika deer may either introduce new pathogens, alter the epidemiology of local pathogens, become reservoir hosts, and increase disease risk for humans, along with other species (e.g. by introducing changes in the vector-host-parasite relationship).

A short review of disease and parasites found in sika deer in both its native and alien range, is found in Feldhamer (1980). Feldhamer and Demarais (2008) reported those disease and parasites found in sika in the US. Current and possible future infections of sika deer which may have an impact on livestock and/or human health in the UK are reviewed by Böhm et al. (2007). In Japan, there is direct evidence for zoonotic transmission of hepatitis E virus from sika deer to human beings (Tei et al. 2003) although prevalence in sika deer was found to be lower than the in two other possible reservoirs, pigs and wild boar (Matsuura et al. 2007).

In addition to carrying diseases that can infect humans, sika deer may cause road and rail collisions. In Denmark, a total of 33,605 collision sites between medium-sized and large wildlife and vehicles were recorded from 2003 to 2012, of which 141 sika (Elmeros et al. 2014). Deer/vehicle collisions with sika were estimated at 350-600 per year by Langbein (2007), contributing to about 1% of all deer/vehicle collisions in England and Scotland (Langbein 2007, 2011). Sika do not appear any more prone to involvement in such collisions than other deer species. In Japan, on Hokkaido, the number deer-vehicle traffic accidents increased from 293 in 1993 to 1,474 in 2007, successively 1,628 accidents were reported in 2008 and 1,838 in 2009 (Kawata 2011). In the same region, deer-train collisions involving sika deer were also reported: on a 330 km railway section there were 696 accidents between 1987 and 1995 (Onoyama et al. 1998).

As pointed out by Solarz at al. (2018) there is no available information suggesting that the species has biological, physical and/or chemical properties that are harmful to people, although it cannot be ruled out that because of sika deer size, there may be cases of kicking or injuring with antlers in specific circumstances, i.e. when in contact with humans.

Qu. 4.15. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism in the future for the risk assessment area.

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable).

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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In the event of substantial spread and increase in numbers of sika deer to new parts of the risk assessment area, the impact may be expected to increase accordingly.

For example, it was pointed out In the UK, by the GB Non-Native Species Secretariat (2011), in the event of substantial spread and increase in numbers of sika to new parts of the risk assessment area, increased social harm through deer/vehicle collisions may be expected.

Other impacts

Qu. 4.16. How important is the organism in facilitating other damaging organisms (e.g. diseases) as food source, a host, a symbiont or a vector etc.?

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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The sika deer, as other ungulates, can be a carrier of a number of diseases and parasites that may be harmful to native species. Deer, in general, may transmit infectious diseases directly to other species of deer (as well as to livestock, and to humans), especially if their density is high (Côté et al. 2004). The risk of disease transmission is not limited to the animals in the wild, since sika deer kept in captivity may be acting as a source of infection as well. As Feldhamer and Demarais (2008) noted, disease transmission may occur between captive deer and livestock, and between captive deer and free-ranging wildlife populations adjacent to the fences.

Bovine tuberculosis and avian tuberculosis have been found in sika deer - both in free-living populations and in captivity - which can be infecting both animals, as well as humans (Solarz et al. 2018). In particular, Bovine tuberculosis has been recorded in sika deer in Ireland, the UK (GB Non-Native Species Secretariat 2011, Delahay et al. 2002), and New Zealand (Nugent et al. 2001). In the UK and Ireland sika are not the principal wildlife reservoir for Bovine tuberculosis but have a potential role in perpetuating the disease (Ward and Smith 2012). In the UK, like all other established deer species are carriers of ticks *Ixodes ricinus* which are a vector for Lyme disease (GB Non-Native Species Secretariat 2011).

In Eastern and Central Europe, sika are deemed to play an important role in the epidemiology of the highly pathogenic *Ashworthius sidemi* (Vadlejch et al. 2017, Solarz et al. 2018). In the previous century this non-specific blood-sucking gastrointestinal nematode of Asiatic origin was introduced with sika into Europe, e.g. Slovakia, Czech Republic and France as well as Ukraine (Demiaszkiewicz 2014, Demiaszkiewicz et al. 2013, Ferté et al. 2000, Kotrla and Kotrly 1973) and since then it spread in Poland too (Vadlejch et al. 2017). *Ashworthius sidemi* is known to infect European bison, roe deer, red deer, fallow deer, elk (*Alces alces*) as well as domestic sheep (*Ovis aries*) and cattle (*Bos taurus*) (see Vadlejch et al. 2017, Hoberg 2010, Baiwy et al. 2013, Demiaszkiewicz et al. 2013). This is creating greatest concern particularly for the conservation of European bison, for which diseases are considered a main threat (Plumb et al. 2020). Although there has been no case of death caused by this nematode, it may reduce the condition of bison and lead to death, especially of young animals, according to Solarz et al. (2018).

In Ireland a study on the prevalence and distribution in free-ranging deer, of a number of existing and emerging pathogens - namely bovine viral diarrhoea virus (BVDV), bovine herpesvirus-1 (BoHV-1), Schmallenberg virus (SBV) and bluetongue virus (BTV) - indicates that sika do not represent a risk for the transmission of these viruses to cattle and sheep although they can be infected (Graham et al. 2017). An example is relative to the chronic wasting disease which keeps emerging in Norway and is known to be transmissible to sika, so this species could exacerbate an epidemic (Osterholm et al. 2019).

In conclusion, it is evident that a lot of European diseases and parasites of ungulates may infect sika deer and could potentially be amplified by it (reservoir function) and spillback to native deer. Some of them like bovine tuberculosis and sarcoptic mange may even be at threat for wildlife populations. However, few information is available in the scientific literature about the potential role of sika in this as compared to other deer species, but it has clearly the capacity to increase native disease persistence and prevalence due to the formation of overabundant populations (Baiwy et al. 2013).

Qu. 4.17. How important might other impacts not already covered by previous questions be resulting from introduction of the organism?

RESPONSE	N/A minimal minor moderate major massive	CONFIDENCE	low medium high
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No information has been found.

Qu. 4.18. How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area?

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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As described in Qu.2.4 the risk assessment area is certainly characterised by the presence of potential predators, parasites or pathogens of sika deer. However, there are several species of native and alien deer already occurring, and this does not seem to represent a limiting factor for the relevant populations. In fact, the role of predators in controlling ungulate populations remains uncertain as pointed out by Côté et al. (2004), and is considered not effective, at least in some ecosystems.

Qu. 4.19. Estimate the overall impact in the risk assessment area under current climate conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, in current conditions.

RESPONSE	minimal minor	CONFIDENCE	low medium
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	moderate major massive		high
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The species is known to exert a multifaceted impact on both biodiversity and ecosystem services, by feeding on native vegetation and contributing to the loss of habitat structure and function (hence indirectly affecting other species, including birds, reptiles, invertebrates, etc.). Hybridisation with red deer and competition with other ungulates is documented. The species is known to contribute to the spread of diseases and pathogens affecting wild animals, livestock and humans. It can also damage crops and compete with livestock. Moreover, it can be a threat for human health in relation to possible deer/vehicle collisions. It is worth pointing it out that the species does not even have to establish to generate problems. Even single escapees may be dangerous, particularly with regard to hybridisation and parasite transmission.

The overall impact is deemed similar in all biogeographical regions where the species is known to occur.

Qu. 4.20. Estimate the overall impact in the risk assessment area in foreseeable climate change conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, under future conditions.

- See also guidance to Qu. 4.3.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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In foreseeable climate change conditions, the area suitable for the species in the risk assessment area may increase in the Alpine and Boreal biogeographic regions (see Annex VIII), hence the impact may be expected to increase accordingly. In case of a future expansion of the species range, other habitats and native species may be affected.

RISK SUMMARIES			
	RESPONSE	CONFIDENCE	COMMENT
Summarise Introduction and Entry*	very unlikely unlikely moderately likely likely very likely	low medium high	Sika have been widely introduced to numerous countries in the risk assessment area (in the wild and in confinements). Further introductions for hunting, farming or exhibitions are very likely. Releases or escapes from captive facilities have been documented in the past in the risk assessment area and are very likely to take place again.
Summarise Establishment*	very unlikely unlikely moderately likely likely very likely	low medium high	The species is already established in several EU Member States and in neighbouring countries such as the UK. The species life-history, available habitat conditions and management practices in the EU offer the potential to support self-sustaining populations of sika deer also in other countries and biogeographical regions.
Summarise Spread*	very slowly slowly moderately rapidly very rapidly	low medium high	Natural spread into new parts of the risk assessment area has occurred already and is likely to occur also in the future. Occasional releases from parks and farms may aid dispersing individuals to establish new populations.
Summarise Impact*	minimal minor moderate major massive	low medium high	The species is known to exert a multifaceted impact on both biodiversity and ecosystem services, by feeding on native vegetation and contributing to the loss of habitat structure and function (hence indirectly affecting other species, including birds, reptiles, invertebrates, etc.). Competition with other ungulates is documented, and hybridisation with native red deer <i>Cervus elaphus</i> is representing a major threat due

			to genetic introgression. The species is known to contribute to the spread of diseases and pathogens affecting wildlife, livestock and humans. This could have an impact on native species, for example the European bison (<i>Bison bonasus</i>) as a consequence of the spread of the pathogenic nematode <i>Ashworthius sidemi</i> . Sika can also damage forest (commercial) plantations and crops and can be a threat in relation to possible road and rail collisions.
Conclusion of the risk assessment (overall risk)	low moderate high	low medium high	The sika deer represents a high risk in the risk assessment area, given the ability to establish in the wild, the potential for spread, and the documented impact in most MS where established populations occur and other parts of the introduced range.

*in current climate conditions and in foreseeable future climate conditions

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Distribution Summary

Please answer as follows:

- Yes if recorded, established or invasive
- if not recorded, established or invasive
- ? Unknown; data deficient

The columns refer to the answers to Questions A5 to A12 under Section A.

For data on marine species at the Member State level, delete Member States that have no marine borders. In all other cases, provide answers for all columns.

Member States and the United Kingdom

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Austria	Yes	Yes	Yes	Yes	
Belgium	Yes		Yes	Yes	
Bulgaria			Yes	Yes	
Croatia			Yes	Yes	
Cyprus					
Czech Republic	Yes	Yes	Yes	Yes	Yes
Denmark	Yes	Yes	Yes	Yes	Yes
Estonia	Yes		Yes	Yes	
Finland	Yes		Yes	Yes	
France	Yes	Yes	Yes	Yes	Yes
Germany	Yes	Yes	Yes	Yes	
Greece			Yes	Yes	
Hungary	Yes	Yes	Yes	Yes	
Ireland	Yes	Yes	Yes	Yes	Yes
Italy	Yes	?	Yes	Yes	Yes
Latvia			Yes	Yes	
Lithuania	Yes	Yes	Yes	Yes	Yes
Luxembourg	Yes	?	Yes	Yes	
Malta					
Netherlands	Yes		Yes	Yes	
Poland	Yes	Yes	Yes	Yes	Yes
Portugal			Yes	Yes	
Romania			Yes	Yes	
Slovakia			Yes	Yes	
Slovenia			Yes	Yes	
Spain			Yes	Yes	
Sweden			Yes	Yes	
United Kingdom	Yes	Yes	Yes	Yes	Yes

Biogeographical regions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Alpine	Yes	Yes	Yes	Yes	Yes
Atlantic	Yes	Yes	Yes	Yes	Yes
Black Sea			Yes	Yes	
Boreal	Yes	Yes	Yes	Yes	Yes
Continental	Yes	Yes	Yes	Yes	Yes
Mediterranean	Yes	Yes	Yes	Yes	Yes
Pannonian	Yes	Yes	Yes	Yes	Yes
Steppic			Yes	Yes	

ANNEX I Scoring of Likelihoods of Events

(taken from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Description	Frequency
Very unlikely	This sort of event is theoretically possible, but is never known to have occurred and is not expected to occur	1 in 10,000 years
Unlikely	This sort of event has occurred somewhere at least once in the last millenium	1 in 1,000 years
Moderately likely	This sort of event has occurred somewhere at least once in the last century	1 in 100 years
Likely	This sort of event has happened on several occasions elsewhere, or on at least once in the last decade	1 in 10 years
Very likely	This sort of event happens continually and would be expected to occur	Once a year

ANNEX II Scoring of Magnitude of Impacts

(modified from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Biodiversity and ecosystem impact	Ecosystem Services impact	Economic impact (Monetary loss and response costs per year)	Social and human health impact, and other impacts
	<i>Question 5.1-5</i>	<i>Question 5.6-8</i>	<i>Question 5.9-13</i>	<i>Question 5.14-18</i>
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected ⁵	Up to 10,000 Euro	No social disruption. Local, mild, short-term reversible effects to individuals.
Minor	Some ecosystem impact, reversible changes, localised	Local and temporary, reversible effects to one or few services	10,000-100,000 Euro	Significant concern expressed at local level. Mild short-term reversible effects to identifiable groups, localised.
Moderate	Measureable long-term damage to populations and ecosystem, but reversible; little spread, no extinction	Measureable, temporary, local and reversible effects on one or several services	100,000-1,000,000 Euro	Temporary changes to normal activities at local level. Minor irreversible effects and/or larger numbers covered by reversible effects, localised.
Major	Long-term irreversible ecosystem change, spreading beyond local area	Local and irreversible or widespread and reversible effects on one / several services	1,000,000-10,000,000 Euro	Some permanent change of activity locally, concern expressed over wider area. Significant irreversible effects locally or reversible effects over large area.
Massive	Widespread, long-term population loss or extinction, affecting several species with serious ecosystem effects	Widespread and irreversible effects on one / several services	Above 10,000,000 Euro	Long-term social change, significant loss of employment, migration from affected area. Widespread, severe, long-term, irreversible health effects.

⁵ Not to be confused with “no impact”.

ANNEX III Scoring of Confidence Levels

(modified from Bacher et al. 2017)

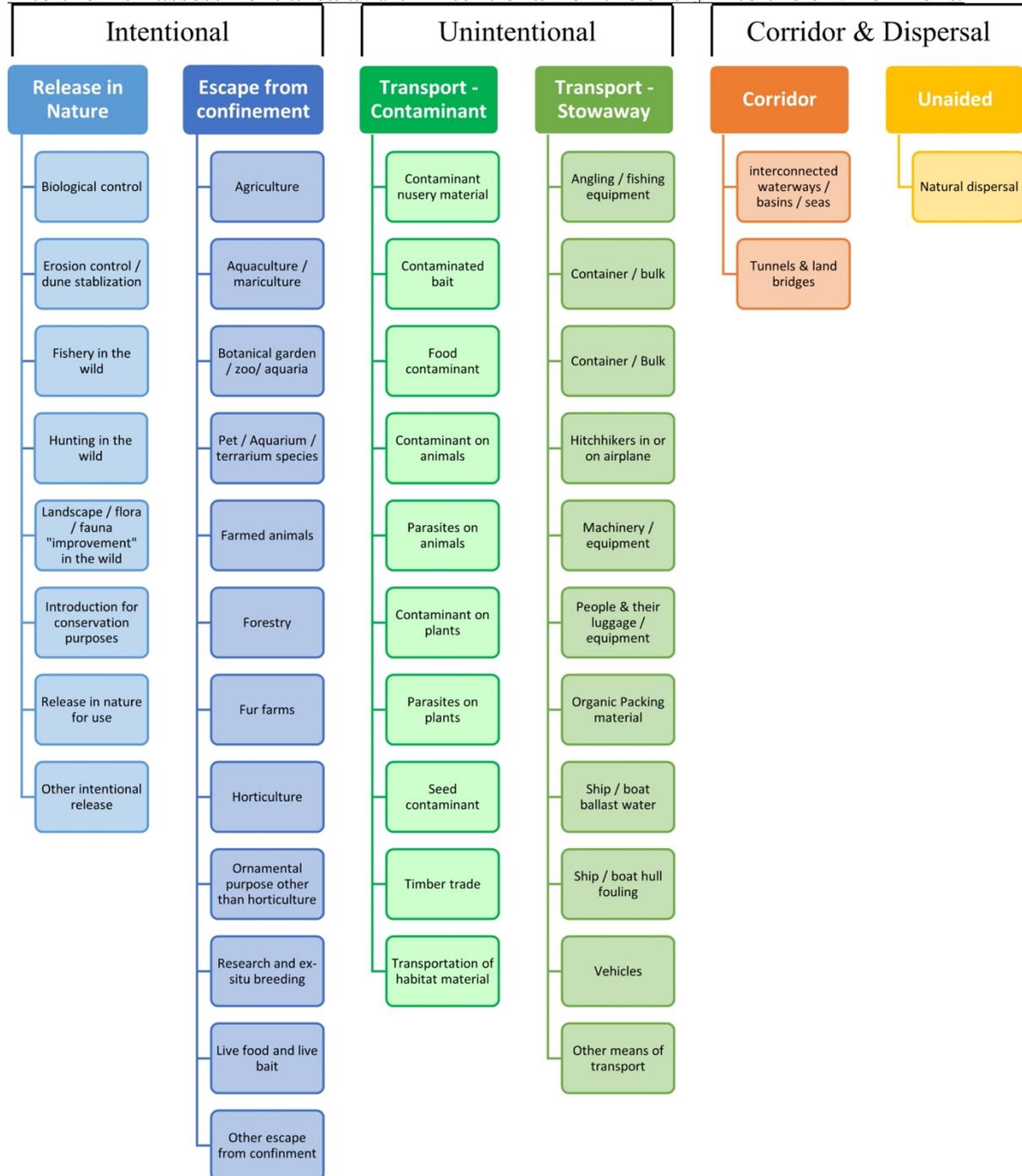
Each answer provided in the risk assessment must include an assessment of the level of confidence attached to that answer, reflecting the possibility that information needed for the answer is not available or is insufficient or available but conflicting.

The responses in the risk assessment should clearly support the choice of the confidence level.

Confidence level	Description
Low	There is no direct observational evidence to support the assessment, e.g. only inferred data have been used as supporting evidence <i>and/or</i> Impacts are recorded at a spatial scale which is unlikely to be relevant to the assessment area <i>and/or</i> Evidence is poor and difficult to interpret, e.g. because it is strongly ambiguous <i>and/or</i> The information sources are considered to be of low quality or contain information that is unreliable.
Medium	There is some direct observational evidence to support the assessment, but some information is inferred <i>and/or</i> Impacts are recorded at a small spatial scale, but rescaling of the data to relevant scales of the assessment area is considered reliable, or to embrace little uncertainty <i>and/or</i> The interpretation of the data is to some extent ambiguous or contradictory.
High	There is direct relevant observational evidence to support the assessment (including causality) <i>and</i> Impacts are recorded at a comparable scale <i>and/or</i> There are reliable/good quality data sources on impacts of the taxa <i>and</i> The interpretation of data/information is straightforward <i>and/or</i> Data/information are not controversial or contradictory.

ANNEX IV CBD pathway categorisation scheme

Overview of CBD pathway categorisation scheme showing how the 44 pathways relate to the six main pathway categories. All of the pathways can be broadly classified into 1) those that involve intentional transport (blue), 2) those in which the taxa are unintentionally transported (green) and 3) those where taxa moved between regions without direct transportation by humans and/or via artificial corridors (orange and yellow). **Note that the pathways in the category “Escape from confinement” can be considered intentional for the introduction into the risk assessment area and unintentional for the entry into the environment.**



ANNEX V Ecosystem services classification (CICES V5.1, simplified) and examples

For the purposes of this risk assessment, please feel free to use what seems as the most appropriate category / level / combination of impact (Section – Division – Group), reflecting information available.

Section	Division	Group	Examples (i.e. relevant CICES “classes”)
Provisioning	Biomass	Cultivated <i>terrestrial</i> plants	<p>Cultivated terrestrial plants (including fungi, algae) grown for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from cultivated plants, fungi, algae and bacteria for direct use or processing (excluding genetic materials); Cultivated plants (including fungi, algae) grown as a <u>source of energy</u></p> <p><i>Example: negative impacts of non-native organisms to crops, orchards, timber etc.</i></p>
		Cultivated <i>aquatic</i> plants	<p>Plants cultivated by in- situ aquaculture grown for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from in-situ aquaculture for direct use or processing (excluding genetic materials); Plants cultivated by in- situ aquaculture grown as an <u>energy source</u>.</p> <p><i>Example: negative impacts of non-native organisms to aquatic plants cultivated for nutrition, gardening etc. purposes.</i></p>
		Reared animals	<p>Animals reared for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from reared animals for direct use or processing (excluding genetic materials); Animals reared to provide <u>energy</u> (including mechanical)</p> <p><i>Example: negative impacts of non-native organisms to livestock</i></p>
		Reared <i>aquatic</i> animals	<p>Animals reared by in-situ aquaculture for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from animals grown by in-situ aquaculture for direct use or processing (excluding genetic materials); Animals reared by in-situ aquaculture as an <u>energy source</u></p> <p><i>Example: negative impacts of non-native organisms to fish farming</i></p>
		Wild plants (terrestrial and aquatic)	<p>Wild plants (terrestrial and aquatic, including fungi, algae) used for <u>nutrition</u>; <u>Fibres and other materials</u> from wild plants for direct use or processing (excluding genetic materials); Wild plants (terrestrial and aquatic, including fungi, algae) used as a <u>source of energy</u></p> <p><i>Example: reduction in the availability of wild plants (e.g. wild berries, ornamentals) due to non-native organisms (competition, spread of disease etc.)</i></p>
		Wild animals (terrestrial and aquatic)	<p>Wild animals (terrestrial and aquatic) used for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from wild animals for direct use or processing (excluding genetic materials); Wild animals (terrestrial and aquatic) used as a <u>source of energy</u></p> <p><i>Example: reduction in the availability of wild animals (e.g. fish stocks, game) due to non-native organisms (competition, predations, spread of disease etc.)</i></p>

	Genetic material from all biota	Genetic material from plants, algae or fungi	<p><u>Seeds, spores and other plant materials</u> collected for maintaining or establishing a population; Higher and lower plants (whole organisms) used to <u>breed new strains or varieties</u>; Individual genes extracted from higher and lower plants for the <u>design and construction of new biological entities</u></p> <p><i>Example: negative impacts of non-native organisms due to interbreeding</i></p>
		Genetic material from animals	<p>Animal material collected for the purposes of maintaining or establishing a population; Wild animals (whole organisms) used to breed new strains or varieties; Individual genes extracted from organisms for the design and construction of new biological entities</p> <p><i>Example: negative impacts of non-native organisms due to interbreeding</i></p>
	Water ⁶	Surface water used for nutrition, materials or energy	<p>Surface water for <u>drinking</u>; Surface water used as a material (<u>non-drinking purposes</u>); Freshwater surface water, coastal and marine water used as an <u>energy source</u></p> <p><i>Example: loss of access to surface water due to spread of non-native organisms</i></p>
		Ground water for used for nutrition, materials or energy	<p>Ground (and subsurface) water for <u>drinking</u>; Ground water (and subsurface) used as a material (<u>non-drinking purposes</u>); Ground water (and subsurface) used as an <u>energy source</u></p> <p><i>Example: reduced availability of ground water due to spread of non-native organisms and associated increase of ground water consumption by vegetation.</i></p>
Regulation & Maintenance	Transformation of biochemical or physical inputs to ecosystems	Mediation of wastes or toxic substances of anthropogenic origin by living processes	<p><u>Bio-remediation</u> by micro-organisms, algae, plants, and animals; <u>Filtration/sequestration/storage/accumulation</u> by micro-organisms, algae, plants, and animals</p> <p><i>Example: changes caused by non-native organisms to ecosystem functioning and ability to filtrate etc. waste or toxics</i></p>
		Mediation of nuisances of anthropogenic origin	<p><u>Smell reduction; noise attenuation; visual screening</u> (e.g. by means of green infrastructure)</p> <p><i>Example: changes caused by non-native organisms to ecosystem structure, leading to reduced ability to mediate nuisances.</i></p>
	Regulation of physical, chemical, biological conditions	Baseline flows and extreme event regulation	<p>Control of <u>erosion</u> rates; Buffering and attenuation of <u>mass movement</u>; <u>Hydrological cycle and water flow regulation</u> (Including flood control, and coastal protection); <u>Wind</u> protection; <u>Fire</u> protection</p> <p><i>Example: changes caused by non-native organisms to ecosystem functioning or structure leading to, for example, destabilisation of soil, increased risk or intensity of wild fires etc.</i></p>
		Lifecycle maintenance , habitat and gene pool protection	<p><u>Pollination</u> (or 'gamete' dispersal in a marine context); <u>Seed dispersal</u>; Maintaining <u>nursery populations and habitats</u> (Including gene pool protection)</p>

⁶ Note: in the CICES classification provisioning of water is considered as an abiotic service whereas the rest of ecosystem services listed here are considered biotic.

			<p><i>Example: changes caused by non-native organisms to the abundance and/or distribution of wild pollinators; changes to the availability / quality of nursery habitats for fisheries</i></p>
		Pest and disease control	<p>Pest control; Disease control</p> <p><i>Example: changes caused by non-native organisms to the abundance and/or distribution of pests</i></p>
		Soil quality regulation	<p><u>Weathering processes</u> and their effect on soil quality; <u>Decomposition and fixing processes</u> and their effect on soil quality</p> <p><i>Example: changes caused by non-native organisms to vegetation structure and/or soil fauna leading to reduced soil quality</i></p>
		Water conditions	<p>Regulation of the <u>chemical condition</u> of freshwaters by living processes; Regulation of the chemical condition of salt waters by living processes</p> <p><i>Example: changes caused by non-native organisms to buffer strips along water courses that remove nutrients in runoff and/or fish communities that regulate the resilience and resistance of water bodies to eutrophication</i></p>
		Atmospheric composition and conditions	<p>Regulation of <u>chemical composition</u> of atmosphere and oceans; Regulation of <u>temperature and humidity</u>, including ventilation and transpiration</p> <p><i>Example: changes caused by non-native organisms to ecosystems' ability to sequester carbon and/or evaporative cooling (e.g. by urban trees)</i></p>
Cultural	Direct, in-situ and outdoor interactions with living systems that depend on presence in the environmental setting	Physical and experiential interactions with natural environment	<p>Characteristics of living systems that that enable activities promoting health, recuperation or enjoyment through <u>active or immersive interactions</u>;</p> <p>Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through <u>passive or observational interactions</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that make it attractive for recreation, wild life watching etc.</i></p>
		Intellectual and representative interactions with natural environment	<p>Characteristics of living systems that enable <u>scientific investigation</u> or the creation of traditional ecological knowledge;</p> <p>Characteristics of living systems that enable <u>education and training</u>;</p> <p>Characteristics of living systems that are resonant in terms of <u>culture or heritage</u>;</p> <p>Characteristics of living systems that enable <u>aesthetic experiences</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have cultural importance</i></p>
	Indirect, remote, often indoor interactions with living systems that do not require presence in the environmental setting	Spiritual, symbolic and other interactions with natural environment	<p>Elements of living systems that have <u>symbolic meaning</u>;</p> <p>Elements of living systems that have <u>sacred or religious meaning</u>;</p> <p>Elements of living systems used for <u>entertainment or representation</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have sacred or religious meaning</i></p>
		Other biotic characteristics that have a non-use value	<p>Characteristics or features of living systems that have an <u>existence value</u>;</p>

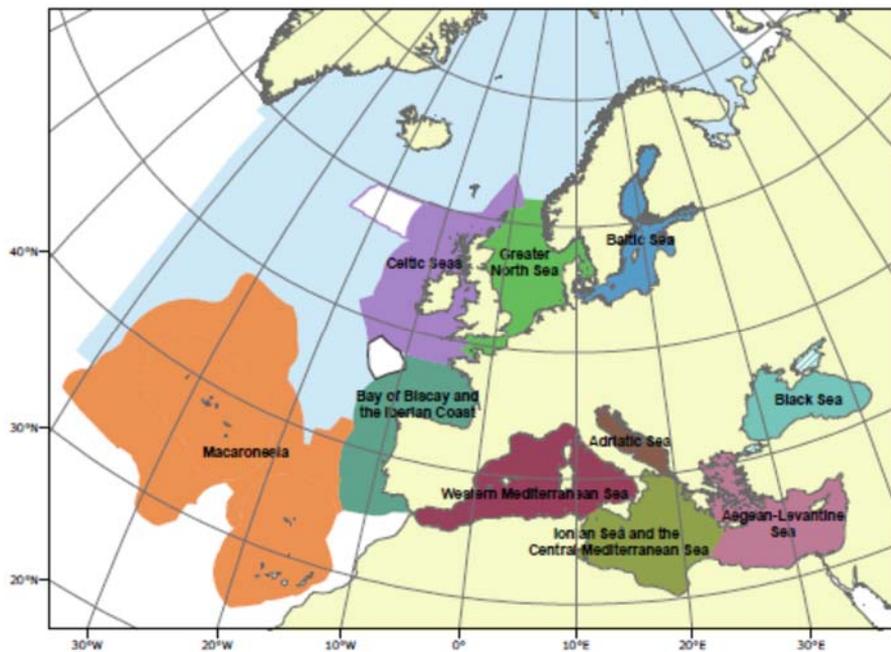
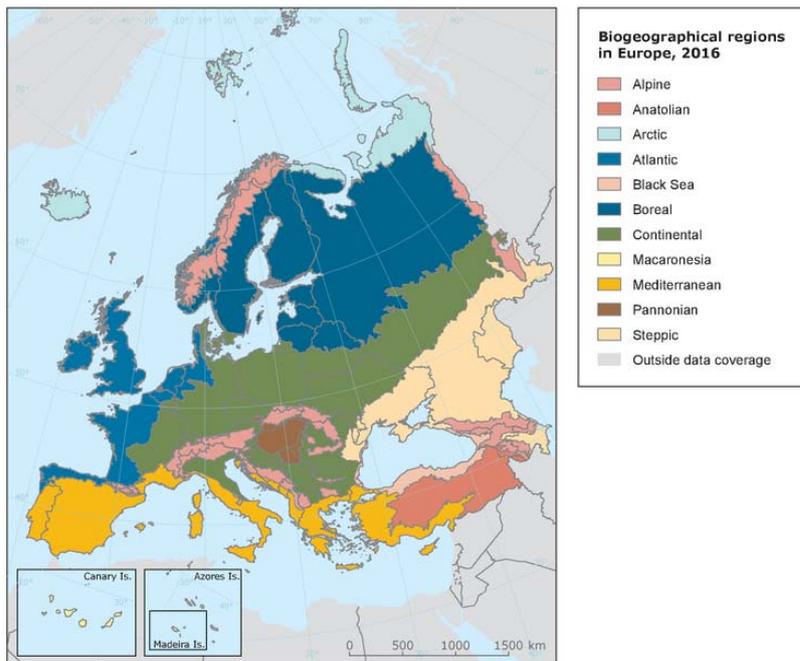
			<p>Characteristics or features of living systems that have an <u>option or bequest value</u></p> <p><i>Example: changes caused by non-native organisms to ecosystems designated as wilderness areas, habitats of endangered species etc.</i></p>
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ANNEX VI EU Biogeographic Regions and MSFD Subregions

See <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2> ,
http://ec.europa.eu/environment/nature/natura2000/biogeog_regions/

and

<https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions-1/technical-document/pdf>



ANNEX VII Delegated Regulation (EU) 2018/968 of 30 April 2018

see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>

ANNEX VIII Projection of environmental suitability for sika deer *Cervus nippon* establishment in Europe

Björn Beckmann, Riccardo Scalera, Tim Adriaens, Wolfgang Rabitsch and Dan Chapman

31 May 2021

Aim

To project the suitability for potential establishment of *Cervus nippon* in Europe, under current and predicted future climatic conditions.

Data for modelling

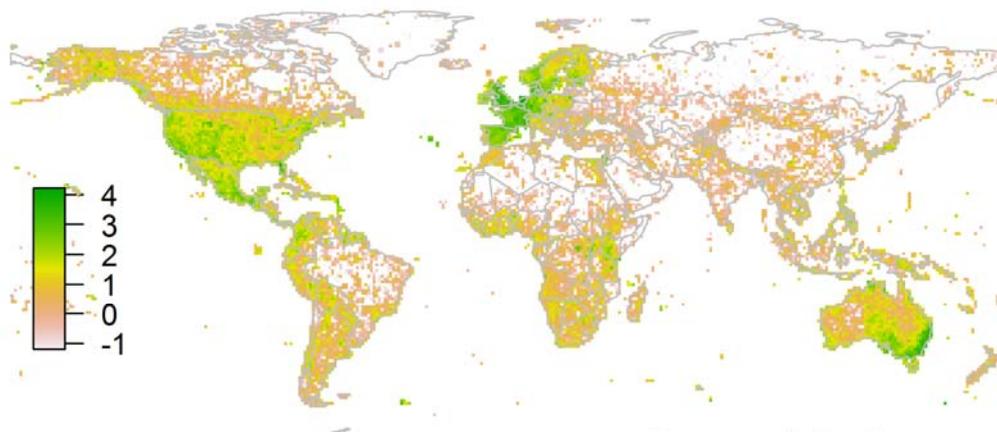
Species occurrence data were obtained from the Global Biodiversity Information Facility (GBIF) (5114 records), iNaturalist (793 records), the Biodiversity Information Serving Our Nation database (BISON) (31 records), the Atlas of Living Australia (14 records), and additional records from the risk assessment team. We scrutinised occurrence records from regions where the species is not known to be established and removed any dubious records or where the georeferencing was too imprecise (e.g. records referenced to a country or island centroid) or outside of the coverage of the predictor layers (e.g. small island or coastal occurrences). We removed records dated pre-1950, as these might refer to populations no longer in existence. The remaining records were gridded at a 0.25 x 0.25 degree resolution for modelling, yielding 550 grid cells with occurrences (Figure 1a). As a proxy for recording effort, the density of Mammalia records held by GBIF was also compiled on the same grid (Figure 1b).

Figure 1. (a) Occurrence records obtained for *Cervus nippon* and used in the modelling, showing native and invaded distributions. (b) The recording density of Mammalia on GBIF, which was used as a proxy for recording effort.

(a) Species distribution used in modelling



(b) Estimated recording effort (log10-scaled)

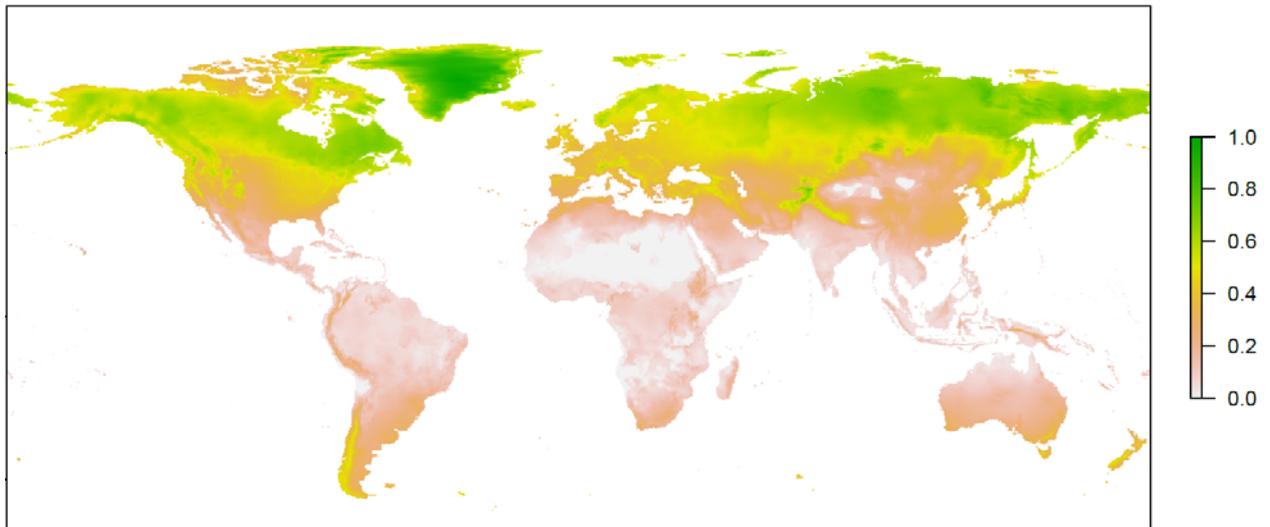


Climate data were selected from the 'Bioclim' variables contained within the WorldClim database (Hijmans et al., 2005), originally at 5 arcminute resolution (0.083 x 0.083 degrees of longitude/latitude) and aggregated to a 0.25 x 0.25 degree grid for use in the model.

Based on the biology of *Cervus nippon*, the following climate variables were used in the modelling:

- Minimum temperature of the coldest month (Bio6)
- Mean temperature of the warmest quarter (Bio10)
- Annual precipitation (Bio12)
- As a proxy for potential depth and duration of snow cover (Snow), the inverse of the mean temperature of the coldest quarter (Bio11) was multiplied with mean precipitation of the coldest quarter (Bio19), to produce a number that is greatest in areas where winters are both cold and have high precipitation. The numbers were scaled to values between 0 and 1 (Fig. 2).

Figure 2. Proxy for potential snow cover (Snow): the inverse of the mean temperature of the coldest quarter (Bio11) was multiplied with mean precipitation of the coldest quarter (Bio19), to produce a number that is greatest in areas where winters are both cold and have high precipitation. The numbers were scaled to values between 0 and 1.



To estimate the effect of climate change on the potential distribution, equivalent modelled future climate conditions for the 2070s under the Representative Concentration Pathways (RCP) 2.6 and 4.5 were also obtained. These represent low and medium emissions scenarios, respectively. The above variables were obtained as averages of outputs of eight Global Climate Models (BCC-CSM1-1, CCSM4, GISS-E2-R, HadGEM2-AO, IPSL-CM5A-LR, MIROC-ESM, MRI-CGCM3, NorESM1-M), downscaled and calibrated against the WorldClim baseline (see http://www.worldclim.org/cmip5_5m).

The following habitat layers were also used:

- Tree cover (Tree): This was estimated from the MODerate-resolution Imaging Spectroradiometer (MODIS) satellite continuous tree cover raster product, produced by the Global Land Cover Facility (<http://glcf.umd.edu/data/vcf/>). The raw product contains the percentage cover by trees in each 0.002083 x 0.002083 degree grid cell. We aggregated this to the mean cover in our 0.25 x 0.25 degree grid cells.

Species distribution model

A presence-background (presence-only) ensemble modelling strategy was employed using the BIOMOD2 R package version 3.4.6 (Thuiller et al., 2020, Thuiller et al., 2009). These models contrast the environment at the species' occurrence locations against a random sample of the global background environmental conditions (often termed 'pseudo-absences') in order to characterise and project suitability for occurrence. This approach has been developed for distributions that are in equilibrium with the environment. Because invasive species' distributions are not at equilibrium and subject to dispersal constraints at a global scale, we took care to minimise the inclusion of locations suitable for the species but where it has not been able to disperse to (Chapman et al. 2019). Therefore the background sampling region included:

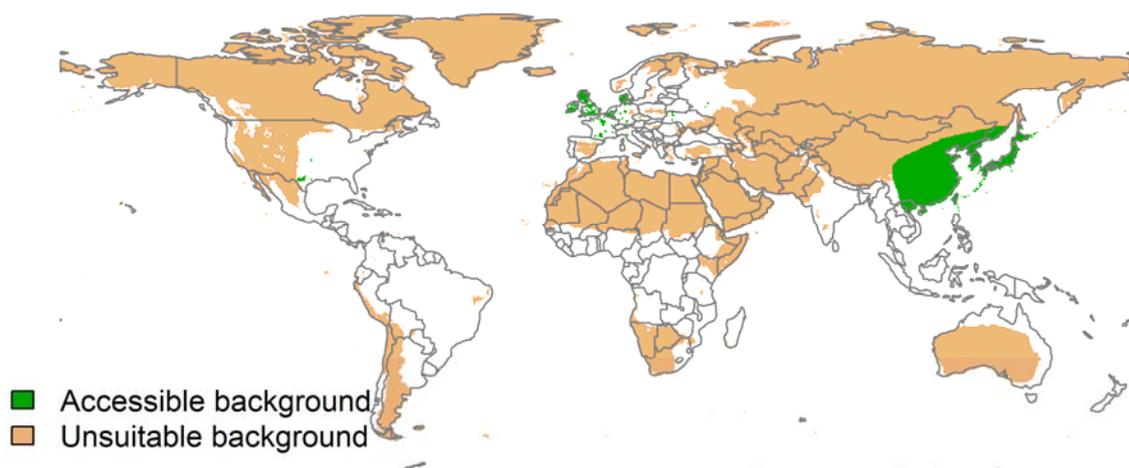
- The area accessible by native *Cervus nippon* populations, in which the species is likely to have had sufficient time to disperse to all locations. Based on presumed maximum dispersal distances, the accessible region was defined as a 300km buffer around the native range occurrences; AND

- A 30km buffer around the non-native occurrences, encompassing regions likely to have had high propagule pressure for introduction by humans and/or dispersal of the species; AND
- Regions where we have an *a priori* expectation of high unsuitability for the species so that absence is assumed irrespective of dispersal constraints (see Figure 2). The following rules were applied to define a region expected to be highly unsuitable for *Cervus nippon* at the spatial scale of the model:
 - Minimum temperature of the coldest month (Bio6) < -18°C
 - Mean temperature of the warmest quarter (Bio10) < 9°C
 - Annual precipitation (Bio12) < 545mm
 - Proxy for potential snow cover (Snow) > 0.6

Altogether, only 1.1% of occurrence grid cells were located in the unsuitable background region.

Within the unsuitable background region, 10 samples of 5000 randomly sampled grid cells were obtained. In the accessible background (comprising the accessible areas around native and non-native occurrences as detailed above), the same number of pseudo-absence samples were drawn as there were presence records (550), weighting the sampling by a proxy for recording effort (Figure 2).

Figure 2. The background from which pseudo-absence samples were taken in the modelling of *Cervus nippon*. Samples were taken from a 300km buffer around the native range and a 30km buffer around non-native occurrences (together forming the accessible background), and from areas expected to be highly unsuitable for the species (the unsuitable background region). Samples from the accessible background were weighted by a proxy for recording effort (Figure 1(b)).



Each dataset (i.e. combination of the presences and the individual background samples) was randomly split into 80% for model training and 20% for model evaluation. With each training dataset, seven statistical algorithms were fitted with the default BIOMOD2 settings and rescaled using logistic regression, except where specified below:

- Generalised linear model (GLM)

- Generalised boosting model (GBM)
- Generalised additive model (GAM) with a maximum of four degrees of freedom per smoothing spline
- Artificial neural network (ANN)
- Multivariate adaptive regression splines (MARS)
- Random forest (RF)
- Maxent

Since the total background sample was larger than the number of occurrences, prevalence fitting weights were applied to give equal overall importance to the occurrences and the background. Normalised variable importance was assessed and variable response functions were produced using BIOMOD2's default procedure.

Model predictive performance was assessed by the following three measures:

- AUC, the area under the receiver operating characteristic curve (Fielding & Bell 1997). Predictions of presence-absence models can be compared with a subset of records set aside for model evaluation (here 20%) by constructing a confusion matrix with the number of true positive, false positive, false negative and true negative cases. For models generating non-dichotomous scores (as here) a threshold can be applied to transform the scores into a dichotomous set of presence-absence predictions. Two measures that can be derived from the confusion matrix are sensitivity (the proportion of observed presences that are predicted as such, quantifying omission errors), and specificity (the proportion of observed absences that are predicted as such, quantifying commission errors). A receiver operating characteristic (ROC) curve can be constructed by using all possible thresholds to classify the scores into confusion matrices, obtaining sensitivity and specificity for each matrix, and plotting sensitivity against the corresponding proportion of false positives (equal to $1 - \text{specificity}$). The use of all possible thresholds avoids the need for a selection of a single threshold, which is often arbitrary, and allows appreciation of the trade-off between sensitivity and specificity. The area under the ROC curve (AUC) is often used as a single threshold-independent measure for model performance (Manel, Williams & Ormerod 2001). AUC is the probability that a randomly selected presence has a higher model-predicted suitability than a randomly selected absence (Allouche et al. 2006).
- Cohen's Kappa (Cohen 1960). This measure corrects the overall accuracy of model predictions (ratio of the sum of true presences plus true absences to the total number of records) by the accuracy expected to occur by chance. The kappa statistic ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random. Advantages of kappa are its simplicity, the fact that both commission and omission errors are accounted for in one parameter, and its relative tolerance to zero values in the confusion matrix (Manel, Williams & Ormerod 2001). However, Kappa has been criticised for being sensitive to prevalence (the proportion of sites in which the species was recorded as present) and may therefore be inappropriate for comparisons of model accuracy between species or regions (McPherson, Jetz & Rogers 2004, Allouche et al. 2006).
- TSS, the true skill statistic (Allouche et al. 2006). TSS is defined as sensitivity + specificity - 1, and corrects for Kappa's dependency on prevalence. TSS compares the

number of correct forecasts, minus those attributable to random guessing, to that of a hypothetical set of perfect forecasts. Like kappa, TSS takes into account both omission and commission errors, and success as a result of random guessing, and ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random (Allouche et al. 2006).

An ensemble model was created by first rejecting poorly performing algorithms with relatively extreme low AUC values and then averaging the predictions of the remaining algorithms, weighted by their AUC. To identify poorly performing algorithms, AUC values were converted into modified z-scores based on their difference to the median and the median absolute deviation across all algorithms (Iglewicz & Hoaglin 1993). Algorithms with $z < -2$ were rejected. In this way, ensemble projections were made for each dataset and then averaged to give an overall suitability, as well as its standard deviation.

Projections were classified into suitable and unsuitable regions using a “lowest presence threshold” (Pearson et al. 2007), setting the cut-off as the lowest value at which 98% of all presence records are classified correctly under the current climate (here 0.64). In order to express the sensitivity of classifications to the choice of this threshold, thresholds at which 95% and 99% of records are classified correctly (here 0.73 and 0.49 respectively) were used in the calculation of error bars in Figs. 9 and 10 below in addition to taking account of uncertainty in the projections themselves.

We also produced a limiting factor map for Europe following Elith et al. (2010). For this, projections were made separately with each individual variable fixed at a near-optimal value. These were chosen as the median values at the occurrence grid cells. Then, the most strongly limiting factors were identified as the ones resulting in the highest increase in suitability in each grid cell.

Results

The ensemble model suggested that suitability for *Cervus nippon* was most strongly determined by Annual precipitation (Bio12), accounting for 53.8% of variation explained, followed by Minimum temperature of the coldest month (Bio6) (21.3%), Proxy for potential snow cover (Snow) (12.3%), Mean temperature of the warmest quarter (Bio10) (11.4%) and Global tree cover (Tree) (1.2%) (Table 1, Figure 3).

Table 1. Summary of the cross-validation predictive performance (AUC, Kappa, TSS) and variable importance of the fitted model algorithms and the ensemble (AUC-weighted average of the best performing algorithms). Results are the average from models fitted to 10 different background samples of the data.

Algorithm	AUC	Kappa	TSS	Used in the ensemble	Variable importance (%)				
					Annual precipitation (Bio12)	Minimum temperature of the coldest month (Bio6)	Proxy for potential snow cover (Snow)	Mean temperature of the warmest quarter (Bio10)	Global tree cover (Tree)
GLM	0.958	0.611	0.887	yes	56	15	15	12	1
GAM	0.961	0.618	0.890	yes	50	19	18	12	0
GBM	0.957	0.611	0.887	yes	58	25	3	13	0
ANN	0.960	0.608	0.882	yes	50	26	8	13	2
MARS	0.954	0.600	0.883	yes	60	19	17	4	0
RF	0.941	0.592	0.883	no	59	19	7	10	5
Maxent	0.955	0.611	0.881	yes	48	23	13	13	3
Ensemble	0.961	0.615	0.890		54	21	12	11	1

Figure 3. Partial response plots from the fitted models. Thin coloured lines show responses from the algorithms in the ensemble, while the thick black line is their ensemble. In each plot, other model variables are held at their median value in the training data. Some of the divergence among algorithms is because of their different treatment of interactions among variables.

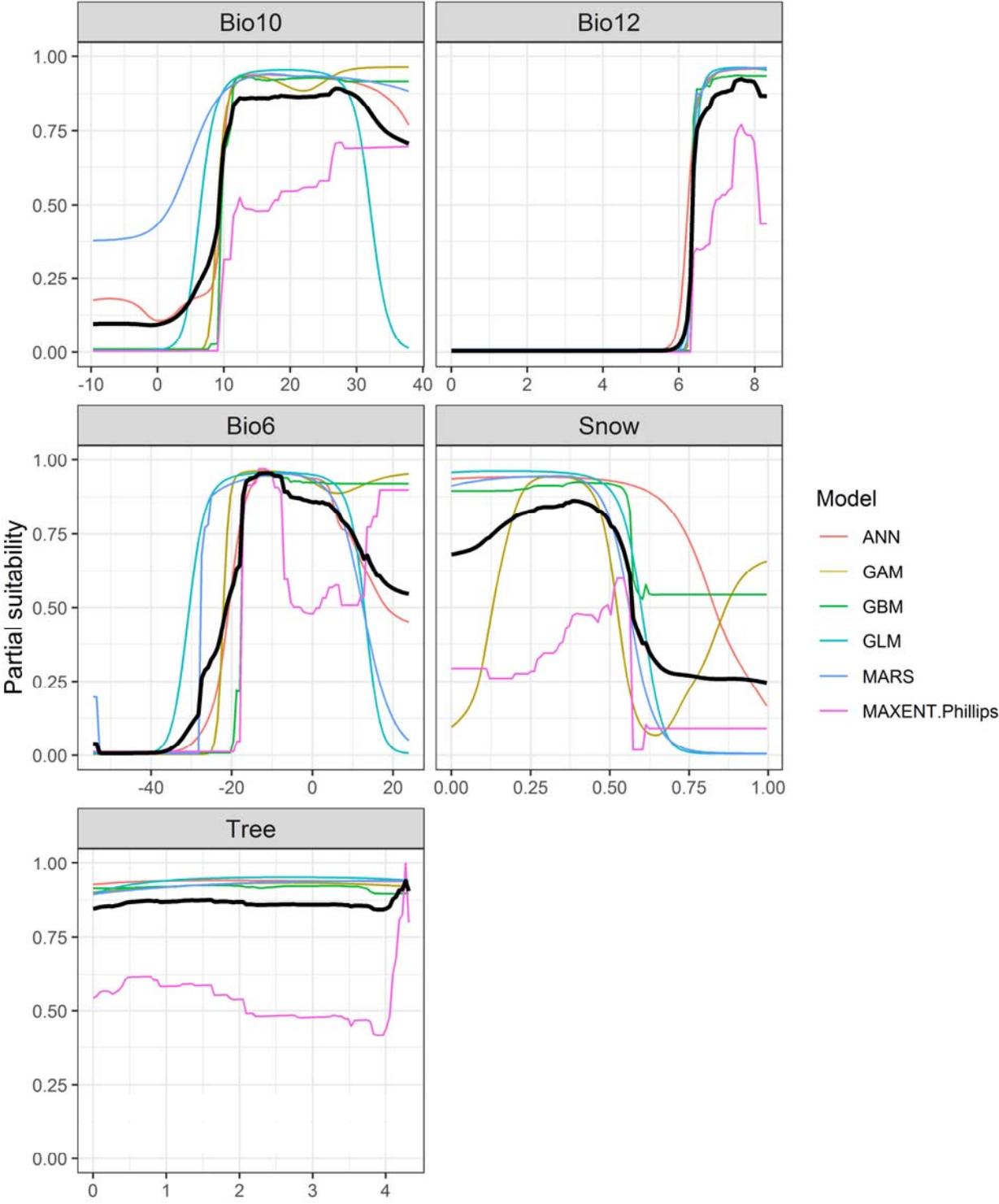
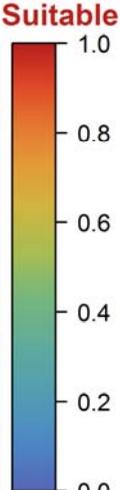
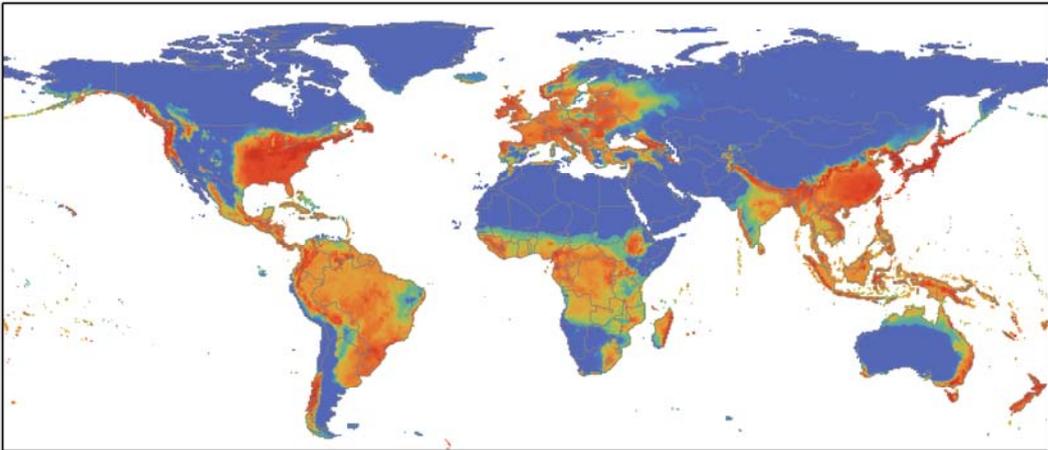


Figure 4. (a) Projected global suitability for *Cervus nippon* establishment in the current climate. For visualisation, the projection has been aggregated to a 0.5 x 0.5 degree resolution, by taking the maximum suitability of constituent higher resolution grid cells. Values > 0.64 are suitable for the species, with 98% of global presence records above this threshold. Values below 0.64 indicate lower relative suitability. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

(a) Projected suitability



(b) Standard deviation in projected suitability

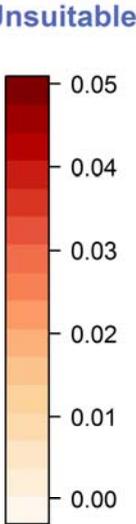
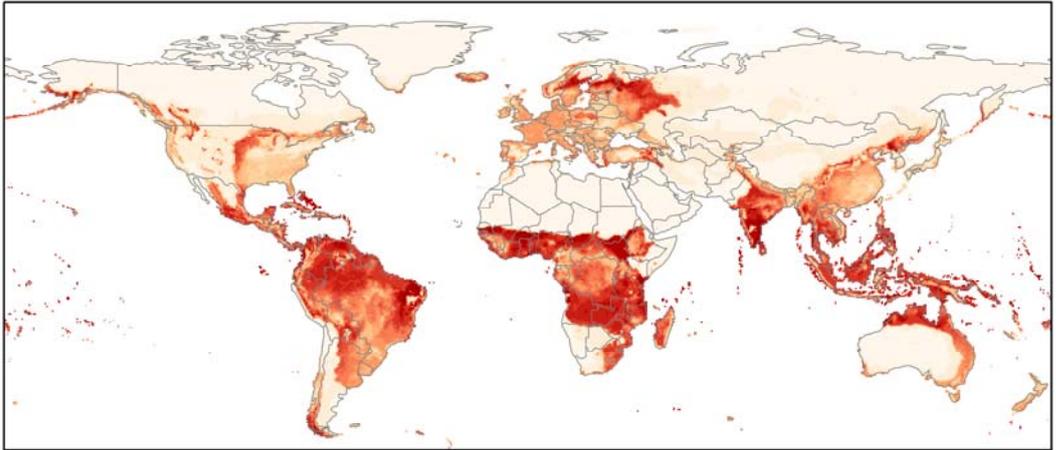


Figure 5. (a) Projected current suitability for *Cervus nippon* establishment in Europe and the Mediterranean region. Values > 0.64 are suitable for the species, with 98% of global presence records above this threshold. Values below 0.64 indicate lower relative suitability. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

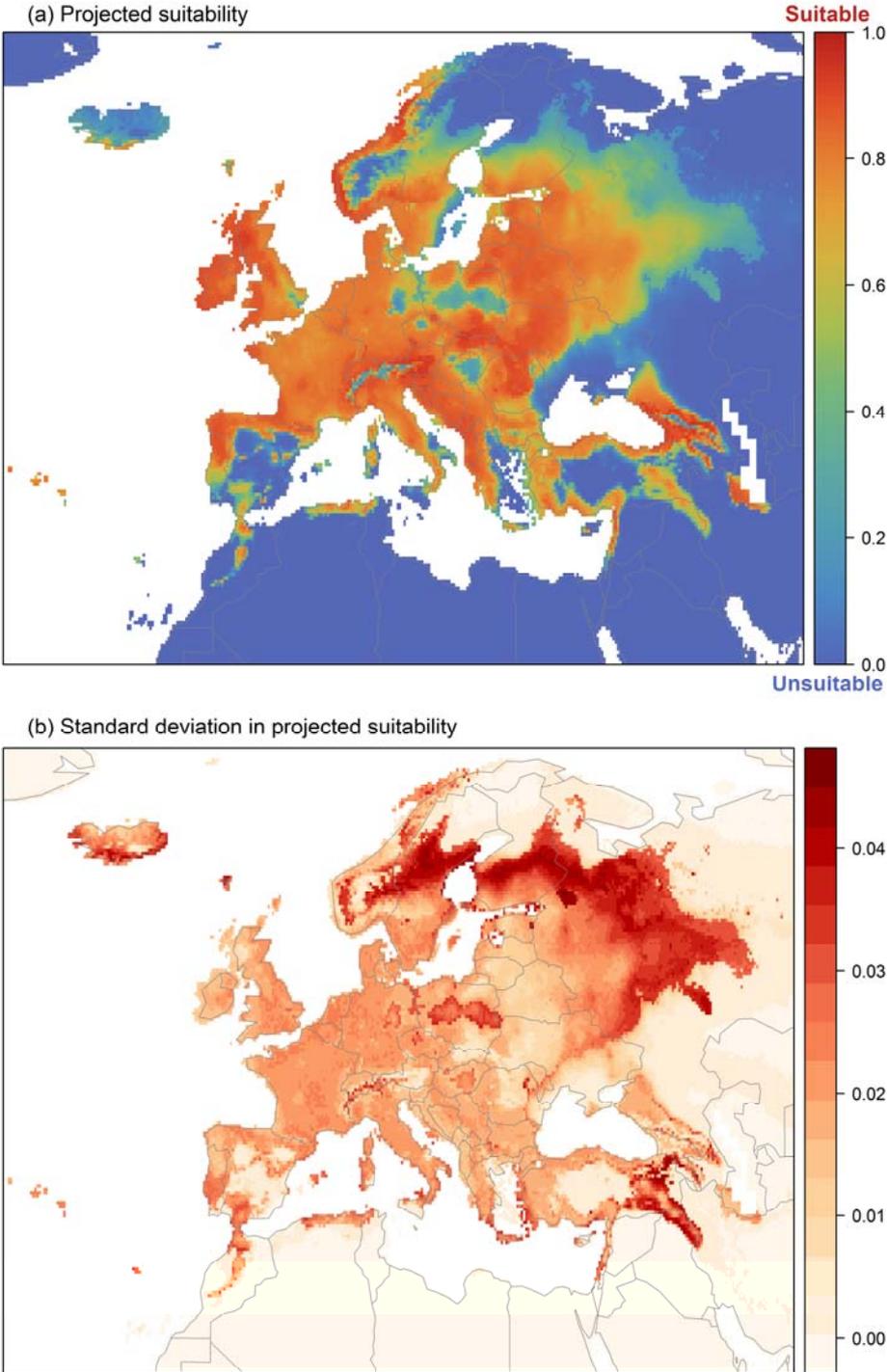


Figure 6. The most strongly limiting factors for *Cervus nippon* establishment estimated by the model in Europe and the Mediterranean region in current climatic conditions.

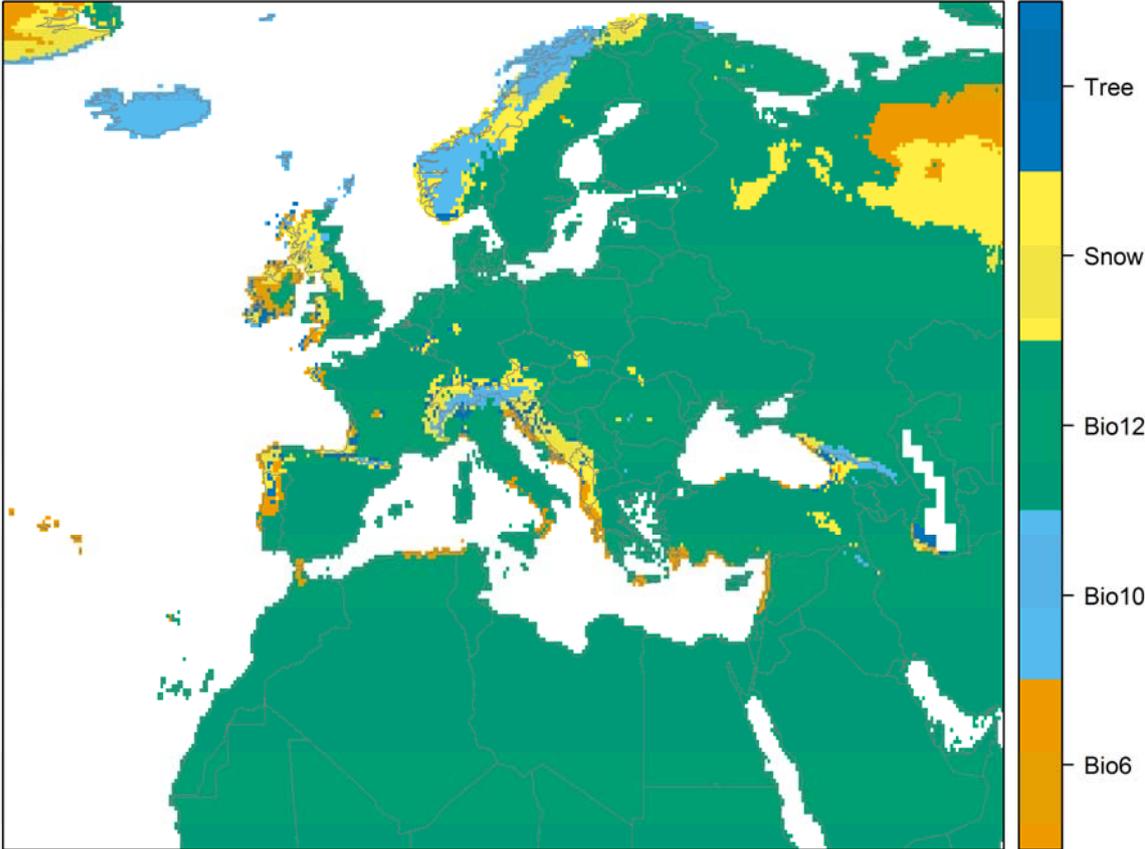


Figure 7. (a) Projected suitability for *Cervus nippon* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP2.6. Values > 0.64 are suitable for the species, with 98% of global presence records above this threshold under current climate. Values below 0.64 indicate lower relative suitability. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

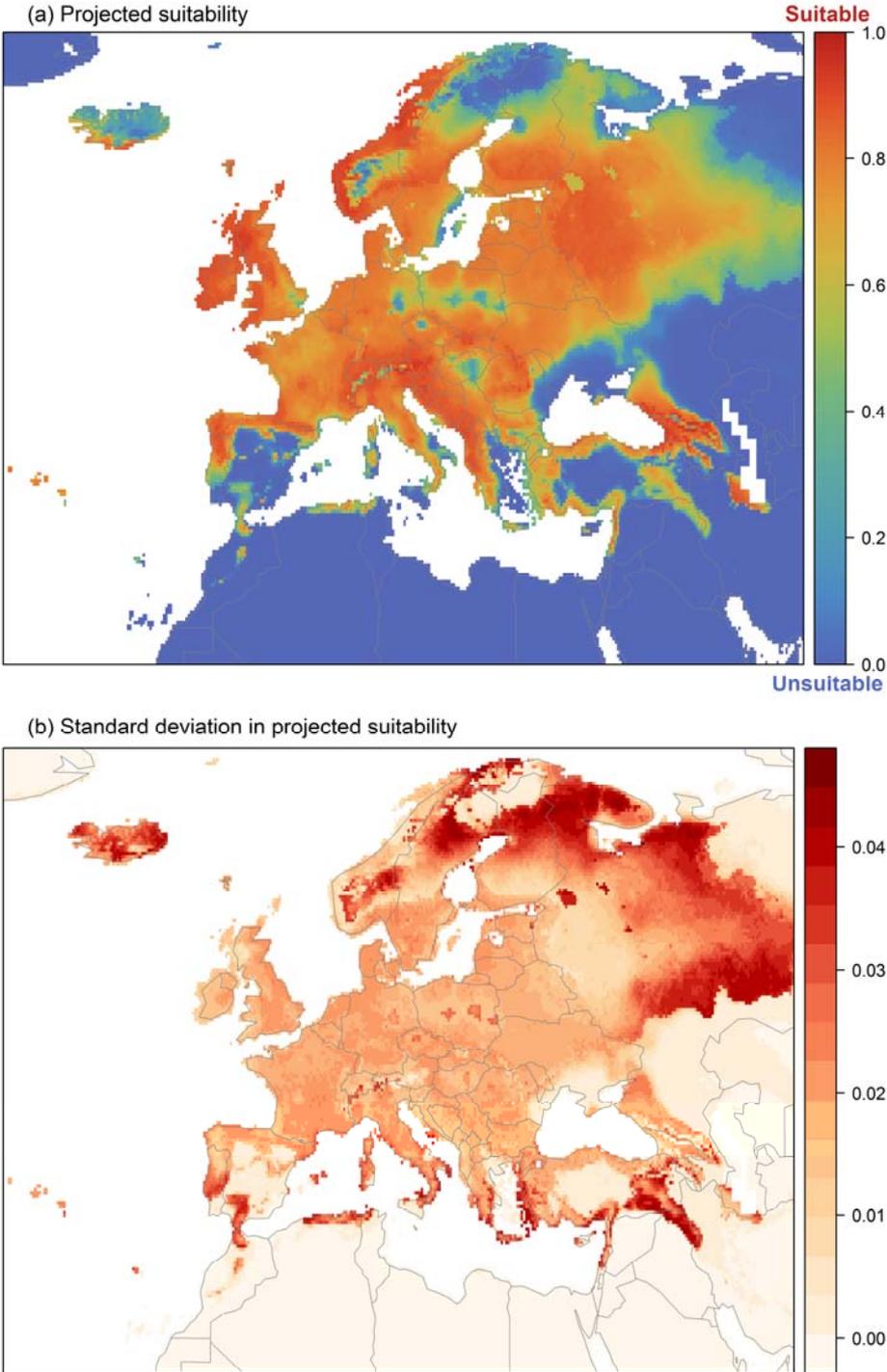


Figure 8. (a) Projected suitability for *Cervus nippon* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP4.5. Values > 0.64 are suitable for the species, with 98% of global presence records above this threshold under current climate. Values below 0.64 indicate lower relative suitability. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

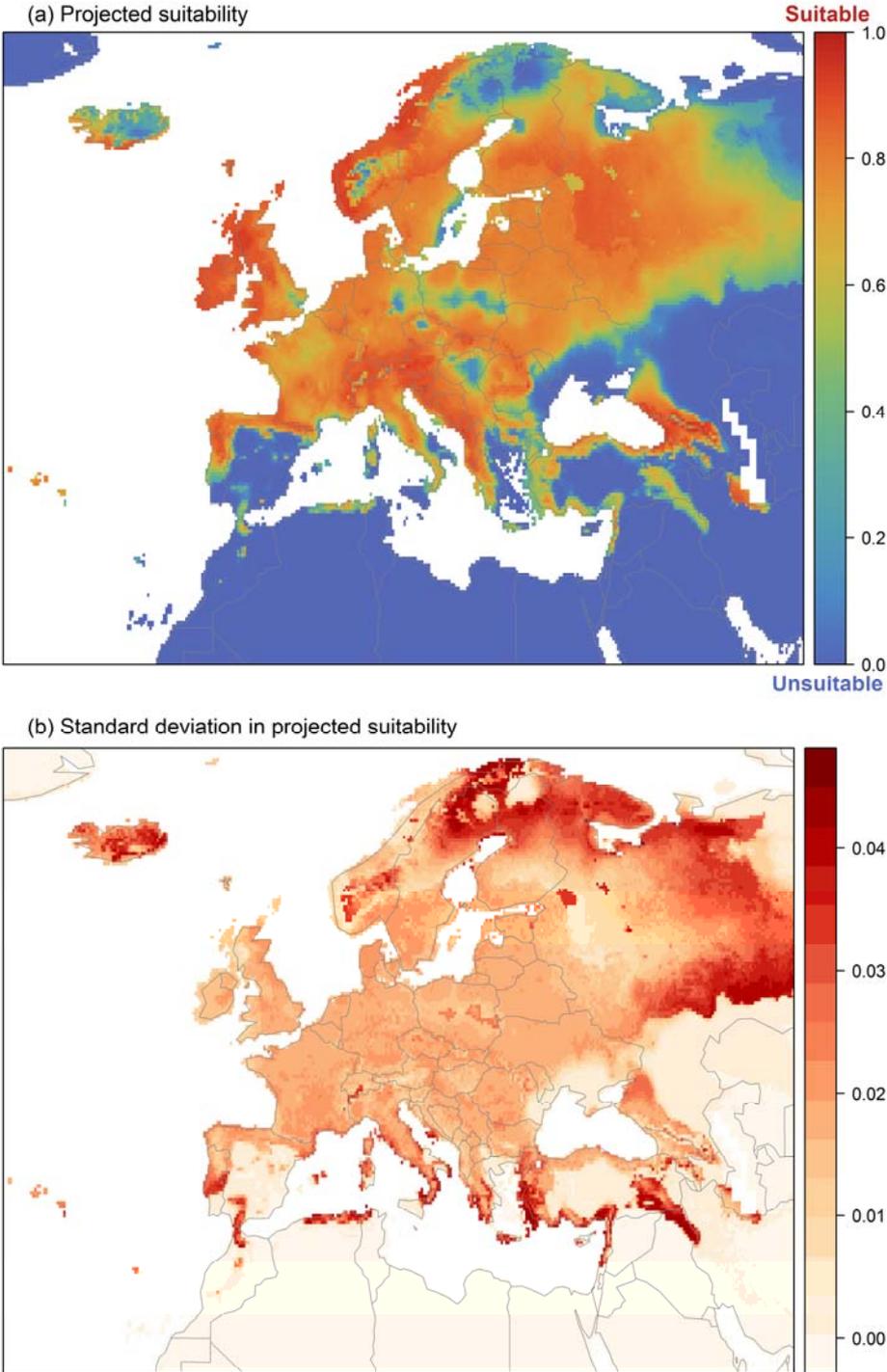


Figure 9. Variation in projected suitability for *Cervus nippon* establishment among Biogeographical Regions of Europe (<https://www.eea.europa.eu/data-and-maps/data/biogeographical-regions-europe-3>). The bar plots show the proportion of grid cells in each region classified as suitable (with values > 0.64) in the current climate and projected climate for the 2070s under two RCP emissions scenarios. Error bars indicate uncertainty due to both the choice of classification threshold (cf. p.6) and uncertainty in the projections themselves (cf. part (b) of Figs. 5,7,8). The location of each region is also shown. The Arctic and Macaronesian regions are not part of the study area, but are included for completeness.

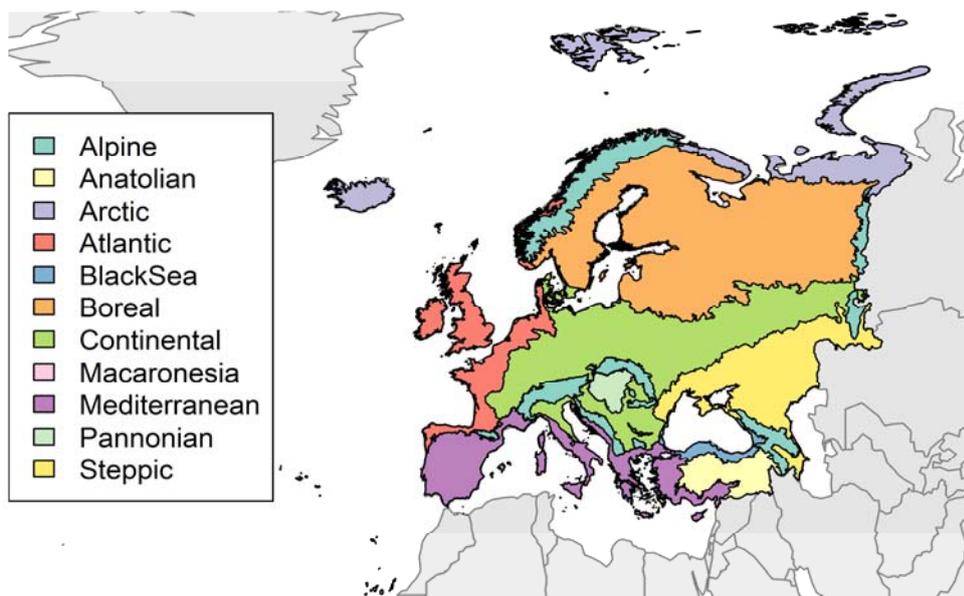
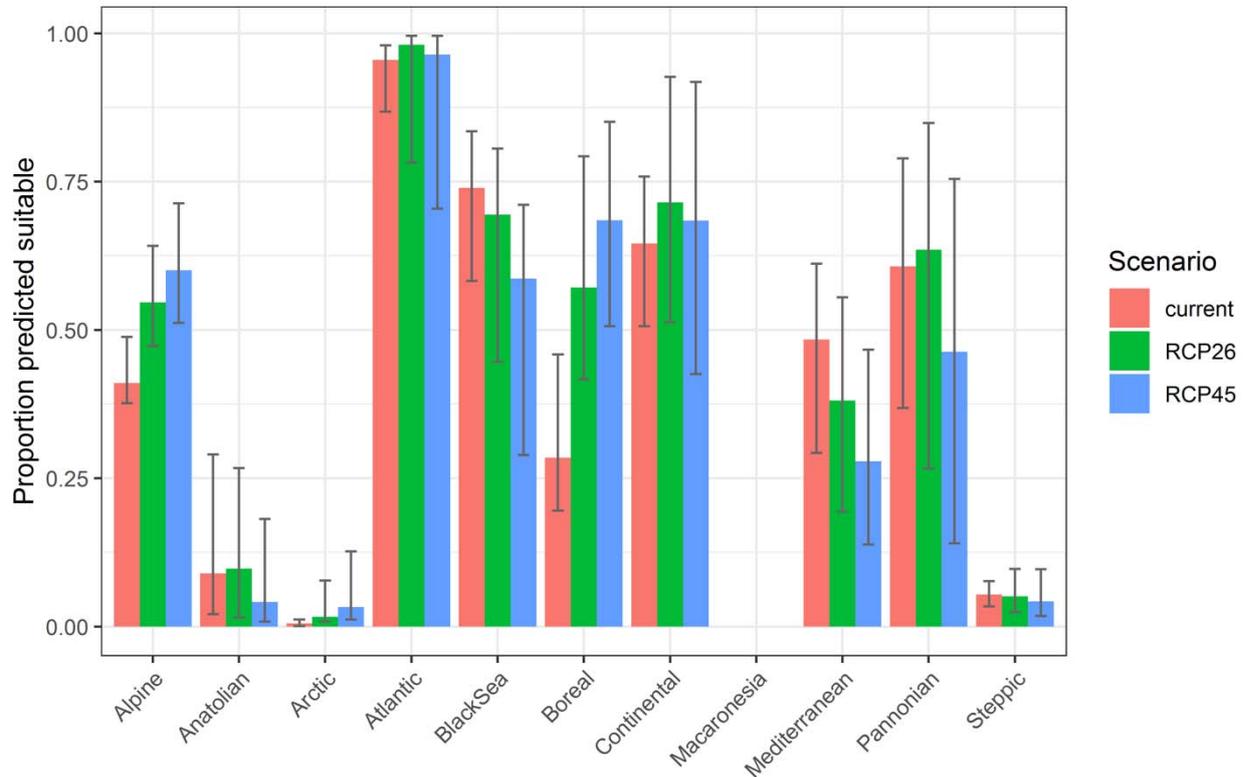


Table 2. Variation in projected suitability for *Cervus nippon* establishment among Biogeographical regions of Europe (numerical values of Figure 9 above). The numbers are the proportion of grid cells in each region classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. The Arctic and Macaronesian biogeographical regions are not part of the study area, but are included for completeness.

	current climate			2070s RCP2.6			2070s RCP4.5		
	lower	central estimate	upper	lower	central estimate	upper	lower	central estimate	upper
Alpine	0.38	0.41	0.49	0.47	0.55	0.64	0.51	0.60	0.71
Anatolian	0.02	0.09	0.29	0.02	0.10	0.27	0.01	0.04	0.18
Arctic	0.00	0.01	0.01	0.01	0.02	0.08	0.01	0.03	0.13
Atlantic	0.87	0.96	0.98	0.78	0.98	1.00	0.70	0.96	1.00
BlackSea	0.58	0.74	0.83	0.45	0.69	0.81	0.29	0.59	0.71
Boreal	0.20	0.28	0.46	0.42	0.57	0.79	0.51	0.68	0.85
Continental	0.51	0.65	0.76	0.51	0.71	0.93	0.43	0.68	0.92
Macaronesia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mediterranean	0.29	0.48	0.61	0.19	0.38	0.56	0.14	0.28	0.47
Pannonian	0.37	0.61	0.79	0.27	0.64	0.85	0.14	0.46	0.75
Steppic	0.03	0.05	0.08	0.02	0.05	0.10	0.02	0.04	0.10

Figure 10. Variation in projected suitability for *Cervus nippon* establishment among European Union countries and the UK. The bar plots show the proportion of grid cells in each country classified as suitable (with values > 0.64) in the current climate and projected climate for the 2070s under two RCP emissions scenarios. Error bars indicate uncertainty due to both the choice of classification threshold (cf. p.6) and uncertainty in the projections themselves (cf. part (b) of Figs. 5,7,8).

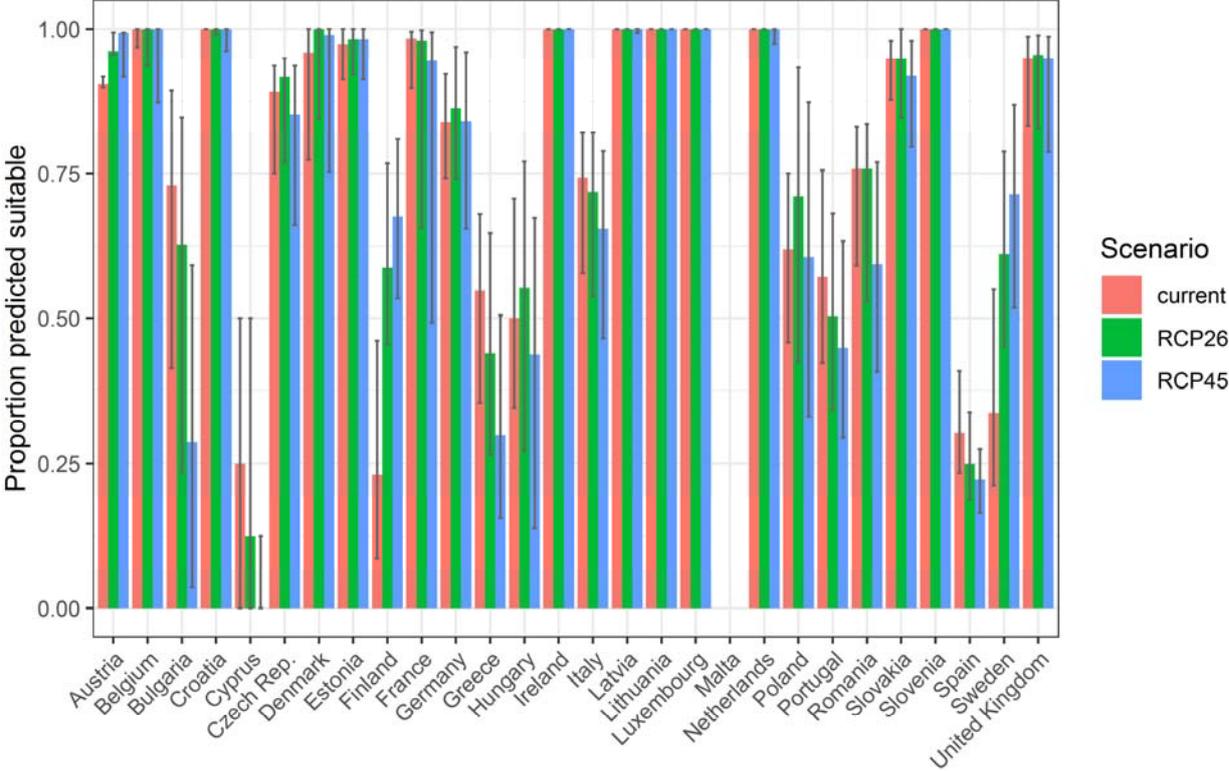


Table 3. Variation in projected suitability for *Cervus nippon* establishment among European Union countries and the UK (numerical values of Figure 10 above). The numbers are the proportion of grid cells in each country classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios.

	current climate			2070s RCP2.6			2070s RCP4.5		
	lower	central estimate	upper	lower	central estimate	upper	lower	central estimate	upper
Austria	0.90	0.90	0.92	0.96	0.96	0.99	0.92	0.99	0.99
Belgium	0.97	1.00	1.00	0.94	1.00	1.00	0.87	1.00	1.00
Bulgaria	0.41	0.73	0.89	0.23	0.63	0.85	0.04	0.29	0.59
Croatia	1.00	1.00	1.00	0.99	1.00	1.00	0.96	1.00	1.00
Cyprus	0.00	0.25	0.50	0.00	0.12	0.50	0.00	0.00	0.12
Czech Rep.	0.75	0.89	0.94	0.77	0.92	0.95	0.66	0.85	0.94
Denmark	0.77	0.96	1.00	0.85	1.00	1.00	0.75	0.99	1.00
Estonia	0.91	0.97	1.00	0.92	0.98	1.00	0.91	0.98	1.00
Finland	0.09	0.23	0.46	0.46	0.59	0.77	0.53	0.68	0.81
France	0.90	0.98	1.00	0.66	0.98	1.00	0.49	0.95	0.99
Germany	0.74	0.84	0.92	0.74	0.86	0.97	0.65	0.84	0.96
Greece	0.35	0.55	0.68	0.26	0.44	0.65	0.16	0.30	0.50
Hungary	0.34	0.50	0.71	0.27	0.55	0.77	0.14	0.44	0.67
Ireland	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Italy	0.58	0.74	0.82	0.54	0.72	0.82	0.47	0.65	0.79
Latvia	1.00	1.00	1.00	1.00	1.00	1.00	0.99	1.00	1.00
Lithuania	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Luxembourg	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Malta	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Netherlands	1.00	1.00	1.00	1.00	1.00	1.00	0.97	1.00	1.00
Poland	0.46	0.62	0.75	0.42	0.71	0.93	0.33	0.61	0.87
Portugal	0.42	0.57	0.76	0.34	0.50	0.68	0.29	0.45	0.63
Romania	0.59	0.76	0.83	0.53	0.76	0.84	0.41	0.59	0.77
Slovakia	0.88	0.95	0.98	0.85	0.95	1.00	0.80	0.92	0.98
Slovenia	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Spain	0.23	0.30	0.41	0.19	0.25	0.34	0.16	0.22	0.27
Sweden	0.21	0.34	0.55	0.45	0.61	0.79	0.52	0.71	0.87
UK	0.83	0.95	0.99	0.83	0.95	0.99	0.79	0.95	0.99

Caveats to the modelling

To remove spatial recording biases, the selection of the background sample from the accessible background was weighted by the density of Mammalia records on the Global Biodiversity Information Facility (GBIF). While this is preferable to not accounting for recording bias at all, it may not provide the perfect measure of recording bias.

There was substantial variation among modelling algorithms in the partial response plots (Figure 3). In part this will reflect their different treatment of interactions among variables. Since partial plots are made with other variables held at their median, there may be values of a particular variable at which this does not provide a realistic combination of variables to predict from.

Other variables potentially affecting the distribution of the species, such as types of land cover other than trees were not included in the model.

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Annex with evidence on measures and their implementation cost and cost-effectiveness

Species (scientific name)	<i>Cervus nippon</i>
Species (common name)	Sika Deer
Author(s)	Peter Robertson
Reviewer	Alastair Ward, Wojciech Solarz
Date Completed	15/10/2021

Summary¹

Highlight of measures that provide the most cost-effective options to prevent the introduction, achieve early detection, rapidly eradicate and manage the species, including significant gaps in information or knowledge to identify cost-effective measures.

Sika deer have been widely introduced globally through escapes from ornamental collections, deer farms and releases to supplement hunting. The adoption and enforcement of appropriate legislation and codes of best practice to reduce the risks posed by these pathways should reduce the probability of further introductions in Europe. This can be assisted by raising public awareness of the impacts of non-native deer and invasive alien species in general. The effective reporting of new incursions, with records obtained from citizen science initiatives and through existing hunting networks, will reduce the costs of rapid response to new incursions. However, Sika deer are now present in many different European countries including Austria, Belgium, Denmark, Estonia, Finland, France, Germany, Ireland, Latvia, Lithuania, Netherlands, Norway, Poland, Russia, Romania, Sweden, Switzerland, and the United Kingdom. The further spread of these widely established species within Europe probably poses a greater threat than further introductions from elsewhere.

Shooting with a rifle is the most widely used and cost-effective method used to control deer, including Sika. There is little specific evidence available on the eradication of Sika deer, although there are current plans for its eradication from a forested region in New Zealand.

However, there are published examples of the successful eradication of other *Cervus* species from areas of up to 222km² in size. More widely, eradication programmes for other deer species and ungulates have relied on shooting and have been successful over large areas of up to 6,000km². Globally, the cost-effectiveness of ungulate eradications has been improved through the use of helicopter shooting, judas animals to locate the last remaining individuals in the controlled populations, night shooting combined with infra-red cameras, fences and natural barriers to break larger areas into smaller components, and dogs to help locate animals. Acoustic sensors to detect the calls of particular species may also have applications. However, the use of these methods may be restricted in particular European member states and may attract opposition from the public and hunters on a case specific basis. Traps and toxins have been used as supporting methods in some eradication programmes. There are no toxins approved for use on deer in the EU.

The long-term management of deer populations is widespread in Europe, with established hunting traditions which value deer as a resource, and programmes to reduce impacts, such as local damage to crops and forestry, to acceptable levels. Current deer management practice relies on shooting and this is the primary method used to manage Sika deer. The potential use of contraceptives as a deer management tool attracts frequent public interest, but current approaches rely on catch and inject methods. There are no examples of contraceptives being used to achieve eradication, and only a small number of limited examples of their use on free-living populations of other deer. The use of fences, scaring methods and deterrents provide useful additional methods to reduce local damage.

One particular concern associated with introduced populations of Sika deer is hybridisation with native *Cervus* species, in particular Red Deer *Cervus elaphus*. The presence of hybrids complicates the prospects for management and programmes to support the selective culling of animals with hybrid characteristics may go some way to reducing rates of gene flow into the native populations. However, the frequency of hybridisation is highly variable between different populations, while many hybrids cannot reliably be determined from external examination in the field, reducing the effectiveness of selective culling. To date there are no effective methods available to prevent hybrids from an established stock eventually spreading and their genes introgressing into neighbouring red deer populations.

Detailed assessment			
	Description of measures ²	Assessment of implementation cost and cost-effectiveness (per measure) ³	Level of confidence ⁴
Methods to achieve prevention of intentional and unintentional introduction⁵	Sika deer have been introduced to numerous Member States, primarily as an ornamental species from collections or escapes from deer farms, or as an addition for hunting. The adoption and enforcement of appropriate legislation and codes of best practice to reduce the risks posed by these pathways should reduce the probability of further introductions.	The enforcement of existing legislation to limit the introduction of this species will reduce the risk of further introductions into the EU. The costs of current enforcement measures are not known, However, the risk from new introductions must be considered in light of the already widespread distribution of this species in Europe.	High
	Managing pathways	Managing pathways	High

	<p>Spread from these initial introductions has been through a combination of natural dispersal, possibly assisted by the relocation of individuals by hunters, although there is little documented evidence.</p>	<p>Public education of the risks posed by this species together with the enforcement of existing legislation to limit the illegal transport of animals or relocations should help limit this risk. Natural spread from the existing populations is probably now the main pathway of spread</p>	
	<p>Raising awareness Raising public awareness of the risks posed by invasive alien species in general and Sika deer in particular can reduce the risks of further intentional spread and increase support for control. This can include the production of targeted publicity and identification material.</p>	<p>Raising awareness A number of information sources and sheets are available on-line which can assist with the production of new material to raise public awareness. Examples specific to Sika deer include: British Deer Society https://www.bds.org.uk/wp-content/uploads/2020/12/Sika-Deer_web.pdf Scottish Natural Heritage (2018) https://www.bestpracticeguides.org.uk/wp-content/downloads/ecology_sika.pdf Deer hunting and woodland conservation organisations exist in most European countries and provide a route for targeted information dissemination</p>	High
<p>Methods to achieve early detection⁶</p>	<p>Effective surveillance and reporting of new incursions Sika deer are a distinctive species. Encouraging rapid reporting of new incursions increases the likely success of rapid response before the species can become established. Citizen science provides a useful route for the reporting of IAS.</p>	<p>Effective surveillance and reporting of new incursions Citizen-science species occurrence datasets are increasingly recognized as a useful tool for monitoring the occurrence and spread of invasive alien species across large spatial and temporal scale. They are dependent on citizen-scientists who collect and upload data, typically from 'opportunistic sampling' with no underlying scientific survey design which can limit the conclusions that can be drawn from these data. Most parts of north-west Europe have an extensive network of volunteer observers although this is less true of</p>	High

	<p>Deer are widely hunted and reports from the hunting community may be of particular value to detect new species in an area.</p> <p>The presence of native <i>Cervus</i> species and the possibility of hybrids may complicate the identification of Sika.</p>	<p>southern and especially eastern Europe. Nevertheless, the focus on native species may lead to disregard the presence of non-native species, and consequently to a delay in detecting a new presence of this non-native species. However, this naturalist community also provides an opportunity for developing an effective surveillance system. Unstructured citizen-science data do not reliably allow the estimation of species abundance or population trends, yet in an early-warning scenario it is likely sufficient to know where a species is present and these data limitations are thus of a lesser concern.</p> <p>For deer, reports from hunters can be an effective source of information on occurrence and distribution. Hunter records are often collated nationally to produce statistics on key species (Apollonio et al. 2010) although the methods and formats used vary between Member States. Updating this assessment and increasing standardisation of reporting would improve the usefulness of this approach.</p> <p>Sika deer are broadly similar in appearance to native red deer and the two species may be confused. Hybrids regularly occur in some parts of the risk assessment area with variable mixtures of characteristics from the two species. Many hybrids cannot reliably be determined as such from external examination in the field and genetic methods may be required (Senn and Pemberton 2009). Information of species identification is available through Wildlife On-Line</p> <p>https://www.wildlifeonline.me.uk/animals/article/red-deer-genetic-diversity-hybridization</p>	
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<p>Methods to achieve eradication⁷</p>	<p>Overview of Eradication Programmes</p> <p>No accounts of the successful eradication of Sika deer have been reported. However, a proposed plan to eradicate them from a wooded area in New Zealand has been published (Latham 2020) and there are accounts of the successful eradication of other <i>Cervus</i> species, together with wider experience with other deer and ungulates.</p>	<p>There is only one reported eradication programme for Sika deer, work currently under consideration in New Zealand. Sika deer in the Russell Forest in New Zealand have been controlled since they were first established, but improved land access is thought necessary if eradication is to be achieved. Shooting would provide the main control method alongside supporting methods such as the use of trained dogs, infra-red cameras, Judas animals, faecal DNA surveillance and possibly acoustic sensors (Latham 2020).</p> <p>Considering all deer species, the DIISE (2018) database includes details of 10 successful eradications from islands. All of the cases that give details refer to shooting as the primary method used. These include a number of eradications of other <i>Cervus</i> species from various sites in New Zealand including from two islands (11.7 and 1.1km²) in Preservation Inlet (Brown 2013); Anchor (11.6km²) and Secretary (79.9km²) Islands (Crouchley et al 2011); and Lake Manapouri (2.5km²) (Whitehead 2009). A further example is of the eradication of <i>Cervus timorensis</i> from a 7.9km² island in New Caledonia (Genovesi and Carnevali 2011).</p> <p>The largest reported successful deer eradications are of Mule deer <i>Odocoileus hemionus</i> and Red deer <i>Cervus elaphus</i> from the Santa Rosa Channel Island off the Californian coast, an area of 222km² (Cruz et al 2009). No information on the costs of these deer eradications are available. However, Parkes (1990) and Carrion et al. (2011) describe the costs and methods of large-scale goat eradications from islands in some detail. These include successful removals from islands of up to 6000km² at a cost</p>	<p>Medium</p> <p>Eradications of similar species have been successfully achieved on increasingly large areas of land, mostly islands, of up to 6000km². However, there are no published evidence accounts of the successful eradication of this species.</p>
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		<p>of around US\$10,500,000 (US\$1,750 per km²) at 2011 prices, including the use of aerial hunting and Judas animals (Carrion et al. 2011). The costs per unit area are known to vary in relation to the size of the area managed (Robertson et al. 2017), the costs per unit area from smaller programmes will be substantially higher.</p>	
	<p>Shooting to achieve eradication Shooting is the most widely used and effective method to control deer (Côté et al. 2004). This underpins most current management of deer populations in Europe (Apollonio et al. 2010) and is the most frequent method used for deer and other ungulate eradications worldwide (DIISE 2018). Deer are typically shot for sport with rifles by solitary hunters firing from a fixed position or by stalking the animals. Ungulate control programmes worldwide have used a wider variety of methods including the use of automatic weapons, night shooting, thermal imaging, use of fences and natural barriers to break up populations, dogs to locate animals, shooting from vehicles and from helicopters.</p>	<p>Shooting to achieve eradication Sika deer are actively managed through shooting across most of their introduced range, using similar methods to those used to manage native Red deer. Shooting is typically carried out with centre-fire rifles with expanding ammunition. The use of shotguns for deer control is not recommended. Shooting with bows occurs in the USA, but is not lawful in the EU. Shooting is recognised as the main method used for the control of deer, including Sika (McCullough et al. 2008, Pérez-Espona et al. 2009). Guidelines for the management of deer populations, including Sika in their native (Takatsuki 2009, Kaji et al. 2010) and introduced ranges (Ratcliffe 1989, McCullough et al. 2008), are widely available. Eradication requires the removal of all individuals from an area, and this typically requires other methods to increase the effectiveness of control as densities are reduced, and specialised methods to remove the last few remaining individuals. Latham (2020) describes the potential use of automatic and semi-automatic firearms, night shooting, thermal imaging, together with shooting from vehicles and helicopters. The use of single shot rifles as used by sportsmen may be generally accepted, while the use of other these methods are likely to all raise public concern,</p>	<p>High. Shooting is widely used for controlling deer species, including Sika deer. It has been the primary method used to support deer eradication programmes.</p>

		<p>including opposition from hunters who may normally be prohibited from their use. Although shooting is already widely used to control deer across Europe, its use in public places is likely to bring opposition and raise particular concerns. This is particularly the case for species with a high public profile, as can be the case for some deer populations.</p> <p>The use of different firearms, its calibre, number of cartridges and weight of ammunition together with the times of day and location where deer shooting is permitted is heavily regulated in Europe, and the details vary between Member States. These are likely to restrict the nature of the weapon, the requirements for the operator and the times and locations where they may be used. Many Member States require those using shooting to control deer to undertake a specialist training programme to ensure they are competent and safe before issuing a license. Local authorities in the Member State must be consulted before their use.</p> <p>The use of firearms brings risks to health and safety which need to be considered. The use of lead bullets has been restricted in some areas due to environmental concerns, although non-toxic alternatives are available.</p> <p>In some cases, Sika deer have been introduced to an area for hunting purposes. Attitudes to deer control also vary widely between stakeholders (Moriarty 2004a). Differing stakeholders may therefore present a barrier to eradication.</p>	
	<p>Use of Dogs Trained dogs can be an effective tool to locate animals,</p>	<p>Use of Dogs Dogs are widely used by hunters to help locate animals, and to assist with the retrieval of shot animals. The skills and</p>	<p>Medium</p>

	<p>particularly in habitats such as woodland which may otherwise provide concealment. This approach can be used to locate the animals themselves, or the presence of their signs, and can be particularly effective to assist with the removal of the last few remaining animals during an eradication, and to help confirm the absence of animals from an area.</p> <p>In some regions of the world, professional ground hunting with appropriately trained dogs may be used to control deer under conditions where conventional ground shooting would be difficult (Moriarty 2004b).</p>	<p>training required are widely available in many areas. Specialist training may be required to focus the dogs on a specific species, and to ensure that the dogs only locate and do not attack the animal.</p> <p>Dogs which bring an animal to bay rather than attack would allow humane management of deer in circumstances which would otherwise make eradication impossible (Moriarty 2004b).</p> <p>However, the use of dogs to kill prey is heavily restricted by law in many European countries. Local and national legislation would need to be consulted before considering the use of this method. This method has also been the focus of public protest in a number of countries and its use is likely to attract opposition. Deer hunting using trained dogs is less selective than shooting (Novak et al. 1991), while dogs can also cause greater disturbance which may decrease deer sighting rates (Godwin et al. 2013).</p>	<p>This method has been used for the control of other deer, but no published evidence for its use with this species or in areas where hybrids may be present</p>
	<p>Judas Animals</p> <p>Judas animals are marked individuals that are used to identify the locations of other of their species to assist with control by locating remaining groups or individuals. Judas animals are typically captured, radio-tagged, and then released to join a social group. This group</p>	<p>Judas Animals</p> <p>There are no published reports of the use of this method on Sika deer. However, Latham (2020) considers the use of Judas deer for the proposed eradication of Sika deer from Russell Forest in New Zealand.</p> <p>The Judas method for deer has only been trialled on red deer in New Zealand, suggesting it may be a useful tool for the latter stages of an eradication (Crouchley et al. 2011).</p> <p>The method is likely to have the greatest utility for deer species that are highly social, because they will be more</p>	<p>Medium</p> <p>Judas animals have been used successfully to assist the eradication of other ungulates, but no reports involve Sika deer or the use of this method in</p>

	<p>can then be followed using the radio-transmitter to assist with lethal control, typically by shooting. By sparing the Judas animal, it can then be left to locate and join another social group and the process repeated. The ability of Judas goats to attract conspecifics has also been enhanced through the use of chemically induced oestrous (Campbell et al. 2007), to lure otherwise wary males. Originally used to improve the efficiency of feral goat control (Parkes 1990, Taylor & Katahira 1988, Campbell & Donlan 2005), these approaches have now been used on a range of social mammals including deer (Crouchley et al. 2011) to improve the efficiency of eradication programmes.</p>	<p>likely to seek out conspecifics than solitary or species showing low social behaviour. However, Sika deer in New Zealand and elsewhere within their native and introduced ranges are considered only a moderately social species. Thus, it is debatable whether deploying Judas female Sika deer would be as useful as it has proven to be on more social species (Parkes 1990; Crouchley et al. 2011). However, Sika deer stags use a number of behavioural strategies to acquire mating opportunities during the rut. It may be possible to utilise this to help locate females during the rut.</p> <p>Latham (2020) concludes that using Judas Sika deer in Russell Forest would probably be expensive, and only achieve minor gains in terms of increased detection and kill rates. However, assessing the efficacy of this method before using it as a mop-up tool may be beneficial.</p> <p>The effectiveness of this method may be limited in areas where other <i>Cervus</i> species or their hybrids are present. No costs for the use of Judas deer are available. However, Campbell & Donlan (2005) describe large improvements in the cost-effectiveness of goat eradications since the first application of this approach.</p> <p>This method has been predominantly used on uninhabited islands, although it is now also widely used in Scandinavia to assist the control of raccoon dogs (Kuczynski 2019). Little information is available on the public reaction to this approach. However, an adverse public reaction remains a possibility.</p>	<p>areas where hybrids are present.</p>
	<p>Traps</p>	<p>Traps There are no accounts of deer eradication programmes</p>	<p>Medium</p>

	<p>A wide variety of traps and nets have been used to catch deer. However, these are typically used for live capture rather than as the primary control method during eradications. A variety of designs such as drop-nets (Ramsey 1968, Conner et al. 1987), Stephenson traps, clover traps, rocket nets and darting (Haulton et al. 2001) are all described in the literature, with information on their success and associated rates of injury. Deer may also be captured in corrals.</p>	<p>based on the use of traps as the primary control method. However, Crouchley et al. (2011) used traps as a subsidiary method during the eradication of red deer from an island of New Zealand. Trapping may also be a useful control method for use in areas where shooting is not feasible. Live capture is needed for the Judas animal method, and may form a useful part of eradication programmes based on this method. Given the wide variety of trap designs only general costs can be given, but most designs are likely to cost a few hundred Euros to construct.</p> <p>The live capture of deer risks stress and injury to the animals, requiring experienced personnel to reduce the risks. Conner et al. (1987) estimated White tailed deer (<i>Odocoileus virginianus</i>) mortality associated with drop traps to be between 1 and 7% within a week of capture. Haulton et al. (2001) reports between 2-20% trap related mortality.</p> <p>While trapping may remove deer, it is likely that remaining trap-shy individuals would still need to be removed by other means, for example shooting is likely to be required at some stage if complete eradication is the objective (Moriarty 2004b).</p>	<p>Traps have been used as a minor method to support deer eradication programmes and the live capture of Judas animals. However, no documented accounts of their use to manage Sika deer have been found.</p>
	<p>Toxins Toxins have not been widely used for the management of deer in other parts of the world. The toxin 1080 (sodium monofluoroacetate) has been incorporated into a gel and</p>	<p>Toxins No toxins are currently approved for use on deer in the EU. The use of toxins to manage deer in the EU could be expected to raise considerable public opposition and can be criticised for its likely impact on non-target species (Moriarty 2004b).</p>	<p>Medium</p> <p>Although toxins have been used for deer management, this has typically been to support other</p>

	<p>smear on leaves as a deer toxin in New Zealand (Batcheler & Challies 1988). DIISE (2018) includes two New Zealand reports applying aerially based Brodifacoum baits as a secondary method to assist with deer eradication, but shooting forming the primary method in each case.</p>	<p>The New Zealand experience suggests that 1080 was expensive when applied over large areas, but it was used successfully to control localised populations of deer that could not be killed by other means (Moriarty 2004b) although actual costs are unavailable.</p>	<p>methods such as shooting. No data is available related to their use on Sika deer.</p>
	<p>Surveillance during eradication Monitoring the reduction in population size and identifying the number of remaining animals is an important component of an eradication programme. Many programmes have used declines in catch, signs or sightings per unit effort to assess progress. More recently, programmes have collected DNA from culled animals and from faeces (pellet) found in the field to estimate the number of remaining animals in a population (Nugent et al. 2005, Crouchley et al. 2011). Thermal imaging (TI) cameras have been used for monitoring wildlife for over 20 years (Gill et al. 1997; Havens & Sharp 1998).</p>	<p>Surveillance during eradication DNA The use of DNA to estimate the size of any remaining population during eradication offers improvements in cost-effectiveness. However, animals need to be sufficiently genetically distinct for the method to be effective. Small populations derived from a limited founder stock may be too similar for the method to be effective (Crouchley et al. 2011). Latham (2020) discusses the potential use of this method to help achieve the eradication of Sika deer from Russell Forest, concluding that in that area information yielded by faecal DNA may marginally improve control efficacy, but he considers that it cannot currently help achieve eradication in this area.</p> <p>Signs Pellet counts are widely used to monitor ungulates but rely on the assumption that pellets of different species are correctly identified in the field. However recent eDNA studies revealed high rates of misidentification in diverse</p>	<p>Medium</p> <p>Given the absence of successful sika deer eradications, the effectiveness of different surveillance methods to support that objective have not been assessed,.</p>

	<p>They offer improvements when determining the presence or absence of a species in an area, and can be used to help locate animals for control using methods such as shooting. TI relies on the differences in temperature between the animal and the surrounding habitat. Its use is therefore most effective when the habitat is cool, for example at night, in the early morning or in shaded habitats such as woodland.</p> <p>Acoustic devices are becoming increasingly used in wildlife ecology and management (Wall et al. 2014; Buxton et al. 2018). For example, they can provide 'real-time' directional tracking of animal sounds and gunshot detection, both of which have immediate application for wildlife management (Wall et al. 2014, Hill et al 2018).</p>	<p>European ungulate communities monitored through pellet counts (Spitzer et al. 2019).</p> <p>Thermal Imaging Hand-held TI units and rifle scopes are increasingly being used for ground-based shooting of mammalian pests including Sika deer (Latham 2020). The use of thermal imaging equipment to assist with the control of Sika deer may be restricted in some countries. Proposals for its use may be opposed by existing hunter groups to whom this technology, or permission to shoot at night, may not be available, or on ethical grounds.</p> <p>Acoustic Sensors Latham (2020) also discussed the potential utility of acoustic monitoring devices for Sika deer management in Russell Forest. Five devices were trialled for two weeks and were able to detect calls emitted by Sika deer. However, the utility of these devices was limited because data had to be downloaded from each device, rather than being retrieved remotely in real-time. Although this study confirmed that Sika deer calls could be detected using acoustic monitoring devices, the information was not received quickly enough to be useful for Sika deer control. Receiving data in real time would mean that control staff could respond immediately to a Sika deer call, thereby increasing the likelihood of locating and killing the individual detected by the device. There is also the potential to link acoustic monitoring devices to artificial intelligence (AI) systems that can learn to recognise the target species and then alert an appropriate person in real time when a deer is heard. This AI approach is at an early stage of development</p>	
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		and would need to be developed specifically for detecting Sika deer.	
Methods to achieve management⁸	All of the methods described to support eradication can also be used to manage existing deer populations.		
	<p>Shooting Shooting is by far the most common and main control method used for the long-term management of Sika deer throughout its native and introduced range. This includes control to limit impacts on agriculture, forestry, biodiversity and to reduce hybridisation with native <i>Cervids</i>. While the situation varies widely dependent on Sika deer numbers and local conditions, there are frequent reports that current control efforts are insufficient to effectively limit the impacts of Sika and other deer species in many areas and their populations are generally increasing (McCullough et al. 2008, Takatsuki 2009, Kaji et al. 2010).</p>	<p>Shooting Shooting is recognised as the main method used for the control of deer, including Sika (McCullough et al. 2008, Pérez-Espona et al. 2009). Guidelines for the management of deer populations, including Sika in their native (Takatsuki 2009, Kaji et al. 2010) and introduced ranges (Ratcliffe 1989 McCullough et al. 2008), are widely available.</p>	<p>High</p> <p>Shooting is widely used for the control of deer species, including Sika deer in a range of countries.</p>
	Contraceptives	Contraceptives	High

	<p>A wide variety of contraceptive agents have been considered for use on deer, including surgical implants, injectable contraceptives and orally delivered products. These can work through a number of different mechanisms, including supplementing or replacing natural hormones; inducing an immune response to elements of the reproductive system, such as sperm, egg surface proteins or particular hormones involved in reproduction.</p> <p>Two products have received particular attention. Injectable Porcine Zona Pallucida (PZP) raises an auto-immune response in the female to egg surface proteins, preventing fertilisation. GonaCon is another injectable product that raises an auto-immune response to GnRH, the hormone that provides high level regulation of other sex hormones; suppression of GnRH maintains the animal in a non-breeding or juvenile condition. The products and their potential</p>	<p>The use of contraceptives is of proven effectiveness for individual animals, but to reduce a population a significant proportion of the reproductive animals must be treated. This raises complex issues of scale, cost and practicality that have limited the widespread use of these products to date. There are currently few examples of the successful use of wildlife contraception beyond small, closed populations such as on islands, in suburbia or in fenced enclosures such as parks (IUCN 2017). There are also no examples of the use of contraceptives to eradicate an established population of deer or to reduce deer abundance over any significant area. Beyond surgical implants, currently the only commercially available immune-contraceptive products are PZP and GonaCon although their use is limited to particular species and this varies between countries. Neither is currently specifically licensed for use on Sika deer.</p> <p>There may be a variety of welfare consequences for the treated animal dependent on the product chosen. For example, females treated with PZP may undergo prolonged oestrus together with its associated behaviours. This can lead to stress and loss of body condition in the females from male harassment. Products such as GonaCon may interfere with the development of secondary sexual characters in males, such as antlers (Killian et al. 2005), with welfare implications. These can be avoided by the careful selection of products and their targeted delivery to only one sex, although these can increase the cost and limit the practicality of their use. The injection of immune-contraceptive products may be associated with the formation of abscesses at the injection site (Miller et al.</p>	<p>Despite the large volume of research on wildlife contraception, there are currently few examples of its practical use to control deer or examples of its use to achieve eradication.</p>
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	<p>for use have been reviewed by Fagerstone et al. (2010) and in relation to the management of invasive alien species in Europe (IUCN 2017). The UK Deer Initiative have also reviewed the prospects of deer control using contraceptives in the UK.</p>	<p>2008). The welfare implications of these products are reviewed by IUCN (2017). Proposals to use contraceptives and avoid lethal control are often welcomed by the public and many stakeholders. Proposals for the use of contraceptives often receive opposition from deer hunting interests who are concerned about contamination of deer products from animals that they might subsequently hunt, the perceived impracticality of contraception for use at a large scale, and the lack of recognition for the traditional role of hunters in managing deer populations. The practical costs of contraceptive use are currently high as they are typically based on the use of small numbers of implants or injectable products. These can involve the capture and surgical treatment of individual animals with associated costs and risks to animal welfare. If orally delivered contraceptive are developed, species specific delivery systems perfected and the appropriate licenses gained, then the costs may be dramatically reduced in future. As reported by Gürtler et al. (2018) fertility reduction (contraception) and non-lethal removal have only played marginal roles for deer management, and was considered unsuitable for most situations in a review discussing the efficacy and cost-efficiency of deer management methods in Australia (Davis et al. 2016).</p>	
	<p>Management of Hybridisation Sika deer are known to hybridise with native deer, in particular Red deer <i>Cervus elaphus</i>. The</p>	<p>Management of Hybridisation One particular concern associated with introduced populations of Sika deer is hybridisation with native <i>Cervus</i> species (Swanson & Putman 2009). At one level, the</p>	<p>Medium Selective culling may help slow rates of</p>

	<p>potential loss of <i>C. elaphus</i> populations through hybridisation is a significant conservation concern.</p>	<p>geographical separation of allospecifics can help limit this risk. However, in areas where hybrids occur, programmes to support the selective culling of animals with hybrid characteristics has been used to reducing rates of gene flow into the native populations. However, the frequency of hybridisation is highly variable between different populations (Senn & Pemberton 2009). Hybrids regularly occur with variable mixtures of characteristics from the two species. Many hybrids cannot reliably be determined as such from external examination in the field and genetic methods may be required. The lack of an effective method to identify hybrids reduces the effectiveness of selective culling (Pérez-Espona et al. 2009). Management approaches based on the geographic separation of the two species offer some advantages (Wildlife On-Line), although the long-term sustainability of such approaches remains uncertain. Pérez-Espona et al. (2009) conclude that a management policy on how to prevent hybrids from an established swarm eventually spreading and introgressing neighbouring red deer populations therefore cannot be proposed. although research to understand the processes behind swarm formation of red and sika hybrids continues (Smith et al 2018)</p>	<p>gene spread in native populations, however the difficulties in the identification of hybrids reduces its effectiveness.</p>
	<p>Fencing, Deterrence and Resource Protection Localised damage caused by deer can be managed by the use of deer proof fences. These are widely used in commercial</p>	<p>Fencing, Deterrence and Resource Protection Designs of deer proof fencing, both wire and electric, scaring methods and repellents are available on-line and from commercial companies. Useful sites include: The Internet Centre for Wildlife Damage Management:</p>	<p>High Methods such as fencing are widely used to reduce local deer impacts.</p>

	<p>forestry to protect young plantations. However, they bring problems of reduced access for other purposes, reducing their use to protect agricultural crops or other resources. Individual trees can be protected during early growth through the use of covers or guards to prevent browsing. A wide variety of scaring and deterrent products have been proposed to limit local damage (Côté et al. 2004). The effectiveness of these methods is highly variable and local trails are required to assess the usefulness of each for a specific purpose.</p>	<p>http://icwdm.org/handbook/mammals/Deer.asp</p> <p>There are a number of accounts of the use and consequences of fencing in relation to Sika deer, particularly in relation to forest management in their native range in Japan (Kumar et al. 2006, Honda et al. 2011, Tamura & Nakajima 2017).</p>	
	<p>Biological control Predation by feral and larger wild predators is believed to impact and possibly limit deer populations in some areas (Okarma et al. 1997, Ballard et al. 2001, Moriarty 2004b, Gürtler et al. 2018). However, the role of predators in controlling ungulate populations remains uncertain, particularly in the human-influenced habitats found across</p>	<p>Biological control The main evidence for the limitation of deer populations by predation comes from relatively undisturbed habitats with a natural suite of predators. There is little evidence that predators currently limit deer numbers in many of the more human-influenced habitats common across Europe. While the reintroduction of predators may introduce some element of predation, the evidence that this will limit deer numbers in human-influenced habitats is limited, and there are significant conflicts associated with the reintroduction of predators. The reintroduction of predators would also potentially impact native as well as introduced deer, as</p>	<p>Low</p> <p>Although wild deer are impacted by predators, there is little evidence of the successful use of this approach to manage deer populations and no evidence to support their practical use to</p>

	most of Europe (Côté et al. 2004).	would the introduction of deer specific pathogens (Williams 2005). There are no other biological controls that appear useful as a control agent for deer (Moriarty 2004b).	manage Sika deer in Europe.
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Guidelines for Completing the Annex

1 Provide a brief summary description of the most cost-effective methods drawing on the reviews in the detailed assessments

2 Provide a description of the potential method. This should be based on the available key scientific evidence which should be gathered from sources including articles and reviews in technical and scientific journals, internet searches, online databases, grey literature and relevant books and personal communications from scientists, stakeholders, conservation practitioners and governmental bodies. This information should include a full bibliographic list detailing the literature and sources considered.

3 Provide an assessment of the likely cost and effectiveness of the method. Where information is available, consider the following range of questions, accepting that not all questions will be appropriate in all circumstances.

- How effective has this approach proven to be in the past or in an analogous situation?
- How publically acceptable is the approach likely to be?
- Over what period of time would this approach need to be applied to be effective?
- What is the direct cost of implementing this approach?
- How likely are the methods used in the approach to be available?
- How likely is it that relevant licences or other approvals to undertake the approach would be difficult to obtain?
- How likely is it that health and safety issues would prevent the use of this approach?
- How significant is the environmental harm caused by this approach?
- How significant is the economic harm caused by this approach?
- How significant is the social harm caused by this approach?
- How likely is it that the approach will be criticised on welfare grounds?
- How likely is it that the approach will be acceptable to other stakeholders?

Where available, factual information on the costs of specialist equipment, or case studies of management costs from across the Union or third countries should be provided. When describing case studies, if the information is available then provide both total cost and the area over which control was undertaken so that a cost per unit area might be derived. Where such quantitative information is not available, then any qualitative information from the literature is acceptable to help guide decision making. It is accepted that in the majority of cases the information required to assess the potential total cost of management at a member state level is unlikely to be available. This would normally require information on the extent and abundance of the species which is beyond the scope of this assessment. Assessors are not expected to extrapolate the potential total costs of management at a member state level, only to report on the information provided within the literature.

4 Provide an overall assessment of the confidence that can be applied to the information provided for this method. This confidence should relate to the quality of the available information using the guidance below. It should NOT relate to the confidence in the effectiveness of the method

The confidence scores are:

- **High:** Information comes from published material, or current practices based on expert experience applied in one of the EU countries or third country with similar environmental, economic and social conditions.
- **Medium:** Information comes from published data or expert opinion, but it is not commonly applied, or it is applied in regions that may be too different from Europe (e.g. tropical regions) to guarantee that the results will be transposable.
- **Low:** data are not published in reliable information sources and methods are not commonly practiced or are based solely on opinion; This is for example the case of a novel situation where there is little evidence on which to base an assessment.

If there are further factors beyond these that have determined the chosen level of confidence, then provide a brief written description to support the choice of the level of confidence.

5 Measures for preventing the species being introduced intentionally or unintentionally into the territory of a Member State (cf. Articles 7(1)(a), 7(2), 13, 15), i.e. preventing a species entering by blocking its pathways, including pre-border prevention and prevention of escape or release into the environment and secondary spread whenever relevant. Measures for preventing the species reproducing while in containment should be included, if appropriate. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

6 Measures to run an effective surveillance system for achieving an early detection of a new occurrence (cf. Article 16). This section assumes that the species is not currently present in a Member State, or part of a Member State's territory.

7 Measures to achieve eradication. Preferably, but not exclusively, used at an early stage of invasion, after an early detection of a new occurrence (cf. Article 17). Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

8 Measures to achieve management of the species once it has become widely spread within a Member State territory (cf. Article 19). These measures can be aimed at eradication, population control or containment of a population of the species. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

The development and completion of this template forms part of the EU project **No 07.0202/2020/834529/ETU/ENV.D.2** Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention.

**Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention"
Contract No 07.0202/2020/834529/ETU/ENV.D.2¹**

Name of organism: *Cherax destructor* Clark, 1936

Author(s) of the assessment:

Elena Tricarico, Department of Biology, University of Florence, Italy

Risk Assessment Area: The risk assessment area is the territory of the European Union 27 and the United Kingdom, excluding the EU-outermost regions.

Peer review 1: Antonín Kouba, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Faculty of Fisheries and Protection of Waters, University of South Bohemia in České Budějovice, Vodňany, Czech Republic

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Date of completion: 15/10/2021

¹ This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA). A number of amendments have been introduced to ensure compliance with Regulation (EU) 1143/2014 on IAS and relevant legislation, including the Delegated Regulation (EU) 2018/968 of 30 April 2018, supplementing Regulation (EU) No 1143/2014 of the European Parliament and of the Council with regard to risk assessments in relation to invasive alien species (see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>).

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SECTION A – Organism Information and Screening

A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?

including the following elements:

- the taxonomic family, order and class to which the species belongs;
- the scientific name and author of the species, as well as a list of the most common synonym names;
- names used in commerce (if any)
- a list of the most common subspecies, lower taxa, varieties, breeds or hybrids

As a general rule, one risk assessment should be developed for a single species. However, there may be cases where it may be justified to develop one risk assessment covering more than one species (e.g. species belonging to the same genus with comparable or identical features and impact). It shall be clearly stated if the risk assessment covers more than one species, or if it excludes or only includes certain subspecies, lower taxa, hybrids, varieties or breeds (and if so, which subspecies, lower taxa, hybrids, varieties or breeds). Any such choice must be properly justified.

Response: The species is a single taxonomic entity, and it can be distinguished from other entities.

Cherax destructor Clark, 1936 (Malacostraca, Decapoda, Parastacidae)

Main synonyms: There are no formal synonyms²

Common names: common yabby, yabby, yabbies (EN), Australische kreeft; yabby (NL), ecrevisse bleue; ecrevisse de Murray; yabbie (FR); yabby (IT); Blauer Yabby (DE), rak ničivý (CZE), cangrejo australiano (ES), cranc australiá (Catalan), karramarro australiarra (Basque)

The species could hybridize with congeneric *Cherax albidus*, resulting in only male progeny: this can be used especially for controlling reproduction in ponds in Australia, limiting population size (Lawrence et al. 2000; Souty-Grosset et al. 2006). There is some debate about the presence of several subspecies belonging to *C. destructor*. There have been suggestions of a synonymy with *C. albidus*, but this has not been confirmed (Souty-Grosset et al. 2006). For the present Risk Assessment, *C. destructor* (and its different colour morphs in trade such as the blue one) is considered a unique species.

A2. Provide information on the existence of other species that look very similar [that may be detected in the risk assessment area, either in the environment, in confinement or associated with a pathway of introduction]

Include both native and non-native species that could be confused with the species being assessed, including the following elements:

- other alien species with similar invasive characteristics, to be avoided as substitute species (in this case preparing a risk assessment for more than one species together may be considered);

² Crandall & De Grave (2017) reported *Cherax davisi* Clark, 1941a and *Cherax esculus* Riek, 1956 as possible synonyms but they were not mentioned in the current literature or other websites.

- other alien species without similar invasive characteristics, potential substitute species;
- native species, potential misidentification and mis-targeting

Response: Native and non-native crayfish species present in the risk assessment area can be distinguished based on morphological and colour characteristics (Souty-Grosset et al. 2006; Kouba et al. 2014). From Souty-Grosset et al. (2006), the species is described as follows: the colour body is green-beige to almost black, blue-grey being common in individuals kept in captivity or even orange in the yabby volcano³. Chelae dorsally showing the same colour as the body but with a marbled-like pattern, underside dirty-white or grey coloured. Colour shows a wide variability depending on the location, season and water conditions and may vary from individual to individual in a single location (Withnall 2000). The carapace is smooth with a single pair of post-orbital ridges forming two keels and no spines on shoulders. The rostrum is short, broad-based, triangular and smooth: no spines present along margins. Median carina indistinct. The chelae, usually robust and a peculiarity of the species, are smooth, elongated and large; there is a mat of setae along proximal cut edges of the chelar fingers. Eyes are relatively small. Membranous posterior half of telson.

Another typical features of *C. destructor*, as in other crayfish species native to the Southern Hemisphere (Parastacoidea: Parastacidae), are the morphological differences between sexes when compared with crayfish from the Northern Hemisphere (Astacoidea: Astacidae, Cambaridae, and Cambaroididae). Overall, in crayfish, females have gonopores located at the base of the third pairs of pereopods, while male genital papillae are at the base of the fifth pair of pereopods, nearest the abdomen. In Astacoidea, males have also the first and second pleopods (ventral appendages) modified in gonopods, while in Parastacoidea males lack of these modified gonopods: the first pleopod is vestigial or missing in both sexes. Moreover, in Cambaridae the females have also a seminal receptacle (*annulus ventralis*), absent in other Astacoidea and Parastacoidea (IUCN 2018). Thus, to identify the sex of *Cherax* individuals, the position of abovementioned gonopores and genital papillae should be assessed.

The congeneric *Cherax quadricarinatus* is also present in aquaculture facilities and internet trade in Europe (Haubrock et al. 2021), and in the wild in Slovenia (thermal waters: Kouba et al. 2014), Hungary (Weiperth et al. 2019), Malta (Deidun et al. 2018) and Spain (thermale waters: Arias & Torralba-Burrial 2021).

Several *Cherax* species, primarily of New Guinean origin, are sold in the European pet trade. Some of these have been already found introduced in the European wild, occupying thermal localities. Evidence on their establishment is, however, missing (Weiperth et al. 2020). Despite the generally similar morphological appearance, they can be usually distinguish from *C. destructor* for their diverse colouration. Moreover, the presence of the abovementioned two keels helps to distinguish *C. destructor* from *C. quadricarinatus*.

The identification of *C. destructor* juveniles could require the help of an expert or even the use of DNA barcoding.

³ <https://www.pixtastock.com/photo/38959707>

A3. Does a relevant earlier risk assessment exist? Give details of any previous risk assessment, including the final scores and its validity in relation to the risk assessment area.

Response: For Europe, the species has been screened using FI-ISK, a tool for identifying potentially invasive freshwater invertebrates, considering as risk assessment area Italy (Tricarico et al. 2010); Czech Republic (Patoka et al. 2014); Greece (Papavlasopoulou et al. 2014); Germany (Chucholl 2013; Chucholl & Wendler 2017), Ukraine (Kotovska et al. 2016), Caspian region (Vodovsky et al. 2017) and Hungary (Weiperth et al. 2019). For all these RAs, the final outcome for *C. destructor* was high risk of invasiveness due to its potential negative impacts, feeding habits and adaptability to the local conditions.

A similar outcome (high risk) has been reported for Kazakhstan (Uderbayev et al. 2017) using FI-ISK, while for New Guinea with the EICAT (Environmental Impact Classification of Alien Taxa) the species has been ranked in the moderate category (Yonvitner et al. 2020).

For USA, the species has been screened by U.S. Fish and Wildlife Service (2019) with high risk as outcome again for its high climate match, potential impacts and history of invasiveness. The species was then officially listed as an injurious wildlife species by the U.S. Fish and Wildlife Service in 2016 under the Lacey Act (18.U.S.C.42) (U.S. Fish and Wildlife Service 2016). Many US states listed it as a prohibited species (U.S. Fish and Wildlife Service 2019).

A4. Where is the organism native?

including the following elements:

- an indication of the continent or part of a continent, climatic zone and habitat where the species is naturally occurring
- if applicable, indicate whether the species could naturally spread into the risk assessment area

Response: The species is native to central-eastern Australia (New South Wales, Victoria, eastern South Australia and southern parts of Northern Territory and Queensland) (Souty-Grosset et al. 2006), inhabiting areas with temperate and tropical climates (characterized by high summer temperatures and low annual rainfall). It can colonize a wide range of habitats (alpine streams, subtropical creeks, springs, billabongs, ephemeral lakes, swamps, irrigation ditches: Souty-Grosset et al. 2006). It can construct burrows (0.5 - 2 m deep) that help surviving during the drought (Withnall 2000; Kouba et al. 2016). It is adapted to a wide range of water temperatures (1 - 35 °C). It does not grow at water temperatures below 15 °C and above 34 °C; the optimal growth is around 28 °C (Souty-Grosset et al. 2006). It starts dying at 36 °C and with temperatures below 16 °C it can undergo to “hibernation” (i.e. metabolism and feeding cease) (Mills 1983; Morrissy et al. 1990; Merrick & Lambert 1991; Morrissy & Cassells 1992; Withnall 2000). However, Veselý et al. (2015) found that yabby can withstand low winter temperatures with foraging activities being observed even in the coldest period of the experiment (2 - 3 °C) with mentioned temperatures lasting for ~ 3 months. It can tolerate salinity (but growth stops at 8 ppt and mortality starts at 16 ppt: Mills & Geddes 1980) and hypoxic waters (oxygen concentration <1 mg L⁻¹: Mills 1983; Morrissy & Cassells 1992). Optimal pH is comprised between 7.5 and 8.5 (with a tolerance up to 7 and 9) (CABI 2011). The species is usually found in turbid waters with muddy or silted bottoms (Withnall 2000).

It can naturally disperse within the same basin; anecdotal reports suggest that *C. destructor* emerges from hypoxic water and migrates between waterbodies (Morris & Callaghan 1998), but no studies confirmed this ability. A physiological study showed that the species appeared able to breathe air (Morris & Callaghan 1998) and this can be expected, being a drought resilience species such as *Procambarus* species (Kouba et al. 2016; Guo et al. 2019). Similar to its congeneric *C. quadricarinatus* (Haubrock et al. 2021), the species is omnivorous and opportunistic (Souty-Grosset et al. 2006), with plant material and detritus being the main component of its diet, followed by a low proportion of arthropods, algae and fungi (Lawrence & Jones 2002; Souty-Grosset et al. 2006; Linton et al. 2009). Predation on small fish has been documented as well (Beatty 2006).

A5. What is the global non-native distribution of the organism outside the risk assessment area?

Response: The species has been introduced in eastern drainages of the non-native range of New South Wales and in Western Australia (Coughran et al. 2009; McCormack 2014), where it became invasive. The species was also introduced in Tasmania, where it established and is spreading after an illegal introduction (Lynas et al. 2007).

A6. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species been recorded and where is it established? The information needs to be given separately for recorded (including casual or transient occurrences) and established occurrences. “Established” means the process of an alien species successfully producing viable offspring with the likelihood of continued survival⁴.

A6a. Recorded: List regions

A6b. Established: List regions

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

- Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea.

Comment on the sources of information on which the response is based and discuss any uncertainty in the response.

For delimitation of EU biogeographical regions please refer to <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2> (see also Annex VI).

⁴ Convention on Biological Diversity, Decision VI/23

For delimitation of EU marine regions and subregions consider the Marine Strategy Framework Directive areas; please refer to <https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions/technical-document/pdf> (see also Annex VI).

Response (6a): Mediterranean and Atlantic (Souty-Grosset et al. 2006; Kouba et al. 2014; Deidun et al. 2018; Vigneron et al. 2019; Collas 2020; Julian Reynolds, pers. comm.). The response is based on publications reporting the presence of the species in these biogeographic regions, so the level of confidence is high.

Response (6b): Mediterranean and Atlantic (Souty-Grosset et al. 2006; Kouba et al. 2014; Deidun et al. 2018; Vigneron et al. 2019; Collas 2020; Julian Reynolds, pers. comm.). The response is based on publications reporting the establishment of the species in these biogeographic regions, so the level of confidence is high.

A7. In which biogeographic region(s) or marine subregion(s) in the risk assessment area could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A7a. Current climate: List regions

A7b. Future climate: List regions

With regard to EU biogeographic and marine (sub)regions, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (7a): Atlantic, Black Sea (very few areas), Continental (very few areas), Mediterranean

Response (7b): 2070 RCP 2.6 Atlantic (increase), Black Sea (still very few areas), Continental (still very few areas), Mediterranean (small decrease); 2070 RCP 4.5 Atlantic (increase), Black Sea and Continental (still very few areas), Mediterranean (small decrease)

These responses are based on the Species Distribution Model (Annex VIII). The ensemble model suggested that suitability for *C. destructor* was most strongly determined by temperature seasonality, accounting for 36.1% of variation explained, followed by minimum temperature of the coldest month (-3°C; 23.2%), and maximum temperature of the warmest month (21 °C; 16.5%). The minimum

temperature of the coldest month is currently limiting the establishment in the northern and coldest biogeographic regions. The temperature increase will favour the species establishment in the future, even if the range increase forecasted under RCP 4.5 would decrease the percentage of suitable areas in the Mediterranean biogeographic region, probably becoming too dry for the species. The importance of temperature as predictor has also been confirmed by distribution models conducted on the species for Iberian Peninsula (Capinha & Anastácio 2011), with colder areas being less suitable. However, from a laboratory study, Veselý et al. (2015) showed that *C. destructor* can withstand low winter temperatures typical of lentic habitats in the European temperate zone, thus posing a risk of establishment also to these areas.

A8. In which EU Member States has the species been recorded and in which EU Member States has it established? List them with an indication of the timeline of observations. The information needs be given separately for recorded and established occurrences.

A8a. Recorded: List Member States

A8b. Established: List Member States

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden

The description of the invasion history of the species shall include information on countries invaded and an indication of the timeline of the first observations, establishment and spread.

Response (8a): The first known introduction of the species (30 individuals from California) from California was in Catalonia, Spain, in 1983 for aquaculture purposes, although earlier or later introductions cannot be ruled out. Due to the lack of permits for aquaculture, these crayfish were released in an irrigation pond (Bolea 1996; Souty-Grosset et al. 2006). In Italy, the species was introduced at the end of 1980s in closed systems for aquaculture purposes (D'Agaro et al. 1999) and the first wild population was found in 2008 (Scalici et al. 2009). In France, the species has been found in the wild in 2018 (Vignon et al. 2019), probably introduced for aquaculture in ponds connected to open waters (Collas 2020). The species has also been reported for southern Ireland in 2018 where it was probably introduced in 2010 (Julian Reynolds, pers. comm.).

Response (8b): Spain, Italy, France, Ireland (Kouba et al. 2014; Deidun et al. 2018; Collas 2020; Julian Reynolds, pers. comm.). In Spain, the species became established in the autonomous communities Navarra and Aragón; then four populations were intentionally eradicated using crayfish plague (Souty-Grosset et al. 2006; Kouba et al. 2014), but at least another established population has been reported in a small irrigation pond close to Bagüés, province Zaragoza, Aragón, and the hypothesis that this population originated from individuals translocated within Spain cannot be discarded (Bolea 1996; Kouba et al. 2014). In Italy, the only established population in Latium disappeared, probably after the crayfish plague was transmitted by the red swamp crayfish *Procambarus clarkii* spreading in the area (Mazza et al. 2018). Still, another population has been reported in 2016 in Sicily in the province of Siracusa (Deidun et al. 2018). In France, the species is currently reported in a river in the north of Finstère (Brittany) (Collas 2020). The species has also been reported for southern Ireland in the county of Cork in a quarry pool (Julian Reynolds, pers. comm.).

A9. In which EU Member States could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A9a. Current climate: List Member States

A9b. Future climate: List Member States

With regard to EU Member States, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (9a): Croatia (very few areas), France, Greece, Italy, Portugal, Spain, UK

Response (9b): 2070 RCP 2.6 Belgium, France (slight increase), Germany (very few areas), Greece (decrease), Ireland, Italy (decrease), Portugal (slight decrease), Spain (decrease), and the United Kingdom (increase); 2070 RCP 4.5: Belgium, France (similar), Germany (very few areas), Greece (decrease), Italy (decrease), Netherlands, Portugal (slight decrease), Spain (decrease), and the United Kingdom (increase).

These responses are based on the SDM (Annex VIII). The ensemble model suggested that suitability for *C. destructor* was most strongly determined by temperature seasonality (please see Annex VIII for details). The minimum temperature of the coldest month is currently limiting the establishment in the northern and coldest biogeographic regions. The temperature increase will favour the species establishment in the future, even if the rise forecasted under RCP 4.5 would decrease the percentage of suitable areas in the Member States of Mediterranean biogeographic region which probably would become too dry for the species, even if the species is rather drought tolerant. The importance of temperature as predictor has been confirmed also by distribution models conducted on the species for the Iberian Peninsula (Capinha & Anastácio 2011), with colder areas being the less suitable. However, from laboratory study, Veselý, et al. (2015) showed that *C. destructor* can withstand low winter temperatures typical of lentic habitats (and many lotic waters with temperatures that do not fall below 2.5 °C during the winter) in the European temperate zone, thus posing a severe threat also to these areas.

A10. Is the organism known to be invasive (i.e. to threaten or adversely impact upon biodiversity and related ecosystem services) anywhere outside the risk assessment area?

Response: In Australia, in the non-native range, it is reported to have destroyed macrophytes, caused siltation, and displaced the endemic crayfish *Euastacus dharawalus* (Coughran & Daily 2012). In addition, other potential impacts on fish, amphibians, freshwater turtles, dragonflies and other invertebrates have been suggested in the non-native Australian range (Bradsell et al. 2002; Lynas et al. 2004; Coughran & Daily 2012; Cerato et al. 2019).

A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea

Response: Up to now, no impacts have been reported in the risk assessment area.

A12. In which EU Member States has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden

Response: Up to now, no impacts have been reported in the risk assessment area.

A13. Describe any known socio-economic benefits of the organism.

including the following elements:

- Description of known uses for the species, including a list and description of known uses in the risk assessment area and third countries, if relevant.
- Description of social and economic benefits deriving from those uses, including a description of the environmental, social and economic relevance of each of those uses and an indication of

associated beneficiaries, quantitatively and/or qualitatively depending on what information is available.

If the information available is not sufficient to provide a description of those benefits for the entire risk assessment area, qualitative data or different case studies from across the risk assessment area or third countries shall be used, if available.

Response: In Australia, it has a relatively high commercial value as food. In its native central-eastern area, it represents about 90% of Australian crayfish production and from 2000 the majority of production derived from the translocated population in Western Australia (Piper 2000; CABI 2011). It is interesting to note that the production and value of the common yabby in aquaculture in Australia could have promoted the introduction of the species elsewhere outside the native range. Moreover, it is commonly used as bait by recreational fishers (Nguyen 2005). It is cultured in several countries in aquaculture facilities as a delicacy and also as ornamental species in aquaria being easy to maintain (CABI 2011).

In Europe, no recent detailed economic data have been found on the use of the species in aquaculture and ornamental pet trade, even though its presence in such activities has frequently been reported (D'Agaro et al. 1999; Chucholl 2013; Kouba et al. 2014; Chucholl & Wendler 2017; Deidun et al. 2018) as well its availability in several e-commerce sites (e.g. ebay for 10-30 € per specimen). D'Agaro et al. (1999) reported in 1995 a price of 8-14 US\$ per kg per *Cherax* sp. (without distinguishing *C. destructor* from *C. quadricarinatus*) sold in Italy and a rough estimate of production of *Cherax* spp. of 5 t per year. *Cherax* species (*C. destructor*, *C. quadricarinatus* and other New Guinean species) are currently becoming more important in the pet trade (Weiperth et al. 2020), considering also the ban of the red swamp crayfish *Procambarus clarkii* and marbled crayfish *P. virginalis*.

SECTION B – Detailed assessment

Important instructions:

- In the case of lack of information the assessors are requested to use a standardized answer: “No information has been found.” In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable).
- With regard to the scoring of the likelihood of events or the magnitude of impacts see Annexes I and II.
- With regard to the confidence levels, see Annex III.
- Highlight the selected response score and confidence level in **bold** but keep the other scores in normal text (so that the selected score is evident in the final document).

1 PROBABILITY OF INTRODUCTION AND ENTRY

Important instructions:

- **Introduction** is the movement of the species into the risk assessment area (it may be either in captive conditions and/or in the environment, depending on the relevant pathways).
- **Entry** is the release/escape/arrival in the environment, i.e. occurrence in the wild
- Introduction and entry may coincide for species entering through pathways such as “corridor” or “unaided”, but it also may differ. If different, please consider all relevant pathways, both for the introduction into the risk assessment area and the entry in the environment.
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used (see Annex IV). For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document⁵ and the provided key to pathways⁶.
- For organisms which are already present (recorded or established) in the risk assessment area, the likelihood of introduction and entry should be scored as “very likely” by default.
- Repeated (independent) introductions and entries at separate locations in the risk assessment area should be considered here (see Qu. 1.7).

Qu. 1.1. List relevant pathways through which the organism could be introduced into the risk assessment area and/or enter into the environment. Where possible give details about the specific origins and end points of the pathways as well as a description of any associated commodities.

For each pathway answer questions 1.2 to 1.8 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 1.2a, 1.3a, etc. and then 1.2b, 1.3b etc. for the next pathway.

In this context a pathway is the route or mechanism of introduction and/or entry of the species.

⁵ <https://circabc.europa.eu/sd/a/7e5f0bd4-34e8-4719-a2f7-c0cd7ec6a86e/2020-CBD-pathways-interpretation.pdf>

⁶ <https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf>

The description of commodities with which the introduction of the species is generally associated shall include a list and description of commodities with an indication of associated risks (e.g. the volume of trade; the likelihood of a commodity being contaminated or acting as vector).

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 1.2-1.9.

Pathway name: Escape from confinement: **Aquaculture / mariculture; Pet/aquarium/terrarium species** (including live food for such species); **Live food and bait**.

The species has been introduced in Spain, France and Italy for aquaculture purposes (Bolea 1996; D’Agaro et al. 1999; Souty-Grosset et al. 2006; Scalici et al. 2009; Kouba et al. 2014; Collas 2020). The species is considered as a delicacy, being sold live for restaurants in Germany and other countries such as Switzerland and England (Souty-Grosset et al. 2006).

The “ornamental” trade became active after its first introduction in Europe (Chucholl 2013; Weiperth et al. 2019) and could lead to repeated introductions (as it probably happened for Ireland: Julian Reynolds, pers. comm) as the specimens for this purpose could be reproduced and sold within Europe. Indeed, hobbyists are always looking for new crayfish species/morphs to have in their aquaria, with the Czech Republic being the hub for freshwater ornamental animals in Europe (Patoka et al. 2015).

Escape from confinement: Aquaculture / mariculture

Qu. 1.2a. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional	CONFIDENCE	low
	unintentional		medium
			high

Response: The species was introduced into the risk assessment area for aquaculture purposes, and it is still farmed in some countries such as Italy (Kouba et al. 2014). The entry into the environment could have been intentional too, but unintentional escapes cannot be ruled out.

Qu. 1.3a. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent

establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Even if the introduction of crayfish as food and bait in Europe has decreased in importance through the years (Kouba et al. 2014), the species is still farmed in some areas. Considering the possibility of repeated and independent introductions via this pathway, the probability of introducing a large number of individuals is moderately likely. However, we cannot discard the possibility that also few individuals would be sufficient to establish a new viable population, as happened in other invasive alien crayfish in Europe (e.g. *Faxonius immunis*; Kouba et al. 2014). Source of these repeated introductions could be individuals from Australia or other parts of the world where the species is farmed (e.g. Asia) or from individuals present in Europe (the last two situations happened for Spain; Souty-Grosset et al. 2006; Kouba et al. 2014). However, in Spain, this species is currently listed in the List of IAS of National Concern, thus, in theory, further introductions from and within this state should be very unlikely. According to Bolea (1996), 30 individuals were imported to Spain from Los Angeles in the introduction of 1983. No other data are available on numbers of crayfish introduced for aquaculture into other parts of the risk assessment area. The likelihood of reinvasion is also moderately likely because of the possible repeated use in aquaculture.

Qu. 1.4a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species is established in the wild, proving that it can survive during the transport along this pathway (Kouba et al. 2014; Deidun et al. 2018). Reproduction and increase in number during the transport are unlikely, but it can reproduce once in the aquaculture facility.

Qu. 1.5a. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely	CONFIDENCE	low medium
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	moderately likely likely very likely		high
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Response: No biosecurity measures target the species before and during the transport; on the contrary, there could be practices aimed at its long-term survival for further commercial exploitation. There could be border inspections for checking the introduction of crayfish already in the list of species of Union or Member States (e.g. Spain) concern, and possibly during these checks also *C. destructor* could be intercepted. But up to now no interceptions have been reported for this species. Also, there are no biosecurity measures for the entry, except for the procedures foreseen by the Regulation (EC) No 708/2007 when used in aquaculture.

Qu. 1.6a. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Theoretically, through this pathway, the introduction of the species into the risk assessment area should be detected as the final purpose is its breeding and trade. However, the species was previously detected in the wild only after its establishment (Bolea 1996; Scalici et al. 2009; Deidun et al. 2018), so there is high probability of being undetected in the wild in the first stages of the invasion process. It will be very likely to detect it only after establishing or when spreading. Its correct identification can also require an expert to confirm its presence.

Qu. 1.7a. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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Response: Repeated introductions and entry are linked to aquaculture facilities, so possible introduction points and entry can be identified (see for example in Italy: D'Agaro et al. 1999).

Qu. 1.8a. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species entered the risk assessment area via this pathway, and it is still farmed in some areas (e.g. Italy), so we cannot completely discard the possibility that new escapes could be reported in the near future, as it already happened in Sicily in 2016 (Deidun et al. 2018).

Escape from confinement: Pet/aquarium/terrarium species (including live food for such species)

Qu. 1.2b. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: For this pathway, as happened for other *Cherax* species and alien crayfish, we consider the possibility of repeated and independent introductions in the risk assessment area and entries into the wild, as the species is present in the European ornamental trade (Chucholl 2013; Weiperth et al. 2019; E. Tricarico, unpublished, 2021). The entry into the environment can be either intentional or unintentional, depending on whether it is the result of deliberate releases or accidental escapes.

Qu. 1.3b. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely	CONFIDENCE	low medium
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	moderately likely likely very likely		high
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Response: The species is used as an ornamental species in Europe (Chucholl 2013; Weiperth et al. 2019), and it is commonly offered in the internet trade being appreciated by the hobbyists (Chucholl & Wendler 2017). Source of these repeated introductions could be specimens from populations established in Europe or present in aquaculture and e-commerce. For introductions and entry, we can hypothesize few crayfish, as they are bought by a single person and then eventually released by them as unwanted pets. We cannot discard the possibility that few individuals can lead to a new viable population that could establish a new viable population as happened in other invasive alien crayfish in Europe (e.g. *Faxonius immunitis*; Kouba et al. 2014). Likelihood of reinvasion is likely because of the use as pet and the possibility of owners dumping unwanted pets into the wild as probably happened in Ireland.

Qu. 1.4b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: As also testified by Irish introduction and other alien crayfish introduced for this purpose (and established in the wild) and the use of the species for ornamental reason (Kouba et al. 2014; Haubrock et al. 2021), it can survive during the transport along this pathway. There is no reproduction or increase in number during transport, but the species could reproduce during storage in aquaria under optimal conditions.

Qu. 1.5b. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: No biosecurity measures target the species before and during the transport; on the contrary, there could be practices aimed at its long-term survival for further commercial exploitation. There could be border inspections for checking the introduction of crayfish already in the list of species of

Union or Member State (e.g. Spain) concern, and possibly during these checks also *C. destructor* could be intercepted. But up to now no interceptions have been reported for this species. Also, there are no biosecurity measures for the entry except for the code of conduct on IAS and pets.

Qu. 1.6b. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Pet owners can purchase the species online without previous indications on the hazards and potential implications of its invasiveness by sellers and can purposely release individuals into the wild, so the species is usually detected only once established. This has happened probably for the Irish introduction and also for many other invasive crayfish present in Europe (e.g. the marbled crayfish *Procambarus virginalis* Kouba et al. 2014, or redclaw crayfish *C. quadricarinatus* Haubrock et al. 2021). Its correct identification can also require an expert to confirm its presence.

Qu. 1.7b. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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Response: As there are no trade restrictions within the risk assessment area (except Spain and the UK) and it can be purchased online, its repeated introductions and entries could be widespread, being linked to release by private citizens potentially in all ponds, lakes, rivers and other suitable water bodies wherever they can have access. Urban and especially thermal waters are in general more prone to the disposal of unwanted pets (Chucholl 2015; Patoka et al. 2016; Weiperth et al. 2017).

Qu. 1.8b. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely	CONFIDENCE	low medium high
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	very likely		
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Response: The species is common in ornamental trade (see for example Italy, Czech Republic, Germany, Hungary: Chucholl & Wendler 2017; Weiperth et al. 2019), so the probability of repeated introductions is likely.

Escape from confinement: Live food and bait

Qu. 1.2c. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional	CONFIDENCE	low
	unintentional		medium
			high

Response: The species has been intentionally sold live for restaurants in Germany and other countries such as Switzerland and England (Souty-Grosset et al. 2006; Kouba et al. 2014). The entry into the environment could be intentional, but unintentional escapes are also possible.

Qu. 1.3c. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely	CONFIDENCE	low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response: Even if the introduction of crayfish as live food and bait in Europe has decreased in importance through the years (Kouba et al. 2014), the species has been reported in fish markets. Considering the possibility of repeated and independent introductions via this pathway, the probability

of introducing a large number of individuals is moderately likely. However, we cannot discard the possibility that also few individuals would be sufficient to establish a new viable population, as happened in other invasive alien crayfish in Europe (e.g., *Faxonius immunis*; Kouba et al. 2014). The source of these repeated introductions could be individuals from aquaculture facilities in native and non-native range in Australia or other parts of the world where the species is farmed (e.g., Asia) or from individuals present in European aquaculture facilities (Souty-Grosset et al. 2006; Kouba et al. 2014). No data are available of numbers of crayfish introduced as live food into the risk assessment area. The likelihood of reinvasion is also moderately likely because of the possible repeated use in aquaculture.

Qu. 1.4c. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species has been reported live in fish markets, proving that it can survive during the transport along this pathway (Kouba et al. 2014). Moreover, other crayfish introduced for this purpose (e.g. the congeneric *Cherax quadricarinatus*) have been reported to survive along this pathway and arrive into the wild (Kouba et al. 2014). Reproduction and increase in number during transport are unlikely.

Qu. 1.5c. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: No biosecurity measures are targeting the species before and during the transport; on the contrary, there could be practices aimed at its long-term survival for further human consumption and commercial exploitation. There could be border inspections for checking the introduction of crayfish already in the list of species of Union and Member State (e.g. Spain) concern, and possibly during these checks also *C. destructor* could be intercepted. But up to now no interceptions have been reported for this species. Also, there are no biosecurity measures for the entry except for eventual veterinary inspections for specimens coming from third countries.

Qu. 1.6c. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Other crayfish species introduced via this pathway (e.g. *Faxonius immunis*: Kouba et al. 2014; *Procambarus clarkii* in France, Oficialdegui et al. 2020) have been detected in the wild only after their establishment. So there is a high probability of the species being detected in the wild only after establishing or when spreading. Its correct identification can also require an expert to confirm its presence.

Qu. 1.7c. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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Response: Repeated introductions and entry are linked to fish markets and individuals released by sellers or fishermen, respectively. Possible points of introduction and entry can be widespread and often close to urban settlements as for unwanted pets (Weiperth et al. 2017).

Qu. 1.8c. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species has already been reported in fish markets in the risk assessment area, so we cannot entirely discard the possibility of an introduction through this pathway, even if aquaculture and pet trade are more common pathways (Kouba et al. 2014).

Qu. 1.9. Estimate the overall likelihood of introduction into the risk assessment area or entry into the environment based on all pathways and specify if different in relevant biogeographical regions in current conditions.

Provide a thorough assessment of the risk of introduction in relevant biogeographical regions in current conditions: providing insight into the risk of introduction into the risk assessment area.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species is already present in aquaculture facilities in the Mediterranean biogeographic region and sold in markets in Continental and Atlantic biogeographic regions (e.g., Kouba et al. 2014; Deidun et al. 2018; Collas 2020). Besides, being a species present in e-commerce, it could be sold all across the risk assessment area, hence all biogeographic regions are (potentially) affected regarding introduction and entry.

Qu. 1.10. Estimate the overall likelihood of introduction into the risk assessment area or entry into the environment based on all pathways in foreseeable climate change conditions?

Thorough assessment of the risk of introduction in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of introduction (e.g. change in trade or user preferences)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely introduction within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: It is a species adapted to a warm climate, we can expect that it could be still used in aquaculture and pet trade also in the future.

2 PROBABILITY OF ESTABLISHMENT

Important instructions:

- For organisms which are already established in parts of the risk assessment area or have previously been eradicated, the likelihood of establishment should be scored as “very likely” by default.
- Discuss the risk also for those parts of the risk assessment area, where the species is not yet established.

Qu. 2.1. How likely is it that the organism will be able to establish in the risk assessment area based on similarity of climatic and abiotic conditions in its distribution elsewhere in the world?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species is already established in the risk assessment area (Kouba et al. 2014; Deidun et al. 2018; Collas 2020). In Australia it inhabits areas with temperate and tropical climates (characterized by high summer temperatures and low annual rainfall: Souty-Grosset et al. 2006). However, from a laboratory study, Veselý et al. (2015) showed that it can withstand low winter temperatures typical of lentic habitats in the European temperate zone, thus posing a risk of establishment also to these areas besides the Mediterranean one.

Qu. 2.2. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area? Consider if the organism specifically requires another species to complete its life cycle.

RESPONSE	very isolated isolated moderately widespread widespread ubiquitous	CONFIDENCE	low medium high
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Response: The habitats required for survival and reproduction are widespread as the species can colonize a wide range of habitats (alpine streams, subtropical creeks, springs, billabongs, ephemeral lakes, swamps, irrigation ditches: Beatty et al. 2005; Souty-Grosset et al. 2006).

Qu. 2.3. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Interspecific aggressive interactions between *C. destructor* and other crayfish species have not yet been studied in the wild in the risk assessment area. However, recent laboratory studies showed that the species is dominant over the marbled crayfish *P. virginalis*, already proved to outcompete other alien crayfish present in Europe such as *P. clarkii*, *Faxonius limosus* and *F. immunis* (e.g. Hossain et al. 2019). Indeed, in interspecific agonistic encounters *C. destructor* won a significantly higher percentage of fights against *P. virginalis* (but not against signal crayfish *Pacifastacus leniusculus*: Fořt et al. 2019) and at 22°C, in mixed stocks of juveniles of *C. destructor* and *P. virginalis*, the former grew faster and reached higher survival rate (Kouba et al. 2021a). In any case, the species did manage to establish successfully in Italy, France, Ireland and Spain. *Cherax destructor* has chelae of considerable size (Austin & Knott 1996), and we can hypothesize that it could outcompete other European native and non-native crayfish.

Qu. 2.4. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: North American crayfish species present in Europe can carry the crayfish plague caused by the oomycete *Aphanomyces astaci*: *C. destructor* is susceptible to the crayfish plague, and, if it comes in contact with them or water contaminated with spores, it can die, as happened in Italy (Mazza et al. 2018). Moreover, four populations in Spain were intentionally eradicated using this disease (Kouba et al. 2014). However, Mrugala et al. (2016) in laboratory experiments found that *C. destructor* individuals exposed to the least virulent *A. astaci* strain (genotype group A) can partly survive and that not all the individuals died after being infected with the two more virulent strains (genotype B and E). Furthermore, compared to the native noble crayfish *Astacus astacus* also tested in the study, the mortality of *C. destructor* was significantly delayed. Based on these results, the authors suggested that under favourable conditions (e.g. fluctuations of spore concentration), *C. destructor* may survive and contribute to crayfish plague spread in Central Europe.

As with other crayfish species, birds, fish, terrapins, and aquatic mammals can predate the species (Withnall 2000), but they do not significantly impact the establishment of populations, as they usually

do not cause its extinction. Eventually, the established populations in Spain, France, Ireland and Italy demonstrate that they can establish permanent populations despite resident fish and birds, or mammals (otters).

Qu. 2.5. How likely is the organism to establish despite existing management practices in the risk assessment area? Explain if existing management practices could facilitate establishment.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Management practices that promote the commercial use of alien crayfish as a product, e.g. for human consumption or pet trade, may facilitate establishment (Kouba et al. 2014). Management practices in the environment, e.g. ecosystem restoration via the creation of ecological corridors and connections between water bodies, can favour the spread and establishment of the species, as, for example, happened for the red swamp crayfish *Procambarus clarkii* in Italy (Mazza et al. 2018).

Qu. 2.6. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The likelihood of surviving eradication campaigns depends on the invaded habitat and the applied eradication methodology. As with all crayfish species, eradication is very challenging: this species is difficult to detect at low densities, it cannot be easily removed by only physical means, and the use of crayfish plague as mean of eradication could have several impacts on native crayfish (Gherardi et al. 2011). Moreover, the species digs burrows and moves overland that can allow it to survive some physical and chemical management actions (Souty-Grosset et al. 2006), and has a high reproductive rate (Lawrence & Jones 2002) that facilitates the recovery of the population in case of incomplete eradication. Eradication from complex natural habitats, in particular streams and rivers, is not feasible due to a lack of effective targeted methods and prohibitive collateral damage of non-selective methods (e.g., crayfish plague) (Gherardi et al. 2011), plus the risk of recolonisation.

Qu. 2.7. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?

including the following elements:

- a list and description of the reproduction mechanisms of the species in relation to the environmental conditions in the risk assessment area
- an indication of the propagule pressure of the species (e.g. number of gametes, seeds, eggs or propagules, number of reproductive cycles per year) of each of those reproduction mechanisms in relation to the environmental conditions in the risk assessment area.
- If relevant, comment on the likelihood of establishment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in establishment whereas for others high propagule pressure (many thousands of individuals) may not.
- If relevant, comment on the adaptability of the organism to facilitate its establishment and if low genetic diversity in the founder population would have an influence on establishment.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species is omnivorous and opportunistic (Souty-Grosset et al. 2006). Plant material and detritus seem to be the main component of its diet, followed by a low proportion of arthropods, algae and fungi (Lawrence & Jones 2002; Souty-Grosset et al. 2006; Linton et al. 2009), but predation on small fish has been documented as well (Beatty 2006). It is an r-selected species: it has a high growth rate, with early maturity occurring also before one year of age (Lawrence & Jones 2002). The life span is three years, possibly up to six years (Souty-Grosset et al. 2006). In its native range in Australia, with water temperature above 15°C, the species reproduce from early spring to early summer: a female can produce from 30 to 450 eggs, for an average of 350 (Lawrence & Jones 2002). In case of constant water temperatures between 18°C and 20°C and artificial light of 14 hours, a female can spawn five times per year (Lawrence & Jones 2002; Souty-Grosset et al. 2006). It can construct burrows (0.5-2m deep) to survive during the drought (Withnall 2000). It is adapted to a wide range of water temperatures (1°C-35°C), and it can naturally disperse within the same basin (Souty-Grosset et al. 2006). Anecdotal reports suggest that *C. destructor* emerges from hypoxic water and migrates between waterbodies (Morris & Callaghan 1998). From laboratory study, Veselý, et al. (2015) showed that *C. destructor* can withstand low winter temperatures typical of lentic habitats in the European temperate zone, thus posing a serious threat to these areas. We do not have any data on propagule pressure. Still, we cannot discard the possibility that few individuals can establish viable populations. Thus, the founder's genetics could not influence its establishment as happened in other introduced crayfish in Europe (e.g. the parthenogenetic marbled crayfish *P. virginialis* and the spinycheek crayfish *F. limosus*: Kouba et al. 2014).

Beatty et al. (2005) studied the population and reproductive biology of a translocated Western Australian population of *C. destructor*: the species matured at the end of its first year of life, have a protracted spawning period (July-January), a high fecundity (210 eggs on average) and fast growth rate.

Qu. 2.8. If the organism does not establish, then how likely is it that casual populations will continue to occur?

Consider, for example, a species which cannot reproduce in the risk assessment area, because of unsuitable climatic conditions or host plants, but is present because of recurring introduction, entry and release events. This may also apply for long-living organisms.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Continuous escape from aquaculture and/or escape/release of the species as unwanted pet in climatically unsuitable regions is likely to occur and may lead to casual populations in these areas as happened to the congeneric *Cherax quadricarinatus* (Kouba et al. 2014; Haubrock et al. 2021).

Qu. 2.9. Estimate the overall likelihood of establishment in the risk assessment area under current climatic conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under current climatic conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in current conditions: providing insight in the risk of establishment in (new areas in) the risk assessment area.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species is already established in the Mediterranean and Atlantic biogeographic region (Kouba et al. 2014). According to the model developed on current climate (Annex VIII), the species could find many suitable areas to establish permanent populations in the Atlantic and Mediterranean biogeographic regions, while very few suitable areas are present in the Black Sea and Continental biogeographic regions, even if the importance of thermal waters in these areas as sink/source of alien crayfish should not be discarded.

Qu. 2.10. Estimate the overall likelihood of establishment in the risk assessment area under foreseeable climate change conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under foreseeable climate change conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of establishment (e.g. increase in average winter temperature, increase in drought periods)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely establishment within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species is adapted to a warm climate and, according to the SDM (2070 RCP 2.6/RCP 4.5; Annex VIII), the areas suitable for the species will increase in the Atlantic biogeographic region (while a small decrease could happen in the Mediterranean biogeographic region), while they will remain almost the same for the Black Sea and Continental biogeographic regions. The minimum temperature of the coldest month is currently limiting the establishment in the northern and coldest biogeographic regions (but apparently not in the coastal areas of some Member States such as Ireland and France), so with a warmer climate we can expect an increase in the establishment.

3 PROBABILITY OF SPREAD

Important instructions:

- Spread is defined as the expansion of the geographical distribution of an alien species within the risk assessment area.
- Repeated releases at separate locations do not represent continuous spread and should be considered in the probability of introduction and entry section (Qu. 1.7).

Qu. 3.1. How important is the expected spread of this organism within the risk assessment area by natural means? (List and comment on each of the mechanisms for natural spread.)

including the following elements:

- a list and description of the natural spread mechanisms of the species in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of spread discussed in relation to the species biology and the environmental conditions in the risk assessment area.

The description of spread patterns here refers to the CBD pathway category “Unaided (Natural Spread)”. It should include elements of the species life history and behavioural traits able to explain its ability to spread, including: reproduction or growth strategy, dispersal capacity, longevity, dietary requirements, environmental and climatic requirements, specialist or generalist characteristics.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Currently, the species is restricted to a few locations in the risk assessment area. There are no detailed studies on its locomotory activity, but it can naturally disperse within the same basin (Souty-Grosset et al. 2006). In the introduced range of Western Australia, it is reported that in 1982 it was found for the first time in the wild, and in three years colonized many sites, showing a continuous spread (Austin 1985; Lynas et al. 2004). Its current distribution occurs from Hutt River in the north to Esperance in the southeast of Australia (Morrissy & Cassells 1992; Horwitz & Knott 1995; Beatty et al. 2005). As reported, anecdotal reports suggest that *C. destructor* emerges from hypoxic water and migrates between waterbodies (Morris & Callaghan 1998), which may increase chances to spread across different water bodies.

The species can colonize a wide range of habitats (alpine streams, subtropical creeks, springs, billabongs, ephemeral lakes, swamps, irrigation ditches: Beatty et al. 2005; Souty-Grosset et al. 2006), digging burrows to survive during the drought and the winter (Withnall 2000; Veselý et al. 2015; Kouba et al. 2016). It is adapted to a wide range of water temperatures (1°C - 35°C). It can tolerate salinity (up to 8 ppt: Mills & Geddes 1980) and hypoxic waters (oxygen concentration <1 mg L⁻¹: Morrissy & Cassells 1992). Optimal pH is comprised between 7.5 and 8.5 (CABI 2011). The species is omnivorous and opportunistic (Souty-Grosset et al. 2006); it has a high growth rate, with early maturity also occurring before one year of age (Lawrence & Jones 2002). The life span is three years,

possibly up to six years (Souty-Grosset et al. 2006), and a female can produce from 30 to 450 eggs, for an average of 350 (Lawrence & Jones 2002).

Qu. 3.2a. List and describe relevant pathways of spread other than "unaided". For each pathway answer questions 3.3 to 3.9 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 3.3a, 3.4a, etc. and then 3.3b, 3.4b, etc. for the next pathway.

including the following elements:

- a list and description of pathways of spread with an indication of their importance and associated risks (e.g. the likelihood of spread in the risk assessment area, based on these pathways; likelihood of survival, or reproduction, or increase during transport and storage; ability and likelihood of transfer from the pathway to a suitable habitat or host) in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of spread for each pathway discussed in relation to the species biology and the environmental conditions in the risk assessment area.
- All relevant pathways of spread (except “Unaided (Natural Spread)”, which is assessed in Qu. 3.1) should be considered. The classification of pathways developed by the Convention of Biological Diversity shall be used (see Annex IV).

Pathway name: Corridor (Interconnected waterways/basins/seas)

Qu. 3.3a. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional	CONFIDENCE	low
	unintentional		medium
			high

Response: There are interconnected rivers and basins in the risk assessment area throughout human infrastructures (e.g., canals) so we cannot discard the possibility of the species spreading through this pathway, as happened for other crayfish species (e.g. *Faxonius* and *Procambarus* spp. Kouba et al. 2014).

Qu. 3.4a. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway

- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: There are no data on the propagule pressure, but, as shown by the natural spread of the species in the invaded range in Australia (Lynas et al. 2004), the species could also spread through this pathway. Some traits mentioned above – such as successful reproduction – could facilitate the spread. Reinvasion could happen, as the species seems capable to disperse also overland (Morris & Callaghan 1998).

Qu. 3.5a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species could survive along this pathway, as shown by its active dispersal in the invaded range in Australia (Lynas et al. 2004). It could even reproduce while spreading, as happened for other macroinvertebrates (Leuven et al. 2009).

Qu. 3.6a. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Some barriers along the interconnected water bodies could slow the spread of species, but they would not affect its survival as showed in other alien crayfish (e.g., Krieg & Zenker 2020). Water drainage could potentially affect the survival of individuals, even if the species can withstand drought conditions remaining in the burrows..

Qu. 3.7a. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Interconnected canals and rivers allowed the undetected spread of many invasive species in Europe (Panov et al. 2007, 2009); this could also happen for *C. destructor*.

Qu. 3.8a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: During natural dispersal in Australia (Lynas et al. 2004), the species usually arrives and settles in suitable habitats or moves on; the same could happen using this pathway.

Qu. 3.9a. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area. (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: No quantitative data are available on the spread rate. Based on the situation observed in Australia (Lynas et al. 2004), we could hypothesise a moderate rate of dispersal using this pathway.

Qu. 3.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?

RESPONSE	very easy easy with some difficulty difficult very difficult	CONFIDENCE	low medium high
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Response: Once in an aquatic system, it could be difficult to contain the species. Physical barriers have been tested on other alien crayfish, but they can also stop native species movement (Gherardi et al. 2011). Containment could be hypothesized for closed or almost confined systems: e.g. ponds or areas that could be fenced. The use of pyrethroids is not always feasible for their toxicity to other non-target species and to be effective against *C. destructor* a higher dose than other alien crayfish is required (Lidova et al. 2019).

Qu. 3.11. Estimate the overall potential rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (indicate any key issues and provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in current conditions, providing insight in the risk of spread into (new areas in) the risk assessment area.

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: According to what was reported for invaded areas in Australia (Lynas et al. 2004), we can hypothesize that species can moderately spread by natural means in the Mediterranean biogeographic region where it is currently present. Moreover, we should also consider that this area is occupied by North American crayfish species, especially *P. clarkii*, carrying highly virulent strains of *A. astaci* to which *C. destructor* is susceptible and that could act as a sort of barrier for the species dispersal.

Qu. 3.12. Estimate the overall potential rate of spread in relevant biogeographical regions in foreseeable climate change conditions (provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if rates of spread are likely slowed down or accelerated.

RESPONSE	very slowly	CONFIDENCE	low
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	slowly moderately rapidly very rapidly		medium high
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Response: As climate change will favour the species, we can hypothesize that in the future the species can spread by natural means at least similar to the current spread rates, considering also the presence of North American crayfish carrying highly virulent *A. astaci* strain.

4 MAGNITUDE OF IMPACT

Important instructions:

- Questions 4.1-4.5 relate to biodiversity and ecosystem impacts, 4.6-4.8 to impacts on ecosystem services, 4.9-4.13 to economic impact, 4.14-4.15 to social and human health impact, and 4.16-4.18 to other impacts. These impacts can be interlinked, for example, a disease may cause impacts on biodiversity and/or ecosystem functioning that leads to impacts on ecosystem services and finally economic impacts. In such cases the assessor should try to note the different impacts where most appropriate, cross-referencing between questions when needed.
- Each set of questions starts with the impact elsewhere in the world, then considers impacts in the risk assessment area (=EU27+UK excluding outermost regions) separating known impacts to date (i.e. past and current impacts) from potential future impacts (including foreseeable climate change).
- Only negative impacts are considered in this section (socio-economic benefits are considered in Qu. A.7)
- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable).

Biodiversity and ecosystem impacts

Qu. 4.1. How important is the impact of the organism on biodiversity at all levels of organisation caused by the organism in its non-native range excluding the risk assessment area?

including the following elements:

- Biodiversity means the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems
- impacted chemical, physical or structural characteristics and functioning of ecosystems

RESPONSE		CONFIDENCE	
	minimal		low
	minor		medium
	moderate		high
	major		
	massive		

Response: In non-native range in Australia, it is reported to have destroyed macrophytes, caused siltation, displaced the endemic crayfish *Euastacus dharawalus* and threatened 11 endemic crayfish of Western Australia (Gherardi 2010; Coughran & Daily 2012). Other potential impacts on fish, amphibians, dragonflies, snails and other invertebrates have been suggested (Beatty 2006; Lynas et al. 2004; Coughran & Daily 2012; Cerato et al. 2019). Moreover, laboratory studies conducted in Australia showed its aggressive and predatory behaviour towards tortoise hatchlings, hypothesizing possible negative impact on native endangered freshwater turtles (Bradsell et al. 2002).

Qu. 4.2. How important is the current known impact of the organism on biodiversity at all levels of organisation (e.g. decline in native species, changes in native species communities, hybridisation) in the risk assessment area (include any past impact in your response)?

Discuss impacts that are currently occurring or are likely occurring or have occurred in the past in the risk assessment area. Where there is no direct evidence of impact in the risk assessment area (for example no studies have been conducted), evidence from outside of the risk assessment area can be used to infer impacts within the risk assessment area.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: In the risk assessment area, up to now, no impacts have been reported. The species is possibly resistant to crayfish plague in some cases, becoming an additional vector of this disease that is lethal for European native crayfish (Mrugala et al. 2016). Moreover, we cannot discard other potential impacts as possible competition with native crayfish, considering its bigger chelae, and possible negative impacts on macroinvertebrates and macrophytes, being an omnivorous species (Souty-Grosset et al. 2006).

Qu. 4.3. How important is the potential future impact of the organism on biodiversity at all levels of organisation likely to be in the risk assessment area?

See comment above. The potential future impact shall be assessed only for the risk assessment area. A potential increase in the distribution range due to climate change does not *per se* justify a higher impact score.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: According to the SDM (Annex VIII), the species will find more suitable climatic areas in the risk assessment area for its establishment in the future, especially in the Atlantic biogeographic region. Considering that the species is better adapted to warm climates, we cannot discard the hypothesis of a higher impact in the risk assessment area than under current conditions. Being an omnivorous species, it can cause a decrease in macrophyte cover, macroinvertebrates abundance and diversity, changing trophic interaction and community composition (Beatty 2006; Lynas et al. 2004; Coughran & Daily 2012; Cerato et al. 2019).

Qu. 4.4. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism currently in the risk assessment area?

including the following elements:

- native species impacted, including red list species, endemic species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: In the risk assessment area, up to now, no impacts have been reported. The species is possibly resistant to crayfish plague in some cases, becoming an additional vector of this disease that is lethal for European native crayfish (Mrugala et al. 2016), listed in the Habitat Directive, such as the white-clawed crayfish, *Austropotamobius pallipes*, the stone crayfish *A. torrentium* and the noble crayfish *Astacus astacus*.

Qu. 4.5. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.3. and 4.4.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: According to the SDM (Annex VIII), the species will find more suitable climatic areas in the risk assessment area in the future, so we can expect a major impact on conservation concern species and habitats as listed below.

Impact on native crayfish species listed in the Habitat Directive, such as the white-clawed crayfish, *Austropotamobius pallipes*, the stone crayfish *A. torrentium* and the noble crayfish *Astacus astacus*, could be significant through disease if *C. destructor* can survive and transmit the crayfish plague, lethally affecting native species (Mrugala et al. 2016). Moreover, several other diseases causing severe mortalities (specifically viruses) and other parasites are known mainly in aquaculture and pet trade of *Cherax* species, *C. destructor* included (e.g. Haubrock et al. 2021; Ložek et al. 2021) but their potential spillover on native crayfish has not yet been assessed and cannot be completely discarded.

Finally, *C. destructor* has chelae of considerable size (Austin & Knott 1996), and, based on laboratory experiments conducted on interspecific conditions with other alien crayfish (Fořt et al. 2019; Kouba et al. 2021a), we can hypothesize that it can outcompete native crayfish. The impact could also be significant on native macroinvertebrates (e.g. snails, odonates *Leucorrhinia caudalis*, *Ophiogomphus cecilia*, both species in the European Red List of Dragonflies and in the Habitat Directive), amphibians and reptiles (e.g. *Triturus* spp., *Rana* spp. in the Habitat Directive). All invaded habitats could be affected due to the species consumption of macroinvertebrates and macrophytes, in addition to burrowing and siltation. In this way, it could alter the ecological status of water bodies according to the Water Framework Directive. It could have negative effects on protected areas, potentially affecting habitats and species listed in the Habitat Directive (e.g. 3150 Natural eutrophic lakes with *Magnopotamion* or *Hydrocharition*; 3270 Rivers with muddy banks with *Chenopodion rubri* pp and *Bidention* pp vegetation; abovementioned dragonflies, reptiles and crayfish).

Ecosystem Services impacts

Qu. 4.6. How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?

- For a list of services use the CICES classification V5.1 provided in Annex V.
- Impacts on ecosystem services build on the observed impacts on biodiversity (habitat, species, genetic, functional) but focus exclusively on reflecting these changes in relation to their links with socio-economic well-being.
- Quantitative data should be provided whenever available and references duly reported.
- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE		CONFIDENCE	
	minimal		low
	minor		medium
	moderate		high
	major		
	massive		

Response: In the Australian introduced range, being a burrowing species, together with damage to riverbanks, siltation is reported together with destruction of macrophytes (Coughran & Daily 2012) with possible cascading effects on native species and ecosystem services (e.g. regulating services: baseline flows and extreme event regulation, water quality, soil quality regulation; provisioning services: wild plants and wild animals; cultural services: intellectual and representative interactions with natural environment considering the impact on local fauna and flora, and on native endangered crayfish species important for local people).

Qu. 4.7. How important is the impact of the organism on provisioning, regulating, and cultural services currently in the different biogeographic regions or marine sub-regions where

the species has established in the risk assessment area (include any past impact in your response)?

- See guidance to Qu. 4.6.

RESPONSE	N/A minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No information has been found on the issue. In the risk assessment area, up to now no impacts on ecosystem services have been reported.

Qu. 4.8. How important is the impact of the organism on provisioning, regulating, and cultural services likely to be in the different biogeographic regions or marine sub-regions where the species can establish in the risk assessment area in the future?

- See guidance to Qu. 4.6.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: According to the predictive climatic models, the species will find more suitable climatic areas in the risk assessment area in the future (see Annex VIII), so we can expect major impacts on ecosystem services. Indeed, being a burrowing species, it can damage riverbanks leading to soil erosion and changes in sediments (regulating services: baseline flows and extreme event regulation; soil quality regulation), while the increase in turbidity can have a cascade effect on native species, primary production and trophic chains (provisioning services: wild plants and wild animals) and water quality (regulating services: water conditions, and provisioning services: water quality), as recorded for other invasive alien crayfish intensively burrowing in Europe (e.g. *Procambarus clarkii*; Souty-Grosset et al. 2016). Also, cultural services can be impacted because turbid ponds do not attract people (physical and experiential interactions with natural environment), and the species alters the pristine characteristics of the habitat and community composition (intellectual and representative interactions with natural environment).

Economic impacts

Qu. 4.9. How great is the overall economic cost caused by the organism within its current area of distribution (excluding the risk assessment area), including both costs of / loss due to damage and the cost of current management.

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the world these should be reported here. The assessment of the potential costs of / loss due to damage shall describe those costs quantitatively and/or qualitatively depending on what information is available. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage. As far as possible, it would be useful to separate costs of / loss due to the organism from costs of current management.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: For Australia, Frost (1975) reported that “the burrows could destroy the integrity of dam walls” and Williams (1980) reported that “Many...construct burrows into which they retreat with the onset of unfavourable conditions. It is these burrows which are often so much of a nuisance in farm dams where they may cause the collapse of retaining walls and drainage canals”. “There are, however, indications that the reported damage to irrigation facilities occurs only under certain conditions” (de Moor 2002). No detailed information has been found for the costs of damaged banks and their management (see Kouba et al. 2021b). It is also considered a threat for the fishery of the congeneric *C. tenuimanus* and *C. cainii* (Souty-Grosset et al. 2006).

Qu. 4.10. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism currently in the risk assessment area (include any past costs in your response)?

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the EU these should be reported here. Assessment of the potential costs of damage on human health, safety, and the economy, including the cost of non-action. A full economic assessment at EU scale might not be possible, but qualitative data or different case studies from across the EU (or third countries if relevant) may provide useful information to inform decision making. In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable). Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	N/A minimal	CONFIDENCE	low medium
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	minor moderate major massive		high
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Response: No information has been found on the issue. In the risk assessment area, up to now, no economic impacts have been reported.

Qu. 4.11. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.10.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: This depends on the time elapsed between the first introduction and when (and if) action is taken to control the species. If action is taken immediately to mitigate its spread, then costs could be relatively low (for example damage to banks would be limited), but if left to spread the costs would potentially be major (the costs linked to riverbanks could increase; increase of turbidity could also affect fish populations with a potential consequence on fishing activities).

Qu. 4.12. How great are the economic costs / losses associated with managing this organism currently in the risk assessment area (include any past costs in your response)?

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable).

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Even if no detailed costs have been found on the issue in the risk assessment area (Kouba et al. 2021b, we can hypothesize a minimal cost for the eradication of the species in Spain using crayfish plague: indeed, cages containing individuals of either *C. destructor* infected in the laboratory or signal crayfish *Pacifastacus leniusculus* with severe signs of infection were introduced into the invaded ponds (Gherardi et al. 2011).

Qu. 4.13. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.12.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: If action is taken immediately to mitigate its spread, then costs could be relatively low, but if left to spread, the costs would potentially be major. Considering management activities on other invasive alien crayfish in Europe, intensive trapping an area of 10,000 m² with 120 traps for 171 trap days could cost approximately 30,000 euro to reduce species abundance by at least 70%. But intensive trapping should be permanently maintained through time. Eradication of crayfish from a water body of 19,000 m² could cost around 46,500 euro of biocides. Drainage at least once of a pond of approximately 400,000 m³ could cost around 40-50,000 euro (E. Tricarico, unpublished, 2021). Costs are estimated based on the experience with measures targeting other invasive crayfish present in the risk assessment area. Costs of using crayfish plague could be lower, but with potentially adverse effects on native European crayfish.

Social and human health impacts

Qu. 4.14. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism for the risk assessment area and for third countries, if relevant (e.g. with similar eco-climatic conditions).

The description of the known impact and the assessment of potential future impact on human health, safety and the economy, shall, if relevant, include information on

- illnesses, allergies or other affections to humans that may derive directly or indirectly from a species;
- damages provoked directly or indirectly by a species with consequences for the safety of people, property or infrastructure;
- direct or indirect disruption of, or other consequences for, an economic or social activity due to the presence of a species.

Social and human health impacts can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal minor moderate major	CONFIDENCE	low medium high
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	massive		
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Response: No human health impacts have been reported up to now. The risk of flooding might increase if dykes are destabilized by crayfish burrowing (e.g. *Procambarus clarkii*: Haubrock et al. 2019). Possible risks could come from consumption of crayfish caught in contaminated waters by e.g. heavy metals as happened in other crayfish (e.g. the red swamp crayfish *P. clarkii*: Anandkumar et al. 2020).

Qu. 4.15. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism in the future for the risk assessment area.

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable).

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: As in the future, the suitable areas for the species will increase we can hypothesize that risk of flooding might increase for crayfish burrowing because they actively dig, and this could lead to instability/collapse of banks, as already happened for a similar burrowing species *Procambarus clarkii* (Haubrock et al. 2019). The risk of consuming contaminated individuals could also persist similar to the current one.

Other impacts

Qu. 4.16. How important is the organism in facilitating other damaging organisms (e.g. diseases) as food source, a host, a symbiont or a vector etc.?

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: If the species survives the crayfish plague, it could transmit it to European native crayfish (Mrugala et al. 2016). Moreover, we cannot discard the potential spillover of other diseases and parasites transmitted by the species.

Qu. 4.17. How important might other impacts not already covered by previous questions be resulting from introduction of the organism?

RESPONSE	N/A minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No information has been found on the issue.

Qu. 4.18. How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area?

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Even if fish and birds could predate on the species, they regulate the population to a low density level but do not cause extinction. Other invasive crayfish through the transmission of crayfish plague may slow the establishment and spread of the species, and maybe the impacts, but further studies are needed to assess this aspect (also because the species seems resistant to this disease in some cases).

Qu. 4.19. Estimate the overall impact in the risk assessment area under current climate conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, in current conditions.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No specific studies are available for the risk assessment area. However, studies from the Australian invaded range demonstrated potential and actual relevant ecological and economic impacts (reduction of macroinvertebrate abundance; decline of macrophytes; highly burrowing activity with damages to banks, impacts on fishery: Bradsell et al. 2002; Beatty 2006; Lynas et al. 2004; Coughran & Daily 2012; Cerato et al. 2019). Moreover, laboratory studies conducted in Europe showed the potential to withstand low temperatures and survival of crayfish plague, becoming an additional vector of the disease (Veselý et al. 2015; Kouba et al. 2016; Mrugala et al. 2016, 2019), and the possibility to outcompete native crayfish (e.g. Fořt et al. 2019; Kouba et al. 2021a). Thus, we can hypothesise moderate impacts under current climate in the Mediterranean and Atlantic biogeographic region.

Qu. 4.20. Estimate the overall impact in the risk assessment area in foreseeable climate change conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, under future conditions.

- See also guidance to Qu. 4.3.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: As the species will find more suitable areas for establishment in the future (see Annex VIII), we can expect its impacts to be major in the future, especially in Atlantic and Mediterranean biogeographic regions.

RISK SUMMARIES			
	RESPONSE	CONFIDENCE	COMMENT
Summarise Introduction and Entry*	very unlikely unlikely moderately likely likely very likely	low medium high	The species was already introduced in the risk assessment area via pet trade, aquaculture, and live food. It also entered into the wild intentionally and unintentionally. It is expected that in the future, due to its tolerance to warm climate and the increasing use of colourful crayfish species in aquaria, it could be still used in aquaculture and pet trade.
Summarise Establishment*	very unlikely unlikely moderately likely likely very likely	low medium high	The species is already established in the Mediterranean and Atlantic biogeographic regions of the risk assessment area (France, Ireland, Spain and Italy since the late 1980s), and it is likely that under future climate it could establish also more in the Atlantic biogeographic region (and partly in few areas of the Black Sea and Continental biogeographic regions).
Summarise Spread*	very slowly slowly moderately rapidly very rapidly	low medium high	The species is currently reported from few locations in the risk assessment area. Considering the favourable current and future climatic conditions and its capability to disperse unaided and via interconnected water basins, the species would be able to moderately spread as shown in the invaded range in Australia. In addition, their susceptibility (or not) to crayfish plague could interfere with the survival of individuals as they spread into other habitats already colonized by North American crayfish species.
Summarise Impact*	minimal minor moderate major massive	low medium high	No studies on impacts are available for the few locations of introduction in the risk assessment area. The studies on the impacts are from its Australian invaded range, but demonstrated ecological and economic impacts (reduction of macroinvertebrate abundance; decline of macrophytes; highly burrowing activity with damages to banks; impacts on fishery). A

			<p>study in Europe confirmed its susceptibility to crayfish plague with a certain resistance (that could allow the species to transmit it to European native crayfish with lethal consequences). The potential spillover of other lethal diseases and parasites carried by the species on native European crayfish cannot be completely discarded. Considering the current climate, we can expect moderate impacts in the risk assessment area, but even major impacts under the future climate.</p>
<p>Conclusion of the risk assessment (overall risk)</p>	<p>low moderate high</p>	<p>low medium high</p>	<p>Based on the literature from Australia, its adaptability and the still active pathways (e.g. aquaculture and pet trade), we can expect that the species may establish wild populations and could pose a high risk to the biodiversity of the risk assessment area and cause also moderate economic damages in the future.</p>

*in current climate conditions and in foreseeable future climate conditions

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Distribution Summary

Please answer as follows:

- Yes if recorded, established or invasive
- if not recorded, established or invasive
- ? Unknown; data deficient and/or?

The columns refer to the answers to Questions A5 to A12 under Section A.

For data on marine species at the Member State level, delete Member States that have no marine borders. In all other cases, provide answers for all columns.

Member States and the United Kingdom

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Austria	-	-			-
Belgium	-	-		YES	-
Bulgaria	-	-			-
Croatia	-	-	YES		-
Cyprus	-	-			-
Czech Republic	-	-			-
Denmark	-	-			-
Estonia	-	-			-
Finland	-	-			-
France	YES	YES	YES	YES	-
Germany	-	-		YES	-
Greece	-	-	YES	YES	-
Hungary	-	-			-
Ireland	YES	YES		YES	-
Italy	YES	YES	YES	YES	-
Latvia	-	-			-
Lithuania	-	-			-
Luxembourg	-	-			-
Malta	-	-			-
Netherlands	-	-		YES	-
Poland	-	-			-
Portugal	-	-	YES	YES	-
Romania	-	-			-
Slovakia	-	-			-
Slovenia	-	-			-
Spain	YES	YES	YES	YES	-
Sweden	-	-		YES	-
United Kingdom	-	-	YES		-

Biogeographical regions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Alpine	-	-	-	-	-
Atlantic	YES	YES	YES	YES	-
Black Sea	-	-	YES	YES	-
Boreal	-	-	-	-	-
Continental	-	-	YES	YES	-
Mediterranean	YES	YES	YES	YES	-
Pannonian	-	-	-	-	-
Steppic	-	-	-	-	-

ANNEX I Scoring of Likelihoods of Events

(taken from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Description	Frequency
Very unlikely	This sort of event is theoretically possible, but is never known to have occurred and is not expected to occur	1 in 10,000 years
Unlikely	This sort of event has occurred somewhere at least once in the last millenium	1 in 1,000 years
Moderately likely	This sort of event has occurred somewhere at least once in the last century	1 in 100 years
Likely	This sort of event has happened on several occasions elsewhere, or on at least once in the last decade	1 in 10 years
Very likely	This sort of event happens continually and would be expected to occur	Once a year

ANNEX II Scoring of Magnitude of Impacts

(modified from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Biodiversity and ecosystem impact	Ecosystem Services impact	Economic impact (Monetary loss and response costs per year)	Social and human health impact, and other impacts
	<i>Question 5.1-5</i>	<i>Question 5.6-8</i>	<i>Question 5.9-13</i>	<i>Question 5.14-18</i>
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected ⁷	Up to 10,000 Euro	No social disruption. Local, mild, short-term reversible effects to individuals.
Minor	Some ecosystem impact, reversible changes, localised	Local and temporary, reversible effects to one or few services	10,000-100,000 Euro	Significant concern expressed at local level. Mild short-term reversible effects to identifiable groups, localised.
Moderate	Measureable long-term damage to populations and ecosystem, but reversible; little spread, no extinction	Measureable, temporary, local and reversible effects on one or several services	100,000-1,000,000 Euro	Temporary changes to normal activities at local level. Minor irreversible effects and/or larger numbers covered by reversible effects, localised.
Major	Long-term irreversible ecosystem change, spreading beyond local area	Local and irreversible or widespread and reversible effects on one / several services	1,000,000-10,000,000 Euro	Some permanent change of activity locally, concern expressed over wider area. Significant irreversible effects locally or reversible effects over large area.
Massive	Widespread, long-term population loss or extinction, affecting several species with serious ecosystem effects	Widespread and irreversible effects on one / several services	Above 10,000,000 Euro	Long-term social change, significant loss of employment, migration from affected area. Widespread, severe, long-term, irreversible health effects.

⁷ Not to be confused with “no impact”.

ANNEX III Scoring of Confidence Levels

(modified from Bacher et al. 2017)

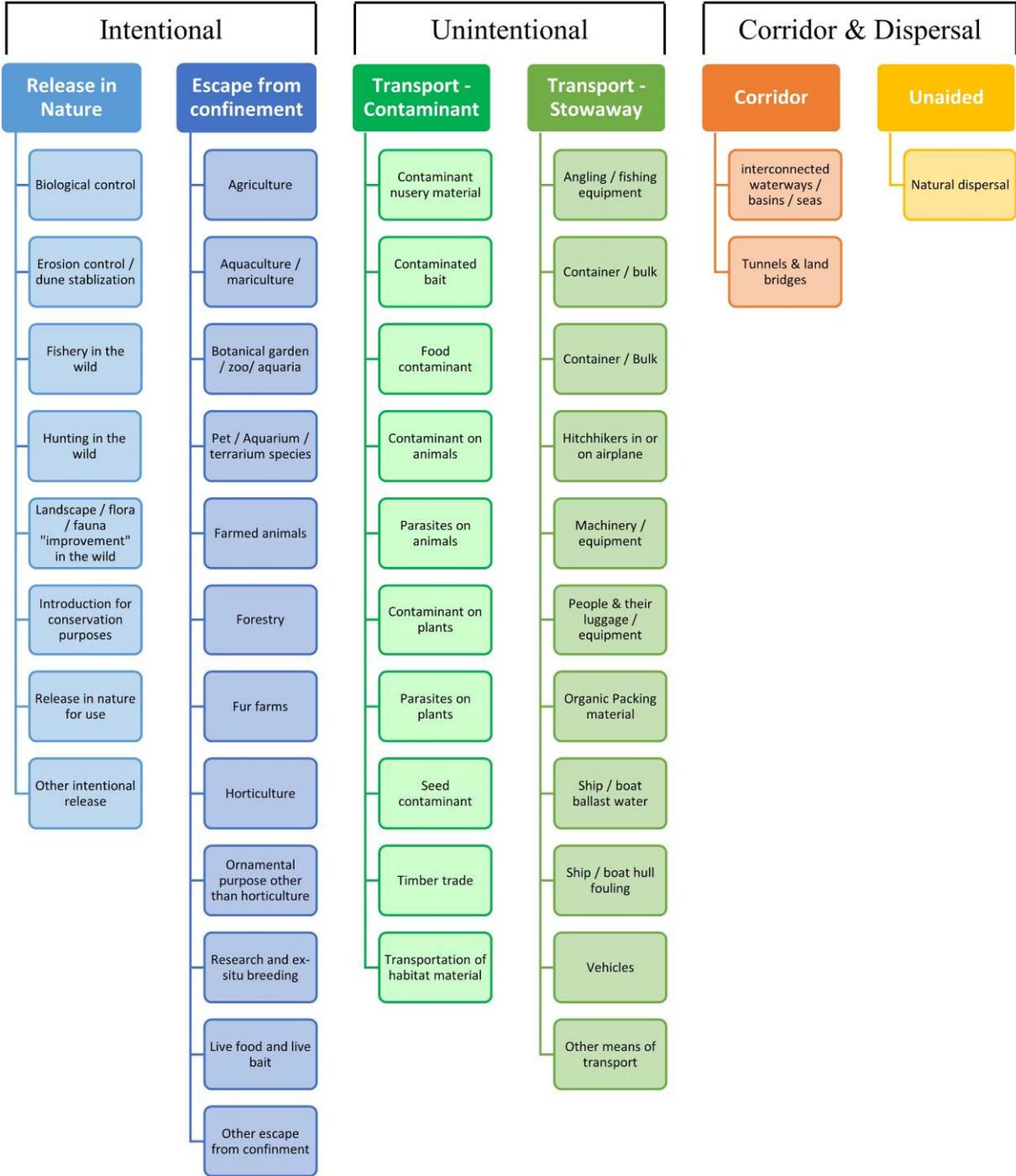
Each answer provided in the risk assessment must include an assessment of the level of confidence attached to that answer, reflecting the possibility that information needed for the answer is not available or is insufficient or available but conflicting.

The responses in the risk assessment should clearly support the choice of the confidence level.

Confidence level	Description
Low	There is no direct observational evidence to support the assessment, e.g. only inferred data have been used as supporting evidence <i>and/or</i> Impacts are recorded at a spatial scale which is unlikely to be relevant to the assessment area <i>and/or</i> Evidence is poor and difficult to interpret, e.g. because it is strongly ambiguous <i>and/or</i> The information sources are considered to be of low quality or contain information that is unreliable.
Medium	There is some direct observational evidence to support the assessment, but some information is inferred <i>and/or</i> Impacts are recorded at a small spatial scale, but rescaling of the data to relevant scales of the assessment area is considered reliable, or to embrace little uncertainty <i>and/or</i> The interpretation of the data is to some extent ambiguous or contradictory.
High	There is direct relevant observational evidence to support the assessment (including causality) <i>and</i> Impacts are recorded at a comparable scale <i>and/or</i> There are reliable/good quality data sources on impacts of the taxa <i>and</i> The interpretation of data/information is straightforward <i>and/or</i> Data/information are not controversial or contradictory.

ANNEX IV CBD pathway categorisation scheme

Overview of CBD pathway categorisation scheme showing how the 44 pathways relate to the six main pathway categories. All of the pathways can be broadly classified into 1) those that involve intentional transport (blue), 2) those in which the taxa are unintentionally transported (green) and 3) those where taxa moved between regions without direct transportation by humans and/or via artificial corridors (orange and yellow). **Note that the pathways in the category “Escape from confinement” can be considered intentional for the introduction into the risk assessment area and unintentional for the entry into the environment.**



ANNEX V Ecosystem services classification (CICES V5.1, simplified) and examples

For the purposes of this risk assessment, please feel free to use what seems as the most appropriate category / level / combination of impact (Section – Division – Group), reflecting information available.

Section	Division	Group	Examples (i.e. relevant CICES “classes”)
Provisioning	Biomass	Cultivated terrestrial plants	Cultivated terrestrial plants (including fungi, algae) grown for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from cultivated plants, fungi, algae and bacteria for direct use or processing (excluding genetic materials); Cultivated plants (including fungi, algae) grown as a <u>source of energy</u> <i>Example: negative impacts of non-native organisms to crops, orchards, timber etc.</i>
		Cultivated aquatic plants	Plants cultivated by in- situ aquaculture grown for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from in-situ aquaculture for direct use or processing (excluding genetic materials); Plants cultivated by in- situ aquaculture grown as an <u>energy source</u> . <i>Example: negative impacts of non-native organisms to aquatic plants cultivated for nutrition, gardening etc. purposes.</i>
		Reared animals	Animals reared for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from reared animals for direct use or processing (excluding genetic materials); Animals reared to provide <u>energy</u> (including mechanical) <i>Example: negative impacts of non-native organisms to livestock</i>
		Reared aquatic animals	Animals reared by in-situ aquaculture for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from animals grown by in-situ aquaculture for direct use or processing (excluding genetic materials); Animals reared by in-situ aquaculture as an <u>energy source</u> <i>Example: negative impacts of non-native organisms to fish farming</i>
		Wild plants (terrestrial and aquatic)	Wild plants (terrestrial and aquatic, including fungi, algae) used for <u>nutrition</u> ; <u>Fibres and other materials</u> from wild plants for direct use or processing (excluding genetic materials); Wild plants (terrestrial and aquatic, including fungi, algae) used as a <u>source of energy</u> <i>Example: reduction in the availability of wild plants (e.g. wild berries, ornamentals) due to non-native organisms (competition, spread of disease etc.)</i>
		Wild animals (terrestrial and aquatic)	Wild animals (terrestrial and aquatic) used for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from wild animals for direct use or processing (excluding genetic materials); Wild animals (terrestrial and aquatic) used as a <u>source of energy</u>

			<i>Example: reduction in the availability of wild animals (e.g. fish stocks, game) due to non-native organisms (competition, predations, spread of disease etc.)</i>
	Genetic material from all biota	Genetic material from plants, algae or fungi	<p><u>Seeds, spores and other plant materials</u> collected for maintaining or establishing a population; Higher and lower plants (whole organisms) used to <u>breed new strains or varieties</u>; Individual genes extracted from higher and lower plants for the <u>design and construction of new biological entities</u></p> <p><i>Example: negative impacts of non-native organisms due to interbreeding</i></p>
		Genetic material from animals	<p>Animal material collected for the purposes of maintaining or establishing a population; Wild animals (whole organisms) used to breed new strains or varieties; Individual genes extracted from organisms for the design and construction of new biological entities</p> <p><i>Example: negative impacts of non-native organisms due to interbreeding</i></p>
	Water ⁸	Surface water used for nutrition, materials or energy	<p>Surface water for <u>drinking</u>; Surface water used as a material (<u>non-drinking purposes</u>); Freshwater surface water, coastal and marine water used as an <u>energy source</u></p> <p><i>Example: loss of access to surface water due to spread of non-native organisms</i></p>
		Ground water for used for nutrition, materials or energy	<p>Ground (and subsurface) water for <u>drinking</u>; Ground water (and subsurface) used as a material (<u>non-drinking purposes</u>); Ground water (and subsurface) used as an <u>energy source</u></p> <p><i>Example: reduced availability of ground water due to spread of non-native organisms and associated increase of ground water consumption by vegetation.</i></p>
Regulation & Maintenance	Transformation of biochemical or physical inputs to ecosystems	Mediation of wastes or toxic substances of anthropogenic origin by living processes	<p><u>Bio-remediation</u> by micro-organisms, algae, plants, and animals; <u>Filtration/sequestration/storage/accumulation</u> by micro-organisms, algae, plants, and animals</p> <p><i>Example: changes caused by non-native organisms to ecosystem functioning and ability to filtrate etc. waste or toxics</i></p>
		Mediation of nuisances of anthropogenic origin	<p><u>Smell reduction</u>; <u>noise attenuation</u>; <u>visual screening</u> (e.g. by means of green infrastructure)</p> <p><i>Example: changes caused by non-native organisms to ecosystem structure, leading to reduced ability to mediate nuisances.</i></p>
	Regulation of physical, chemical, biological conditions	Baseline flows and extreme event regulation	<p>Control of <u>erosion</u> rates; Buffering and attenuation of <u>mass movement</u>; <u>Hydrological cycle and water flow regulation</u> (Including flood control, and coastal protection); <u>Wind</u> protection; <u>Fire</u> protection</p> <p><i>Example: changes caused by non-native organisms to ecosystem functioning or structure leading to, for example,</i></p>

⁸ Note: in the CICES classification provisioning of water is considered as an abiotic service whereas the rest of ecosystem services listed here are considered biotic.

			<p><i>destabilisation of soil, increased risk or intensity of wild fires etc.</i></p>
		<p>Lifecycle maintenance, habitat and gene pool protection</p>	<p><u>Pollination</u> (or 'gamete' dispersal in a marine context); <u>Seed dispersal</u>; Maintaining <u>nursery populations and habitats</u> (Including gene pool protection)</p> <p><i>Example: changes caused by non-native organisms to the abundance and/or distribution of wild pollinators; changes to the availability / quality of nursery habitats for fisheries</i></p>
		<p>Pest and disease control</p>	<p>Pest control; Disease control</p> <p><i>Example: changes caused by non-native organisms to the abundance and/or distribution of pests</i></p>
		<p>Soil quality regulation</p>	<p><u>Weathering processes</u> and their effect on soil quality; <u>Decomposition and fixing processes</u> and their effect on soil quality</p> <p><i>Example: changes caused by non-native organisms to vegetation structure and/or soil fauna leading to reduced soil quality</i></p>
		<p>Water conditions</p>	<p>Regulation of the <u>chemical condition</u> of freshwaters by living processes; Regulation of the chemical condition of salt waters by living processes</p> <p><i>Example: changes caused by non-native organisms to buffer strips along water courses that remove nutrients in runoff and/or fish communities that regulate the resilience and resistance of water bodies to eutrophication</i></p>
		<p>Atmospheric composition and conditions</p>	<p>Regulation of <u>chemical composition</u> of atmosphere and oceans; Regulation of <u>temperature and humidity</u>, including ventilation and transpiration</p> <p><i>Example: changes caused by non-native organisms to ecosystems' ability to sequester carbon and/or evaporative cooling (e.g. by urban trees)</i></p>
<p>Cultural</p>	<p>Direct, in-situ and outdoor interactions with living systems that depend on presence in the environmental setting</p>	<p>Physical and experiential interactions with natural environment</p>	<p>Characteristics of living systems that that enable activities promoting health, recuperation or enjoyment through <u>active or immersive interactions</u>;</p> <p>Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through <u>passive or observational interactions</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that make it attractive for recreation, wild life watching etc.</i></p>
		<p>Intellectual and representative interactions with natural environment</p>	<p>Characteristics of living systems that enable <u>scientific investigation</u> or the creation of traditional ecological knowledge;</p> <p>Characteristics of living systems that enable <u>education and training</u>;</p> <p>Characteristics of living systems that are resonant in terms of <u>culture or heritage</u>;</p> <p>Characteristics of living systems that enable <u>aesthetic experiences</u></p>

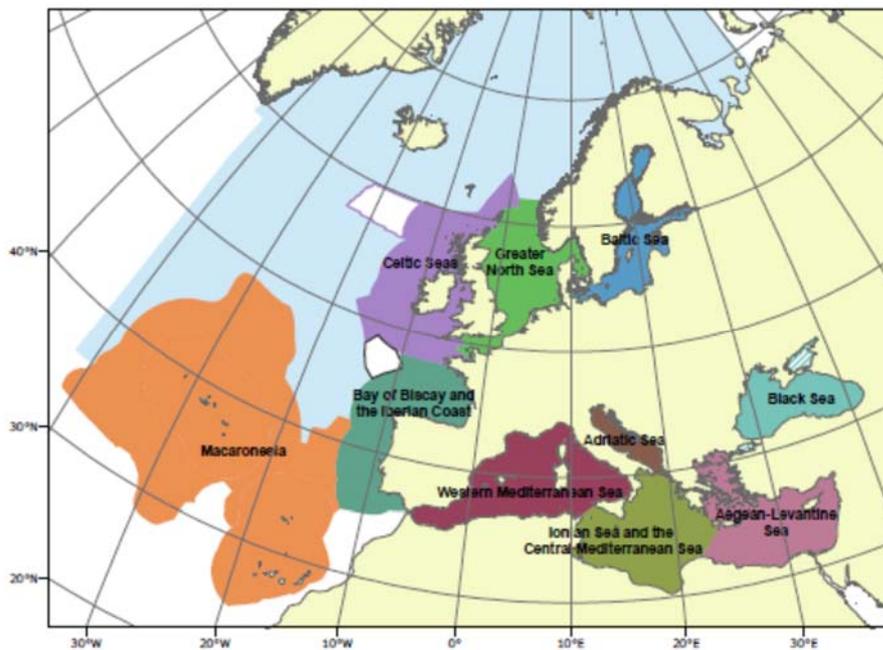
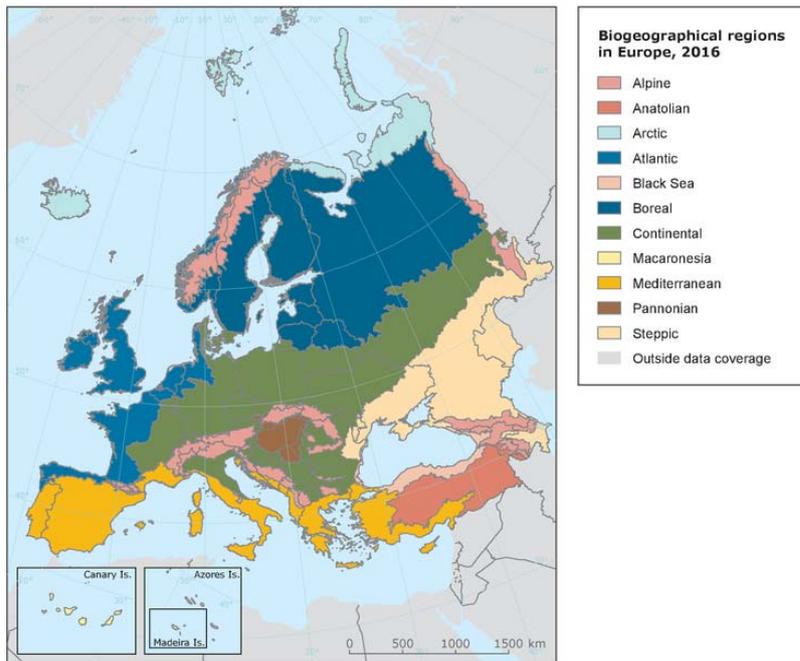
			<i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have cultural importance</i>
	Indirect, remote, often indoor interactions with living systems that do not require presence in the environmental setting	Spiritual, symbolic and other interactions with natural environment	Elements of living systems that have <u>symbolic meaning</u> ; Elements of living systems that have <u>sacred or religious meaning</u> ; Elements of living systems used for <u>entertainment or representation</u> <i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have sacred or religious meaning</i>
		Other biotic characteristics that have a non-use value	Characteristics or features of living systems that have an <u>existence value</u> ; Characteristics or features of living systems that have an <u>option or bequest value</u> <i>Example: changes caused by non-native organisms to ecosystems designated as wilderness areas, habitats of endangered species etc.</i>

ANNEX VI EU Biogeographic Regions and MSFD Subregions

See <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2>,
http://ec.europa.eu/environment/nature/natura2000/biogeog_regions/

and

<https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions-1/technical-document/pdf>



ANNEX VII Delegated Regulation (EU) 2018/968 of 30 April 2018

see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>

ANNEX VIII Species Distribution Model

Projection of environmental suitability for *Cherax destructor* establishment in Europe

Björn Beckmann, Elena Tricarico and Dan Chapman

23 September 2021

Aim

To project the suitability for potential establishment of *Cherax destructor* in Europe, under current and predicted future climatic conditions.

Data for modelling

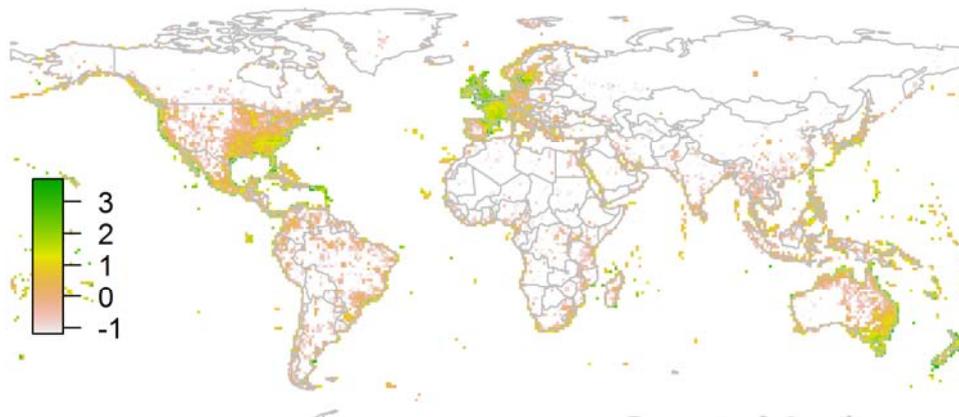
Species occurrence data were obtained from the Global Biodiversity Information Facility (GBIF) (3995 records), the Atlas of Living Australia (976 records), iNaturalist (64 records), the Integrated Digitized Biocollections (iDigBio) (1 records), and additional records from the risk assessment team. We scrutinised occurrence records from regions where the species is not known to be established and removed any dubious records or where the georeferencing was too imprecise (e.g. records referenced to a country or island centroid) or outside of the coverage of the predictor layers (e.g. small island or coastal occurrences). The remaining records were gridded at a 0.25 x 0.25 degree resolution for modelling, yielding 348 grid cells with occurrences (Figure 1a). As a proxy for recording effort, the density of terrestrial Decapoda records held by GBIF was also compiled on the same grid (Figure 1b).

Figure 1. (a) Occurrence records obtained for *Cherax destructor* and used in the modelling, showing native and invaded distributions. (b) The recording density of terrestrial Decapoda on GBIF, which was used as a proxy for recording effort.

(a) Species distribution used in modelling



(b) Estimated recording effort (log10-scaled)



Climate data were selected from the ‘Bioclim’ variables contained within the WorldClim database (Hijmans et al. 2005), originally at 5 arcminute resolution (0.083 x 0.083 degrees of longitude/latitude) and aggregated to a 0.25 x 0.25 degree grid for use in the model.

Based on the biology of *Cherax destructor*, the following climate variables were used in the modelling:

- Temperature seasonality (Bio4)
- Maximum temperature of the warmest month (Bio5)
- Minimum temperature of the coldest month (Bio6)
- Mean temperature of the wettest quarter (Bio8)
- Annual precipitation (Bio12)
- Precipitation of the wettest month (Bio13)
- Precipitation seasonality (Bio15)

To estimate the effect of climate change on the potential distribution, equivalent modelled future climate conditions for the 2070s under the Representative Concentration Pathways (RCP) 2.6 and 4.5 were also obtained. These represent low and medium emissions scenarios, respectively. The above variables were obtained as averages of outputs of eight Global Climate Models (BCC-CSM1-1, CCSM4, GISS-E2-R, HadGEM2-AO, IPSL-CM5A-LR, MIROC-ESM, MRI-CGCM3, NorESM1-M), downscaled and calibrated against the WorldClim baseline (see http://www.worldclim.org/cmip5_5m).

Species distribution model

A presence-background (presence-only) ensemble modelling strategy was employed using the BIOMOD2 R package version 3.4.6 (Thuiller et al. 2020; Thuiller et al. 2009). These models contrast the environment at the species' occurrence locations against a random sample of the global background environmental conditions (often termed 'pseudo-absences') in order to characterise and project suitability for occurrence. This approach has been developed for distributions that are in equilibrium with the environment. Because invasive species' distributions are not at equilibrium and subject to dispersal constraints at a global scale, we took care to minimise the inclusion of locations suitable for the species but where it has not been able to disperse to (Chapman et al. 2019). Therefore the background sampling region included:

- The area accessible by native *Cherax destructor* populations, in which the species is likely to have had sufficient time to disperse to all locations. Based on presumed maximum dispersal distances, the accessible region was defined as a 400km buffer around the native range occurrences; AND
- A 30km buffer around the non-native occurrences, encompassing regions likely to have had high propagule pressure for introduction by humans and/or natural dispersal of the species; AND
- Regions where we have an *a priori* expectation of high unsuitability for the species so that absence is assumed irrespective of dispersal constraints (see Figure 2). The following rules were applied to define a region expected to be highly unsuitable for *Cherax destructor* at the spatial scale of the model:
 - Minimum temperature of the coldest month (Bio6) < -3°C
 - Maximum temperature of the warmest month (Bio5) < 20°C
 - Annual precipitation (Bio12) < 200mm

Altogether, 1.1% of occurrence grid cells were located in the unsuitable background region.

Within the unsuitable background region, 10 samples of 5000 randomly sampled grid cells were obtained. In the accessible background (comprising the accessible areas around native and non-native occurrences as detailed above), the same number of pseudo-absence samples were drawn as there were presence records (348), weighting the sampling by a proxy for recording effort (Figure 2).

Figure 2. The background from which pseudo-absence samples were taken in the modelling of *Cherax destructor*. Samples were taken from a 400km buffer around the native range and a 30km buffer around non-native occurrences (together forming the accessible background), and from areas expected to be highly unsuitable for the species (the unsuitable background region). Samples from the accessible background were weighted by a proxy for recording effort (Figure 1(b)).



Each dataset (i.e. combination of the presences and the individual background samples) was randomly split into 80% for model training and 20% for model evaluation. With each training dataset, seven statistical algorithms were fitted with the default BIOMOD2 settings and rescaled using logistic regression, except where specified below:

- Generalised linear model (GLM)
- Generalised boosting model (GBM)
- Generalised additive model (GAM) with a maximum of four degrees of freedom per smoothing spline
- Artificial neural network (ANN)
- Multivariate adaptive regression splines (MARS)
- Random forest (RF)
- Maxent

Since the total background sample was larger than the number of occurrences, prevalence fitting weights were applied to give equal overall importance to the occurrences and the background. Normalised variable importance was assessed and variable response functions were produced using BIOMOD2's default procedure.

Model predictive performance was assessed by the following three measures:

- AUC, the area under the receiver operating characteristic curve (Fielding & Bell 1997). Predictions of presence-absence models can be compared with a subset of records set aside for model evaluation (here 20%) by constructing a confusion matrix with the number of true positive, false positive, false negative and true negative cases. For models generating non-

dichotomous scores (as here) a threshold can be applied to transform the scores into a dichotomous set of presence-absence predictions. Two measures that can be derived from the confusion matrix are sensitivity (the proportion of observed presences that are predicted as such, quantifying omission errors), and specificity (the proportion of observed absences that are predicted as such, quantifying commission errors). A receiver operating characteristic (ROC) curve can be constructed by using all possible thresholds to classify the scores into confusion matrices, obtaining sensitivity and specificity for each matrix, and plotting sensitivity against the corresponding proportion of false positives (equal to $1 - \text{specificity}$). The use of all possible thresholds avoids the need for a selection of a single threshold, which is often arbitrary, and allows appreciation of the trade-off between sensitivity and specificity. The area under the ROC curve (AUC) is often used as a single threshold-independent measure for model performance (Manel et al. 2001). AUC is the probability that a randomly selected presence has a higher model-predicted suitability than a randomly selected absence (Allouche et al. 2006).

- Cohen's Kappa (Cohen 1960). This measure corrects the overall accuracy of model predictions (ratio of the sum of true presences plus true absences to the total number of records) by the accuracy expected to occur by chance. The kappa statistic ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random. Advantages of kappa are its simplicity, the fact that both commission and omission errors are accounted for in one parameter, and its relative tolerance to zero values in the confusion matrix (Manel et al. 2001). However, Kappa has been criticised for being sensitive to prevalence (the proportion of sites in which the species was recorded as present) and may therefore be inappropriate for comparisons of model accuracy between species or regions (McPherson et al. 2004; Allouche et al. 2006).
- TSS, the true skill statistic (Allouche et al. 2006). TSS is defined as $\text{sensitivity} + \text{specificity} - 1$, and corrects for Kappa's dependency on prevalence. TSS compares the number of correct forecasts, minus those attributable to random guessing, to that of a hypothetical set of perfect forecasts. Like kappa, TSS takes into account both omission and commission errors, and success as a result of random guessing, and ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random (Allouche et al. 2006).

An ensemble model was created by first rejecting poorly performing algorithms with relatively extreme low AUC values and then averaging the predictions of the remaining algorithms, weighted by their AUC. To identify poorly performing algorithms, AUC values were converted into modified z-scores based on their difference to the median and the median absolute deviation across all algorithms (Iglewicz & Hoaglin 1993). Algorithms with $z < -2$ were rejected. In this way, ensemble projections were made for each dataset and then averaged to give an overall suitability, as well as its standard deviation. The projections were then classified into suitable and unsuitable regions using a "minimum presence threshold", setting the cut-off as the lowest value at which 98% of all presence records are classified correctly.

Projections were classified into suitable and unsuitable regions using a "lowest presence threshold" (Pearson et al. 2007), setting the cut-off as the lowest value at which 98% of all presence records are classified correctly under the current climate (here 0.68). In order to express the sensitivity of classifications to the choice of this threshold, thresholds at which 95% and 99% of records are classified correctly (here 0.81 and 0.55 respectively) were used in the calculation of error bars in Figs. 9 and 10 below in addition to taking account of uncertainty in the projections themselves.

We also produced limiting factor maps for Europe following Elith et al. (2010). For this, projections were made separately with each individual variable fixed at a near-optimal value. These were chosen as the median values at the occurrence grid cells. Then, the most strongly limiting factors were identified as the one resulting in the highest increase in suitability in each grid cell.

Results

The ensemble model suggested that suitability for *Cherax destructor* was most strongly determined by Temperature seasonality (Bio4), accounting for 36.1% of variation explained, followed by Minimum temperature of the coldest month (Bio6) (23.2%), Maximum temperature of the warmest month (Bio5) (16.5%), Annual precipitation (Bio12) (11.3%), Precipitation of the wettest month (Bio13) (5.7%), Precipitation seasonality (Bio15) (4.7%) and Mean temperature of the wettest quarter (Bio8) (2.6%) (Table 1, Figure 3).

Table 1. Summary of the cross-validation predictive performance (AUC, Kappa, TSS) and variable importance of the fitted model algorithms and the ensemble (AUC-weighted average of the best performing algorithms). Results are the average from models fitted to 10 different background samples of the data.

Algorithm	AUC	Kappa	TSS	Used in the ensemble	Variable importance (%)						
					Temperature seasonality (Bio4)	Minimum temperature of the coldest month (Bio6)	Maximum temperature of the warmest month	Annual precipitation (Bio12)	Precipitation of the wettest month (Bio13)	Precipitation seasonality (Bio15)	Mean temperature of the wettest quarter (Bio8)
GLM	0.985	0.742	0.935	yes	28	23	14	8	16	10	2
GAM	0.984	0.743	0.938	yes	37	24	12	14	5	5	4
GBM	0.985	0.731	0.939	yes	56	4	24	15	0	1	0
ANN	0.985	0.750	0.935	yes	28	29	20	5	7	5	5
MARS	0.983	0.716	0.926	yes	31	37	13	14	0	3	2
RF	0.979	0.729	0.920	no	20	24	28	18	3	4	4
Maxent	0.976	0.711	0.911	no	29	20	18	19	4	5	6
Ensemble	0.986	0.745	0.939		36	23	17	11	6	5	3

Figure 3. Partial response plots from the fitted models. Thin coloured lines show responses from the algorithms in the ensemble, while the thick black line is their ensemble. In each plot, other model variables are held at their median value in the training data. Some of the divergence among algorithms is because of their different treatment of interactions among variables.

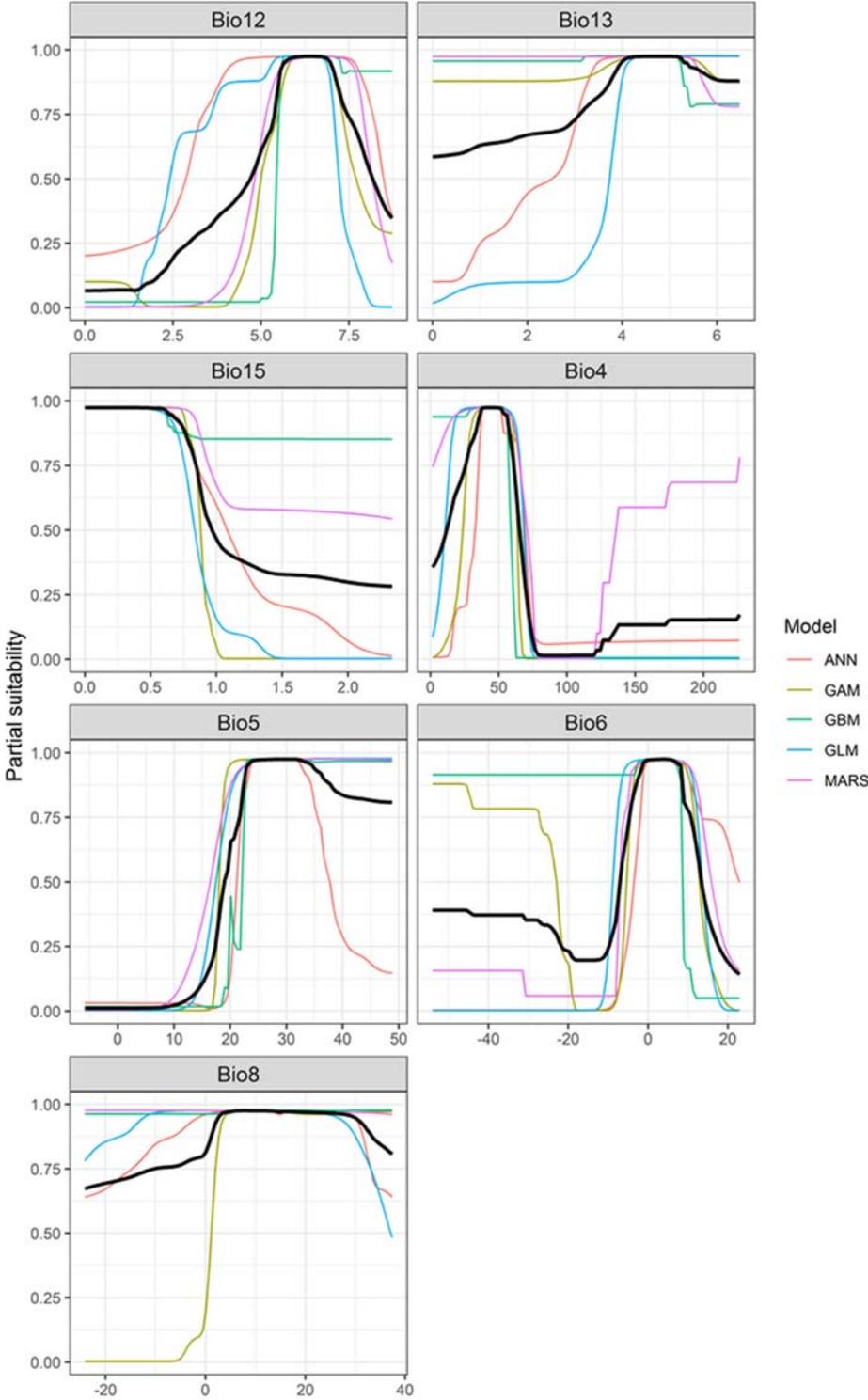


Figure 4. (a) Projected global suitability for *Cherax destructor* establishment in the current climate. For visualisation, the projection has been aggregated to a 0.5 x 0.5 degree resolution, by taking the maximum suitability of constituent higher resolution grid cells. Values > 0.68 are suitable for the species, with 98% of global presence records above this threshold. Values below 0.68 indicate lower relative suitability. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

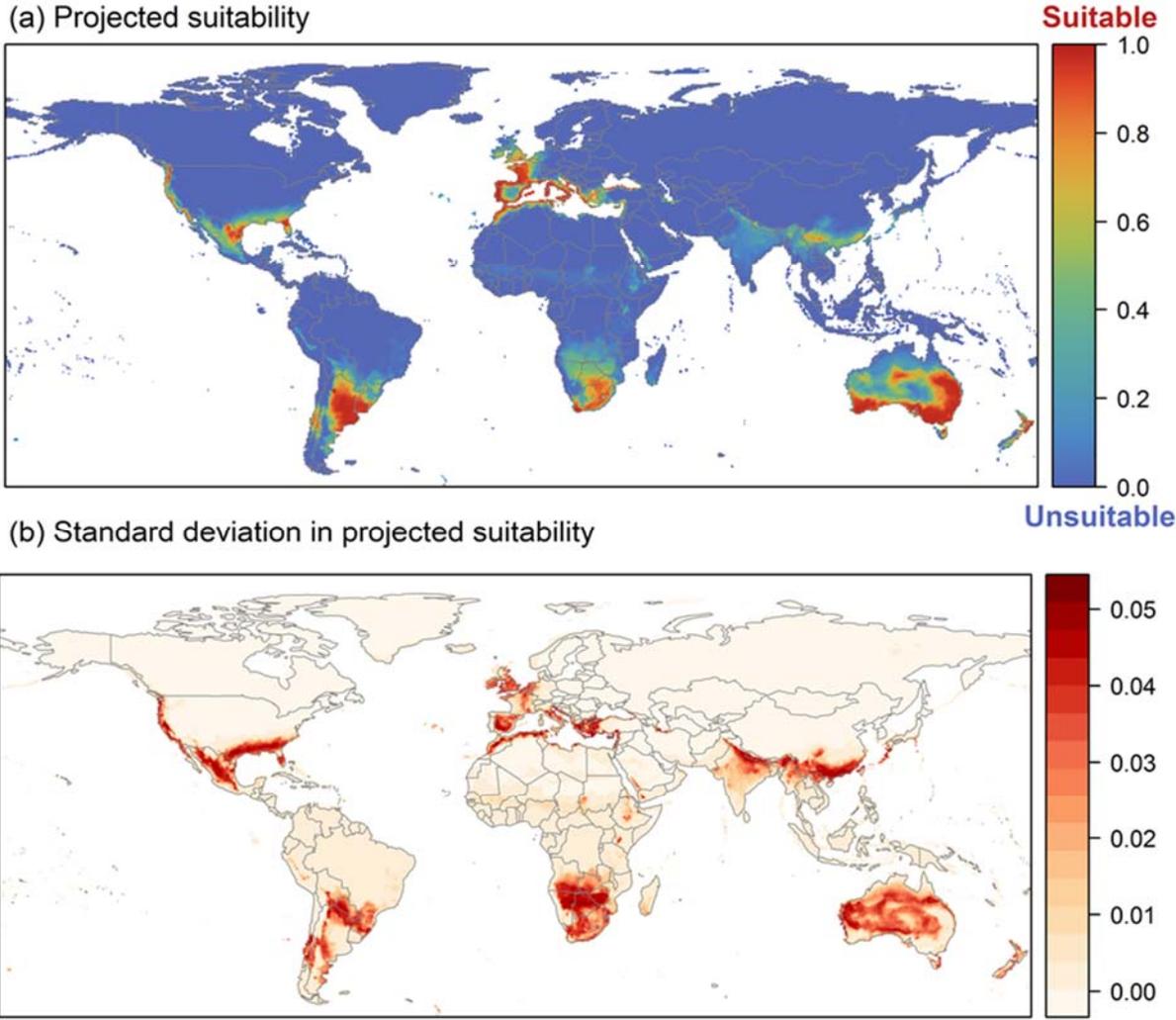


Figure 5. (a) Projected current suitability for *Cherax destructor* establishment in Europe and the Mediterranean region. Values > 0.68 are suitable for the species, with 98% of global presence records above this threshold. Values below 0.68 indicate lower relative suitability. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

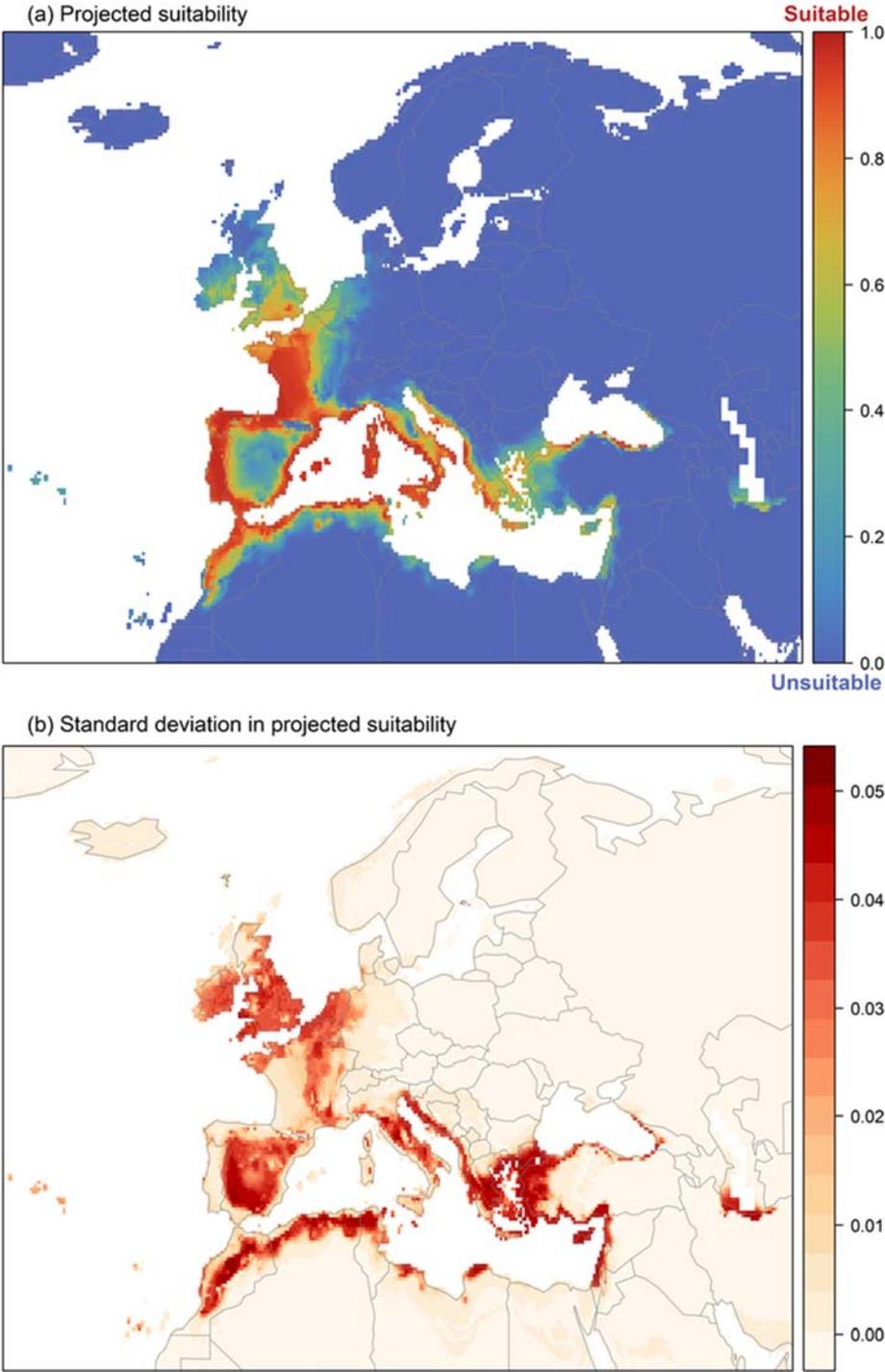


Figure 6. The most strongly limiting factors for *Cherax destructor* establishment estimated by the model in Europe and the Mediterranean region in current climatic conditions.



Figure 7. (a) Projected suitability for *Cherax destructor* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP2.6. Values > 0.68 are suitable for the species, with 98% of global presence records above this threshold under current climate. Values below 0.68 indicate lower relative suitability. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

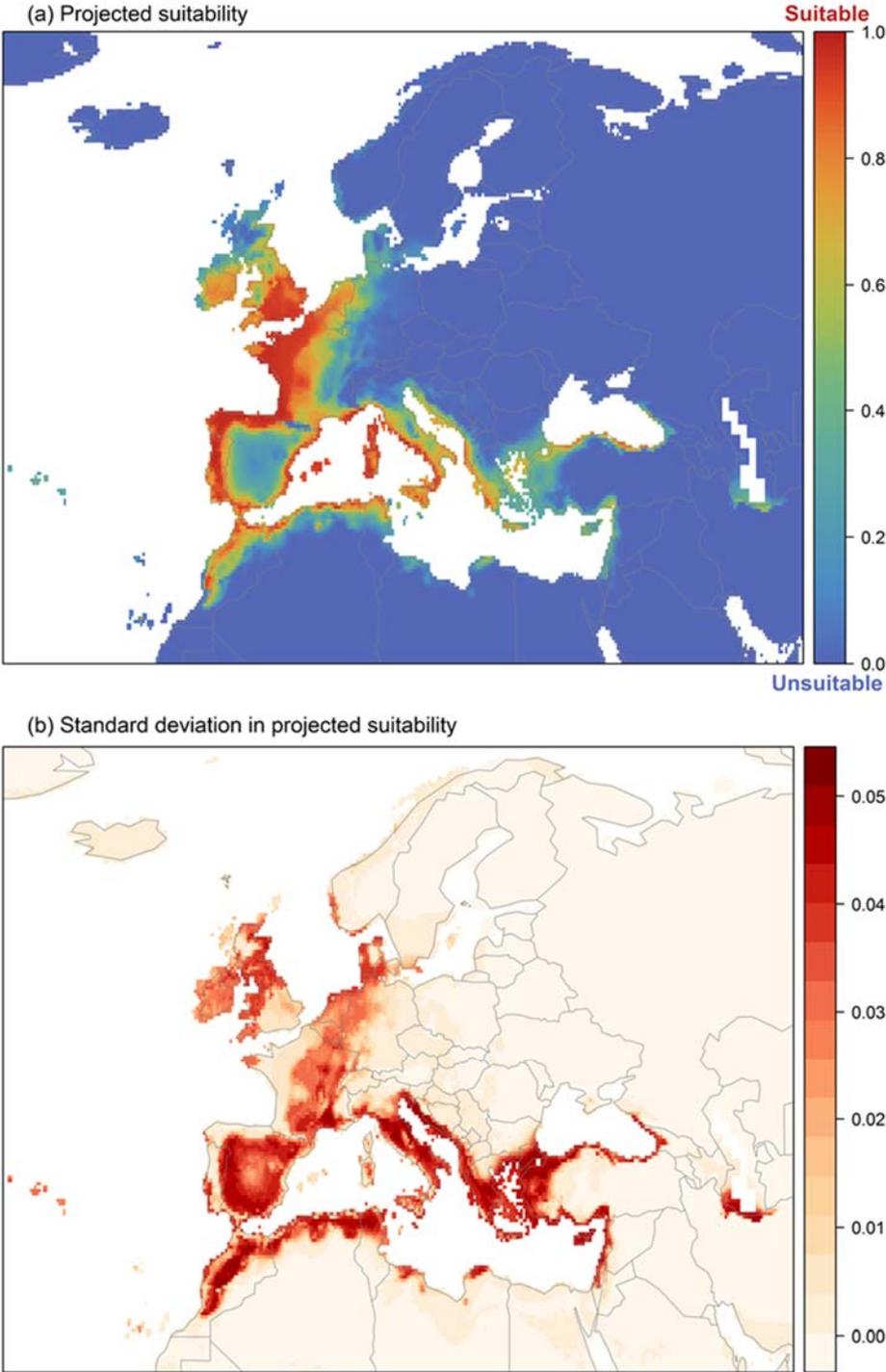


Figure 8. (a) Projected suitability for *Cherax destructor* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP4.5. Values > 0.68 are suitable for the species, with 98% of global presence records above this threshold under current climate. Values below 0.68 indicate lower relative suitability. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

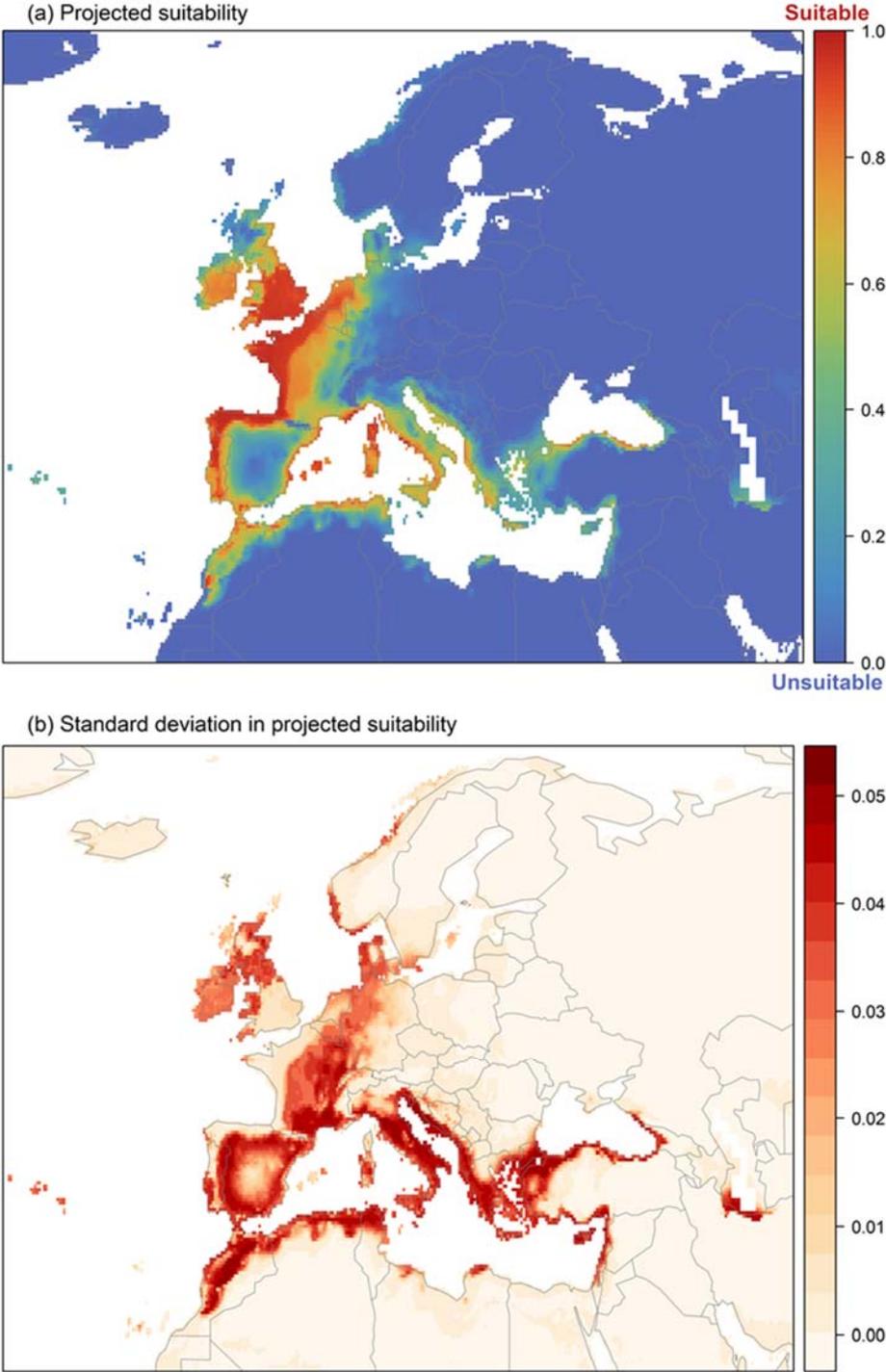


Figure 9. Variation in projected suitability for *Cherax destructor* establishment among Biogeographical Regions of Europe (<https://www.eea.europa.eu/data-and-maps/data/biogeographical-regions-europe-3>). The bar plots show the proportion of grid cells in each region classified as suitable (with values > 0.68) in the current climate and projected climate for the 2070s under two RCP emissions scenarios. Error bars indicate uncertainty due to both the choice of classification threshold (cf. p.5/6) and uncertainty in the projections themselves (cf. part (b) of Figures 5, 7 and 8). The location of each region is also shown. The Arctic and Macaronesian regions are not part of the study area, but are included for completeness.

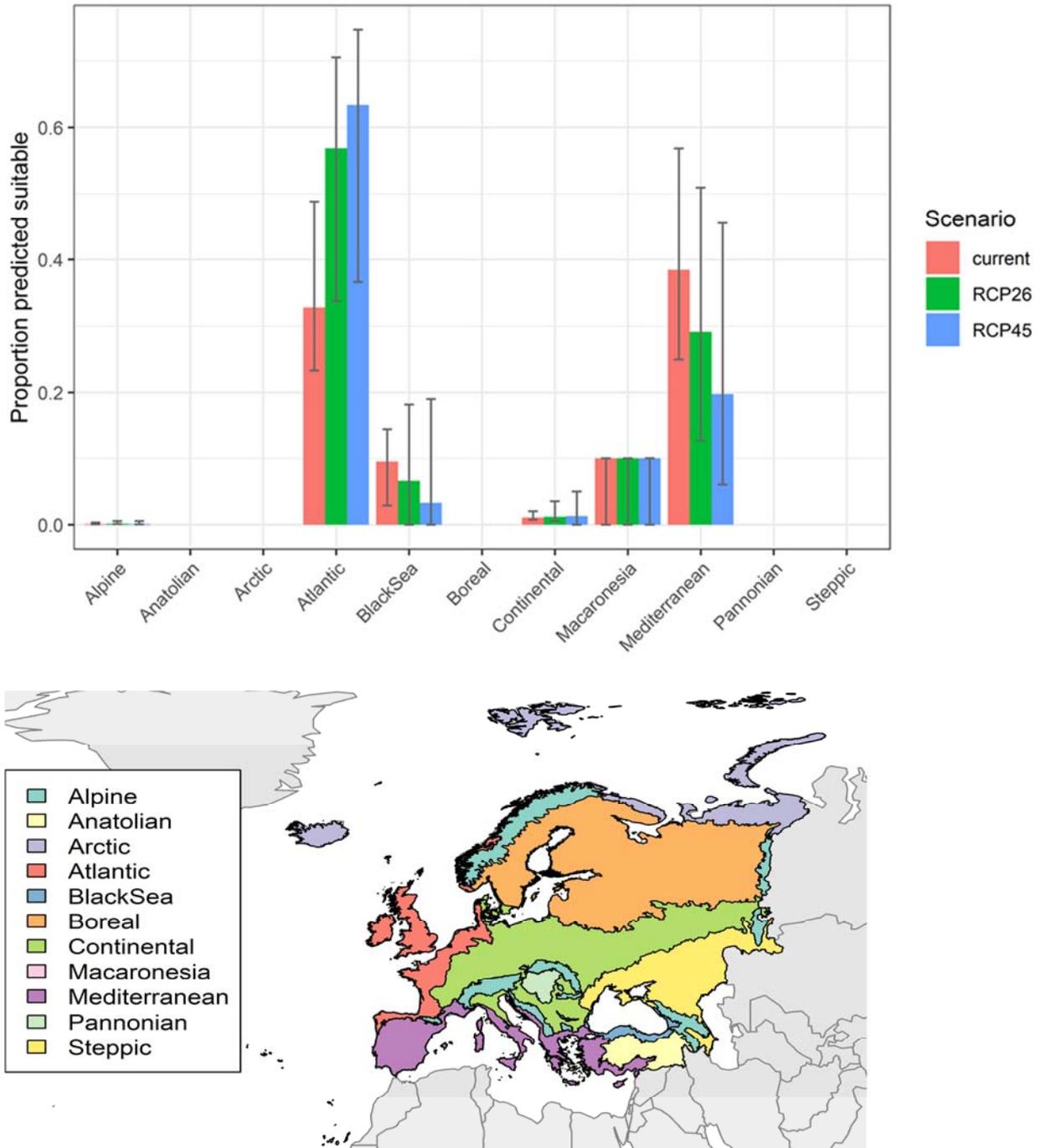


Table 2. Variation in projected suitability for *Cherax destructor* establishment among Biogeographical regions of Europe (numerical values of Figure 9 above). The numbers are the proportion of grid cells in each region classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. The Arctic and Macaronesian biogeographical regions are not part of the study area, but are included for completeness.

	current climate			2070s RCP2.6			2070s RCP4.5		
	lower	central estimate	upper	lower	central estimate	upper	lower	central estimate	upper
Alpine	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01
Anatolian	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Arctic	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Atlantic	0.23	0.33	0.49	0.34	0.57	0.71	0.37	0.63	0.75
Black Sea	0.03	0.10	0.14	0.00	0.07	0.18	0.00	0.03	0.19
Boreal	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Continental	0.01	0.01	0.02	0.01	0.01	0.04	0.00	0.01	0.05
Macaronesia	0.00	0.10	0.10	0.00	0.10	0.10	0.00	0.10	0.10
Mediterranean	0.25	0.38	0.57	0.13	0.29	0.51	0.06	0.20	0.46
Pannonian	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Steppic	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Figure 10. Variation in projected suitability for *Cherax destructor* establishment among European Union countries and the UK. The bar plots show the proportion of grid cells in each country classified as suitable (with values > 0.68) in the current climate and projected climate for the 2070s under two RCP emissions scenarios. Error bars indicate uncertainty due to both the choice of classification threshold (cf. p.5/6) and uncertainty in the projections themselves (cf. part (b) of Figures 5, 7 and 8).

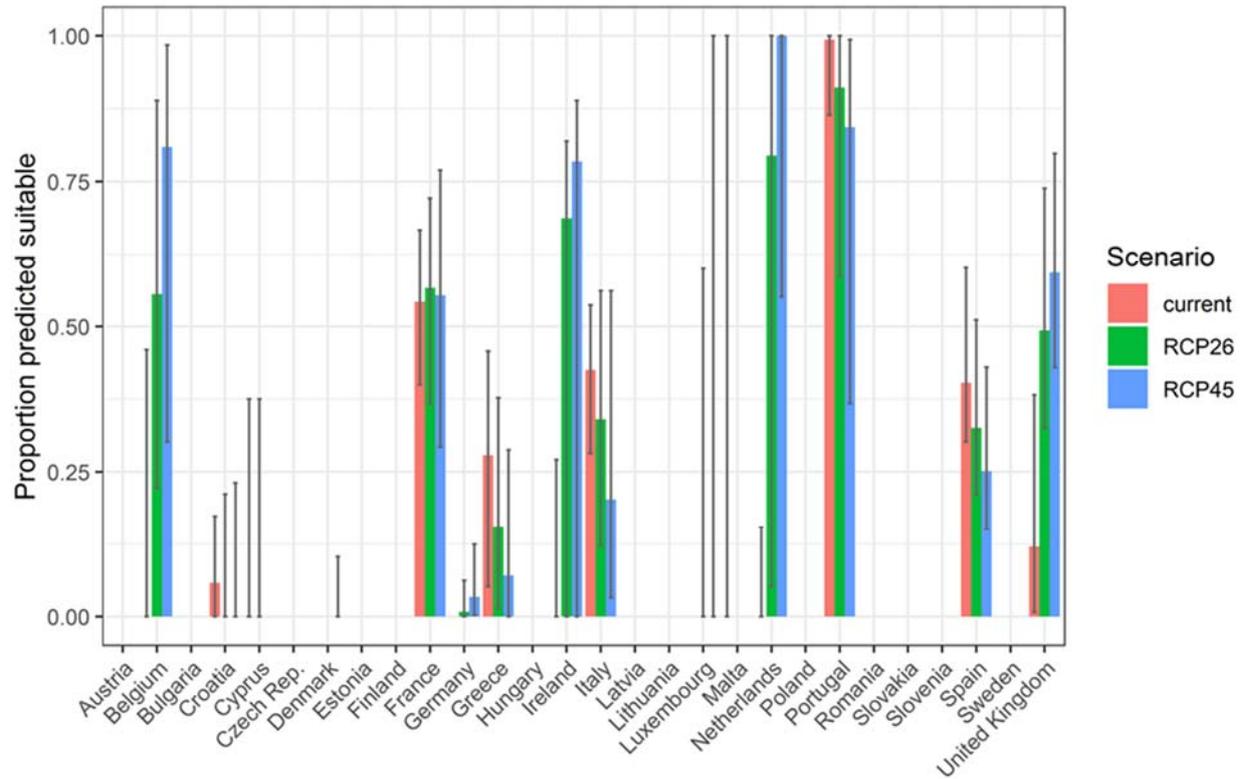


Table 3. Variation in projected suitability for *Cherax destructor* establishment among European Union countries and the UK (numerical values of Figure 10 above). The numbers are the proportion of grid cells in each country classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios.

	current climate			2070s RCP2.6			2070s RCP4.5		
	lower	central estimate	upper	lower	central estimate	upper	lower	central estimate	upper
Austria	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Belgium	0.00	0.00	0.46	0.22	0.56	0.89	0.30	0.81	0.98
Bulgaria	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Croatia	0.00	0.06	0.17	0.00	0.00	0.21	0.00	0.00	0.23
Cyprus	0.00	0.00	0.38	0.00	0.00	0.38	0.00	0.00	0.00
Czech Rep.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Denmark	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10
Estonia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Finland	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
France	0.40	0.54	0.67	0.37	0.57	0.72	0.29	0.55	0.77
Germany	0.00	0.00	0.00	0.00	0.01	0.06	0.00	0.03	0.12
Greece	0.05	0.28	0.46	0.01	0.16	0.38	0.00	0.07	0.29
Hungary	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Ireland	0.00	0.00	0.27	0.00	0.69	0.82	0.00	0.78	0.89
Italy	0.28	0.43	0.54	0.12	0.34	0.56	0.03	0.20	0.56
Latvia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Lithuania	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Luxembourg	0.00	0.00	0.00	0.00	0.00	0.60	0.00	0.00	1.00
Malta	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
Netherlands	0.00	0.00	0.15	0.05	0.79	1.00	0.55	1.00	1.00
Poland	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Portugal	0.86	0.99	1.00	0.59	0.91	1.00	0.37	0.84	0.99
Romania	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Slovakia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Slovenia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Spain	0.30	0.40	0.60	0.21	0.33	0.51	0.15	0.25	0.43
Sweden	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
UK	0.01	0.12	0.38	0.32	0.49	0.74	0.43	0.59	0.80

Caveats to the modelling

To remove spatial recording biases, the selection of the background sample from the accessible background was weighted by the density of Decapoda records on the Global Biodiversity Information Facility (GBIF). While this is preferable to not accounting for recording bias at all, it may not provide the perfect measure of recording bias.

There was substantial variation among modelling algorithms in the partial response plots (Figure 3). In part this will reflect their different treatment of interactions among variables. Since partial plots are made with other variables held at their median, there may be values of a particular variable at which this does not provide a realistic combination of variables to predict from.

Other variables potentially affecting the distribution of the species, such as types of land cover were not included in the model.

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Annex with evidence on measures and their implementation cost and cost-effectiveness

Species (scientific name)	<i>Cherax destructor</i> Clark, 1936
Species (common name)	Common yabby, yabby
Author(s)	Elena Tricarico
Reviewer	Peter Robertson, Antonín Kouba, Francisco J. Oficialdegui
Date completed	15/10/2021

Summary¹

Highlight of measures that provide the most cost-effective options to prevent the introduction, achieve early detection, rapidly eradicate and manage the species, including significant gaps in information or knowledge to identify cost-effective measures.

Cherax destructor is an Australian crayfish species reported in Europe in the wild in Spain, France, Ireland and Italy. In general, eradication and management of aquatic invasive species are very challenging. Prevention remains the best approach, which also applies to this species. The measures reported in this Annex also consider the European experience on other invasive alien crayfish. A combination of techniques is recommended to manage the species as there is no single “silver bullet” for every situation.

Prevention can be achieved by implementing the legislation (banning the species for the pathways of introduction: aquaculture and e-commerce) coupled with powerful and effective public awareness campaigns.

Early detection foresees effective surveillance and reporting by monitoring areas using environmental DNA, or citizen science. Traps can be used for surveillance and monitoring, even if less effective than eDNA, when the species is at low population densities.

Eradication and management practices can be more effective at the early stage of invasion in closed water bodies (timescale of the intervention depends on the situation), but not in open (connected) systems. An Integrated Pest Management approach is the most recommended method, being the best and most effective way to target different crayfish life stages. Chemical control can be used to rapidly eradicate a population in closed waterbodies. Chemical eradication of already established populations in the catchment scale is usually unfeasible. Only non-selective products are available, causing environmental damage and problems with public opinion. These are not approved for use in all Member States and can only be applied in very particular situations (their use in protected areas should be rigorously evaluated, for example). As the species is a burrowing crayfish, like the red swamp crayfish (*Procambarus clarkii*), biocides should be applied to the water using a method that reaches the individuals in the burrows, possibly with more than one application.

The combined use of intensive trapping and native predators has been proved to be effective in tackling invasive crayfish, so it applies to *C. destructor* as well. It almost eradicated (reduction of 99%) the rusty crayfish (*Faxonius rusticus*) in a US lake after eight years of activities

(without a population recovery for the subsequent four years). Moreover, in two years, the combined use of intensive trapping and sterile male techniques has led to a decrease of 87% in a red swamp crayfish population inhabiting an Italian lake, without a complete eradication. Drainage of closed waterbodies may be used in the case of confined populations, but to be effective, waterbodies should be left dried for a prolonged time (at least one year). Habitat destruction/modification (i.e. filling of ponds) and subsequent restoration could be considered for very specific cases.

The use of crayfish plague to eradicate *C. destructor* has been proven effective, but it can lead to significant risks for native European crayfish (and also a chronically infected population of *C. destructor* can establish, serving as a reservoir of the pathogen).

Besides managing the pathways and increasing public awareness, if the species is established, physical barriers can halt or slow down the spread of the species. However, as a side effect, they can also impede the movement of native species.

For management, the same listed measures for eradication (chemical, trapping) can be used, plus the construction of barriers that may be used in the case of confined populations. Barriers can be costly and impede the movement of native species.

Recently, new autocidal techniques, such as the creation of monosex populations, silencing of key hormones through RNA interference and oral delivery of neuropeptides, have also been suggested as promising methods to control crayfish populations. However, they have not proven entirely effective in practice yet, except in aquaculture.

Detailed assessment			
	Description of measures ²	Assessment of implementation cost and cost-effectiveness (per measure) ³	Level of confidence ⁴
Methods to achieve prevention of intentional and unintentional introduction⁵	Managing the pathways: Common yabby is already present in Europe (Spain, France, Ireland and Italy) where it was introduced for aquaculture purposes (Bolea 1996; D'Agaro et al. 1999). The species also became an ornamental species in the European online trade, which is another potential pathway of its introduction (Chucholl 2013). The species has been reported to be sold live in markets (i.e. Germany).	Legislation can be a very effective method of limiting the risk of introduction but requires sufficient sustained enforcement effort. A ban on trade would be an effective means of limiting the risk of introducing the species through the pathways identified (aquaculture, pet trade and use as live food). Still, it is necessary to properly educate and communicate with the wider public (Patoka et al. 2018). Bans are an accepted tool to limit trade and are already used worldwide for this and other species. <i>Cherax destructor</i> is a common species in the ornamental trade (e.g. Chucholl 2013; Chucholl & Wendler 2017) and is still	High

	<p>The adoption and enforcement of appropriate legislation and codes of best practice could reduce the likelihood of introduction in other EU countries.</p>	<p>farmed, so some opposition by stakeholders can be expected, even if other colourful <i>Cherax</i> spp. would still be sold/exchanged online as replacement species. Costs of implementation could be kept to a minimum by utilising existing enforcement processes, for example, those in place for the other crayfish (up to now five species) already present in the list of Invasive Alien Species of Union concern of the EU Regulation 1143/2014. Some specific training for relevant authorities would be necessary to recognise the species. Implementation of legislation should be coupled with a targeted public awareness campaign and spread of codes of conduct to be more effective, involving the most relevant stakeholders (e.g. pet retailers, farmers, and online marketplaces).</p>	
	<p>Increasing public awareness: Dumping from citizens can cause the introduction of this species as already recorded for other crayfish sold for ornamental purposes (e.g. the marbled crayfish; Kouba et al. 2014, and the common yabby in Ireland). Information campaigns are necessary to increase public awareness and avoid new introductions. Guidance material can be produced and distributed for species identification (e.g. IUCN 2018).</p>	<p>Campaigns to educate and increase awareness on invasive alien species (IAS) are an effective way to curb illegal introductions, especially those targeted at specific sectors (e.g. pet retailers and owners). Public awareness campaigns, however, do need to be maintained, so they do not drop out of the collective consciousness, but are also renewed periodically to avoid fatigue. Implementing public campaigns as a preventative measure for the introduction of IAS is generally well accepted by stakeholders and the general public. The costs are possibly related to labour costs and of the production of communication materials and of communication campaigns. They can vary a lot, depending on the communication channel (radio, depending on how the information newspapers, websites or TV, or field</p>	<p>High</p>

		campaigns) ¹ . Despite being a highly recommended measure, up to now, the effectiveness in reducing introductions of crayfish has not been measured and detailed costs are not available. No strong opposition by stakeholders is expected.	
Methods to achieve early detection⁶	<p>Effective surveillance and reporting: there are other <i>Cherax</i> species already present in Europe, especially in internet trade (Kouba et al. 2014; Chucholl & Wendler 2017), and the congeneric <i>C. quadricarinatus</i> is already reported in the wild for Slovenia, Malta, Spain, and Hungary (Kouba et al. 2014; Deidun et al. 2018; Weiperth et al. 2019; Arias & Torralba-Burrial 2021).</p> <p>There are different methods that can be used for surveillance, such as eDNA, citizen science, traps.</p> <p>1) eDNA has been used to successfully detect other alien crayfish in USA and Europe, also at low population densities (Mauvisseau et al. 2018, 2019; Manfrin et al. 2019; Rusch et al. 2020).</p> <p>2) Citizen science could be promoted to monitor the possible introduction and spread of the species and has been proved to be effective in detecting the arrival of IAS (e.g. involving anglers:</p>	<p>Usually, these methods do not elicit any problems with acceptance by the public and stakeholders. The costs vary.</p> <p>1) For eDNA, its development and testing have high initial costs, but its regular use can reduce the total costs considerably (Manfrin et al. 2019; Rusch et al. 2020). According to Zogaris (2017), “setting up the lab (PCR equipment etc.) would cost a minimum of € 20,000, while c. €30,000 could be estimated for annual operational costs (€24,000 personnel and travel + €6,000 lab consumables), referring to six months development, 12 months sampling campaign and six months for analysis. The method requires collecting water samples (1 to 10 L of water) from strategically placed sampling sites to search for the targeted species”.</p> <p>2) For citizen science, costs are related to individual training of citizens, validating data and maintaining the e-platform where data are collected. For example, as Adriaens et al. (2015) reported, an app developed by the RINSE project cost € 20,262, so a rough estimate of maintenance costs could be between € 3,000 and 4,000 per year. Already existing apps could also be used. Costs of people screening, validating and managing the collected data should be included: a part-time position could be sufficient, with</p>	High

¹ <https://www.shropshirewildlifetrust.org.uk/shropshire-invasive-non-native-species-initiative>

	<p>Zukowski et al. 2018). A simple and clear identification sheet could be also drafted and distributed to different stakeholders (e.g. anglers, aquarists) to increase the probability of an early detection and rapid response (e.g. IUCN 2018)².</p> <p>3) Traps can be used for surveillance and monitoring, even if not always effective when the species is at low densities (Gherardi et al. 2011).</p>	<p>personnel managing data also collected on other species targeted by the citizen science initiative (the cost of the position depends on labour costs of Member States³: in 2020, in the EU, the average hourly labour cost was € 28.5). eDNA may have advantages over citizen science for use in areas where both native and alien crayfish co-occur, due to the risk of accidentally spreading crayfish plague by untrained citizens (so strict biosecurity protocols should be enforced or activities be limited).</p> <p>3) For surveillance trapping, the costs depend on the number of traps used (anglers and other stakeholders could be involved to check them). The cost of one trap can vary between € 8 and 15, cat food used in the traps as bait costs € 0.25 per can. Trapped animals should be disposed of according to the national legislation. Moreover, there could be associated environmental costs if non-target species are captured in the traps (e.g., reptiles, amphibians, fishes,...), so they should be set semi-submerged and frequently checked.</p> <p>All the three listed methods are already used for other aquatic IAS (thus, costs can be reduced). No relevant health and safety issues for personnel involved in citizen science and eDNA procedures, while all health and safety procedures should be strictly followed for trapping, including, e.g. wearing protective clothing and life jackets, especially if using boats.</p>	
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² <https://circabc.europa.eu/sd/a/8062f840-b609-4926-ac63-3ca1b2b0a926/2018%20-%20Field%20guide%20-%20surveillance.pdf>

³ https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Wages_and_labour_costs

<p>Methods to achieve eradication⁷</p>	<p>Eradication of aquatic species is very challenging and is usually only feasible in small, enclosed water bodies. This places the emphasis on prevention as the most effective management tool. Management practices can be more effective at the early stage of invasion in closed but not in open waterbodies. An integrated approach is recommended (Gherardi et al. 2011; Stebbing et al. 2014; Stebbing 2016; Manfrin et al. 2019).</p>	<p>The suggested methods are those developed from European experience on other invasive alien crayfish.</p>	<p>High</p>
	<p>Mechanical removal: the use of baited traps of various designs (Swedish traps, Evo-traps, collapsible traps, fyke nets, seine nets, etc. https://picclick.co.uk/Swedish-Crayfish-Trap-Otter-Friendly-UK-123356021888.html), ART (Artificial Refuge Traps consisting of a series of tubes of different diameters and lengths that are attached to a metal baseplate and that mimic the burrows; Green et al. 2018) or by electrofishing can reduce the density of the populations.</p>	<p>Traps are very simple and user friendly, and the public and stakeholders generally accept their use. Traps should be set semi-submerged to avoid the capture or harm of non-target organisms (such as amphibians, grass snakes, turtles). Frequent emptying of traps can increase capture rates since previously captured crayfish prevent other crayfish from entering the trap (Gherardi et al. 2011; Stebbing 2016). However, juveniles and ovigerous females can be trap shy and thus less trapped. The use of ART should be considered (Green et al. 2018) together with traditional traps. Trapping over extended periods is often required, which brings significant man-power costs. Setting traps when crayfish are most active (usually in summer and at night) increases trapping efficiency and therefore reduces costs for sampling effort in periods where the catch rate is very low (Dana et al. 2011a). The scale of this is dependent on the type of water body: e.g. in Italy two people on a boat in three hours covered a lake of 10,000 m² with 120 traps to manage the</p>	<p>High</p>

		<p>red swamp crayfish. The costs referred of trap checking over 171 days was around € 8,000 for traps and baits, and € 20,000 for manpower. Usually, mechanical removal (e.g. trapping) is coupled with other techniques to achieve the eradication (or near eradication). Autocidal methods such as sterilisation and biological methods (see descriptions below) can enhance the effectiveness of physical removal (Gherardi et al. 2011; Stebbing et al. 2014; Manfrin et al. 2019). Sex pheromones could be used in the traps, even if their identification is still far from being completed. Traps, if properly set, usually do not elicit any public concern. Electrofishing is seldom used and limited in its application to certain habitats (e.g. shallow, clear water; small water systems; Stebbing 2016). Costs are related to the equipment (between € 6,000 and 12,000) and manpower (this depends on labour costs of Member States, see above).</p> <p>Animals caught in traps or by electrofishing should be disposed of according to the national legislation.</p> <p>All health and safety procedures should be strictly followed when trapping and electrofishing, including e.g. wearing protective clothing and life jackets, especially if using boats. Special training and permission are required for electrofishing to prevent any damages to operators and species.</p>	
	<p>Biocides: chemicals (BETAMAX VET, Pyblast) can be used in certain cases. Many effective maintenance management projects employ</p>	<p>The synthetic pyrethroid BETAMAX VET was used in Europe to successfully eradicate the signal crayfish from some ponds (five small ponds, the largest measuring approximately 2000 m² surface area; Sandodden & Johnsen 2010). Similarly, the natural pyrethrum Pyblast was used</p>	<p>High</p>

	<p>chemicals, alone or in concert with mechanical or physical methods.</p>	<p>with varying success to eradicate the signal crayfish and the red swamp crayfish in different areas of Europe (Gherardi et al. 2011). For example, in Scotland, signal crayfish were treated in an isolated gravel-pit, c. 9,000 m³; three dammed ponds, c. 5,000 m³ and a leaking, offline pond, c. 6,000 m³ (Peay et al. 2006), and then in a quarry pond of 18,776 m² (Ballantyne et al. 2019), while in Italy the red swamp crayfish was treated from a small part of a ditch (c 700 m²) (Cecchinelli et al. 2011). Other chemicals have been tested and suggested to control invasive alien crayfish (Gherardi et al. 2011), but no chemicals have been used on <i>C. destructor</i> in the wild. However, laboratory trials on the species using Cyperkill 25 EC (active compound 250 g/l cypermethrin) and Decis Mega (active compound 50 g/l deltamethrin) showed that the values of Cyperkill 25 EC and Decis Mega inducing a 50% mortality after 96 hrs were 0.30 µg/l and 0.27 µg/l, respectively, higher than those required for other alien crayfish (Lidova et al 2019). However, these chemicals are not aquatic species selective, can be expensive and application protocols according to species biology and recipient environments are not established. Moreover, they can cause environmental damage and problems with public opinion; they are not approved for use in all the Member States and can only be applied in very particular situations. Pyblast could be better perceived, being natural and less toxic. Non-target species (e.g. fish, amphibians, reptiles) should be removed before their application, following strict and detailed protocols to recover them. As the species is a burrowing crayfish, like the red swamp crayfish, biocides should be applied in the water so that it also reaches the</p>	
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		<p>individuals in the burrows (Cecchinelli et al. 2011), possibly requiring more than one application.</p> <p>Chemicals have been proved to be successful in closed small water bodies (< 25,000 m²: Peay et al. 2019) or confined systems. Costs are related to the chemicals (1 l of Pyblast costs € 75; Ballantyne et al. 2019 used 620 l; synthetic pyrethroids containing cypermethrin or deltamethrin should be cheaper, e.g. 250 ml of Decis mega costs € 8-9), boat (estimate of € 200-440 per day) and manpower (this depends on labour costs of Member States, see above). All health and safety procedures should be strictly followed when applying chemicals.</p>	
	<p>Physical methods: drainage of closed waterbodies may be used in the case of confined populations. Habitat destruction/modification (e.g., filling the waterbody) and restoration could also be considered for small, isolated ponds, which cannot be drained.</p>	<p>In cases where a single closed pond has been invaded, it may be possible to drain the pond, destroying the population, but also the habitat (Poulet 2014). Moreover, repeated draining could be necessary before achieving the eradication (Poulet 2014) and water should be released in a safe place (e.g. pasture, arable land) far away from other waterbodies. Sieving could be considered only in very special and rare cases (very low water volumes). However, drying out cannot be effective to eradicate some species, especially those that burrow like common yabby (Gherardi et al. 2011). So, the water body should be left dry for at least one year to achieve the eradication. This may not always be possible, depending on the use/destination of the water body (e.g. if used for irrigation or fisheries, there would be an interest to restore as soon as possible).</p> <p>Draining a pond of approximately 400,000 m³ could cost around € 40-50,000 (E. Tricarico, unpublished). Costs could</p>	<p>High</p>

		<p>be lower in case of planned draining for other reasons (e.g. fish harvesting).</p> <p>Habitat destruction/modification and restoration might work for small, isolated ponds, which cannot be drained and where the species tends to cause severe ecological damage. Re-invasion after restoration can be prevented with fencing or using tree-trunks (C. Chucholl, pers. comm. 2020). In Germany, a combination of techniques (traps, nets, bricks, gravel covering the ground to avoid burrowing and tree trunks as fence around the pond to avoid species dispersion) was used to manage <i>Faxonius immunis</i> in a pond of 763 m² (A. Martens, pers. comm. 2020). No details are available of the results and the costs.</p> <p>Depending on the use of the pond (e.g. fishing, aquaculture), stakeholders could have concerns about complete destroying the pond, and compensatory measures should be considered (e.g. creating a new pond in another area).</p> <p>Caught animals when draining a pond should be disposed of according to the national legislation. Health and safety procedures should be followed when draining a pond (e.g. protective clothing).</p>	
	<p>Autocidal methods: they include the sterile male release technique (SMRT) and the use of sex pheromones. SMRT is based on capturing or rearing, sterilising, and releasing large numbers of males into the wild to mate with females, who will then produce non-viable eggs. It has been successful in the</p>	<p>In a pond (70,000 m²) located in North Italy, a two-year SMRT programme using males sterilised with X-rays coupled with intensive trapping led to a reduction of 87% in the abundance of red swamp crayfish (Aquiloni & Zanetti 2014). The SMRT technique has the advantage of being species-specific, not harmful to the environment and generally acceptable to the public and stakeholders. Costs depend on the number of animals to be sterilised but are usually low,</p>	<p>Medium</p>

	<p>control of some insect pests and aquatic vertebrates, such as sea lamprey <i>Petromyzon marinus</i>. Up to now, SMRT in crayfish has been successfully used only in the red swamp crayfish. Sex pheromones are widely used to control insect pests and research is in progress to identify them in crayfish.</p>	<p>depending on the cost of manpower (labour costs vary according to the different Member States). Its use is more effective in relatively small, closed systems. However, manual removal of gonopods (i.e. modified appendages in males used for reproduction) cannot be considered for <i>Cherax destructor</i>, lacking typical male gonopods present in members of other crayfish families. Moreover, even if intersex individuals (with both male and female genital openings) are present (and thus the identification of only males could be more difficult), SMRT could work on common yabby (but no studies have been conducted so far).</p> <p>The presence of sex pheromones has been proved in crayfish (Gherardi et al. 2011; Stebbing 2016). However, their purification and identification have not yet been achieved, even if their use in managing invasive alien crayfish could be very successful. Sex pheromones can be used in the traps to attract and kill reproductive individuals (El-Sayed et al. 2009). Caught animals should be disposed of according to the national legislation.</p> <p>The public and stakeholders accept both methods, and there are no relevant health and safety procedures to follow (except when using traps).</p>	
	<p>Autocidal methods: novel methods There are other promising methods (monosex populations, silencing of key hormones through RNA interference, oral delivery of neuropeptides) successfully tested in the laboratory on</p>	<p>These methods are species-specific and should not harm any other species; they should be generally accepted, even if the genetic modification could elicit some concerns in public opinion. Their effectiveness in the field should be assessed. Costs are related to manpower (this depends on the labour costs of Member States) and genetics/chemical consumables (no detailed info). For <i>C. destructor</i>, hybrids</p>	<p>Medium</p>

	red swamp crayfish but not yet verified in the field (Manfrin et al. 2019).	with the congeneric <i>C. albidus</i> result in only male progeny (Lawrence et al. 2000). This technique has been used to control the reproduction in stocked ponds in Australia.	
	Biological control methods: predators. Several studies have shown that fish predation has an impact on crayfish populations. Fish usually predate juveniles, soft molted and small-sized crayfish, being complementary to trapping. In Europe, native fish as the eel <i>Anguilla anguilla</i> , the pike <i>Esox lucius</i> , the perch <i>Perca fluviatilis</i> and the pikeperch <i>Stizostedion lucioperca</i> have been observed to prey on invasive alien crayfish (Gherardi et al. 2011; Stebbing 2016; Manfrin et al. 2019).	In the USA, in an isolated lake in Wisconsin, Sparkling Lake (1,000,000 m ²), during a long-term management action (8 years) involving the combination of intensive trapping and the protection of local smallmouth bass from fishing pressure led to a 99% decline in crayfish abundance with no population recovery in the subsequent four years (Hein et al. 2006, 2007; Hansen et al. 2013). This method is generally acceptable to the public or does not cause environmental damage, although moving fish between waterbodies would also require certification of fish health and maintenance of biosecurity protocols. There could be a potential conflict with anglers who want to catch and remove the fish, but a catch and release approach can be promoted in these cases. The cost is related mainly to traps (see above for mechanical methods) and manpower (this depends on labor costs of Member States). Native European fish should be used to control crayfish. They can be reintroduced or restocked (after having assessed the feasibility, i.e. potential effects on non-target species should be considered). There are no relevant health and safety procedures to follow.	High
	Biological control methods: pathogens. The species is susceptible to crayfish plague caused by the oomycete <i>Aphanomyces astaci</i> . Four populations of the species have been eradicated with this method in Spain (Souty-Grosset et al. 2006).	<i>Cherax destructor</i> infected in the laboratory or chronically infected signal crayfish <i>Pacifastacus leniusculus</i> were introduced into the invaded ponds and the entire <i>C. destructor</i> invasive populations died after 30 days and 120 days, respectively (Souty-Grosset et al. 2006). There are no relevant health and safety procedures to follow, and the method is not costly. However, its use might generate the	High

		risk of its indirect spreading to the native crayfish populations, that are all protected, elicited major concerns by public and stakeholders. Similarly, a chronically infected population can establish, serving as a source of the pathogen. Moreover, legal requirements (including a risk assessment) could be necessary to use pathogens as a control method in the wild in some Member States.	
Methods to achieve management⁸	All the methods described above for spread and eradication can also be used to manage established populations of common yabby. An integrated approach is always recommended.	See above	See above
	Physical methods: diversion of rivers, and construction of barriers (e.g. weirs, small dams) may be used in the case of confined populations or to exclude the invasive alien species from habitats of protected or imperilled native species.	In some cases, exclusion barriers have been effective to contain invasive crayfish populations (e.g. preventing them from spreading into headwater reaches; Dana et al. 2011b; Frings et al. 2013). However, they can also impede the dispersal of native species like fish (thus eliciting some concerns in some stakeholders, such as anglers and conservationists) and be costly. Dams can also be effective, because they halt the spread of the species, but they should be maintained, and their cost could be high. No health and safety issues for personnel involved.	Medium

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Zukowski S, Asmus M, Whiterod N, Conallin A, Campbell J, Fisher I, Bright T. 2018. Collaborating with recreational fishers to inform fisheries management: Estimating population abundance for an iconic freshwater crayfish. *Ecological Management and Restoration* 19: 85-88.

Guidelines for Completing the Annex

1 Provide a brief summary description of the most cost-effective methods drawing on the reviews in the detailed assessments

2 Provide a description of the potential method. This should be based on the available key scientific evidence which should be gathered from sources including articles and reviews in technical and scientific journals, internet searches, online databases, grey literature and relevant books and personal communications from scientists, stakeholders, conservation practitioners and governmental bodies. This information should include a full bibliographic list detailing the literature and sources considered.

3 Provide an assessment of the likely cost and effectiveness of the method. Where information is available, consider the following range of questions, accepting that not all questions will be appropriate in all circumstances.

- How effective has this approach proven to be in the past or in an analogous situation?
- How publically acceptable is the approach likely to be?
- Over what period of time would this approach need to be applied to be effective?
- What is the direct cost of implementing this approach?
- How likely are the methods used in the approach to be available?
- How likely is it that relevant licences or other approvals to undertake the approach would be difficult to obtain?
- How likely is it that health and safety issues would prevent the use of this approach?
- How significant is the environmental harm caused by this approach?
- How significant is the economic harm caused by this approach?
- How significant is the social harm caused by this approach?
- How likely is it that the approach will be criticised on welfare grounds?
- How likely is it that the approach will be acceptable to other stakeholders?

Where available, factual information on the costs of specialist equipment, or case studies of management costs from across the Union or third countries should be provided. When describing case studies, if the information is available then provide both total cost and the area over which control was undertaken so that a cost per unit area might be derived. Where such quantitative information is not available, then any qualitative information from the literature is acceptable to help guide decision making. It is accepted that in the majority of cases the information required to assess the potential total cost of management at a member state level is unlikely to be available. This would normally require information on the extent and abundance of the species which is beyond the scope of this assessment. Assessors are not expected to extrapolate the potential total costs of management at a member state level, only to report on the information provided within the literature.

4 Provide an overall assessment of the confidence that can be applied to the information provided for this method. This confidence should relate to the quality of the available information using the guidance below. It should NOT relate to the confidence in the effectiveness of the method

The confidence scores are:

- **High:** Information comes from published material, or current practices based on expert experience applied in one of the EU countries or third country with similar environmental, economic and social conditions.
- **Medium:** Information comes from published data or expert opinion, but it is not commonly applied, or it is applied in regions that may be too different from Europe (e.g. tropical regions) to guarantee that the results will be transposable.
- **Low:** data are not published in reliable information sources and methods are not commonly practiced or are based solely on opinion; This is for example the case of a novel situation where there is little evidence on which to base an assessment.

If there are further factors beyond these that have determined the chosen level of confidence, then provide a brief written description to support the choice of the level of confidence.

5 Measures for preventing the species being introduced intentionally or unintentionally into the territory of a Member State (cf. Articles 7(1)(a), 7(2), 13, 15), i.e. preventing a species entering by blocking its pathways, including pre-border prevention and prevention of escape or release into the environment and secondary spread whenever relevant. Measures for preventing the species reproducing while in containment should be included, if appropriate. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

6 Measures to run an effective surveillance system for achieving an early detection of a new occurrence (cf. Article 16). This section assumes that the species is not currently present in a Member State, or part of a Member State's territory.

7 Measures to achieve eradication. Preferably, but not exclusively, used at an early stage of invasion, after an early detection of a new occurrence (cf. Article 17). Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

8 Measures to achieve management of the species once it has become widely spread within a Member State territory (cf. Article 19). These measures can be aimed at eradication, population control or containment of a population of the species. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

The development and completion of this template forms part of the EU project **No 07.0202/2020/834529/ETU/ENV.D.2** Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention.

**Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention"
Contract No 07.0202/2020/834529/ETU/ENV.D.2¹**

Name of organism: *Delawarea odorata* Lem.

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Risk Assessment Area: The risk assessment area is the territory of the European Union 27 and UK, excluding the outermost regions.

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Date of completion: 15/10/2021

¹ This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA). A number of amendments have been introduced to ensure compliance with Regulation (EU) 1143/2014 on IAS and relevant legislation, including the Delegated Regulation (EU) 2018/968 of 30 April 2018, supplementing Regulation (EU) No 1143/2014 of the European Parliament and of the Council with regard to risk assessments in relation to invasive alien species (see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>).

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SECTION A – Organism Information and Screening

A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?

including the following elements:

- the taxonomic family, order and class to which the species belongs;
- the scientific name and author of the species, as well as a list of the most common synonym names;
- names used in commerce (if any)
- a list of the most common subspecies, lower taxa, varieties, breeds or hybrids

As a general rule, one risk assessment should be developed for a single species. However, there may be cases where it may be justified to develop one risk assessment covering more than one species (e.g. species belonging to the same genus with comparable or identical features and impact). It shall be clearly stated if the risk assessment covers more than one species, or if it excludes or only includes certain subspecies, lower taxa, hybrids, varieties or breeds (and if so, which subspecies, lower taxa, hybrids, varieties or breeds). Any such choice must be properly justified.

Response: This risk assessment covers one species, the Cape ivy *Delairea odorata* Lem.

Taxonomy

Kingdom	<i>Plantae</i>
Phylum	<i>Tracheophyta</i>
Class	<i>Magnoliopsida</i>
Order	<i>Asterales</i>
Family	<i>Asteraceae</i> Dumort.
Subfamily	<i>Asteroideae</i> (Cass.) Lindl.
Genus	<i>Delairea</i> Lem.
Species	<i>Delairea odorata</i> Lem.

Delairea odorata Lem., in Ann. Sci. Nat., Bot. 1: 379 (1844), is the valid name of the species with an origin in the Cape region of South Africa in the genus *Delairea* (family *Asteraceae*) (Euro+Med 2006-; IPNI 2021; Plants of the World Online 2021; Roskov et al. 2021; The Plant List 2013). This vine was first described by Lemaire (1844) as *Delairea odorata*, but transferred to *Senecio* (as *S. mikanioides*) by

Harvey (1865) and returned to the original *D. odorata* by Jeffrey (1986). For a long period, the genus *Delairea* was considered monospecific. Recently, Funez et al. (2021) newly described *Delairea aparadensis*, an endemic species in the highlands of southern Brazil.

Synonyms

After cross-checking the information available at IPNI (2021), Plants of the World Online (2021), Euro+Med (2006-), Roskov et al. (2021), Iamónico (2017), and the African Plant Database (2021) it can be concluded that main synonyms for the species are:

- *Delairea scandens* (DC.) Lem.;
- *Mikania scandens* Lem. [*non* (L.) W.];
- *Senecio mikanioides* Otto ex Walp., in C.F.Otto & A.Dietrich, Allg. Gartenzeitung 13: 42. 1845;
- *Senecio scandens* Juss. ex DC. *non* Buch.-Ham. ex D.Don, *nom. illeg., p.p.*];
- *Breonia palmata* Hort. [*non* A.Rich.];
- *Ipomoea hederacea* Hort.

Common names

According to CABI (2021), EPPO (2021), Euro+Med (2006-), FinBIF (2021) and Roskov et al. (2021) common names in the European region are:

- Croatian: staračac
- English: Cape ivy, German ivy, climbing senecio, parlor-ivy
- Finnish: murattivillakko
- French: lierre d'Allemagne
- German: Kap-Efeu, Efeugreiskraut, Salonefeu
- Italian: Senecione profumato, Senecione mikanioide
- Portuguese: erva-de-são-tiago
- Russian: крестовник миканиевидный
- Spanish: yedra alemana
- Swedish: sommarmurgröna
- Welsh: Creulys Dail Eiddew

According to Cal-IPC (2021a), Italian ivy, ivy groundsel, parlor ivy and water ivy are additional common names in the USA.

Most common subspecies, lower taxa, varieties, breeds or hybrids

Subspecies, lower taxa, breeds or hybrids are not known. Two morphological varieties (stipulate and exstipulate) of Cape-ivy exist in the native region in South Africa as well in the invaded regions of California and Oregon (Reddy & Mehelis 2015). The stipulate variety has ear-like, flattened auricles at the base of the leaf petioles, whereas the exstipulate variety lacks auricles. The stipulate variety is the most common variety reported in all collections of Cape-ivy in South Africa (Robison & DiTomaso, 2010). In contrast, the exstipulate variety is the most common variety in California (Robison & DiTomaso, 2010).

Assumptions made by the authors on the reproductive mechanisms of *D. odorata* in the risk assessment area

Delairea odorata is an obligate outcrossing species that can reproduce sexually by seed which are wind dispersed and spreads vegetatively from stem fragments that readily root at nodes (Robison, 2006) and by the extension of the plant through stolons (Alvarez 1998). Viable seed has only been reported from the plants' native range (South Africa) and from Australia (Rolando 2000) and the USA (Hawaii, California and Oregon) (Robison 2006, Alvarez, 1998, Rolando, 2000).

It is interesting to note that viable seed was not reported in the USA before 2000, despite relevant studies. Bossard (2000) and Bossard et al. (2000) tested thousands of seeds from 26 populations in the USA and did not find any viable seeds. Additionally, Young (2000) tested 100 seeds at temperatures between 5 and 40 °C using various treatments and only one seed germinated. However, Robison (2006) showed that *D. odorata* was self-incompatible and able to produce viable seed throughout California. Over a three-year period, seed from a wide geographic range of populations was sampled and 66% of 104 locations produced viable seed. In addition, viable seed is reported from Oregon (Robison 2006).

Robison (2006) suggests that this indicates, at least in California, viable seed was not produced in populations in the natural environment prior to 2001. The author suggests that this could be due to two reasons:

1. Because the flowers of *D. odorata* are self-incompatible, only a few genetic lines have been introduced, which inhibits viable seed production. But potentially, additional genetic lines have been introduced.
2. A factor inhibiting viable seed production occurs in the risk assessment area.

Robison (2006) does however highlight that it is not known whether sexual or asexual reproduction contributes more to the spread of *D. odorata* in North America. Robison (2006) notes that although viable seed is produced in some populations in the USA, most populations are sterile and clonal expansions.

In the risk assessment area, vegetative reproduction is considered the main, if not the only reproductive mechanism. Vegetative reproduction can occur at any time when the nodes of the stem, stolon, or leaf petiole are in contact with the soil. Small fragments of 1.3 cm can root easily and quickly.

Seed production has been observed in the risk assessment area (for example, pers. obs. E. Marchante, Portugal), but scientific studies are lacking to confirm seed viability.

The authors of this risk assessment have assumed that at the current time in the risk assessment area, *D. odorata* does not produce:

- (1) viable seed, or
- (2) Seedlings are not produced in the natural environment, or
- (3) Seedlings do not persist to grow into mature plants.

The authors base these assumptions on two main arguments. Firstly, there is no scientific evidence to suggest one or more of the above occur in the risk assessment area. Secondly, although the species is established in the risk assessment area, evidence of high spread rates and establishment of satellite populations, as would be expected from a wind dispersed species, is lacking.

Therefore, throughout the risk assessment, only vegetative reproduction is considered when answering and scoring the questions (with the exception of some questions on entry when potentially viable seed could be imported). However, when providing the overall score for the risk assessment and the associated confidence, the uncertainty of viable seeds has been taken into consideration.

If point 1, 2 and 3 occur in the future this may increase the scores in the spread sections. This may require a revision of the spread scores. The authors do not consider that the lack of seed production in itself is a limitation to invasiveness.

A2. Provide information on the existence of other species that look very similar [that may be detected in the risk assessment area, either in the environment, in confinement or associated with a pathway of introduction]

Include both native and non-native species that could be confused with the species being assessed, including the following elements:

- other alien species with similar invasive characteristics, to be avoided as substitute species (in this case preparing a risk assessment for more than one species together may be considered);
- other alien species without similar invasive characteristics, potential substitute species;
- native species, potential misidentification and mis-targeting

Response: *Delairea odorata* is a fleshy, perennial, evergreen vine with a woody base. Roots arise from the nodes of stolons and rhizomes, and are simple and shallow, frequently scarcely penetrating the loose surface layer of organic material. Stolons and rhizomes are shiny, with thick cuticles, and the latter frequently have dark purple blotches, or are entirely purple. Stems are twining and can ascend to 8 m. Leaves are green, glabrous, alternate, broadly deltate to “ivy-shaped”, 3-10 cm long, 3.6 cm wide with 3 to 10 lobes. Both leaves and stems store water. Capitula with yellow florets, grouped on terminal and axillary cymes, disk florets 4-5 mm long arranged in clusters, ray flowers absent. Fruits are cypselas about 2 mm long, pappus 5–6 mm long, readily falling or fragile, white; bristles minutely scabrid-barbellate (CABI 2021a; EPPO 2012; Manning, & Goldblatt 2012).

Existence of other non-native species that look very similar

Senecio angulatus (*Asteraceae*; creeping groundsel, climbing groundsel) is a vigorous fast-growing climber with semi-succulent stems and leaves native to South Africa. Its form is a dense tangled shrub 2 meters tall or a climber that can reach 6 meters high. The leaves are rhombic to ovate, 3.7 to 22

centimeters long and 1 to 14 centimeters wide and occur in 1-4 pairs. They are thick, glossy, fleshy and coarsely toothed (but not entirely), with one to three teeth each side and bluntly lobed, with upper leaves becoming smaller with fewer teeth or none at all. Flower heads radiate, in branched corymbs or panicles, yellow, involucre calyced (Manning, & Goldblatt 2012). According to Euro+Med (2006- b) and CABI (2021b), the species is present in the following European countries: Albania, Croatia, France, Italy, Malta, Portugal and Spain.

Senecio tamoides (*Asteraceae*; Canary creeper, parlor ivy) is a climbing *Asteraceae* species native to Southern Africa. It is a fast-growing, scrambling, mostly evergreen perennial climber with semi-succulent stems and leaves that creeps along the ground or climbs several meters into the trees. It grows up to a height of 4 metres. The light green, shiny, fleshy leaves resemble those of ivy in that they are roughly triangle-shaped with unequal lobes. According to CABI (2021c), the species is present in Portugal.

Roldana petasitis (*Asteraceae*; Velvet groundsel) is an erect perennial *Asteraceae* shrub native to Southern Africa. Leaves with lamina are \pm circular to broad-ovate, 10–20 cm long and wide, margins palmately lobed with 9–13 lobes, softly hairy,; petiole at least half as long as lamina, except on leaves near heads. According to Euro+Med (2006- c) and CABI (2021d), the species is present in France, Italy, Portugal and Spain.

Existence of other native species that look very similar

No information about native species looking very similar are found.

A3. Does a relevant earlier risk assessment exist? Give details of any previous risk assessment, including the final scores and its validity in relation to the risk assessment area.

Response: No.

Delairea odorata was added to the EPPO Alert List in 2009 and transferred to the List of Invasive Alien Plants in 2012 (EPPO 2012).

Inside of the risk assessment area

Cyprus

In 2019, a workshop of experts on invasive alien species, prioritised species that may cause a threat to Cyprus. Experts were asked to score each potential IAS within their thematic group for their separate likelihoods of: (i) arrival, (ii) establishment, (iii) magnitude of the potential negative impact on human health or economies. *Delairea odorata* was considered a high threat as this species can negatively impact on both human health and economies. The plant species has a high likelihood of arrival, establishment and human health impacts within Cyprus (Peyton et al. 2020).

Outside of the risk assessment area

United States of America (USA)

USDA (2013) used a PPQ weed risk assessment model (Koop et al. 2012) to evaluate the risk potential of *Delairea odorata*. The species was first introduced into the USA in the 1850s as an ornamental houseplant (Robison et al. 2011). It was first collected from California in 1892 (Robison & DiTomaso, 2010) and naturalized there by the 1960s (Bossard et al. 2000). Since then, this species has become naturalized in all coastal counties in California, one coastal county in Oregon, and much of Hawaii (Kartesz 2021; Robison & DiTomaso 2010). In Hawaii, it was first collected in 1910 and is now considered naturalized (Wagner et al. 1999). In California, it occurs in riparian forest, coastal scrub, salt marsh, oak woodland, conifer forest, agricultural, and non-native forests (Robison & DiTomaso 2010). In California, it readily establishes in riparian areas and then spreads into drier sites (CABI 2021). In Hawaii, it encompasses an elevation range of 1600 to 8200 feet (487 m – 2500 m) (Jacobi & Warschauer 1992). *Delairea odorata* is managed in California and Hawaii (Bossard et al. 2000; Elliott 1994; Motooka et al. 2003). It is commercially grown and sold in the USA, but it does not appear to be sold by any of the major distributors (e.g., Monrovia, Green Leaf, Bailey). It is sold as both Cape ivy and German ivy (CABI 2021). It primarily impacts natural systems. As with other vine species, *D. odorata* forms dense mats that blanket and smother vegetation (Elliott 1994), including small trees (Bossard et al. 2000), and reduces native species diversity (Alvarez & Cushman 2002). It prefers shady disturbed sites with ample year-round moisture (Bossard et al. 2000). In habitats without year-round moisture, it dies back during dry seasons and then regrows during wet seasons (Bossard et al. 2000). *Delairea odorata* is particularly troublesome because of its ability to root at stem nodes (Bossard et al. 2000; Elliott 1994). This species reproduces and spreads vegetatively from stem fragments that readily root at nodes (Robison 2006) and by the extension of the plant through stolons (Alvarez 1998). In summary, the result of the weed risk assessment for *Delairea odorata* is scored as **High Risk**.

Parts of the USA West coast may have similar climatic conditions as well as habitat conditions like some regions in the EU, basic information of e.g. biology, habitat preferences, invasiveness given in this risk assessment can be taken into account.

USA - Hawaii

According to the Hawaiian Ecosystems at Risk project (HEAR 2021) *Delairea odorata* is assessed as **High Risk** by a score of **14**. The relevant scores are:

Question	Score
Has the species become naturalized where grown?	
Species suited to tropical or subtropical climate(s) (0-low; 1-intermediate; 2-high)	2
Quality of climate match data (0-low; 1-intermediate; 2-high)	
Broad climate suitability (environmental versatility)	Yes
Native or naturalized in regions with tropical or subtropical climates	Yes
Does the species have a history of repeated introductions outside its natural range?	Yes

Question	Score
Naturalized beyond native range	Yes
Environmental weed	Yes
Toxic to animals	Yes
Causes allergies or is otherwise toxic to humans	Yes
Climbing or smothering growth habit	Yes
Geophyte (herbaceous with underground storage organs -- bulbs, corms, or tubers)	Yes
Produces viable seed	yes
Reproduction by vegetative fragmentation	Yes
Propagules likely to be dispersed unintentionally (plants growing in heavily trafficked areas)	Yes
Propagules dispersed intentionally by people	Yes
Propagules adapted to wind dispersal	Yes
Tolerates, or benefits from, mutilation, cultivation, or fire	Yes

Hawaii has different climatic conditions than regions in the EU, though basic information on e.g. biology, habitat preferences, given in this risk assessment can be taken into account, though the assessment of e.g. invasiveness has to be reviewed accordingly to different climate conditions.

USA - Oregon

Delairea odorata meets the criteria of an “A” listed noxious weed as defined by the Oregon Department of Agriculture Noxious Weed Policy and Classification System (ODA 2014). *Delairea odorata* scored **58** out of a potential score of 90 using an adapted USDA-APHIS PPQ rating system. Using the ODA Noxious Weed Rating system, the species scored **19**, indicating an “A” listing. Noxious weeds are non-native plants with scores of 11 points or higher. The determination is based on two independent risk assessments.

Issue	Answer and score

Invasive in other areas.	Known to be invasive in geographically similar areas (California Coastal Riparian Areas) - 6
Habitat availability: Are there susceptible habitats for this species and how common or widespread are they?	High - 6
Environmental factors: Do abiotic (non-living) factors in the environment effect establishment and spread of the species? (e.g., precipitation, drought, temperature, nutrient availability, soil type, slope, aspect, soil moisture, standing or moving water).	Medium – Moderately confined by environmental factors (Needs moisture year round to become established, can survive drought once established) - 2
Reproductive traits: How does this species reproduce? Traits that may allow rapid population increase both on and off site.	Medium – Reproduction is vegetative (e.g., by root fragments, rhizomes, bulbs, stolons) - 3
Biological factors: Do biotic (living) factors restrict or aid establishment and spread of the species?	High – Few biotic interactions restrict growth and reproduction. Species expresses full growth and reproductive potential (No evidence indicating biotic factors limiting spread on the west coast.) - 4
Reproductive potential and spread after establishment - Non-human Factors.	Medium - Moderate potential for natural spread with either high reproductive potential or highly mobile propagules (Can be moved in waterways during flood events, seeds can be wind blown via wispy pappus on achenes) - 3
Economic Impact: What impact does/can the species have on Oregon's agriculture and economy?	Medium – Potential to, or causes moderate impacts to urban areas, right-of-way maintenance, property values, recreational activities, reduces rangeland productivity (Similar impacts to that of English Ivy, which include additional right of way maintenance costs, forest regeneration costs, and control costs to public and private landowners. These impacts are most likely to occur in the coastal zones. Leaves are toxic to livestock, animals) - 5
Environmental Impact: What risks or harm to the environment does this species pose? Plant may cause negative impacts on ecosystem function, structure, and biodiversity of plant or fish and wildlife habitat; may put desired species at risk.	High - Species can or does cause significant impacts in several of the above categories. Plant causes severe impacts to limited or priority habitats (Significant impacts to riparian and some inland habitats, alters entire canopy and understory diversity.) - 6

Parts of Oregon may have similar climatic conditions as well as habitat conditions like some regions in the EU, basic information of e.g. biology, habitat preferences, invasiveness given in this risk assessment can be taken into account.

USA - California

Cal-IPC (2021a) assesses *D. odorata* as a perennial vine found along the coast of California and in the San Gabriel Mountains. *Delawarea odorata* is especially problematic in coastal riparian areas, though it may also invade inland riparian areas, moist forests, and oak woodlands. Vines are known to form dense mats of vegetation over trees and shrubs, killing plants underneath. It is toxic to animals and fish can be killed when plant materials are soaking in waterways. Stem, rhizome and stolon fragments resprout if left in the ground after treatment. Can occasionally reproduce by seeds in some areas. The Cal-IPC rating is **high**. These species have severe ecological impacts on physical processes, plant and animal communities, and vegetation structure. Their reproductive biology and other attributes are conducive to moderate to high rates of dispersal and establishment. The California Department of Food and Agriculture (CDFA) Rating is **B***. A pest with rating B is of known economic or environmental detriment and, if present in California, it is of limited distribution and is included in the CCR Section 4500 list of California State Noxious Weeds Cal-IPC (2021a). The impact on abiotic ecosystem processes, plant communities and on higher trophic levels are assessed as severe. Role of anthropogenic and natural disturbance in establishment is assessed as severe as well. *Delawarea odorata* takes over habitats regardless of anthropogenic or natural disturbance. *Delawarea odorata* on the US west coast grows vigorously in physically challenging environments such as streamside thickets, willows and poison oak (*Toxicodendron diversilobum*). Severe invasive potential. This species can establish independently of natural or anthropogenic disturbance. The distribution of *D. odorata* ranges along the entire Californian coast and some mesic areas of the Central Valley. Over 500,000 acres (202342 ha) are infested in California, and on Catalina Island, 13 populations were detected with six in riparian habitats and one in island scrub oak chaparral near a riparian area. *Delawarea odorata* spreads primarily by vegetative means, breaks apart easily and both stem nodes and leaf petioles are capable of rooting. Seeds disperse with wind, water, and soil movement. If there is a *D. odorata* source upstream, high water flows in the winter can be expected to transport pieces of plants down-stream, which can begin new colonies (2021b).

Parts of California may have similar climatic conditions as well as habitat conditions like some regions in the EU, basic information of e.g. biology, habitat preferences, invasiveness given in this risk assessment can be taken into account.

Australia - Victoria

The Weed Risk Assessment (WRA) developed by the Biosciences Research Division of the Department of Primary Industries, Victoria, is a prioritisation process or risk assessment, based on the Analytic Hierarchy Process (AHP), which ranks weeds by (1) assessing the plant’s invasiveness, (2) comparing the plant’s present and potential distribution and (3) determining the impacts of the plant on social, economic, and environmental values. Accordingly Agriculture Victoria (2021a) conducted an invasiveness (2021b) and impact (2021c) assessment of *Delawarea odorata* and modelled the species’ distribution (2021d). During the assessment of biological data, criteria are assigned intensity ratings (criteria ratings) of high (H), medium-high (MH), medium (M), medium-low (ML), and low (L), to score each species.

Relevant high respectively medium-high rated criterions regarding biological characteristics of *D. odorata* in the invasiveness assessment (2021b) are:

Establishment

Germination requirements	Germination is reported to be rare, occurring in autumn. However vegetative propagules (pieces of stem and stolons) which is the main form of reproduction can root and grow when conditions are suitable (Blood 2001) - High
Establishment requirements	Reported to invade rainforest (Carr et al. 1992). Therefore it can establish under low light conditions. Cut stems can survive months, then set roots and grow when exposed to suitable conditions (Blood 2001). Therefore it has some requirements for establishment. – Medium-High
How much disturbance is required?	Establishes in undisturbed /minor disturbed natural ecosystems: heath land: sclerophyll forest and woodland: riparian vegetation: rainforest (Carr et al. 1992). - High
Growth/Competitive	
Tolerates herb pressure?	Contains pyrrolizidine alkaloids and xanthenes that make it unsuitable forage for most fauna and if slashed to ground level, plants can regrow from rootstock (Bossard et al. 2000). Bio-Control agents have been researched in South Africa and a program has been developed in California, however no such efforts are reported in Australia (Bossard et al. 2000). - High
Normal growth rate?	Reported to be a fast growing vine (Weber 2003). In California it has been reported as spreading more rapidly than any other weed species. Average growth rates of plants around San Francisco have been calculated to one foot of growth per month. (Alvarez 1998). - High
Stress tolerance to frost, drought, w/logg, sal. etc?	Tolerates some salinity (recorded on salt exposed bluffs) (Alvarez 1998). Drought tolerant (Blood 2001). Reported in seasonal wetlands, therefore tolerant of waterlogging (Bossard et al. 2000). Frost tender (Blood 2001), however hard frosts appear to stimulate seed set, therefore while frost may damage the plant, it may not kill it. Foliage has high moisture content and therefore resistant to burning also plants can regrow from rootstock (Bossard et al. 2000). - High
Reproduction	
Reproductive system	Produces seed, however primary mode of reproduction is vegetative, through stolons and stem fragments (Bossard et al. 2000 and Muyt 2001). - High
Number of propagules produced?	Large plants can produce more than 40,000 seeds annually (Muyt 2001). - High

Reproductive period?	Long lived, can form monocultures (Bossard et al. 2000). - High
Time to reproductive maturity?	Reach sexual maturity within two years (Muyt 2001) – Medium-High
Dispersal	
Number of mechanisms?	Seeds are 2 mm with hairs attached, spread by wind and water (Muyt 2001). Vegetative propagules spread by water and deliberate human actions (Alvarez 1998). - High
How far do they disperse?	Wind dispersed seed can travel distances of more than 1 km. - High

Relevant high or medium-high rated criteria regarding recreation, abiotic characteristics, habitat and fauna in the impact assessment (2021c) are:

Recreation	
Restrict human access?	Can form dense tangled curtains of vegetation, including in riparian areas (Muyt 2001). Access would be difficult and would require significant work to control the species to maintain access due to the species rapid growth. – Medium-High
Injurious to people?	Toxic to people. Toxins include pyrrolizidine alkaloids and xanthones, which are contained in the leaves and present all year (Bossard et al. 2000). No reported fatalities. – Medium-High
Abiotic	
Increase soil erosion?	Can contribute to soil erosion on hillsides (Bossard et al. 2000). – Medium-High
Reduce biomass?	The weight of the ivy can cause trees to fall, and dense canopy of the vine can smother species of the lower strata and reduce growth (Bossard et al. 2000). – Medium-High
Community Habitat	
Impact on composition (a) high value Ecological Vegetation Class (EVC): Riparian Forest	The weight of the ivy can cause trees to fall, the dense canopy of the vine can smother species of the lower strata and reduce growth and regeneration of species and can form monocultures (Bossard et al. 2000). All EVCs - High

(b) medium value EVC: Lowland Forest	
(c) low value EVC: Wet Forest	
Impact on structure?	The weight of the ivy can cause trees to fall, and the dense canopy of the vine can smother species of the lower strata and reduce growth and regeneration of species (Bossard et al. 2000). – Medium-High
Effect on threatened flora?	Can significantly reduce the species richness and diversity of an area, and render habitat in protected reserves for plant species worthless (Bossard et al. 2000). – Medium-High
Fauna	
Effect on threatened fauna?	Can render habitat in protected reserves for animal species worthless (Bossard et al. 2000). – Medium-High
Effect on non-threatened fauna?	Significant alteration of habitat; reducing plant species richness and diversity and therefore diversity of food and shelter available. Invasion by cape ivy can render habitat in protected reserves for animal species worthless (Bossard et al. 2000). – Medium-High
Benefits fauna?	Nothing reported. Insects may visit flowers, dense foliage may provide some shelter. - High
Injurious to fauna?	Foliage contains compounds toxic to mammals, spiders and fish (Bossard et al. 2000). Compounds toxic but not necessarily lethal to rats. - High

In summary, *Delairea odorata* is assessed as **Highly Invasive** (Score 0.6-0.79) by its invasiveness index of 0.72 and a **risk rating V - very serious threat to one or more vegetation formation in Victoria**.

Parts of Victoria may have similar climatic conditions as well as habitat conditions like some regions in the risk assessment, basic information of e.g. biology, habitat preferences, invasiveness given in this risk assessment can be taken into account.

A4. Where is the organism native?

including the following elements:

- an indication of the continent or part of a continent, climatic zone and habitat where the species is naturally occurring
- if applicable, indicate whether the species could naturally spread into the risk assessment area

Response: *Delairea odorata* is native to southern Africa from Uitenhage to KwaZulu-Natal (CABI 2021; Manning & Goldblatt 2012; Red List of South African Plants 2021), i.e. Lesotho and (Eastern and Western) Cape Province and KwaZulu-Natal in South Africa (GRIN-Global 2021; Grobbelaar et al. 1999; Queensland Government 2021; Roskov et al. 2021; USDA 2013).

Hilliard (1977) describes the native distribution as: “*Widespread, but not much collected, from the Cape peninsula through the southern and eastern Cape to Natal as far north as Nkandia, Nhlazatsche Mt. and the Biggarsberg north of Ladysmith; also recorded from Mhaleshoek in Lesotho. Grows on forest margins or in the forest, in Natal between ca. 800 and 1900 m above sea level.*” In its native region, *D. odorata* appears to have originally been confined to 'mist-veldt' regions, such as those in the Drakensberg Mountains, where it is found along forest edges and as an opportunistic vine exploiting openings in the native forest. Its occurrence at more coastal sites in South Africa may be more recent (Balciunas et al. 2004). Some of the coastal sites are quite arid, to less than 100 mm/yr (CABI 2021). According to EPPO (2012), Cape ivy grows in moist mountain forests.

Since the native distribution center in southern Africa and the European continent are separated by a large distance, natural spread into the risk assessment area is very unlikely.

A5. What is the global non-native distribution of the organism outside the risk assessment area?

Response: Table 1 and Figure 1 give an overview about global non-native distribution outside the risk assessment area. Considering the native range in the Cape region of Southern Africa, available spatial and non-spatial data describe the non-native distribution of *Delairea odorata* covering parts of North, Middle and South America, Northern Africa, Asia and Oceania. Occurrence information provided in literature without coordinates are listed in the table, but are not shown in the map.

Delairea odorata

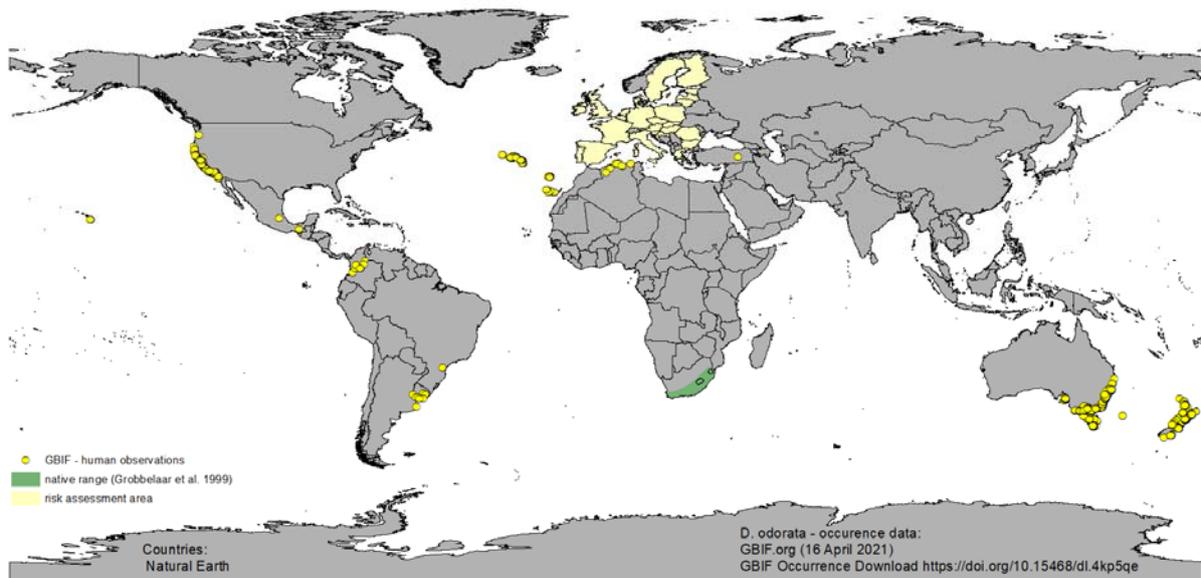


Figure 1 Global non-native occurrence records of *Delairea odorata* outside the risk assessment area (map designed by Umweltbundesamt/Environment Agency Austria - 2021/04/19)

Table 1 countries with *Delairea odorata* occurrences mentioned in references and databases (* references without coordinates not shown in map)

Continent/ region	country	general remarks	source
Africa	Algeria	Present; naturalized	Chatelain (2011), GBIF (2021), GRIN-Global (2021), Euro+Med (2006- a), Quézel & Santa (1963), Roskov et al. 2021
Asia	India*	invasive	Prabu et al. (2012)
Atlantic Islands	Azores, Madeira	Present / localized; naturalized; invasive	Brunel et al. (2010), CABI (2021a), Euro+Med (2006- a), GBIF (2021), GISD (2021), EPPO (2021); Sampaio (1947), Plants of the World online (2021), Roskov et al. 2021, Royal Botanic Garden Edinburgh (2003)
	Canary Islands	Present /localized; naturalized	Brunel et al. (2010), CABI (2021a), GBIF (2021), EPPO (2012), EPPO (2021), EXOS (2021), GISD (2021), GRIN-Global (2021), Plants of the World online

Continent/ region	country	general remarks	source
			(2021), Roskov et al. 2021, Royal Botanic Garden Edinburgh (2003)
	Saint Helena*	Present	CABI (2021a), Hilliard (1977)
North and Middle America (incl. Caribbean)	United States (California, Hawaii, Oregon)	Present / widespread / localized; naturalized; invasive; first reported: 1892 (California), 1909 (Hawaii)	Balciunas et al. (2004), CABI, (2021), EPPO (2012), GBIF (2021), GRIN-Global (2021), Munz (1959), Roskov et al. 2021, Starr et al. (2003), Wagner et al. (1990)
	Costa Rica*		Roskov et al. 2021
	Guatemala*		Roskov et al. 2021
South America	Argentina	Present / localized	CABI (2021a), GBIF (2021), Hilliard (1977), Roskov et al. 2021
	Bolivia*		Plants of the World online (2021), Roskov et al. 2021
	Chile*	Present	CABI (2021a), Rodríguez et al. (2018), Roskov et al. 2021
	Colombia		GBIF (2021), Plants of the World online (2021), Roskov et al. 2021
	Uruguay	Present / naturalized	GBIF (2021), GRIN-Global (2021), Plants of the World online (2021), Roskov et al. 2021
	Venezuela*		Plants of the World online (2021)

Continent/ region	country	general remarks	source
Oceania	Australia	Present / widespread / localized; naturalized; invasive	CABI (2021a), Fagg (1989), EPPO (2012), GISD (2021), GRIN-Global (2021), Hnatiuk (1990), Roskov et al. 2021
	Federal States of Micronesia*	Present	EPPO (2012), GISD (2021), GRIN-Global (2021)
	New Zealand	Present / widespread; naturalized; invasive	CABI (2021a), EPPO (2012), GISD (2021), GRIN-Global (2021), Webb et al. (1988), Plants of the World online (2021), Roskov et al. 2021

A6. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species been recorded and where is it established? The information needs to be given separately for recorded (including casual or transient occurrences) and established occurrences. “Established” means the process of an alien species successfully producing viable offspring with the likelihood of continued survival².

A6a. Recorded: List regions

A6b. Established: List regions

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

- Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea.

Comment on the sources of information on which the response is based and discuss any uncertainty in the response.

For delimitation of EU biogeographical regions please refer to <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2> (see also Annex VI).

² Convention on Biological Diversity, Decision VI/23

For delimitation of EU marine regions and subregions consider the Marine Strategy Framework Directive areas; please refer to <https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions/technical-document/pdf> (see also Annex VI).

Response: The Arctic, Anatolian and Macaronesian biogeographical regions are not part of the risk assessment area, but included for completeness. Table 2 and Figure 2 give an overview about occurrences of the species in the biogeographical regions. In the Steppic region, no occurrence information is available.

Source of human observation data is GBIF (2021), European wide overviews like Euro+Med PlantBase (providing an on-line database and information system for the vascular plants of Europe and the Mediterranean region, against an up-to-date and critically evaluated consensus taxonomic core of the species concerned) as well as national floristic or invasive species references.

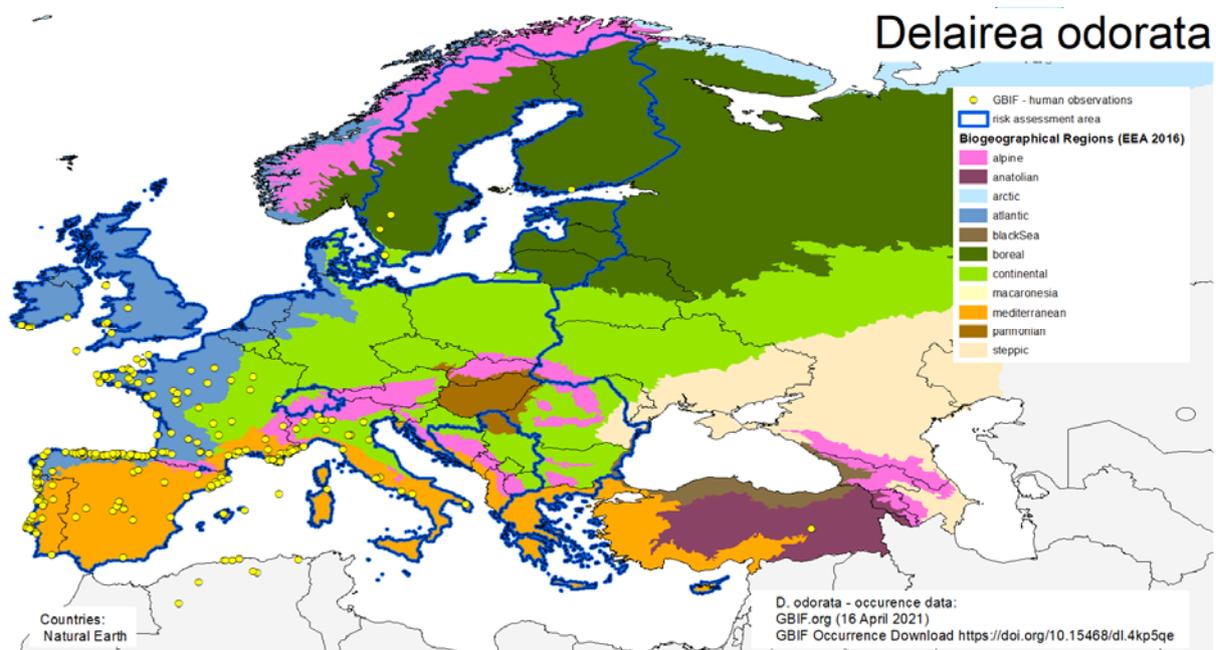


Figure 2 Occurrence records of *Delairea odorata* in the biogeographical regions (map designed by Umweltbundesamt/Environment Agency Austria – 2021/04/19)

Table 2 biogeographical regions with recorded/established *Delawarea odorata* occurrences (grey cell – biogeographical region not covered by the risk assessment area)

biogeographical region	recorded	established	source of recorded occurrences	source of established occurrences
Alpine	Yes	No	GBIF (2021)	
Anatolian	Yes	No	GBIF (2021)	
Arctic				
Atlantic	Yes	Yes	GBIF (2021)	CABI (2021a), EPPO (2012), EPPO (2021), Euro+Med (2006- a), GISD (2021), GRIN-Global (2021), Hilliard (1977), NBN Atlas (2021), Plants of the World online (2021), Reynolds (2002), Roskov et al. 2021, Sell & Murrell (1963), Tutin et al. (1976)
Black Sea	No	No		
Boreal	Yes	No	Euro+Med (2006- a), FinBIF (2021), GBIF (2021),	
Continental	Yes	Yes	Brunel et al. (2010), CABI (2021a), EPPO (2012), EPPO (2021), Euro+Med (2006- a), GBIF (2021), GRIN-Global (2021), Plants of the World online (2021), Roskov et al. 2021	Euro+Med (2006- a), Tutin et al. (1976)
Macaronesian	Yes	Yes	GBIF (2021)	Brunel et al. (2010), CABI (2021a), Euro+Med (2006- a), GISD (2021), EPPO (2021); Sampaio (1947), Plants of the World online (2021), Roskov et al. 2021, Royal Botanic Garden Edinburgh (2003)

biogeographical region	recorded	established	source of recorded occurrences	source of established occurrences
Mediterranean	Yes	Yes	GBIF (2021)	Brunel et al. (2010), CABI (2021a), Catalano et al. (1996), Celesti-Grapow et al. (2009), Conti et al. (2005), EPPO (2012), EPPO (2021), Euro+Med (2006- a), GISD (2021), GRIN-Global (2021), Plants of the World online (2021), Roskov et al. 2021, Royal Botanic Garden Edinburgh (2003), Sampaio (1947), Tutin et al. (1976)
Pannonian	No	No		
Steppic	No	No		

Response (6a): *Delairea odorata* is recorded in the Alpine, Atlantic, Boreal, Continental and Mediterranean biogeographical regions. No recorded occurrence data is available for the Black Sea, Pannonian and Steppic biogeographical regions.

Response (6b): According to the available information, *D. odorata* can be considered as “established” in the Atlantic, Continental and Mediterranean biogeographical regions.

“Established” means the process of an alien species successfully producing viable offspring with the likelihood of continued survival. In the available references (mainly Euro+Med PlantBase), the term “naturalized” is used. In order to answer question 6b, “naturalized” is used as a synonym for “established”. The assessment of established occurrences in biogeographical regions is mainly deduced from the data available on national level (compare Table 3).

A7. In which biogeographic region(s) or marine subregion(s) in the risk assessment area could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A7a. Current climate: List regions

A7b. Future climate: List regions

With regard to EU biogeographic and marine (sub)regions, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

According to SDM results (see also Annex IX) the biogeographical regions *D. odorata* could establish in are as follows:

Response (7a):

Biogeographic region	likeliness	confidence
Alpine	unlikely	high
Anatolian	unlikely	high
Arctic	unlikely	high
Atlantic	likely	high
Black Sea	likely	high
Boreal	unlikely	high
Continental	likely	medium
Macaronesia	likely	high
Mediterranean	likely	high
Pannonian	unlikely	high
Steppic	unlikely	high

Response (7b):

Biogeographic region	Scenario RCP 2.6		Scenario RCP 4.5	
	likelihood	confidence	likelihood	confidence
Alpine	unlikely	medium	likely	medium
Anatolian	unlikely	high	unlikely	high
Arctic	unlikely	high	unlikely	high
Atlantic	likely	high	likely	high
Black Sea	likely	high	likely	high
Boreal	unlikely	high	unlikely	high
Continental	likely	medium	likely	medium
Macaronesia	likely	high	likely	high
Mediterranean	likely	high	likely	high
Pannonian	likely	medium	likely	medium
Steppic	unlikely	high	unlikely	high

A8. In which EU Member States has the species been recorded and in which EU Member States has it established? List them with an indication of the timeline of observations. The information needs be given separately for recorded and established occurrences.

A8a. Recorded: List Member States

A8b. Established: List Member States

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden

The description of the invasion history of the species shall include information on countries invaded and an indication of the timeline of the first observations, establishment and spread.

Response: Figure 2 and Table 3 give an overview about occurrences of the species in the EU Member States and the United Kingdom. Sources of information are observational data (GBIF 2021), European wide overviews like Euro+Med PlantBase as well as national floristic or invasive species references. An indication of the first observation is not possible for every member state but given if possible.

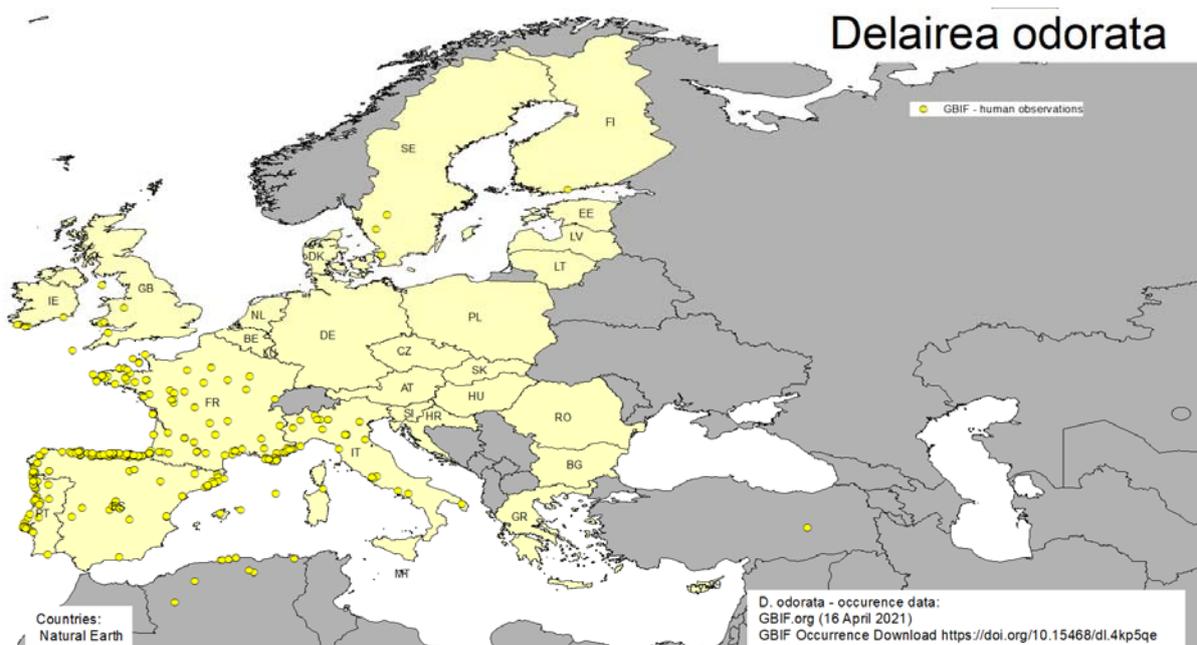


Figure 2 occurrence data records of *Delairea odorata* in the EU member states (including United Kingdom) (map designed by Umweltbundesamt/Environment Agency Austria – 2021/04/19)

Table 3 EU member states and United Kingdom with recorded/established *Delairea odorata* occurrences

country	recorded	established	source recorded	source established	general remarks	timeline of observations
Austria	No	No				
Belgium	No	No				
Bulgaria	No	No				
Croatia	Yes	Yes	Brunel et al. (2010), CABI (2021a), EPPO (2021), Milović et al. 2010, Milović et al. 2014 Roskov et al. 2021, Wallnöfer 2007			
Cyprus	No	No				
Czech Republic	No	No				

country	recorded	established	source recorded	source established	general remarks	timeline of observations
Denmark	No	No				
Estonia	No	No				
Finland	Yes	No	FinBIF (2021), GBIF (2021)		alien, new, ephemeral - at most a soil immigrant, perhaps slightly spreading vegetatively; 2 observations: 1983, 2012	1983 - 2012
France	Yes	Yes	Brunel et al. (2010), CABI (2021a), EPPO (2012), EPPO (2021), Euro+Med (2006- a), GBIF (2021), GRIN-Global (2021), Plants of the World online (2021), Roskov et al. 2021		Present / localized; naturalized	
Germany	No	No				
Greece	No	No				
Hungary	No	No				
Ireland	Yes	Yes	CABI (2021a), EPPO (2012), EPPO (2021), Euro+Med (2006- a), GBIF (2021), GRIN-Global (2021), NBN Atlas (2021), Plants of the World online (2021), Reynolds (2002), Roskov et al. 2021		Present; naturalized	
Italy	Yes	Yes	Brunel et al. (2010), CABI (2021a), Catalano et al. (1996), Celesti-Grapow et al. (2009), Conti et al. (2005), EPPO (2012), EPPO (2021), Euro+Med (2006- a), GBIF (2021), GRIN-Global (2021), Plants of the World online (2021), Roskov et al. 2021		Present / localized; naturalized; invasive	

country	recorded	established	source recorded	source established	general remarks	timeline of observations
Latvia	No	No				
Lithuania	No	No				
Luxembourg	No	No				
Malta	No	No				
Netherlands	No	No				
Poland	No	No				
Portugal	Yes	Yes	Brunel et al. (2010), CABI (2021a), Euro+Med (2006- a), GISD (2021), EPPO (2021), GBIF (2021)Sampaio (1947), Plantas invasoras em Portugal (2020), Plants of the World online (2021), Roskov et al. 2021, Royal Botanic Garden Edinburgh (2003)		Present / localized; naturalized; invasive	
Romania	No	No				
Slovakia	No	No				
Slovenia	No	No				
Spain	Yes	Yes	Brunel et al. (2010), CABI (2021a), EPPO (2012), EPPO (2021), GBIF (2021), GISD (2021), GRIN-Global (2021), Plants of the World online (2021), Roskov et al. 2021, Royal Botanic Garden Edinburgh (2003)		Present / localized; naturalized	

country	recorded	established	source recorded	source established	general remarks	timeline of observations
Sweden	Yes	No	Euro+Med (2006- a), GRIN-Global (2021), Karlsson (1998), Plants of the World online (2021), Roskov et al. 2021		Cultivated	
United Kingdom	Yes	Yes	CABI (2021a), Ecoflora (2021), EPPO (2012), Euro+Med (2006-a), GBIF (2021), GISD (2021), GRIN-Global (2021), Hilliard (1977), NBN Atlas (2021), Plants of the World online (2021), Roskov et al. 2021, Sell & Murrell (1963)		Present / localized; naturalized; invasive	Introduced: 1855; appearance in the wild: 1923

Response (8a): *Delairea odorata* is recorded in 8 out of 27 Member States (Croatia, Finland, France, Ireland, Italy, Portugal, Spain, Sweden) and in the United Kingdom.

Response (8b): Based upon the available data, *Delairea odorata* can be assessed as established in 6 Member States (Croatia, France, Ireland, Italy, Portugal, Spain) and in the United Kingdom.

By using a subset of GBIF records with a spatial accuracy ≤ 250 m, the EUNIS habitat classes at Level 2 (EEA 2019) can be spatially retrieved from the Ecosystem types of Europe 2012 raster data set. *Delairea odorata* in its native distribution seems to underlie some oceanic climate conditions. Therefore, the parameter “distance to sea” is used in the species distribution model (SDM; see Annex VIII). In its non-native range in the Western USA (California, Oregon), the species is described with most occurrences in coastal areas. In order to get some impressions whether there may be differences in EUNIS ecosystems at actual European occurrences, these are roughly classified as directly along coast-line respectively as countryside (Figure 3).

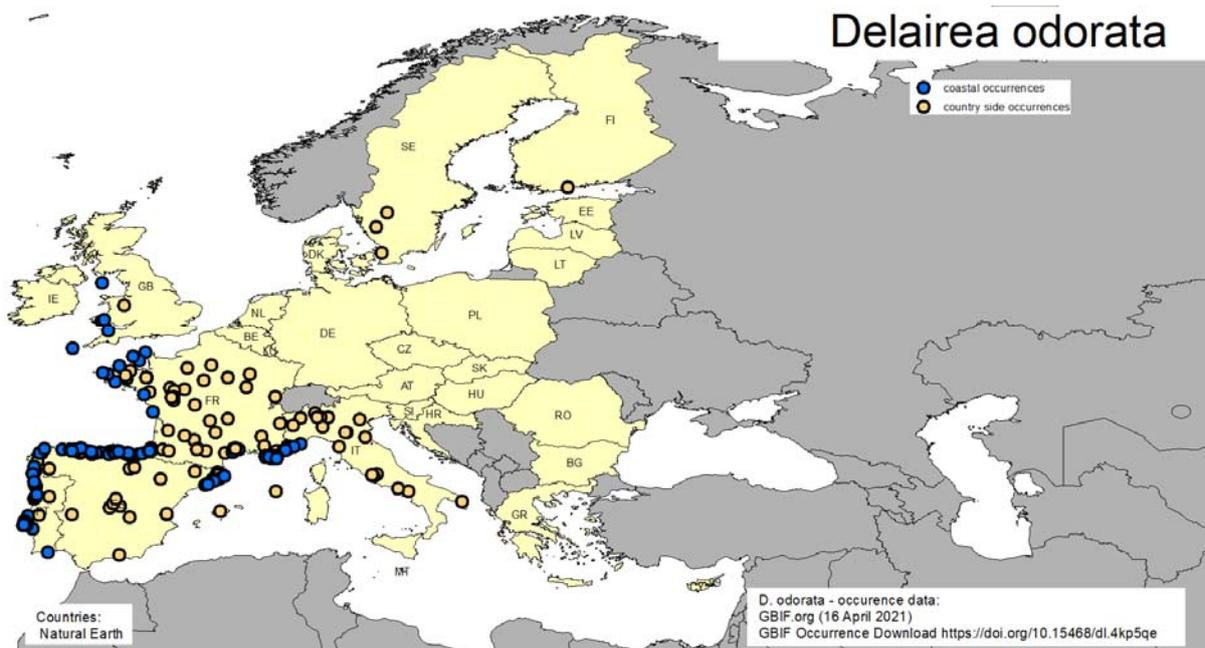


Figure 3 coastal/countryside classified occurrences of *Delairea odorata* (map designed by Umweltbundesamt/Environment Agency Austria – 2021/04/19)

Based upon the available data, there is enough evidence that the used data covers several ecosystem categories from anthropogenic/urban to more natural types (Table 4) in coastal as well as in countryside classified occurrences.

Anthropogenic types are for example *Buildings of cities, towns and villages* or *Low density buildings*; more natural types are woodland (*Broadleaved deciduous woodland* or *Mixed deciduous and coniferous woodland*) and grassland (*Mesic grassland*).

Table 4 *Delairea odorata* GBIF occurrences with coordinate accuracy < 250 m classified as coastal or countryside, and related EUNIS habitat classes at level 2 (EEA 2019)

EUNIS ecosystem level 2	coastal occurrences	countryside occurrences
E2 - Mesic grasslands	18	10
F3 - Temperate and Mediterranean-montane scrub	3	
F5 - Maquis, arborescent matorral and thermo-Mediterranean brushes	1	
FB - Shrub plantations		1
G1 - Broadleaved deciduous woodland	8	8

EUNIS ecosystem level 2	coastal occurrences	countryside occurrences
G2 - Broadleaved evergreen woodland	1	
G3 - Coniferous woodland	4	
G4 - Mixed deciduous and coniferous woodland	5	
G5 - Lines of trees, small anthropogenic woodlands, recently felled woodland, early-stage woodland and coppice	1	
I1 - Arable land and market gardens	15	6
I2 - Cultivated areas of gardens and parks	5	4
J1 - Buildings of cities, towns and villages	28	50
J2 - Low density buildings	13	24
J3 - Extractive industrial sites		1
J4 - Transport networks and other constructed hard-surfaced areas	3	2

A9. In which EU Member States could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A9a. Current climate: List Member States

A9b. Future climate: List Member States

With regard to EU Member States, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

The ensemble model (see Annex IX) suggested that suitability for *D. odorata* was most strongly determined by Minimum temperature of the coldest month (Bio6), accounting for 50.2% of variation explained, followed by Human influence index (HII) (24.8%), Mean temperature of the warmest quarter (Bio10) (10.4%), Distance to the sea (dist_to_sea) (8.6%), Climatic moisture index (CMI) (5.6%) and Global tree cover (Tree) (0.4%)

Response (9a): EU Members States and the United Kingdom with suitability under current conditions

country	likeliness	confidence
Austria	unlikely	high
Belgium	likely	high
Bulgaria	unlikely	high
Croatia	likely	high
Cyprus	likely	high
Czechia	unlikely	high
Denmark	likely	high
Estonia	unlikely	high
Finland	unlikely	high
France	likely	high
Germany	likely	medium

Greece	likely	high
Hungary	unlikely	high
Ireland	likely	high
Italy	likely	high
Latvia	unlikely	high
Lithuania	unlikely	high
Luxembourg	likely	medium
Malta	likely	high
Netherlands	likely	high
Poland	unlikely	high
Portugal	likely	high
Romania	unlikely	high
Slovakia	unlikely	high
Slovenia	likely	medium
Spain	likely	high
Sweden	unlikely	high
United Kingdom	likely	high

Response (9b): EU Members States and the United Kingdom with suitability under projected conditions

country	Scenario RCP 2.6		Scenario RCP 4.5	
	likeliness	confidence	likeliness	confidence
Austria	unlikely	medium	unlikely	high
Belgium	likely	high	likely	high
Bulgaria	likely	high	likely	high
Croatia	likely	high	likely	high
Cyprus	likely	high	likely	high
Czechia	unlikely	medium	likely	medium
Denmark	likely	high	likely	high
Estonia	unlikely	high	unlikely	high
Finland	unlikely	high	unlikely	high
France	likely	high	likely	high
Germany	likely	high	likely	high
Greece	likely	high	likely	high
Hungary	likely	medium	likely	medium
Ireland	unlikely	high	unlikely	high
Italy	likely	high	likely	high
Latvia	unlikely	medium	likely	medium
Lithuania	unlikely	medium	likely	medium
Luxembourg	likely	high	likely	high
Malta	likely	high	likely	high
Netherlands	likely	high	likely	high
Poland	unlikely	medium	likely	medium
Portugal	likely	high	likely	high
Romania	unlikely	high	unlikely	high
Slovakia	unlikely	high	unlikely	high
Slovenia	likely	high	likely	high
Spain	likely	high	likely	high
Sweden	unlikely	high	unlikely	high
United Kingdom	likely	high	likely	high

A10. Is the organism known to be invasive (i.e. to threaten or adversely impact upon biodiversity and related ecosystem services) anywhere outside the risk assessment area?

Response: *Delairea odorata* is invasive in North America (California, Hawaii and Oregon), Australia (New South Wales, Northern Territory, South Australia, Tasmania, Victoria) and New Zealand.

Table 1 gives an overview about invasiveness outside of the risk assessment area. Well-documented invasive behavior of *Delairea odorata* is reported for Australia, New Zealand, and the United States.

In California, it occurs in riparian forest, coastal scrub, salt marsh, oak woodland, conifer forest, agricultural, and non-native forests (Robison & DiTomaso 2010). *Delairea odorata* forms dense mats that blanket and smother vegetation (Elliott 1994), including small trees (Bossard et al. 2000), and reduces native plant species diversity (Alvarez & Cushman 2002). Cape ivy can invade different habitat types ranging from disturbed to native ecosystems and occurs in both dry to moist conditions. Due to a shallow root system, Cape ivy can contribute to soil erosion problems on hillsides and impact flood control functions along streams (Bossard 2000).

In southern Australia, Cape Ivy is a highly invasive environmental weed. It invades waterways, moist gullies, closed forests and margins, open woodlands, roadsides, waste areas and coastal areas and is also commonly found in gardens and along fences and urban areas parks (Weeds Australia 2021).

In New Zealand, Cape Ivy can be found in coastal and lowland regions. The plant occurs in coastal ecosystems as well as in scrub and forest margin communities, shrublands, rocklands, roadsides, quarries, farm hedges, wasteland and house gardens (New Zealand Plant Conservation Network 2021).

A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea

Response: *Delairea odorata* exhibits a widespread distribution in parts of the Atlantic and Mediterranean biogeographical regions (compare Figure 2). There are documented signs of invasiveness in these regions, especially along the coastlines. In Portugal, Cape Ivy frequently invades banks of watercourses, hedgerows and slopes, ruderal environments and communities of thorny shrubs (Plantas invasoras em Portugal 2020). *Delairea odorata* is reported as established in Tuscany (including the Tuscan Archipelago, see Lazzaro et al., 2014) and in Sardinia (Italy) (Iamónico 2017), where it occurs in ruderal habitats only, and in few localities with low density, so that at the time being no significant ecological impacts have been recorded (Bacchetta et al. 2009, Camarda et al. 2016, Portale della Flora d'Italia 2021).

A12. In which EU Member States has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden

Response: There are documented signs of invasiveness in Portugal and Italy. In Portugal, Cape Ivy frequently invades banks of watercourses, hedgerows and slopes, ruderal environments and communities of thorny shrubs (Plantas invasoras em Portugal 2020). *Delairea odorata* is reported as established in Tuscany and in Sardinia (Italy) (Iamónico 2017), where it occurs in ruderal habitats only, and in few localities with low density, so that at the time being no significant ecological impacts have been recorded (Bacchetta et al. 2009, Camarda et al. 2016, Portale della Flora d'Italia 2021).

For the United Kingdom, invasiveness is mentioned in a few references (e.g. CABI 2021a), though no further and detailed information could be found.

A13. Describe any known socio-economic benefits of the organism.

including the following elements:

- Description of known uses for the species, including a list and description of known uses in the risk assessment area and third countries, if relevant.
- Description of social and economic benefits deriving from those uses, including a description of the environmental, social and economic relevance of each of those uses and an indication of associated beneficiaries, quantitatively and/or qualitatively depending on what information is available.

If the information available is not sufficient to provide a description of those benefits for the entire risk assessment area, qualitative data or different case studies from across the risk assessment area or third countries shall be used, if available.

Response: *Delairea odorata* is a popular ornamental climbing vine used in landscaping and gardening (Cal-IPC 2021a, EPPO 2012, ISSG 2021). *Delairea odorata* is traded and used as an ornamental species

in the RA area. However, there is no quantitative data on its value to the horticulture trade and it is not considered to be a popular species in horticulture. This may also be due to the fact that the species can easily be propagated by its ramets. Ornamental use includes the use as a ground cover and also for covering structures; additionally, the species is used as an indoor ornamental.

SECTION B – Detailed assessment

Important instructions:

- In the case of lack of information the assessors are requested to use a standardized answer: “No information has been found.” In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable).
- With regard to the scoring of the likelihood of events or the magnitude of impacts see Annexes I and II.
- With regard to the confidence levels, see Annex III.
- Highlight the selected response score and confidence level in **bold** but keep the other scores in normal text (so that the selected score is evident in the final document).

1 PROBABILITY OF INTRODUCTION AND ENTRY

Important instructions:

- **Introduction** is the movement of the species into the risk assessment area (it may be either in captive conditions and/or in the environment, depending on the relevant pathways).
- **Entry** is the release/escape/arrival in the environment, i.e. occurrence in the wild
- Introduction and entry may coincide for species entering through pathways such as “corridor” or “unaided”, but it also may differ. If different, please consider all relevant pathways, both for the introduction into the risk assessment area and the entry in the environment.
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used (see Annex IV). For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document³ and the provided key to pathways⁴.
- For organisms which are already present (recorded or established) in the risk assessment area, the likelihood of introduction and entry should be scored as “very likely” by default.
- Repeated (independent) introductions and entries at separate locations in the risk assessment area should be considered here (see Qu. 1.7).

Qu. 1.1. List relevant pathways through which the organism could be introduced into the risk assessment area and/or enter into the environment. Where possible give details about the specific origins and end points of the pathways as well as a description of any associated commodities.

For each pathway answer questions 1.2 to 1.8 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 1.2a, 1.3a, etc. and then 1.2b, 1.3b etc. for the next pathway.

In this context a pathway is the route or mechanism of introduction and/or entry of the species.

³ <https://circabc.europa.eu/sd/a/7e5f0bd4-34e8-4719-a2f7-c0cd7ec6a86e/2020-CBD-pathways-interpretation.pdf>

⁴ <https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf>

The description of commodities with which the introduction of the species is generally associated shall include a list and description of commodities with an indication of associated risks (e.g. the volume of trade; the likelihood of a commodity being contaminated or acting as vector).

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 1.2-1.9.

(1): Horticulture (escape from confinement)

(2): Ornamental purpose other than horticulture (escape from confinement)

(3): Transport of habitat material (soil, vegetation) (transport – contaminant)

Pathways considered but excluded from the risk assessment.

The pathway people and their luggage/equipment in particular tourism (transport - stowaway) was considered when writing this risk assessment. However, the authors consider it is very unlikely that *D. odorata* will enter along this pathway. There is no evidence in the literature that tourism is an active pathway, and it is unlikely that the seeds will be moved by this pathway. Seeds do not have barbs or spines which can attach to clothes or recreation equipment.

The authors considered the pathway Landscape / flora / fauna improvement in the wild and concluded that this pathway did not need to be detailed as the species is generally described as growing poorly in full sunlight (Robison et al., 2011). The pathway is not considered further in the risk assessment.

Pathway name: **Horticulture (escape from confinement)**

Qu. 1.2a. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional	CONFIDENCE	low
	unintentional		medium
			high

Response: Horticulture is the intentional introduction of the species into the risk assessment area for commercial culturing. *Delairea odorata* has been utilized as a garden ornamental species in the risk assessment area since the early 1900s (Bollettino 1902).

The pathway includes the movement of plant material via e-commerce.

The Horticulture pathway should be applied to plants escaping from commercial culturing facilities (nurseries, greenhouses) (including the deliberate dumping of plant material) or during transport to/from the nursery trade. Therefore, this pathway includes the bulk shipment of live plants for nurseries and gardens centres, and the intentional introduction of seeds for planting.

García Gallo et al. (2008) detail that *D. odorata* has been intentionally introduced as an ornamental species into the Azores, Madeira and the Canary Islands, most likely from stock in Portugal.

Qu. 1.3a. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species is an ornamental species and grown in the risk assessment area. There are a number of suppliers listed throughout EU Member states.

The pathway ‘Horticulture’ is the deliberate movement of the plant into the risk assessment area for use as an ornamental species. However, it should be noted that the frequency and volume of individuals entering the risk assessment area is potentially low as much of the material sold in nurseries and garden centers is likely to be propagated within the risk assessment area.

The species is available for sale via a limited number of e-commerce sites (often under the name *Senecio mikanioides*) such as: [eBay](#) and others, e.g. https://www.arafloora.nl/p4042/senecio_mikanioides

It is moderately likely that the species will enter the natural environment via this pathway. Potentially, if planted in nurseries and garden centers, plant material may escape from confined areas into the natural environment. Additionally, dumping of material into the natural environment may occur though unlikely with good horticulture industry practice. Dumped waste, containing seeds (though not relevant for the risk assessment area) and stem pieces, has been reported as contributing to its spread in Australia (Northcote City Council & Duggan 1994).

If eradication measures are taken following dumping, there is the potential that the species can re-establish if it is dumped again or if some small shoot or root material is left behind. Plants can regenerate from a small piece of stem, stolon or rhizome as long as a node is included (DiTomaso and Healy 2004).

Qu. 1.4a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The pathway ‘Horticulture’ is the deliberate movement of plant material into the risk assessment area and as such plant material would be maintained and moved to ensure survival.

Delairea odorata is utilized as an ornamental plant in landscaping and gardening (Cal-IPC 2021a, EPPO 2012, ISSG 2021) and also as a houseplant (Robison & DiTomaso2010).

Three types of material can potentially enter the risk assessment area via this pathway (1) seed, (2) live plants and (3) cuttings (including rhizomes and stems).

Seed

Although seed import cannot be ruled out, it is very unlikely seeds of this species will be part of the horticulture pathway for the commercial production of the species.

Seed may survive along the pathway but there is no period of dormancy of the seed. It would not be possible for the species to reproduce or increase during transport and storage along this pathway. There is no detailed analysis on the viability of seed when stored or transported.

Live plants

Live plants will be moved along the pathway and packaged to promote their survival. It is unlikely the plants will reproduce or increase during their transport along this pathway. The end result of the pathway is the planting of the species in a suitable area (garden) to promote its growth.

Shoot or root material

Shoot or root material may be moved along the pathway and packaged to promote survival. There is no data on the survival of this material, but it is likely that it will survive during transportation as industry will ensure they are packaged appropriately to ensure survival. Roots are robust and if placed near the soil surface, this can increase the chances of growth after they are dumped. If plant material is placed in areas where there is disturbance, there may be a greater chance that the species can survive in these habitats. Often dumping of plant material takes place in ruderal habitats.

Qu. 1.5a. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely	CONFIDENCE	low medium
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	moderately likely likely very likely		high
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Response: Plant material (most likely live plants or cuttings) is the commodity itself and it is deliberately moved for sale within the risk assessment area. Therefore, management practices before and during transport are not considered in this question. Management practices during storage at the end point (nursery and garden centre) may affect the survival of the species for example cleaning, temperature storage etc. However, the species is likely to survive.

Qu. 1.6a. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: It is moderately likely that the organism will enter the risk assessment area undetected via this pathway as this concerns the intentional movement of plant material into the risk assessment area. USDA (2013) found no evidence that propagules are likely to be dispersed in trade as contaminants or hitchhikers.

The species could enter the environment undetected as the species may escape from garden centers and nurseries via seed or the dumping of plant material. Robison & DiTomaso (2010) highlight that the species may have been established in California from discarded houseplants. A moderate score has been given with a medium confidence due to potential dumping of the species.

Qu. 1.7a. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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Response: For live plant imports in the risk assessment area (introduction), each EU Member country has official entry points where plant material can be imported into. Therefore, these introduction points are widespread in the risk assessment area.

For the introduction of plant material via e-commerce, the entry points are widespread as anyone with an internet connection and a postal address can obtain seeds of the species from outside the risk assessment area. Therefore, introduction points are widespread within the risk assessment area.

Delaria odorata can enter the environment from nurseries and garden centers (the end point of this pathway) which are widespread within the risk assessment area. This does not mean the species is widely propagated but implies these possible points of introduction are widespread.

Qu. 1.8a. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The pathway ‘Horticulture’ involves the movement of plant material for planting from outside of the risk assessment area via nurseries and garden centres.

This pathway is active though it is unlikely that the species is being moved along the pathway from outside the risk assessment area into the risk assessment area. However, the species is sold within the risk assessment area and nurseries will propagate the species and it is moderately likely for the species to enter the natural environment from these types of facilities.

Live plant material may be imported into the risk assessment area for sale in the horticulture industry. However, it should be noted that this is probably more of a historic pathway as the species is likely to be propagated within the risk assessment area as the species can be easily grown from root fragments.

Pathway name: (2) Ornamental purposes other than horticulture (escape from confinement)

Qu. 1.2b. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	Unintentional intentional	CONFIDENCE	low medium high
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Response: This pathway involves the intentional introduction of the species for ornamental purposes, though entry into the natural environment would be unintentional. This pathway includes the movement of all potential plant parts (live plants, seed and rhizomes) into the risk assessment area. However, it should be noted that there is only evidence of live plants being moved along this pathway.

In Spain, *D. odorata* is an ornamental escape in waste places, abandoned gardens and along paths (Perez 2002).

For escape/entry into the environment, this pathway includes the dumping of plant material into the natural environment. Dumping of garden waste, containing seed, shoots or roots, has been reported as contributing to its spread in Australia (Northcote City Council & Duggan 1994).

Delairea odorata has been utilized as a garden ornamental species for a number of decades in the risk assessment area since the early 1900s (Bollettino SBI 1902, p. 141).

Qu. 1.3b. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: There is no information on frequency or volumes entering the risk assessment area or the environment.

Entry into the natural environment is likely as the species will be planted in gardens, sometimes in close proximity to the natural environment. Shoots can spread into the natural environment from planting in gardens.

If eradication measures are taken following planting, there is the potential that the species can re-establish if replanted. The number of potential areas where the species can escape from increases the difficulty of eradication at a large scale.

Qu. 1.4b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Plant material is the commodity itself and it is deliberately moved for sale within the risk assessment area. Therefore, plant parts or whole parts are likely to survive along the pathway to the end point where it is planted in a garden.

The following section lists the plant parts of concern:

Seed (low concern)

There are no reports of seed in the Risk assessment area.

Live plants (high concern)

Live plants may be dumped into the natural environment and it is likely that these can survive during transport. *D. odorata* can grow in a range of habitats from disturbed to natural areas and occurs in both dry to moist conditions. It also can tolerate a wide range of soil conditions. Robison & DiTomaso (2010) highlight that the species may have been established in California from discarded houseplants.

Shoot and root material (high concern)

Shoot material and to a lesser extent, root material may spread outside the confines of a garden where it is deliberately planted. Additionally, if garden waste is placed on a suitable substrate, it will be able to survive.

Qu. 1.5b. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Management practices before and during transport are very likely to facilitate the survival of the species as it is the commodity itself..

Management practices, i.e. removal and waste disposal may increase the propagule pressure of the species in the environment and facilitate the reproduction of the species. Therefore, management practices such as physical removal or cutting above and below ground material may lead to fragmentation of plant material.

Other management practices (e.g. chemical control of weeds in gardens), may have an impact on the survival and reproduction of the species.

Qu. 1.6b. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response. It is moderately likely the species can enter the environment via the dumping of garden waste. The score is based on the worst case scenario. A moderately likely overall score has been given based on the potential of it entering the natural environment.

Qu. 1.7b. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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Response: Private gardens associated with households are high. For example, data from 2014 shows that 58% of houses have a garden or outside space in France, 81% in the United Kingdom, 80.3% in Poland and 27% in Spain (Statista 2020).

Qu. 1.8b. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Through management practices or dumping of plant material, plant material can enter into the natural environment.

This introduction pathway is active and there is evidence that the species is being moved along the pathway into the risk assessment area. Based on this, a score of likely with medium confidence has been given.

Pathway name (3): Transport – Contaminant (transport of habitat material (soil, vegetation))

Qu. 1.2c. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: Entry via this pathway is unintentional movement of the species via the contamination of habitat material (soil and vegetation).

Qu. 1.3c. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?
including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: There is no information available on the volumes of movement along this pathway. The transport of topsoil, sand or other contaminated habitat material with shoot or root material could facilitate entry into the RA area. However, the pathway is mainly closed within the RA as there are prohibitions of the movement of soil into the EU from many countries.

Such material is likely to be placed in suitable habitats in the risk assessment area for the establishment of the species.

Delairea odorata can produce a large biomass of shoot material which may regenerate into viable plants if broken from the parent plant. Shoot material can become incorporated into soil and included in habitat material which is then transported both into the risk assessment area and within the risk assessment area.

Qu. 1.4c. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Shoot and root material can survive transport along the pathway, especially as it would be moved with habitat material that is likely to retain moisture. It is unlikely that plant material will reproduce or increase along the pathway.

Qu. 1.5c. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species is moderately likely to survive existing management practices along this pathway. However, there is no information available for this pathway.

Qu. 1.6c. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Shoots and root material can be viable at a small size and buried in soil and other material, making them difficult to detect.

Qu. 1.7c. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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Response: There are no specific data on the points of introduction or entry.

Points of introduction can include road routes which join countries and other international transportation hubs. These are widespread throughout the risk assessment area. Urbanization and the management of land is increasing through the risk assessment area.

Qu. 1.8c. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Root and shoot material may be able to move along this pathway, and the commodity is placed within the environment where the propagules have the opportunity to establish. However, there is no direct evidence that the species has moved along this pathway and therefore it is scored moderately likely with medium confidence.

Qu. 1.9. Estimate the overall likelihood of introduction into the risk assessment area or entry into the environment based on all pathways and specify if different in relevant biogeographical regions in current conditions.

Provide a thorough assessment of the risk of introduction in relevant biogeographical regions in current conditions: providing insight in to the risk of introduction into the risk assessment area.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The overall likelihood of *D. odorata* being introduced into the risk assessment area is very likely with a high confidence especially because the species is already present (recorded or established) in the risk assessment area, hence the likelihood of introduction and entry should be scored as “very likely” by default. The species has been known to be introduced into the risk assessment area by ‘Horticulture’ (escape from confinement), and ‘Ornamental purpose other than horticulture’ (e.g. planting by private gardeners). *Delairea odorata* has the potential of being dumped in the environment as the species produces a lot of above ground biomass which can be difficult to dispose of in the proper means.

As the highest occurrences of the species in the environment are in the Atlantic and Mediterranean biogeographical regions, it is these regions that would potentially see a higher likelihood of entry into the environment. The species distribution modelling shows that the Alpine, Boreal, Pannonian and Steppic biogeographical regions are currently largely unsuitable for the establishment of the species (compare SDM in Annex IX) and therefore the likelihood of the species traded for horticultural purposes in these regions is low.

Qu. 1.10. Estimate the overall likelihood of introduction into the risk assessment area or entry into the environment based on all pathways in foreseeable climate change conditions?

Thorough assessment of the risk of introduction in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of introduction (e.g. change in trade or user preferences)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely introduction within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6 °C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0 °C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The main aspects of climatic change which will influence the entry of *D. odorata* into the risk assessment area include increased minimum temperature of winter months, increased spring and summer temperatures.

Climate change (RCP 2.6 2070) will expand the potential distribution the species can establish in, making the species more available to gardeners. For example, more inland areas in the Atlantic and Continental biogeographical regions may become more suitable for growing the species and the horticulture pathway may expand in this area to meet further demand. Ornamental plants traded for private gardens and landscape improvements may also see a higher volume and frequency. A high rating has been given with a medium confidence (due to the fact that this is a projection).

2 PROBABILITY OF ESTABLISHMENT

Important instructions:

- For organisms which are already established in parts of the risk assessment area or have previously been eradicated, the likelihood of establishment should be scored as “very likely” by default.
- Discuss the risk also for those parts of the risk assessment area, where the species is not yet established.

Qu. 2.1. How likely is it that the organism will be able to establish in the risk assessment area based on similarity of climatic and abiotic conditions in its distribution elsewhere in the world?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Delairea odorata* is already established in the natural environment in the risk assessment area (Croatia, France, Ireland, Italy, Portugal, Spain, United Kingdom) (see Qu. A8b). It is likely that further countries where the species could potentially establish are present within the RA area (see Qu. A9a).

The SDM (Annex VIII) shows the potential distribution of the species based on the current occurrences of the species both in the risk assessment area and elsewhere in the world. The model shows that there is a large area of the EU that is suitable for the establishment of *D. odorata*.

In the native and introduced ranges (outside of the EU), *D. odorata* occurs predominantly in the Köppen-Geiger climate classifications of Cfb (Temperate [oceanic climate](#)), Csb ([Warm-summer Mediterranean climate](#)), Csa ([Hot-summer Mediterranean climate](#)) and BSk ([Cold semi-arid climate](#)). MacLeod and Korycinska (2019) estimate that the percentage of 5-minute grid cells for each aforementioned climate category occurring in the EU are as follows: Cfb 48.62 %, Csb 3.8% Csa 10.13 and BSk 1.46. Based on this almost 60% of the EU plus the United Kingdom is currently climatically suitable.

Qu. 2.2. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area? Consider if the organism specifically requires another species to complete its life cycle.

RESPONSE	very isolated isolated	CONFIDENCE	low medium
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	moderately widespread widespread ubiquitous		high
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Response: *Delairea odorata* can invade different habitats ranging from disturbed to natural and occurs in both dry to moist conditions. It can form dense vegetative ground cover mats that can prevent seeding of native plants.

In South Africa, *D. odorata* grows in moist mountain forests. Where it naturalized, it extended its habitat range and is found in coastal areas, riparian zones and wetlands, dry forests and moist forests, shrublands and ruderal habitats, and grows very well in early successional forests. Typically found below 200 m elevation, it prefers shady, disturbed sites with year-round moisture.

According to the CORINE Land Cover nomenclature, these habitats correspond to: natural grassland, mixed forests, broad-leaved forests, sclerophyllous vegetation (e.g. garrigue, maquis), inland wetlands (marshes, EPPO, 2012 peat bogs), continental waters (water courses, water bodies), banks of continental water, riverbanks/canal sides (dry river beds), road and rail networks and associated land, and other artificial surfaces (wastelands).

CABI (2021) lists potential habitats as managed forests, plantations and orchards, managed grasslands, disturbed areas, rail/roadsides, urban/per-urban areas, natural forests, natural grasslands, riverbanks, wetlands and coastal areas.

García Gallo et al. (2008) detail habitats for *D. odorata* in Azores, Madeira and the Canary Islands to be: Waste places, hedgerows, walls, ravines, *Pittosporum* and *Morella* scrubland, Cliffs, heath substituting *Apollonias* laurel forest, heath substituting *Ocotea* laurel forest, *Apollonias* laurel forest (Mediterranean laurel forest), *Ocotea* laurel forest (temperate laurel forest), riparian laurel forest (*Sambucus* woodland, *Persea* laurel forest, *Salix* woodland), cultivated land, anthropogenic vegetation, mountain humid woodlands and urban areas.

According to Iamónico (2017), *D. odorata* is recorded in Sardinia (Italy) in ruderal habitats only. According to Viciani et al. (2020), *D. odorata* communities can be included in the vegetation class *Artemisietea vulgaris* Lohmeyer, Preising & Tüxen ex Von Rochow 1951.

When taking into consideration all of the habitats detailed above, within the EU, habitats that can facilitate the survival, development and multiplication of the *D. odorata* are widespread. However, it should be noted that many of the areas where the species is currently clustered in the risk assessment area are close to the coast and/or at lower altitudes.

Delairea odorata is commonly found in high pH soils characterized by high fertility (Baars et al. 1998) and the species has some tolerance to salt as in the US it has been known to establish on salt exposed bluffs (Starr et al. 2003)

Qu. 2.3. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Competition with native vegetation is unlikely to limit the establishment of *D. odorata* in the risk assessment area. Although there is no scientific information on competition with native species, *D. odorata* can form dense ground cover which can prevent the formation of native vegetation. It can use ground cover vegetation as a support to grow up into the canopy and climb over other species.

Competition may occur with native lianas in managed forests in the risk assessment area; other liana species in Europe, with the exception of *Hedera helix* and *Clematis vitalba*, are limited, but a higher diversity of lianas is present in Mediterranean shrublands and forests.

Qu. 2.4. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: CABI (2021) detail a number of biological control agents including two insect species, the Cape ivy gall fly (*Parafreutreta regalis* Munro) and the Cape ivy stem moth (*Digitivalva delaireae* Gaedike & Kruger). However, these species are not present in the EU. It is very likely that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area. *D. odorata* does not have any host specific natural enemies in the risk assessment area and there is no evidence that native organisms feed or infect the species in substantial levels to inflict any damage on the species.

Qu. 2.5. How likely is the organism to establish despite existing management practices in the risk assessment area? Explain if existing management practices could facilitate establishment.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: As detailed in section (2.2), the establishment of *D. odorata* is suited to a number of different habitat types, including disturbed habitats. Management of urban and semi-urban habitats is likely to increase disturbance of the habitat, which can act to break the shoots of the plant and resprouting of these parts can occur if they have contact with the ground (Starr et al. 2003).

Qu. 2.6. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: In the risk assessment area, vegetative reproduction is considered the main, if not the only reproductive mechanism. Vegetative reproduction can occur at any time when the nodes of the stem, stolon, or leaf petiole are in contact with the soil. Small fragments of 1.3 cm can root easily and quickly. Seed production has been observed in the risk assessment area (for example, pers. obs. E. Marchante, Portugal), but scientific studies are lacking to confirm seed viability. These biological properties do not allow survival from eradication campaigns, but the fact that small pieces of shoot and root material can form viable plants increases the likelihood. In addition, the stems are brittle and easily broken, thus this can further complicate eradication. *D. odorata* uses other plants to support its growth and can climb over structures.

Therefore, unless eradication campaigns are thorough and remove all reproductive material, it is likely that the species can survive during eradication campaigns. A medium confidence is given as there is no published information for the risk assessment area on eradication campaigns.

Qu. 2.7. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?

including the following elements:

- a list and description of the reproduction mechanisms of the species in relation to the environmental conditions in the risk assessment area
- an indication of the propagule pressure of the species (e.g. number of gametes, seeds, eggs or propagules, number of reproductive cycles per year) of each of those reproduction mechanisms in relation to the environmental conditions in the risk assessment area.
- If relevant, comment on the likelihood of establishment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in establishment whereas for others high propagule pressure (many thousands of individuals) may not.
- If relevant, comment on the adaptability of the organism to facilitate its establishment and if low genetic diversity in the founder population would have an influence on establishment.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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In the risk assessment area, vegetative reproduction is considered the main, if not the only reproductive mechanism. Vegetative reproduction can occur at any time when the nodes of the stem, stolon, or leaf petiole are in contact with the soil. Small fragmented pieces of 1.3 cm can root easily and quickly. Parts of the plant can also disperse along water courses. Nelson (1999) reports that pieces of *D. odorata* are transported via flooding along the coast of Big Sur river in California.

The growth habit of *D. odorata* can facilitate its establishment, the species climbs over most other vegetation, forming a solid cover that blocks light and smothers other vegetation.

Qu. 2.8. If the organism does not establish, then how likely is it that casual populations will continue to occur?

Consider, for example, a species which cannot reproduce in the risk assessment area, because of unsuitable climatic conditions or host plants, but is present because of recurring introduction, entry and release events. This may also apply for long-living organisms.

RESPONSE	very unlikely unlikely moderately likely likely	CONFIDENCE	low medium high
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	very likely		
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Response: In areas, where *D. odorata* is not present, casual populations may occur in urban habitats or semi-urban habitats. Casual populations may occur in surrounding habitats. Casual populations are likely to occur in areas outside the optimum climatic conditions. For example, this may include areas in Figure 5 of Appendix 4 at the margin of suitable and unsuitable areas. Countries where casual populations may occur include those in central and eastern Europe (see Qu. A8a).

Qu. 2.9. Estimate the overall likelihood of establishment in the risk assessment area under current climatic conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under current climatic conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in current conditions: providing insight in the risk of establishment in (new areas in) the risk assessment area.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The overall likelihood of *D. odorata* establishing in the risk assessment area is very likely with high confidence. The species is already established in the Mediterranean and Atlantic biogeographical regions. Countries where the species is established include: Croatia, France, Ireland, Italy, Portugal, Spain, and the United Kingdom. The Mediterranean and Atlantic biogeographical regions have a high proportion of area suitable for the establishment of the species and thus further establishment is likely in these areas under the current climatic conditions.

Qu. 2.10. Estimate the overall likelihood of establishment in the risk assessment area under foreseeable climate change conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under foreseeable climate change conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of establishment (e.g. increase in average winter temperature, increase in drought periods)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely establishment within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The overall likelihood of establishing in the risk assessment area under future climatic conditions (2070 RCP. 2.6) is very likely with medium confidence. The species is already established in the Mediterranean, Continental and Atlantic biogeographical regions. The Continental and Atlantic biogeographical regions see a slight increase in establishment potential with future climate change whereas the Mediterranean region shows a slight decrease. The Pannonian region shows a steep increase in suitability. Southern areas of the Boreal biogeographical region show potential for establishment and small coastal areas of Norway (Alpine biogeographical region) under future climatic conditions (see Annex IX).

3 PROBABILITY OF SPREAD

Important instructions:

- Spread is defined as the expansion of the geographical distribution of an alien species within the risk assessment area.
- Repeated releases at separate locations do not represent continuous spread and should be considered in the probability of introduction and entry section (Qu. 1.7).

Qu. 3.1. How important is the expected spread of this organism within the risk assessment area by natural means? (List and comment on each of the mechanisms for natural spread.)

including the following elements:

- a list and description of the natural spread mechanisms of the species in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of spread discussed in relation to the species biology and the environmental conditions in the risk assessment area.

The description of spread patterns here refers to the CBD pathway category “Unaided (Natural Spread)”. It should include elements of the species life history and behavioural traits able to explain its ability to spread, including: reproduction or growth strategy, dispersal capacity, longevity, dietary requirements, environmental and climatic requirements, specialist or generalist characteristics.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: In the USA, *D. odorata* reproduces vegetatively from stem fragments that readily root at nodes (Robison 2006) and by the extension of the plant through stolons (Alvarez 1998). There is no scientific information on the spread rates of this species for the risk assessment area.

In California, *D. odorata* has readily established in riparian areas and then subsequently spread into drier sites (CABI 2013). One California infestation expanded from 9 acres (3.6 ha) to 67 acres (27.1 ha) in nine years (Alvarez 1998). Alvarez & Cushman (2002) stated that rapid growth (0.35 to 1.35 m per month), clonal reproduction, and pronounced tolerance to environmental conditions have contributed to its invasiveness.

Vegetative reproduction can occur at any time when the nodes of the stem, stolon, or leaf petiole are in contact with the soil. Small fragmented pieces of 1.3 cm can root easily and quickly. Parts of the plant can also disperse along water courses. Nelson (1999) reports that pieces of *D. odorata* are transported via flooding along the coast of Big Sur river in California.

Qu. 3.2a. List and describe relevant pathways of spread other than "unaided". For each pathway answer questions 3.3 to 3.9 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 3.3a, 3.4a, etc. and then 3.3b, 3.4b etc. for the next pathway.

including the following elements:

- a list and description of pathways of spread with an indication of their importance and associated risks (e.g. the likelihood of spread in the risk assessment area, based on these pathways; likelihood of survival, or reproduction, or increase during transport and storage; ability and likelihood of transfer from the pathway to a suitable habitat or host) in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of spread for each pathway discussed in relation to the species biology and the environmental conditions in the risk assessment area.
- All relevant pathways of spread (except "Unaided (Natural Spread)", which is assessed in Qu. 3.1) should be considered. The classification of pathways developed by the Convention of Biological Diversity shall be used (see Annex IV).

Starr et al. (2013) state: 'Long distance dispersal is currently mostly by humans spreading the plant in landscaping.

Spread pathways are detailed in order of importance

Ornamental purposes other than horticulture (escape from confinement)

This spread pathway is the intentional spread of the species from private gardening and urban and semi-urban plantings (roadsides and roundabouts for example). This pathway would be the most relevant for spread and include the highest movement of plant propagules.

Transport – Contaminant (transport of habitat material (soil, vegetation))

This spread pathway deals with the potential spread of contaminated habitat material. There is no quantitative evidence/ interceptions to support the movement of the species along this spread pathway but where urbanization has occurred in the Mediterranean region the species has shown significant spread.

Transport – stowaway: Machinery/ equipment

This pathway details the potential spread of contaminated machinery and equipment. There is no quantitative evidence/ interceptions to support the movement of the species along this spread pathway but the potential for the pathway is highlighted in the USA (Alvarez 1997; USDA 2013).

Pathway name: (1) Ornamental purposes other than horticulture (escape from confinement)

Qu. 3.3a. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	Unintentional intentional	CONFIDENCE	low medium high
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Response: *Delairea odorata* is present in the risk assessment area as an ornamental species. Additionally, the species may be planted in urban and semi-urban habitats (for example on roundabouts). In Spain, *D. odorata* is an ornamental escape in waste places, abandoned gardens and along paths (Perez 2002).

Qu. 3.4a. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: There is no quantitative information on the number of sales of the species in the risk assessment area or the numbers of sites where the species has been planted. Sales of the species within the risk assessment area will be mainly whole plants as seeds have variable viability in non-optimal conditions. However, plants that can be very easily vegetatively propagated and are sometimes not so interesting for professional breeders and garden centers, as everyone can very easily propagate the species (by ramets) and plant it in his/her garden, without buying it and paying for it.

Plants can be purchased from garden centers within the risk assessment area and moved to gardens where they are planted. However, *D. odorata* is not a popular ornamental species. The species may also be dumped from waste garden material into the natural environment, though again, there are no details and the volume of movement or frequency.

Qu. 3.5a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: It is very likely that *D. odorata* plant material will survive during transport and storage along this pathway.

Live plants will be moved along the pathway and packaged to promote their survival. It is unlikely the plants will reproduce or increase during their transport along this pathway.

Qu. 3.6a. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Delairea odorata* is likely to survive existing management practises during spread. Management of urban and semi-urban habitats is likely to increase disturbance of the habitat, which can act to break the shoots of the plant and resprouting of these parts can occur if they have contact with the ground (Starr et al. 2003).

Although there is no data on the effect of urbanisation on the spread of the species, small fragments of the plant may be moved which can facilitate spread. If dumped, the species is likely to be deposited in ruderal habitats and potentially on waste ground where management practices may be minimal and the native vegetation sparse, this may make the survival of the species in these areas more likely.

Qu. 3.7a. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Small root fragments (e.g. 2-3 cm in length with a node) can produce a viable plant. These fragments may be spread via dumping of garden waste and therefore may be spread in the risk assessment area undetected.

Qu. 3.8a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species has been shown to spread to ruderal habitats and natural habitats. The presence of the species in urban and semi-urban habitats in the risk assessment area, including waste/abandoned lands highlights the species spread. In Spain, *D. odorata* is an ornamental escape in waste places, abandoned gardens and along paths (Perez 2002).

Qu. 3.9a. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area. (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: *Delairea odorata* is a garden ornamental species which is sold in the risk assessment area. It has also been planted in urban and semi-urban habitats. There is no quantitative analysis on the spread of the species in the risk assessment area.

Pathway name: (2) Transport – Contaminant (transport of habitat material (soil, vegetation))

Qu. 3.3b. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: The spread of *D. odorata* via transport of habitat material (soil, vegetation) is the unintentional spread of the species within the risk assessment area. Both seed material and plant fragments can be spread via this pathway, though seed is unlikely to be viable in the risk assessment area.

Qu. 3.4b. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: There is no information available on the volumes of movement along this pathway. Shoot and root material can be present on/in the soil, in potentially large amounts if such material is taken from an area where the species is present.

Qu. 3.5b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: There is no quantitative evidence for *D. odorata*, for the survival, reproduction or increase as a contaminant of habitat material. Viable shoot and root material can be small and therefore it could

survive during transport along this spread pathway. Habitat material would be suitable for the survival of the species over short periods. Shoot and root material can remain viable within soil material.

Qu. 3.6b. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Careful methodical management practices coupled with inspection would be needed to ensure that the species did not spread with contaminated soil and vegetation. This is often not feasible with such small shoot or root fragments.

Qu. 3.7b. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Soil and other habitat material can be moved throughout the RA area and can be spread within such material. Shoot and root material may be small and buried in soil and other material, making them difficult to detect.

Qu. 3.8b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: It would be very likely that *D. odorata* can transfer to a suitable habitat if shoot and root material of the species is incorporated in soil. Topsoil and habitat material is often physically transferred and deposited in suitable habitats. This fact that the species is recorded in urban development areas further supports the hypothesis that the species can be moved by soil and habitat material.

Qu. 3.9b. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area. (provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: There is no quantitative data on the spread of the species in relation to habitat material.

Habitat material can move freely within the single market in the EU enabling contaminated material to spread over long distances. Shoot or root material may be included as a contaminant. Because of the lack of quantitative data, confidence is medium.

Pathway name: (3) Transport – stowaway: Machinery / equipment

Qu. 3.3c. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: This pathway details the potential spread of the species with contaminated machinery and equipment. There is no quantitative evidence / interceptions to support the movement of the species along this spread pathway but the potential for the pathway is highlighted in the USA (Alvarez 1997; USDA 2013).

Qu. 3.4c. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: There is no information available on the volumes of movement along this pathway. However, any mechanisms involving equipment and vehicles could act to break shoot or root material into smaller fragments and therefore the number of viable stem or root pieces can be high. Only small shoot/root pieces are needed to form a viable plant.

Qu. 3.5c. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Root and shoot material could survive during transport along this spread pathway. The species is unlikely to increase along the pathway until it finds a suitable habitat.

Qu. 3.6c. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Root and shoot material can also remain hidden within crevices of machinery and equipment. ISPM 41 (IPPC 2017) 'Intentional movement of machinery and equipment' provides international measures for cleaning, which can be applied to local level movement also.

Qu. 3.7c. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Shoot and root fragments are relatively small, are easily broken from the main stem or root and potentially can remain hidden in soil in cracks and crevices in machinery and equipment. Such machinery can be moved within the RA area transporting the seed to new areas.

Qu. 3.8c. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: It would be moderately likely that *D. odorata* can transfer to a suitable habitat if the species is a stowaway on machinery/ equipment. Landscaping machinery and construction machinery is used in suitable habitats of the species.

Qu. 3.9c. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area. (provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: Used machinery and equipment can be moved within the RA area at a local and long distances scale. Any seed and root/shoot material attached could be spread at a fast rate. However, it is scored moderately as a number of measures can be adopted against this spread pathway to reduce the spread of the species. Inspections of used machinery and equipment coupled with cleaning will reduce the potential of spread of the species. A low confidence has been given as there is no quantitative data.

Qu. 3.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?

RESPONSE	very easy easy with some difficulty difficult very difficult	CONFIDENCE	low medium high
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Response: Natural spread would be relatively easy to control within the risk assessment area. Though some habitats where the species grows may present more difficulties - e.g. coastal habitats.

As the species can spread as a contaminant of soil and habitat material, machinery and equipment and recreational equipment, biosecurity measures and inspections would need to be adopted where these spread pathways are active, and the species is present. This would involve multiple stakeholders and communication and awareness raising to reduce the spread pathways. A ban on sale could act to prevent further spread of the species within the risk assessment area.

Preventing the spread from already established populations, including those in private gardens will be difficult.

Qu. 3.11. Estimate the overall potential rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (indicate any key issues and provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in current conditions, providing insight in the risk of spread into (new areas in) the risk assessment area.

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: In the risk assessment area, *D. odorata* reproduces and spreads by vegetative reproduction. Therefore, spread by natural means can be assessed as moderately.

Human mediated spread includes intentional movement of the species through horticulture and unintentional movement through contamination of habitat material and used machinery and equipment). For human mediated spread, the rate of spread can be estimated as moderately. Spread would be highest in biogeographical regions where the species is already present, such as Alpine, Atlantic, Boreal, Continental and Mediterranean biogeographical regions. A higher rate of spread may be seen in biogeographical regions where *D. odorata* can be considered as established, e.g. Atlantic, Continental and Mediterranean biogeographical regions.

Qu. 3.12. Estimate the overall potential rate of spread in relevant biogeographical regions in foreseeable climate change conditions (provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if rates of spread are likely slowed down or accelerated.

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: The overall potential rate of spread in the risk assessment area under future climatic conditions (2070 RCP. 2.6) is moderately with a low confidence. Spread would maintain in the areas where the species is already established: Mediterranean, Continental and Atlantic biogeographical regions. The Continental and Atlantic biogeographical regions see a slight increase in establishment potential with future climate change whereas the Mediterranean region shows a slight decrease. The Pannonian region shows a steep increase in suitability. Southern areas of the Boreal biogeographical region show potential for establishment and small coastal areas of Norway (Alpine biogeographical

region) under future climatic conditions (see Annex VIII). All this might also increase the likelihood of human-mediated spread in these regions.

4 MAGNITUDE OF IMPACT

Important instructions:

- Questions 4.1-4.5 relate to biodiversity and ecosystem impacts, 4.6-4.8 to impacts on ecosystem services, 4.9-4.13 to economic impact, 4.14-4.15 to social and human health impact, and 4.16-4.18 to other impacts. These impacts can be interlinked, for example, a disease may cause impacts on biodiversity and/or ecosystem functioning that leads to impacts on ecosystem services and finally economic impacts. In such cases the assessor should try to note the different impacts where most appropriate, cross-referencing between questions when needed.
- Each set of questions starts with the impact elsewhere in the world, then considers impacts in the risk assessment area (=EU excluding outermost regions) separating known impacts to date (i.e. past and current impacts) from potential future impacts (including foreseeable climate change).
- Only negative impacts are considered in this section (socio-economic benefits are considered in Qu. A.7)
- In absence of specific studies or other direct evidence this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable).

Biodiversity and ecosystem impacts

Qu. 4.1. How important is the impact of the organism on biodiversity at all levels of organisation caused by the organism in its non-native range excluding the risk assessment area?

including the following elements:

- Biodiversity means the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems
- impacted chemical, physical or structural characteristics and functioning of ecosystems

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: *Delawarea odorata* has caused considerable biodiversity and ecosystem impacts in its invasive range particularly on the west coast of the USA. The California Invasive Plant Council (Cal-IPC) lists Cape ivy on its High List as a "Species with severe ecological impacts on ecosystems, plant and animal communities, and vegetational structure" (Cal-IPC 2005). Its impacts are broad and Brunel et al. (2010) summed it up as follows: “*D. odorata* can invade different habitats ranging from disturbed to natural and occurs in both dry to moist conditions. It can form dense vegetative ground-cover mats that can prevent seeding of native plants. It can also smother native vegetation and affect regeneration, forming

stands of over 75% cover and competing with other plants for water and nutrients. Native plant species richness can be reduced by about 50 percent, with greater impact on annual than on woody perennial species. The weight of large masses of climbing vines can bring down trees. Higher trophic levels can also be affected, such as several sensitive species of insects and predators.”

In the Golden Gate National Recreation Area, California, USA, Alvarez (1997) found that native plant species richness was significantly lower with increasing cover of Cape ivy, but this was not the case for non-native species. In addition, invasion by Cape ivy was associated with a 31% decrease in species diversity as well as an 88% decrease in the abundance of native seedlings and a 92% decrease in non-native seedlings compared to uninvaded areas. This impact was found to be somewhat reversible in that a 2 yr Cape-ivy reduction experiment resulted in a 10% increase in the richness of native species compared to control plots, but also a 43% increase in the richness of nonnative taxa. Forb species richness increased significantly when Cape-ivy cover was reduced, whereas shrub richness decreased slightly and no effects were detected for ferns and grasses. They also found that Cape-ivy reduction led to a 32% increase in plant species diversity, and importantly an 86% increase in the abundance of native seedlings, and an 85% increase for nonnative seedlings. Lab studies also show the production of biotoxic and bioactive compounds which can inhibit seed germination and root growth as well as having significant impacts on copepod populations (Kozsiz 2013).

According to the US Fish and Wildlife Service (2009a; 2009b) *D. odorata* contributes to the threat posed to two threatened and endangered plant species namely *Thysanocarpus conchuliferus* and *Zanthoxylum dipetalum* var. *tomentosum*

Delairea odorata has been shown to change soil mycobiota to its benefit by increasing functional diversity (Oclaman & Case 2017). There is also some preliminary evidence that there is less microbial metabolic diversity in native coastal scrub soil than in soil under *D. odorata* (Khalil & Case 2013).

Though not a direct impact on species at risk, Robison & DiTomaso (2010) observed that in California three animals vulnerable to Cape Ivy invasion and five vulnerable plants were expected to have 40% reduction of their occurrences within a 500 m buffer to Cape ivy infestations.

Given the wide range of impacts from pulling down trees and shrubs to competing for space and water as well as altering the soil to its benefit across quite a large area, the impact is scored as major with medium confidence.

Qu. 4.2. How important is the current known impact of the organism on biodiversity at all levels of organisation (e.g. decline in native species, changes in native species communities, hybridisation) in the risk assessment area (include any past impact in your response)?

Discuss impacts that are currently occurring or are likely occurring or have occurred in the past in the risk assessment area. Where there is no direct evidence of impact in the risk assessment area (for example no studies have been conducted), evidence from outside of the risk assessment area can be used to infer impacts within the risk assessment area.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: *Delairea odorata* is reported as established in Tuscany and in Sardinia (Italy) (Iamónico 2017), where it occurs in ruderal habitats only, and in few localities with low density, so that at the time being no significant ecological impacts have been recorded (Bacchetta et al. 2009, Camarda et al. 2016). However, in Portugal, Cape Ivy frequently invades banks of watercourses, hedgerows and slopes, ruderal environments and communities of thorny shrubs (Plantas invasoras em Portugal 2020). There is a lack of published information so the score is minor with a low confidence.

Qu. 4.3. How important is the potential future impact of the organism on biodiversity at all levels of organisation likely to be in the risk assessment area?

See comment above. The potential future impact shall be assessed only for the risk assessment area. A potential increase in the distribution range due to climate change does not *per se* justify a higher impact score.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: If its behaviour in other invaded regions is replicated then the impacts on biodiversity could be major but without any evidence of impact at this stage it is hard to place a high potential impact estimate in the medium term. It is expected that the impacts will increase so a moderate score with low confidence is given.

Qu. 4.4. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism currently in the risk assessment area?

including the following elements:

- native species impacted, including red list species, endemic species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats

- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: *D. odorata* is certainly found in areas with high conservation value and there is good evidence of impact in Macaronesian islands which is not included in the risk assessment area. Its recorded presence is likely to devalue status and require management as was the case in the Natura 200 site Cabo Busto-Luanco; where removal of the species was carried out under the LIFE+ project [ARCOS \(2021\)](#).

By overlaying Natura 2000 sites with human observations (GBIF coordinate accuracy <250m), 18 Natura 2000 sites are recorded namely

FR2601012 Gîtes et habitats à chauves-souris en Bourgogne;

ES7020073 Los Acantilados de la Culata;

ES0000143 Marismas de Santoña, Victoria y Joyel y Ría de Ajo;

ES1300007 Marismas de Santoña, Victoria y Joyel; confirmed in Laiseca (2017)

PTCON0061 Ria de Aveiro;

PTZPE0004 Ria de Aveiro;

PTCON0055 Dunas de Mira, Gândara e Gafanhas;

PTZPE0011 Estuário do Sado;

PTCON0011 Estuário do Sado;

ES0000106 Teno;

ES7020096 Teno;

ES1200009 Ponga-Amieva;

ES1200016 Ría del Eo;

ES1200055 Cabo Busto-Luanco;

ES0000318 Cabo Busto-Luanco; where removal of the species was carried out under the LIFE+ project [ARCOS](#)

PTCON0008 Sintra / Cascais;

PTCON0017 Litoral Norte

D. odorata is also recorded as present in the Urdaibai Biosphere Reserve of Biscay, Basque Country, Spain (world species reserve [dataset](#)) [accessed 28.04.2021]

There is some inferred evidence of impact in the risk assessment area and given the multiple sites harbouring the plant and evidence of control measures implemented in at least one Natura 2000 site a score of moderate is given but with a low confidence due to the lack of peer reviewed evidence.

Qu. 4.5. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.3. and 4.4.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: as above the score is kept the same as it is unlikely that major impacts would occur in the time frame considered but confidence remains low.

Ecosystem Services impacts

Qu. 4.6. How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?

- For a list of services use the CICES classification V5.1 provided in Annex V.
- Impacts on ecosystem services build on the observed impacts on biodiversity (habitat, species, genetic, functional) but focus exclusively on reflecting these changes in relation to their links with socio-economic well-being.
- Quantitative data should be provided whenever available and references duly reported.

RESPONSE	minimal minor moderate major	CONFIDENCE	low medium high
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	massive		
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Response: Various ecosystem service impacts are associated with *D. odorata* invasions outside of the risk assessment area and much evidence comes from the USA.

Regulation & Maintenance - Regulation of physical, chemical, biological conditions - Baseline flows and extreme event regulation: In riparian communities, *D. odorata* can increase soil erosion along watercourses due to its shallow root system not capable of holding soil (Brunel et al 2010). It can also contribute to soil erosion on hillsides (Bossard et al. 2000).

Regulation & Maintenance - Regulation of physical, chemical, biological conditions - Water conditions: *Delairea odorata* contains pyrrolizidine alkaloids (liver toxins) and xanthone (Catalano et al, 1996) and can be toxic to animals when ingested; fish can be killed when plant materials are soaking in waterways. It is possible that chemicals could enter the food chain through honey and milk derivatives in the same way as is reported with *Senecio* spp. (see Gottschalk et al, 2020; de Nijs et al, 2017) but no evidence was found of this for *D. odorata*. Flood control function along streams is impacted by *D. odorata* (Bossard et al. 2000).

Regulation & Maintenance - Regulation of physical, chemical, biological conditions - Soil quality regulation: Biotoxicity and bioactive compounds shown to inhibit seed germination and root growth using EPA bioassays as well as having significant impacts on copepod populations (Kozsiz 2013).

Cultural - Direct, in-situ and outdoor interactions with living systems that depend on presence in the environmental setting - natural environment: Can significantly reduce the species richness and diversity of an area, and render habitat in protected reserves for plant species worthless (Bossard et al. 2000). *Delairea odorata* dominates plant communities altering vegetation structure and topples oaks (Knapp 2010). The weight of the ivy can cause trees to fall, and dense canopy of the vine can smother species of the lower strata and reduce growth (Bossard et al. 2000).

Qu. 4.7. How important is the impact of the organism on provisioning, regulating, and cultural services currently in the different biogeographic regions or marine sub-regions where the species has established in the risk assessment area (include any past impact in your response)?

- See guidance to Qu. 4.6.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: The presence of *D. odorata* in vulnerable and/or protected sites and areas of eco-tourism interest across the risk assessment area, coupled with its known behaviour allows us to conclude that it

is likely to be having an effect on multiple ecosystem services but with no direct evidence of ecosystem service impacts in the RA area, a minor score is given with low confidence.

Qu. 4.8. How important is the impact of the organism on provisioning, regulating, and cultural services likely to be in the different biogeographic regions or marine sub-regions where the species can establish in the risk assessment area in the future?

- See guidance to Qu. 4.6.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No information has been found on the issue but as above an expert judgment suggests that a minor score with low confidence would remain in the timescale considered.

Economic impacts

Qu. 4.9. How great is the overall economic cost caused by the organism within its current area of distribution (excluding the risk assessment area), including both costs of / loss due to damage and the cost of current management.

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the world these should be reported here. The assessment of the potential costs of / loss due to damage shall describe those costs quantitatively and/or qualitatively depending on what information is available. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage. As far as possible, it would be useful to separate costs of / loss due to the organism from costs of current management.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: There are references to economic impacts such as reducing forage quality when present in pastures (Weber 2003; GISD 2007) and cattle poisoning in New Zealand (Verdcourt & Trump 1969). However, there are no estimates of financial cost.

In the case of management costs there is reference to Golden Gate National Recreation Area, California, USA which spent over US\$600,000 over three years trying to eradicate this vine (CABI 2021a) This figure justifies the moderate score but it is quite probable that higher costs are incurred by other land owners.

Qu. 4.10. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism currently in the risk assessment area (include any past costs in your response)?

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the EU these should be reported here. Assessment of the potential costs of damage on human health, safety, and the economy, including the cost of non-action. A full economic assessment at EU scale might not be possible, but qualitative data or different case studies from across the EU (or third countries if relevant) may provide useful information to inform decision making. In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable). Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	N/A minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No information has been found on the issue. The question has not been scored.

Qu. 4.11. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.10.

RESPONSE	N/A minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No information has been found on the issue. The question has not been scored.

Qu. 4.12. How great are the economic costs / losses associated with managing this organism currently in the risk assessment area (include any past costs in your response)?

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable).

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: The control measures referred to in the ARCOS Life+ project (<https://www.arcoslife.eu/arcosweb/>) are not costed in the report and the control was also applied to species other than *D. odorata* so it is not possible to estimate costs beyond minimal with low confidence because multiple small scale management efforts would soon exceed the threshold for a minor or even moderate score. Other examples include:

<https://www.gavarres.cat/uploads/imagenes/22-url-1575553537.pdf>

<http://www.comunidadism.es/wp-content/uploads/downloads/2012/07/gestion-de-la-biodiversidad-desde-el-ambito-local.pdf>

https://www.miteco.gob.es/es/ceneam/grupos-de-trabajo-y-seminarios/red-parques-nacionales/Plan%20de%20control%20y%20eliminaci%C3%B3n%20de%20especies%20vegetales%20invasoras%20dunas_tcm30-169318.pdf

Qu. 4.13. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.12.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: In the absence of evidence for current annual costs associated with the management of the species it is difficult to estimate future costs but it is highly likely that across the risk assessment area the costs would exceed 10,000 euros per year in the near future if not already, so a minor score is given with a low confidence.

Social and human health impacts

Qu. 4.14. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism for the risk assessment area and for third countries, if relevant (e.g. with similar eco-climatic conditions).

The description of the known impact and the assessment of potential future impact on human health, safety and the economy, shall, if relevant, include information on

- illnesses, allergies or other affections to humans that may derive directly or indirectly from a species;
- damages provoked directly or indirectly by a species with consequences for the safety of people, property or infrastructure;
- direct or indirect disruption of, or other consequences for, an economic or social activity due to the presence of a species.

Social and human health impacts can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: *Delairea odorata* contains pyrrolizidine alkaloids (liver toxins) and xanthone (Catalano et al, 1996) and can be toxic to animals when ingested (Bossard 2000). Balciunas (2004) reports that *D. odorata* is considered to be poisonous to pets that consume it with the Dog's Trust citing gastrointestinal tract and dermatitis as potential areas of concern (<https://www.dogstrust.org.uk/help-advice/factsheets-downloads/factsheetpoisonoussubstances09.pdf>) No evidence of such impacts were found in the risk assessment area and it is assumed that the impacts are potential rather than common. The fact that park managers in Western USA spend their limited resources managing the plant suggests that the visitor experience would be reduced if such management were not carried out but this is difficult to quantify.

Qu. 4.15. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism in the future for the risk assessment area.

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable).

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: It is not anticipated that any significant change in the importance is caused in the future.

Other impacts

Qu. 4.16. How important is the organism in facilitating other damaging organisms (e.g. diseases) as food source, a host, a symbiont or a vector etc.?

RESPONSE	N/A minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No information has been found on the issue.

Qu. 4.17. How important might other impacts not already covered by previous questions be resulting from introduction of the organism?

RESPONSE	N/A minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No information has been found on the issue.

Qu. 4.18. How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area?

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: *Delairea odorata* does not have any host specific natural enemies in the risk assessment area and there is no evidence that native organisms feed or infect the species in substantial levels to inflict any damage on the species. Given the impacts seen elsewhere in its exotic range, *D. odorata* can be expected to have at least a moderate impact should it replicate its behaviour in California for example but confidence remains low due to lack of current evidence of impact in the risk assessment area.

Qu. 4.19. Estimate the overall impact in the risk assessment area under current climate conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, in current conditions.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: The overall current impact in the risk assessment area can only be scored as minimal due to the limited amount of reliable evidence but confidence in this score is low. In other, more thoroughly researched and reported regions such as California the overall impact of the species is major on various levels of community structure and ecosystem services and it could be considered an ecosystem engineer given the transformations it can achieve through smothering competing vegetation permanently.

Qu. 4.20. Estimate the overall impact in the risk assessment area in foreseeable climate change conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, under future conditions.

- See also guidance to Qu. 4.3.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Under future climate conditions *D. odorata* is likely to exert more impact on habitats and ecosystem services in the risk assessment area but the speed at which this will happen is unclear given the doubts over current and future spread by seed so a slightly elevated score of minor with low confidence is given.

RISK SUMMARIES			
	RESPONSE	CONFIDENCE	COMMENT
Summarise Introduction and Entry*	very unlikely unlikely moderately likely likely very likely	low medium high	The species has been introduced and entered the risk assessment area in the past. A number of active pathways exist (e.g. horticulture, ornamental purposes other than horticulture and contaminant of habitat material). The plant is not a very popular ornamental species in the risk assessment area. The very likely score is given by default as the species is already established in the risk assessment area.
Summarise Establishment*	very unlikely unlikely moderately likely likely very likely	low medium high	The species is established in the risk assessment area and further establishment is likely in both current and future climatic conditions. The species has a wide tolerance of habitats and other abiotic parameters which promotes its establishment.
Summarise Spread*	very slowly slowly moderately rapidly very rapidly	low medium high	Natural spread by vegetative reproduction. There is no scientific evidence to suggest the species spreads by seed in the risk assessment area. Human assisted spread can act to move the species within the RA area.
Summarise Impact*	minimal minor moderate major massive	low medium high	The species has shown significant and extensive impact on biodiversity and ecosystem services in its invaded range outside of the risk assessment area but this has not yet been realised in the risk assessment area yet. However, the significant number of control projects in the risk assessment area means this moderate score could be higher so a low confidence is given
Conclusion of the risk assessment (overall risk)	low moderate high	low medium high	Overall, <i>D. odorata</i> has a moderate risk to the EU with a low confidence. This score takes into account the moderate impact score but also the high likelihood of entry and establishment, coupled with a moderate spread. The low confidence

			score incorporates uncertainty in seed reproduction in the risk assessment area and the potential implications of seed reproduction in the future.
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*in current climate conditions and in foreseeable future climate conditions

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Distribution Summary

Please answer as follows:

- Yes if recorded, established or invasive
- if not recorded, established or invasive
- ? Unknown; data deficient

The columns refer to the answers to Questions A5 to A12 under Section A.

For data on marine species at the Member State level, delete Member States that have no marine borders.

In all other cases, provide answers for all columns.

Member States, and the United Kingdom

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Austria	NO	NO	YES	YES	NO
Belgium	NO	NO	YES	YES	NO
Bulgaria	NO	NO	YES	YES	NO
Croatia	YES	YES	YES	YES	NO
Cyprus	NO	NO	YES	YES	NO
Czech Republic	NO	NO	NO	YES	NO
Denmark	NO	NO	YES	YES	NO
Estonia	NO	NO	NO	YES	NO
Finland	YES	NO	NO	NO	NO
France	YES	YES	YES	YES	NO
Germany	NO	NO	YES	YES	NO
Greece	NO	NO	YES	YES	NO
Hungary	NO	NO	YES	YES	NO
Ireland	YES	YES	YES	YES	NO
Italy	YES	YES	YES	YES	YES
Latvia	NO	NO	NO	YES	NO
Lithuania	NO	NO	YES	YES	NO
Luxembourg	NO	NO	YES	YES	NO
Malta	NO	NO	YES	YES	NO
Netherlands	NO	NO	YES	YES	NO

Poland	NO	NO	YES	YES	NO
Portugal	YES	YES	YES	YES	YES
Romania	NO	NO	YES	YES	NO
Slovakia	NO	NO	NO	YES	NO
Slovenia	NO	NO	YES	YES	NO
Spain	NO	NO	YES	YES	NO
Sweden	YES	NO	YES	YES	NO
United Kingdom	YES	YES	YES	YES	YES

Biogeographical regions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Alpine	YES	NO	YES	YES	NO
Atlantic	YES	YES	YES	YES	YES
Black Sea	NO	NO	YES	YES	NO
Boreal	YES	NO	YES	YES	NO
Continental	YES	YES	YES	YES	YES
Mediterranean	YES	YES	YES	YES	YES
Pannonian	NO	NO	YES	YES	NO
Steppic	NO	NO	YES	YES	NO

ANNEX I Scoring of Likelihoods of Events

(taken from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Description	Frequency
Very unlikely	This sort of event is theoretically possible, but is never known to have occurred and is not expected to occur	1 in 10,000 years
Unlikely	This sort of event has occurred somewhere at least once in the last millenium	1 in 1,000 years
Moderately likely	This sort of event has occurred somewhere at least once in the last century	1 in 100 years
Likely	This sort of event has happened on several occasions elsewhere, or on at least once in the last decade	1 in 10 years
Very likely	This sort of event happens continually and would be expected to occur	Once a year

ANNEX II Scoring of Magnitude of Impacts

(modified from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Biodiversity and ecosystem impact	Ecosystem Services impact	Economic impact (Monetary loss and response costs per year)	Social and human health impact, and other impacts
	<i>Question 5.1-5</i>	<i>Question 5.6-8</i>	<i>Question 5.9-13</i>	<i>Question 5.14-18</i>
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected ⁵	Up to 10,000 Euro	No social disruption. Local, mild, short-term reversible effects to individuals.
Minor	Some ecosystem impact, reversible changes, localised	Local and temporary, reversible effects to one or few services	10,000-100,000 Euro	Significant concern expressed at local level. Mild short-term reversible effects to identifiable groups, localised.
Moderate	Measureable long-term damage to populations and ecosystem, but reversible; little spread, no extinction	Measureable, temporary, local and reversible effects on one or several services	100,000-1,000,000 Euro	Temporary changes to normal activities at local level. Minor irreversible effects and/or larger numbers covered by reversible effects, localised.
Major	Long-term irreversible ecosystem change, spreading beyond local area	Local and irreversible or widespread and reversible effects on one / several services	1,000,000-10,000,000 Euro	Some permanent change of activity locally, concern expressed over wider area. Significant irreversible effects locally or reversible effects over large area.
Massive	Widespread, long-term population loss or extinction, affecting several species with serious ecosystem effects	Widespread and irreversible effects on one / several services	Above 10,000,000 Euro	Long-term social change, significant loss of employment, migration from affected area. Widespread, severe, long-term, irreversible health effects.

⁵ Not to be confused with “no impact”.

ANNEX III Scoring of Confidence Levels

(modified from Bacher et al. 2017)

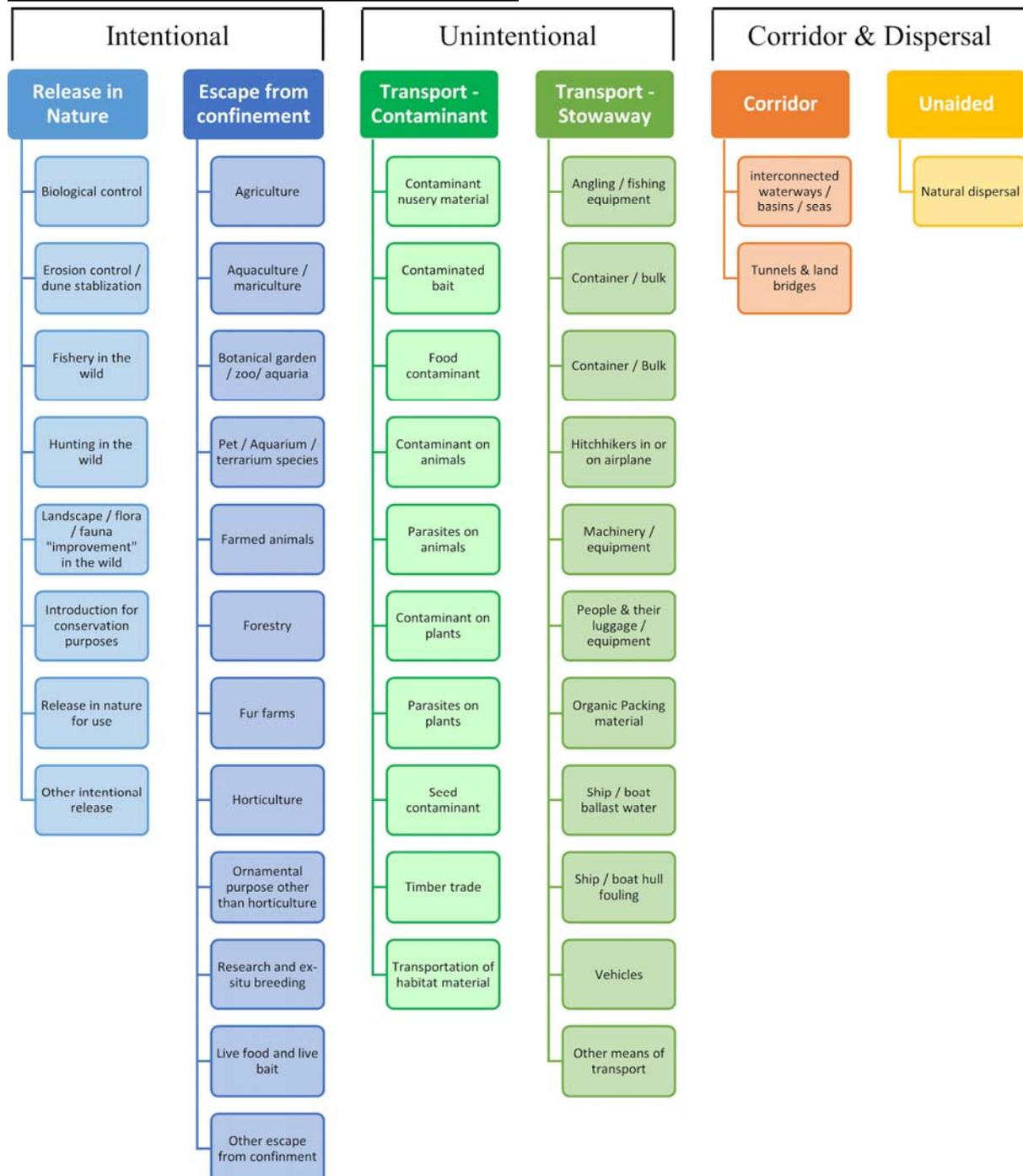
Each answer provided in the risk assessment must include an assessment of the level of confidence attached to that answer, reflecting the possibility that information needed for the answer is not available or is insufficient or available but conflicting.

The responses in the risk assessment should clearly support the choice of the confidence level.

Confidence level	Description
Low	There is no direct observational evidence to support the assessment, e.g. only inferred data have been used as supporting evidence <i>and/or</i> Impacts are recorded at a spatial scale which is unlikely to be relevant to the assessment area <i>and/or</i> Evidence is poor and difficult to interpret, e.g. because it is strongly ambiguous <i>and/or</i> The information sources are considered to be of low quality or contain information that is unreliable.
Medium	There is some direct observational evidence to support the assessment, but some information is inferred <i>and/or</i> Impacts are recorded at a small spatial scale, but rescaling of the data to relevant scales of the assessment area is considered reliable, or to embrace little uncertainty <i>and/or</i> The interpretation of the data is to some extent ambiguous or contradictory.
High	There is direct relevant observational evidence to support the assessment (including causality) <i>and</i> Impacts are recorded at a comparable scale <i>and/or</i> There are reliable/good quality data sources on impacts of the taxa <i>and</i> The interpretation of data/information is straightforward <i>and/or</i> Data/information are not controversial or contradictory.

ANNEX IV CBD pathway categorisation scheme

Overview of CBD pathway categorisation scheme showing how the 44 pathways relate to the six main pathway categories. All of the pathways can be broadly classified into 1) those that involve intentional transport (blue), 2) those in which the taxa are unintentionally transported (green) and 3) those where taxa moved between regions without direct transportation by humans and/or via artificial corridors (orange and yellow). **Note that the pathways in the category “Escape from confinement” can be considered intentional for the introduction into the risk assessment area and unintentional for the entry into the environment.**



ANNEX V Ecosystem services classification (CICES V5.1, simplified) and examples

For the purposes of this risk assessment, please feel free to use what seems as the most appropriate category / level / combination of impact (Section – Division – Group), reflecting information available.

Section	Division	Group	Examples (i.e. relevant CICES “classes”)
Provisioning	Biomass	Cultivated <i>terrestrial</i> plants	<p>Cultivated terrestrial plants (including fungi, algae) grown for <u>nutritional purposes</u>;</p> <p>Fibres and other materials from cultivated plants, fungi, algae and bacteria for direct use or processing (excluding genetic materials);</p> <p>Cultivated plants (including fungi, algae) grown as a <u>source of energy</u></p> <p><i>Example: negative impacts of non-native organisms to crops, orchards, timber etc.</i></p>
		Cultivated <i>aquatic</i> plants	<p>Plants cultivated by in- situ aquaculture grown for <u>nutritional purposes</u>;</p> <p>Fibres and other materials from in-situ aquaculture for direct use or processing (excluding genetic materials);</p> <p>Plants cultivated by in- situ aquaculture grown as an <u>energy source</u>.</p> <p><i>Example: negative impacts of non-native organisms to aquatic plants cultivated for nutrition, gardening etc. purposes.</i></p>
		Reared animals	<p>Animals reared for <u>nutritional purposes</u>;</p> <p>Fibres and other materials from reared animals for direct use or processing (excluding genetic materials);</p> <p>Animals reared to provide <u>energy</u> (including mechanical)</p> <p><i>Example: negative impacts of non-native organisms to livestock</i></p>
		Reared <i>aquatic</i> animals	<p>Animals reared by in-situ aquaculture for <u>nutritional purposes</u>;</p> <p>Fibres and other materials from animals grown by in-situ aquaculture for direct use or processing (excluding genetic materials);</p> <p>Animals reared by in-situ aquaculture as an <u>energy source</u></p> <p><i>Example: negative impacts of non-native organisms to fish farming</i></p>
		Wild plants (terrestrial and aquatic)	<p>Wild plants (terrestrial and aquatic, including fungi, algae) used for <u>nutrition</u>;</p> <p>Fibres and other materials from wild plants for direct use or processing (excluding genetic materials);</p> <p>Wild plants (terrestrial and aquatic, including fungi, algae) used as a <u>source of energy</u></p> <p><i>Example: reduction in the availability of wild plants (e.g. wild berries, ornamentals) due to non-native organisms (competition, spread of disease etc.)</i></p>
		Wild animals (terrestrial and aquatic)	<p>Wild animals (terrestrial and aquatic) used for <u>nutritional purposes</u>;</p>

			<p>Fibres and other <u>materials</u> from wild animals for direct use or processing (excluding genetic materials); Wild animals (terrestrial and aquatic) used as a <u>source of energy</u></p> <p><i>Example: reduction in the availability of wild animals (e.g. fish stocks, game) due to non-native organisms (competition, predations, spread of disease etc.)</i></p>
	Genetic material from all biota	Genetic material from plants, algae or fungi	<p><u>Seeds, spores and other plant materials</u> collected for maintaining or establishing a population; Higher and lower plants (whole organisms) used to <u>breed new strains or varieties</u>; Individual genes extracted from higher and lower plants for the <u>design and construction of new biological entities</u></p> <p><i>Example: negative impacts of non-native organisms due to interbreeding</i></p>
		Genetic material from animals	<p>Animal material collected for the purposes of maintaining or establishing a population; Wild animals (whole organisms) used to breed new strains or varieties; Individual genes extracted from organisms for the design and construction of new biological entities</p> <p><i>Example: negative impacts of non-native organisms due to interbreeding</i></p>
	Water ⁶	Surface water used for nutrition, materials or energy	<p>Surface water for <u>drinking</u>; Surface water used as a material (<u>non-drinking purposes</u>); Freshwater surface water, coastal and marine water used as an <u>energy source</u></p> <p><i>Example: loss of access to surface water due to spread of non-native organisms</i></p>
		Ground water for used for nutrition, materials or energy	<p>Ground (and subsurface) water for <u>drinking</u>; Ground water (and subsurface) used as a material (<u>non-drinking purposes</u>); Ground water (and subsurface) used as an <u>energy source</u></p> <p><i>Example: reduced availability of ground water due to spread of non-native organisms and associated increase of ground water consumption by vegetation.</i></p>
Regulation & Maintenance	Transformation of biochemical or physical inputs to ecosystems	Mediation of wastes or toxic substances of anthropogenic origin by living processes	<p>Bio-remediation by micro-organisms, algae, plants, and animals; <u>Filtration/sequestration/storage/accumulation</u> by micro-organisms, algae, plants, and animals</p> <p><i>Example: changes caused by non-native organisms to ecosystem functioning and ability to filtrate etc. waste or toxics</i></p>
		Mediation of nuisances of anthropogenic origin	<p>Smell reduction; noise attenuation; visual screening (e.g. by means of green infrastructure)</p> <p><i>Example: changes caused by non-native organisms to ecosystem structure, leading to reduced ability to mediate nuisances.</i></p>

⁶ Note: in the CICES classification provisioning of water is considered as an abiotic service whereas the rest of ecosystem services listed here are considered biotic.

	Regulation of physical, chemical, biological conditions	Baseline flows and extreme event regulation	Control of <u>erosion</u> rates; Buffering and attenuation of <u>mass movement</u> ; <u>Hydrological cycle and water flow regulation</u> (Including flood control, and coastal protection); <u>Wind</u> protection; <u>Fire</u> protection <i>Example: changes caused by non-native organisms to ecosystem functioning or structure leading to, for example, destabilisation of soil, increased risk or intensity of wild fires etc.</i>
		Lifecycle maintenance, habitat and gene pool protection	<u>Pollination</u> (or 'gamete' dispersal in a marine context); <u>Seed dispersal</u> ; <u>Maintaining nursery populations and habitats</u> (Including gene pool protection) <i>Example: changes caused by non-native organisms to the abundance and/or distribution of wild pollinators; changes to the availability / quality of nursery habitats for fisheries</i>
		Pest and disease control	Pest control; Disease control <i>Example: changes caused by non-native organisms to the abundance and/or distribution of pests</i>
		Soil quality regulation	<u>Weathering processes</u> and their effect on soil quality; <u>Decomposition and fixing processes</u> and their effect on soil quality <i>Example: changes caused by non-native organisms to vegetation structure and/or soil fauna leading to reduced soil quality</i>
		Water conditions	Regulation of the <u>chemical condition</u> of freshwaters by living processes; Regulation of the chemical condition of salt waters by living processes <i>Example: changes caused by non-native organisms to buffer strips along water courses that remove nutrients in runoff and/or fish communities that regulate the resilience and resistance of water bodies to eutrophication</i>
		Atmospheric composition and conditions	Regulation of <u>chemical composition</u> of atmosphere and oceans; Regulation of <u>temperature and humidity</u> , including ventilation and transpiration <i>Example: changes caused by non-native organisms to ecosystems' ability to sequester carbon and/or evaporative cooling (e.g. by urban trees)</i>
Cultural	Direct, in-situ and outdoor interactions with living systems that depend on presence in the environmental setting	Physical and experiential interactions with natural environment	Characteristics of living systems that that enable activities promoting health, recuperation or enjoyment through <u>active or immersive interactions</u> ; Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through <u>passive or observational interactions</u> <i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that make it attractive for recreation, wild life watching etc.</i>

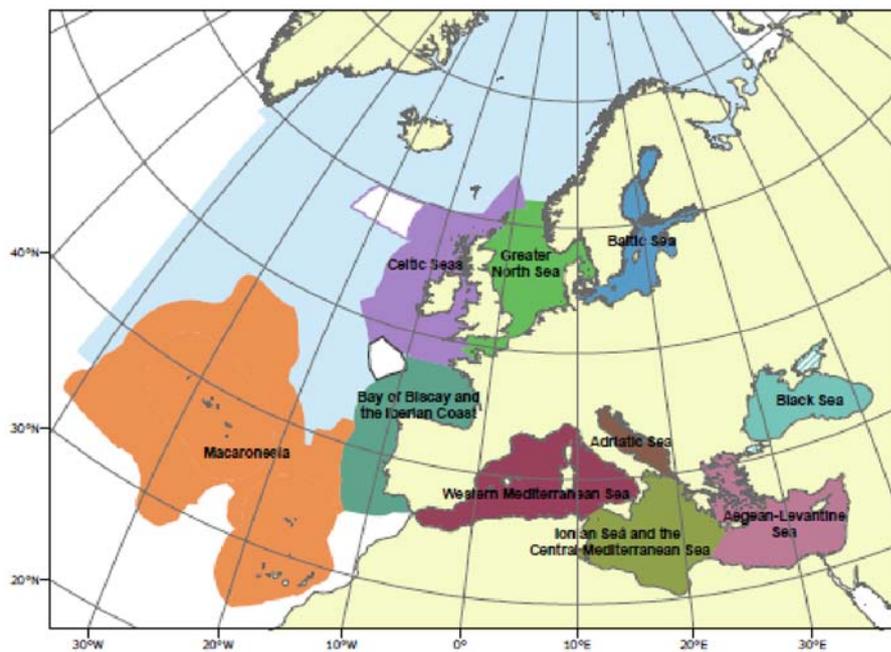
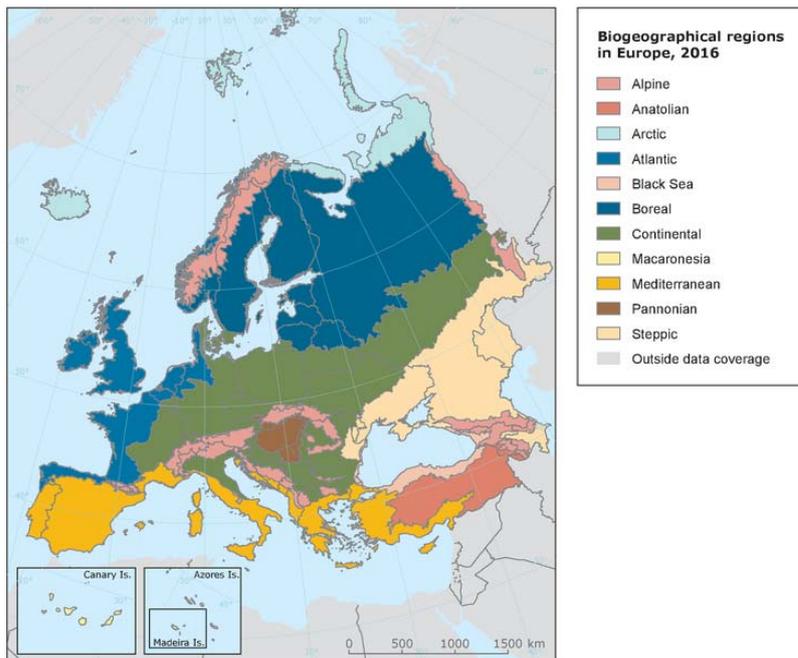
		<p>Intellectual and representative interactions with natural environment</p>	<p>Characteristics of living systems that enable <u>scientific investigation</u> or the creation of traditional ecological knowledge; Characteristics of living systems that enable <u>education and training</u>; Characteristics of living systems that are resonant in terms of <u>culture or heritage</u>; Characteristics of living systems that enable <u>aesthetic experiences</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have cultural importance</i></p>
	<p>Indirect, remote, often indoor interactions with living systems that do not require presence in the environmental setting</p>	<p>Spiritual, symbolic and other interactions with natural environment</p>	<p>Elements of living systems that have <u>symbolic meaning</u>; Elements of living systems that have <u>sacred or religious meaning</u>; Elements of living systems used for <u>entertainment or representation</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have sacred or religious meaning</i></p>
		<p>Other biotic characteristics that have a non-use value</p>	<p>Characteristics or features of living systems that have an <u>existence value</u>; Characteristics or features of living systems that have an <u>option or bequest value</u></p> <p><i>Example: changes caused by non-native organisms to ecosystems designated as wilderness areas, habitats of endangered species etc.</i></p>

ANNEX VI EU Biogeographic Regions and MSFD Subregions

See <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2> ,
http://ec.europa.eu/environment/nature/natura2000/biogeog_regions/

and

<https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions-1/technical-document/pdf>



ANNEX VII Delegated Regulation (EU) 2018/968 of 30 April 2018

see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>

ANNEX VIII Projection of environmental suitability for *Delairea odorata* establishment in Europe

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15 June 2021

Aim

To project the suitability for potential establishment of *Delairea odorata* in Europe, under current and predicted future climatic conditions.

Data for modelling

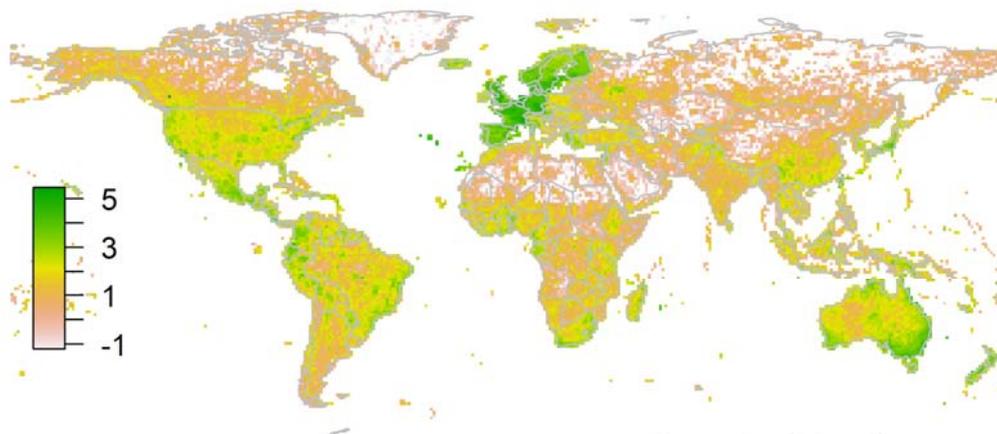
Species occurrence data were obtained from the Global Biodiversity Information Facility (GBIF) (4253 records), iNaturalist (2214 records), the Atlas of Living Australia (1356 records), the Biodiversity Information Serving Our Nation database (BISON) (400 records), and additional records from the risk assessment team. We scrutinised occurrence records from regions where the species is not known to be established and removed any dubious records or where the georeferencing was too imprecise (e.g. records referenced to a country or island centroid) or outside of the coverage of the predictor layers (e.g. small island or coastal occurrences). We removed records dated pre-1950, as these might refer to populations no longer in existence. The remaining records were gridded at a 0.25 x 0.25 degree resolution for modelling, yielding 535 grid cells with occurrences (Figure 1a). As a proxy for recording effort, the density of Tracheophyta records held by GBIF was also compiled on the same grid (Figure 1b).

Figure 1. (a) Occurrence records obtained for *Delairea odorata* and used in the modelling, showing native and invaded distributions. (b) The recording density of Tracheophyta on GBIF, which was used as a proxy for recording effort.

(a) Species distribution used in modelling



(b) Estimated recording effort (log10-scaled)



Climate data were selected from the 'Bioclim' variables contained within the WorldClim database (Hijmans et al., 2005), originally at 5 arcminute resolution (0.083 x 0.083 degrees of longitude/latitude) and aggregated to a 0.25 x 0.25 degree grid for use in the model.

Based on the biology of *Delairea odorata*, the following climate variables were used in the modelling:

- Minimum temperature of the coldest month (Bio6)
- Mean temperature of the warmest quarter (Bio10)
- Climatic moisture index (CMI): ratio of mean annual precipitation to potential evapotranspiration, log+1 transformed. For its calculation, monthly potential evapotranspirations were estimated from the WorldClim monthly temperature data and solar radiation using the simple method of Zomer et al. (2008) which is based on the Hargreaves evapotranspiration equation (Hargreaves, 1994).

To estimate the effect of climate change on the potential distribution, equivalent modelled future climate conditions for the 2070s under the Representative Concentration Pathways (RCP) 2.6 and 4.5 were also obtained. These represent low and medium emissions scenarios, respectively. The above variables were obtained as averages of outputs of eight Global Climate Models (BCC-CSM1-1, CCSM4, GISS-E2-R, HadGEM2-AO, IPSL-CM5A-LR, MIROC-ESM, MRI-CGCM3, NorESM1-M), downscaled and calibrated against the WorldClim baseline (see http://www.worldclim.org/cmip5_5m).

The following habitat layers were also used:

- Tree cover (Tree): This was estimated from the MODerate-resolution Imaging Spectroradiometer (MODIS) satellite continuous tree cover raster product, produced by the Global Land Cover Facility (<http://glcf.umd.edu/data/vcf/>). The raw product contains the percentage cover by trees in each 0.002083 x 0.002083 degree grid cell. We aggregated this to the mean cover in our 0.25 x 0.25 degree grid cells.
- Human influence index (HII): As many non-native invasive species associate with anthropogenically disturbed habitats. We used the Global Human Influence Index Dataset of the Last of the Wild Project (Wildlife Conservation Society - WCS & Center for International Earth Science Information Network - CIESIN - Columbia University, 2005), which is developed from nine global data layers covering human population pressure (population density), human land use and infrastructure (built-up areas, nighttime lights, land use/land cover) and human access (coastlines, roads, railroads, navigable rivers). The index ranges between 0 and 1 and was ln+1 transformed for the modelling to improve normality.
- Distance to the sea (dist_to_sea): As some species require oceanic climates, we calculated distance to the sea as a proxy. Distances are in meters and were ln+1 transformed for modelling to improve normality.

Species distribution model

A presence-background (presence-only) ensemble modelling strategy was employed using the BIOMOD2 R package version 3.4.6 (Thuiller et al., 2020, Thuiller et al., 2009). These models contrast the environment at the species' occurrence locations against a random sample of the global background environmental conditions (often termed 'pseudo-absences') in order to characterise and project suitability for occurrence. This approach has been developed for distributions that are in equilibrium with the environment. Because invasive species' distributions are not at equilibrium and subject to dispersal constraints at a global scale, we took care to minimise the inclusion of locations suitable for the species but where it has not been able to disperse to (Chapman et al. 2019).

Therefore the background sampling region included:

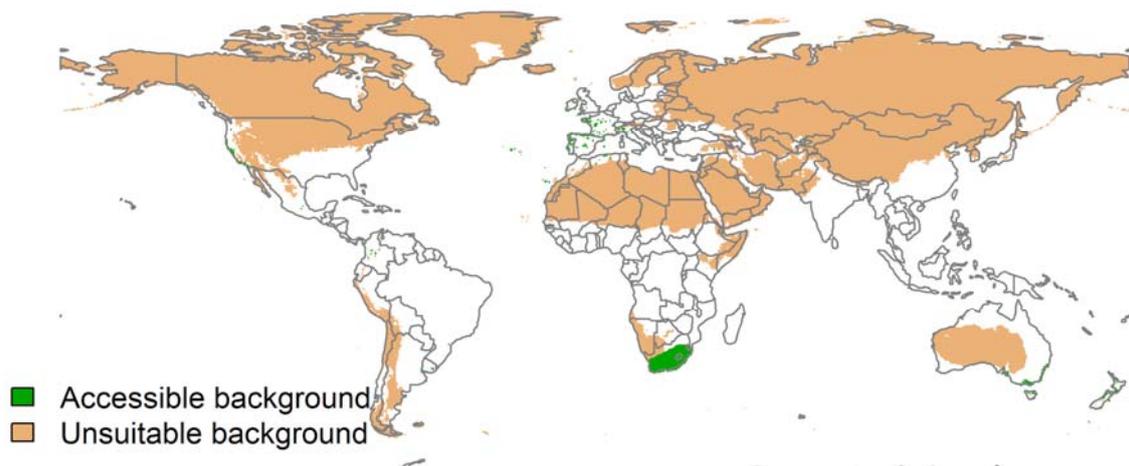
- The area accessible by native *Delairea odorata* populations, in which the species is likely to have had sufficient time to disperse to all locations. Based on presumed maximum dispersal distances, the accessible region was defined as a 400km buffer around the native range occurrences; AND
- A 30km buffer around the non-native occurrences, encompassing regions likely to have had high propagule pressure for introduction by humans and/or dispersal of the species; AND
- Regions where we have an *a priori* expectation of high unsuitability for the species so that absence is assumed irrespective of dispersal constraints (see Figure 2). The following rules were applied to define a region expected to be highly unsuitable for *Delairea odorata* at the spatial scale of the model:

- Minimum temperature of the coldest month (Bio6) < -7.5°C
- Mean temperature of the warmest quarter (Bio10) < 11°C
- Climatic moisture index (CMI) < 0.20

Altogether, only 0.9% of occurrence grid cells were located in the unsuitable background region.

Within the unsuitable background region, 10 samples of 5000 randomly sampled grid cells were obtained. In the accessible background (comprising the accessible areas around native and non-native occurrences as detailed above), the same number of pseudo-absence samples were drawn as there were presence records (535), weighting the sampling by a proxy for recording effort (Figure 2).

Figure 2. The background from which pseudo-absence samples were taken in the modelling of *Delairea odorata*. Samples were taken from a 400km buffer around the native range and a 30km buffer around non-native occurrences (together forming the accessible background), and from areas expected to be highly unsuitable for the species (the unsuitable background region). Samples from the accessible background were weighted by a proxy for recording effort (Figure 1(b)).



Each dataset (i.e. combination of the presences and the individual background samples) was randomly split into 80% for model training and 20% for model evaluation. With each training dataset, seven statistical algorithms were fitted with the default BIOMOD2 settings and rescaled using logistic regression, except where specified below:

- Generalised linear model (GLM)
- Generalised boosting model (GBM)
- Generalised additive model (GAM) with a maximum of four degrees of freedom per smoothing spline
- Artificial neural network (ANN)
- Multivariate adaptive regression splines (MARS)

- Random forest (RF)
- Maxent

Since the total background sample was larger than the number of occurrences, prevalence fitting weights were applied to give equal overall importance to the occurrences and the background. Normalised variable importance was assessed and variable response functions were produced using BIOMOD2's default procedure.

Model predictive performance was assessed by the following three measures:

- AUC, the area under the receiver operating characteristic curve (Fielding & Bell 1997). Predictions of presence-absence models can be compared with a subset of records set aside for model evaluation (here 20%) by constructing a confusion matrix with the number of true positive, false positive, false negative and true negative cases. For models generating non-dichotomous scores (as here) a threshold can be applied to transform the scores into a dichotomous set of presence-absence predictions. Two measures that can be derived from the confusion matrix are sensitivity (the proportion of observed presences that are predicted as such, quantifying omission errors), and specificity (the proportion of observed absences that are predicted as such, quantifying commission errors). A receiver operating characteristic (ROC) curve can be constructed by using all possible thresholds to classify the scores into confusion matrices, obtaining sensitivity and specificity for each matrix, and plotting sensitivity against the corresponding proportion of false positives (equal to $1 - \text{specificity}$). The use of all possible thresholds avoids the need for a selection of a single threshold, which is often arbitrary, and allows appreciation of the trade-off between sensitivity and specificity. The area under the ROC curve (AUC) is often used as a single threshold-independent measure for model performance (Manel, Williams & Ormerod 2001). AUC is the probability that a randomly selected presence has a higher model-predicted suitability than a randomly selected absence (Allouche et al. 2006).
- Cohen's Kappa (Cohen 1960). This measure corrects the overall accuracy of model predictions (ratio of the sum of true presences plus true absences to the total number of records) by the accuracy expected to occur by chance. The kappa statistic ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random. Advantages of kappa are its simplicity, the fact that both commission and omission errors are accounted for in one parameter, and its relative tolerance to zero values in the confusion matrix (Manel, Williams & Ormerod 2001). However, Kappa has been criticised for being sensitive to prevalence (the proportion of sites in which the species was recorded as present) and may therefore be inappropriate for comparisons of model accuracy between species or regions (McPherson, Jetz & Rogers 2004, Allouche et al. 2006).
- TSS, the true skill statistic (Allouche et al. 2006). TSS is defined as sensitivity + specificity - 1, and corrects for Kappa's dependency on prevalence. TSS compares the number of correct forecasts, minus those attributable to random guessing, to that of a hypothetical set of perfect forecasts. Like kappa, TSS takes into account both omission and commission errors, and success as a result of random guessing, and ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random (Allouche et al. 2006).

An ensemble model was created by first rejecting poorly performing algorithms with relatively extreme low AUC values and then averaging the predictions of the remaining algorithms, weighted by their AUC. To identify poorly performing algorithms, AUC values were converted into modified z-scores based on their difference to the median and the median absolute deviation across all

algorithms (Iglewicz & Hoaglin 1993). Algorithms with $z < -2$ were rejected. In this way, ensemble projections were made for each dataset and then averaged to give an overall suitability, as well as its standard deviation.

Projections were classified into suitable and unsuitable regions using a “lowest presence threshold” (Pearson et al. 2007), setting the cut-off as the lowest value at which 98% of all presence records are classified correctly under the current climate (here 0.61). In order to express the sensitivity of classifications to the choice of this threshold, thresholds at which 95% and 99% of records are classified correctly (here 0.77 and 0.36 respectively) were used in the calculation of error bars in Figs. 9 and 10 below in addition to taking account of uncertainty in the projections themselves.

We also produced a limiting factor map for Europe following Elith et al. (2010). For this, projections were made separately with each individual variable fixed at a near-optimal value. These were chosen as the median values at the occurrence grid cells. Then, the most strongly limiting factors were identified as the ones resulting in the highest increase in suitability in each grid cell.

Page Break

Results

The ensemble model suggested that suitability for *Delairea odorata* was most strongly determined by Minimum temperature of the coldest month (Bio6), accounting for 50.9% of variation explained, followed by Human influence index (HII) (24.1%), Mean temperature of the warmest quarter (Bio10) (9.9%), Distance to the sea (dist_to_sea) (8.6%), Climatic moisture index (CMI) (6%) and Global tree cover (Tree) (0.5%) (Table 1, Figure 3).

Table 1. Summary of the cross-validation predictive performance (AUC, Kappa, TSS) and variable importance of the fitted model algorithms and the ensemble (AUC-weighted average of the best performing algorithms). Results are the average from models fitted to 10 different background samples of the data.

Algorithm	AUC	Kappa	TSS	Used in the ensemble	Variable importance (%)					
					Minimum temperature of the coldest month (Bio6)	Human influence index (HII)	Mean temperature of the warmest quarter (Bio10)	Distance to the sea (dist_to_sea)	Climatic moisture index (CMI)	Global tree cover (Tree)
GLM	0.962	0.644	0.895	yes	56	22	15	3	4	0
GAM	0.962	0.640	0.895	yes	47	27	9	15	2	0
GBM	0.959	0.643	0.893	no	51	3	13	12	21	0
ANN	0.963	0.651	0.897	yes	53	24	11	4	7	2
MARS	0.963	0.653	0.896	yes	48	23	5	12	11	0
RF	0.942	0.611	0.882	no	61	7	4	8	16	3
Maxent	0.954	0.638	0.885	no	36	13	13	11	23	4
Ensemble	0.963	0.652	0.897		51	24	10	9	6	1

Figure 3. Partial response plots from the fitted models. Thin coloured lines show responses from the algorithms in the ensemble, while the thick black line is their ensemble. In each plot, other model variables are held at their median value in the training data. Some of the divergence among algorithms is because of their different treatment of interactions among variables.

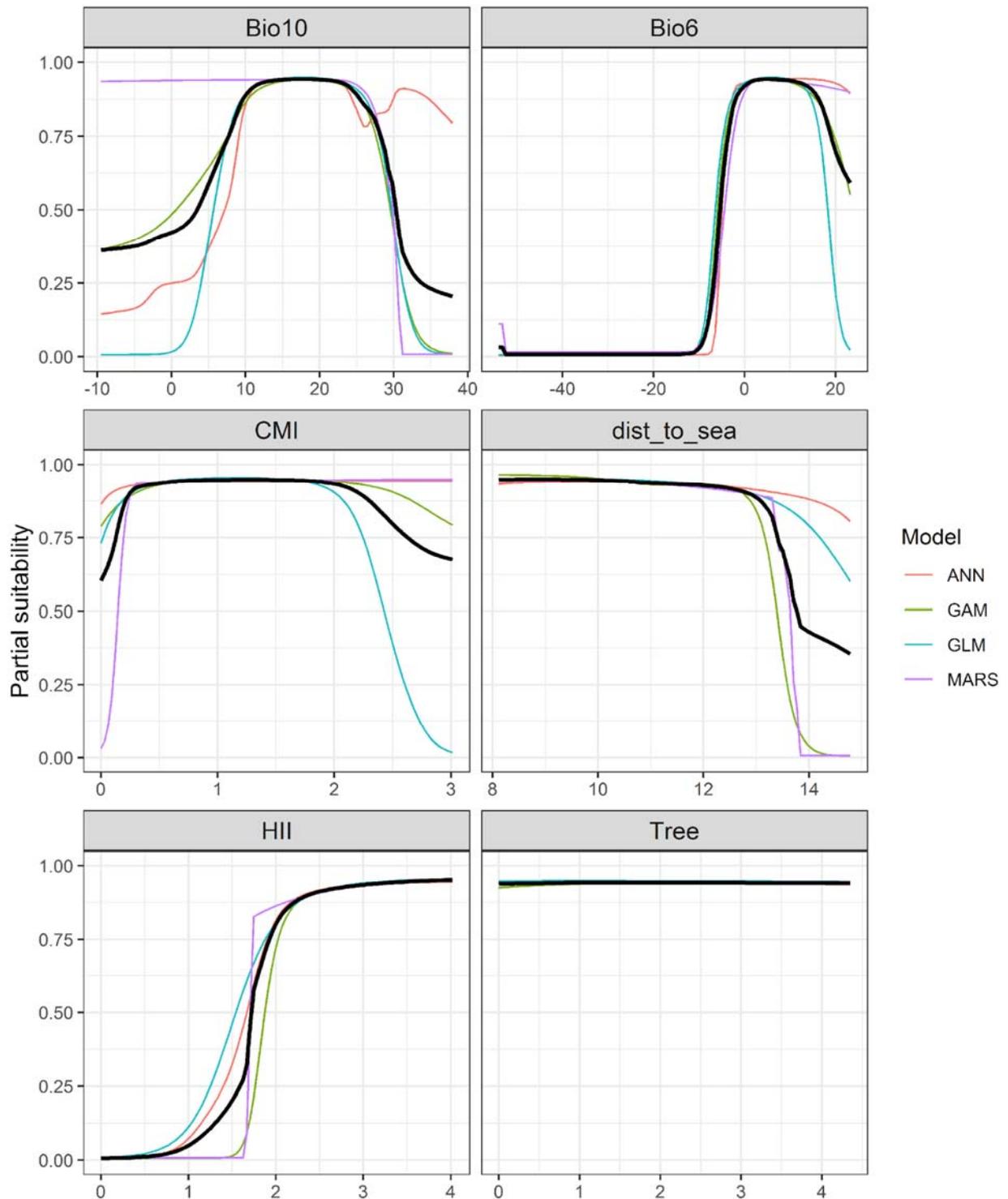
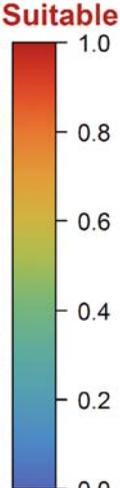
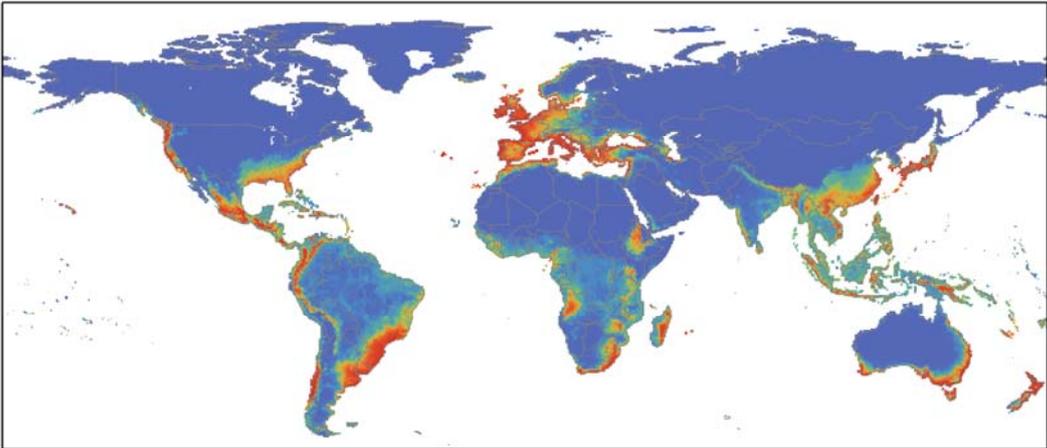


Figure 4. (a) Projected global suitability for *Delairea odorata* establishment in the current climate. For visualisation, the projection has been aggregated to a 0.5 x 0.5 degree resolution, by taking the maximum suitability of constituent higher resolution grid cells. Values > 0.61 are suitable for the species, with 98% of global presence records above this threshold. Values below 0.61 indicate lower relative suitability. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

(a) Projected suitability



(b) Standard deviation in projected suitability

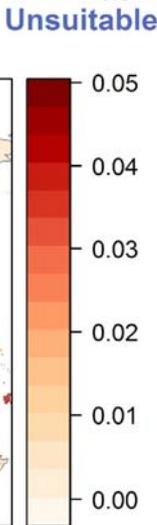
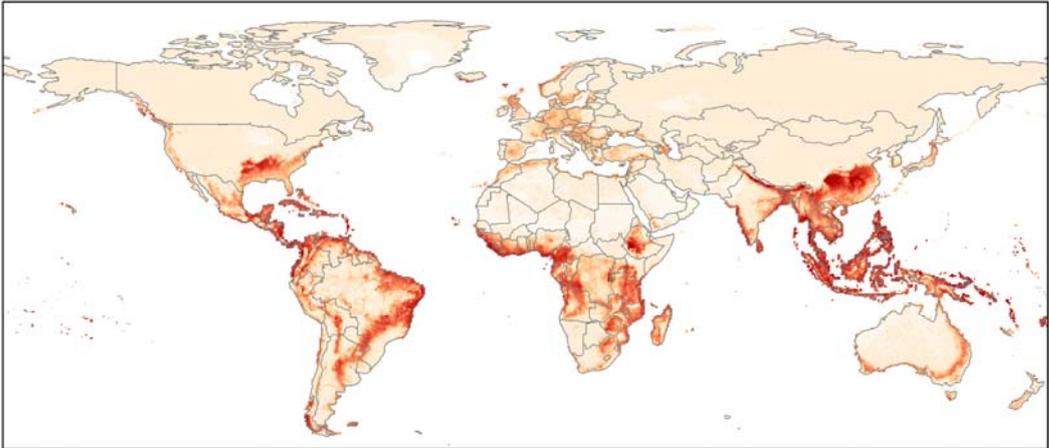


Figure 5. (a) Projected current suitability for *Delairea odorata* establishment in Europe and the Mediterranean region. Values > 0.61 are suitable for the species, with 98% of global presence records above this threshold. Values below 0.61 indicate lower relative suitability. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

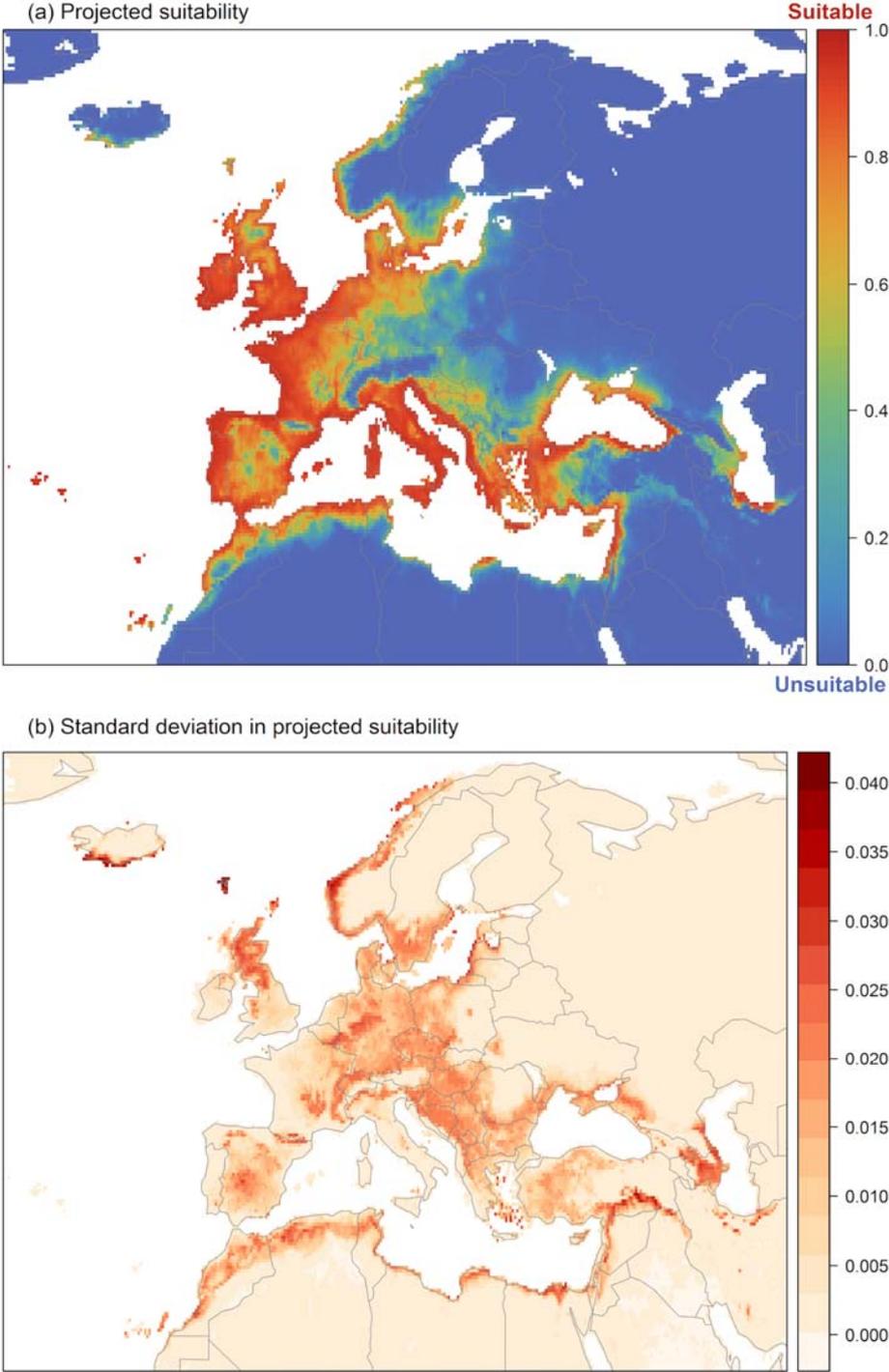


Figure 6. The most strongly limiting factors for *Delairea odorata* establishment estimated by the model in Europe and the Mediterranean region in current climatic conditions.

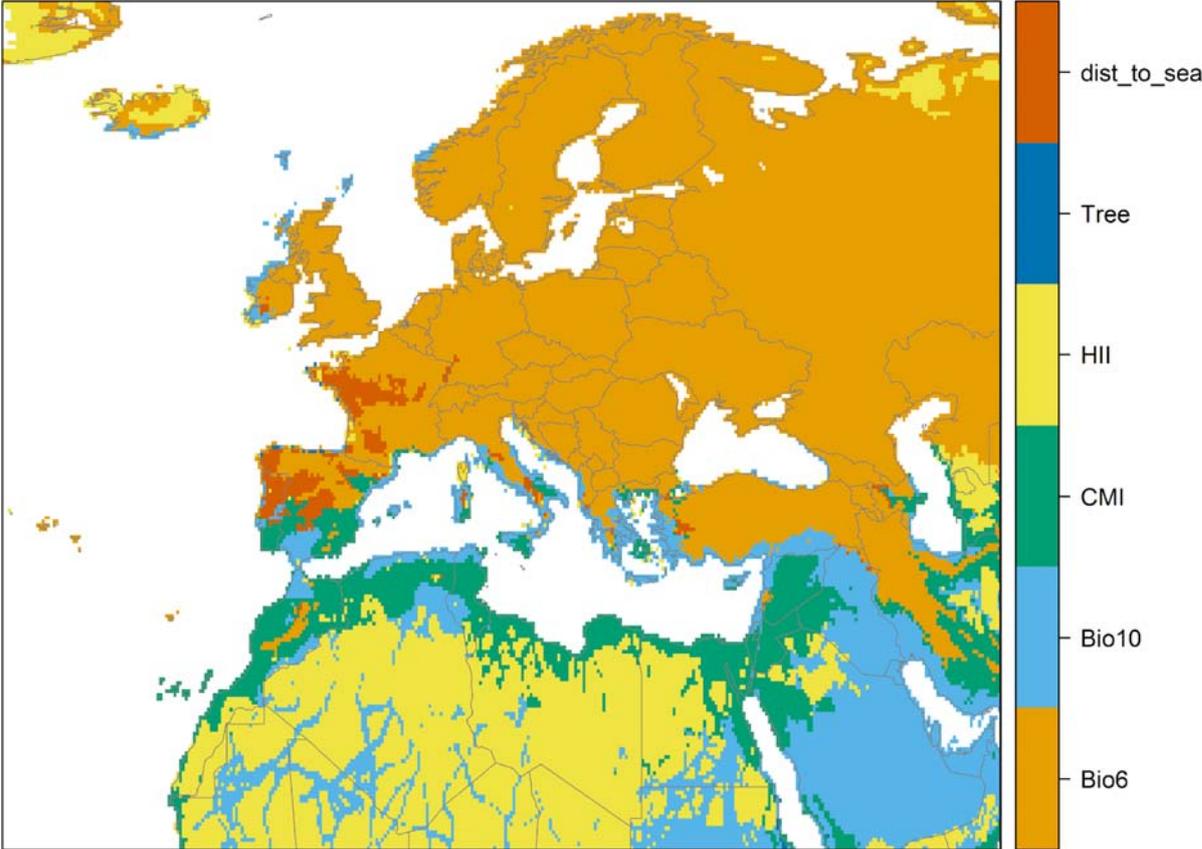


Figure 7. (a) Projected suitability for *Delairea odorata* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP2.6. Values > 0.61 are suitable for the species, with 98% of global presence records above this threshold under current climate. Values below 0.61 indicate lower relative suitability. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

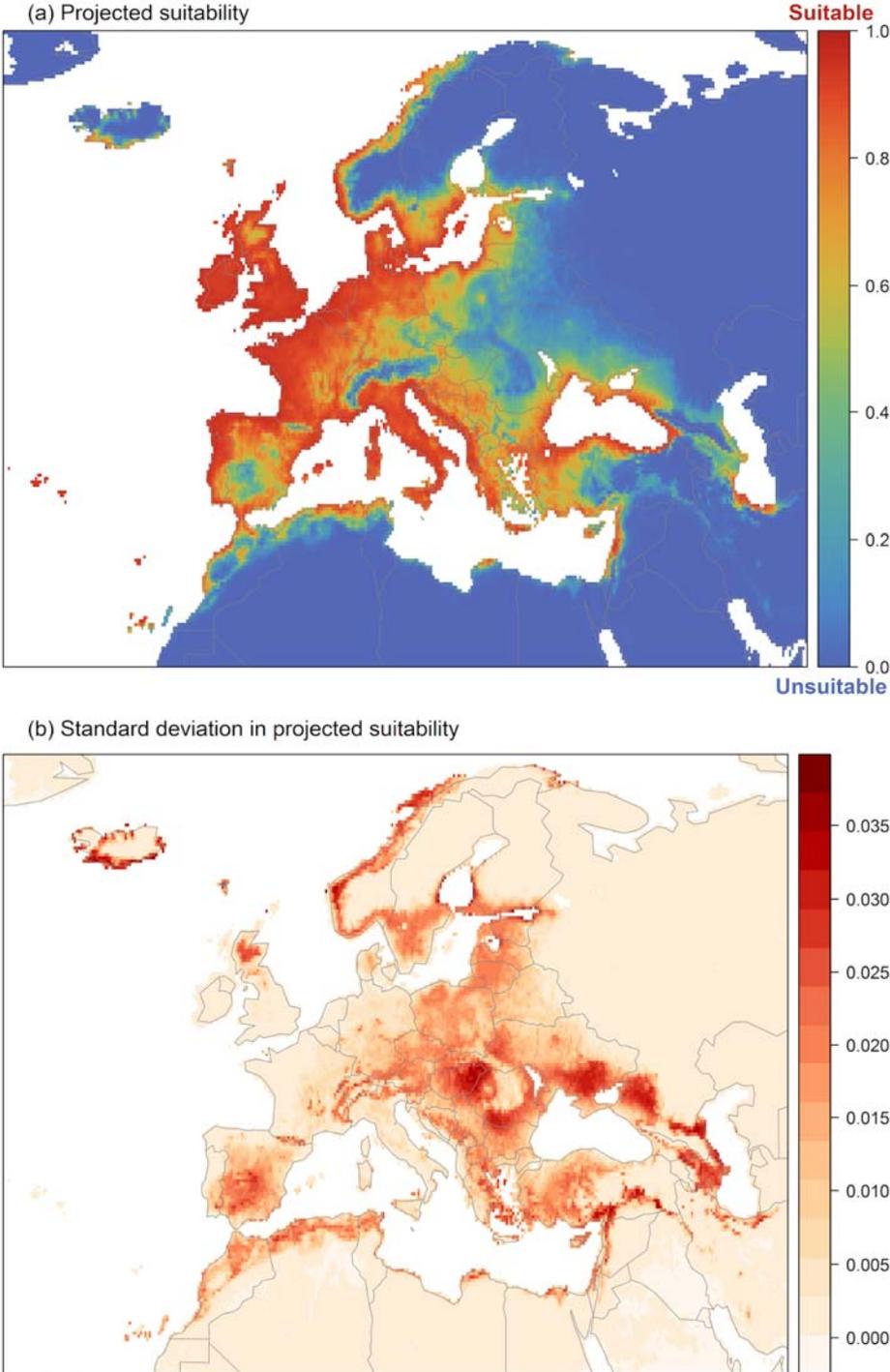


Figure 8. (a) Projected suitability for *Delairea odorata* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP4.5. Values > 0.61 are suitable for the species, with 98% of global presence records above this threshold under current climate. Values below 0.61 indicate lower relative suitability. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

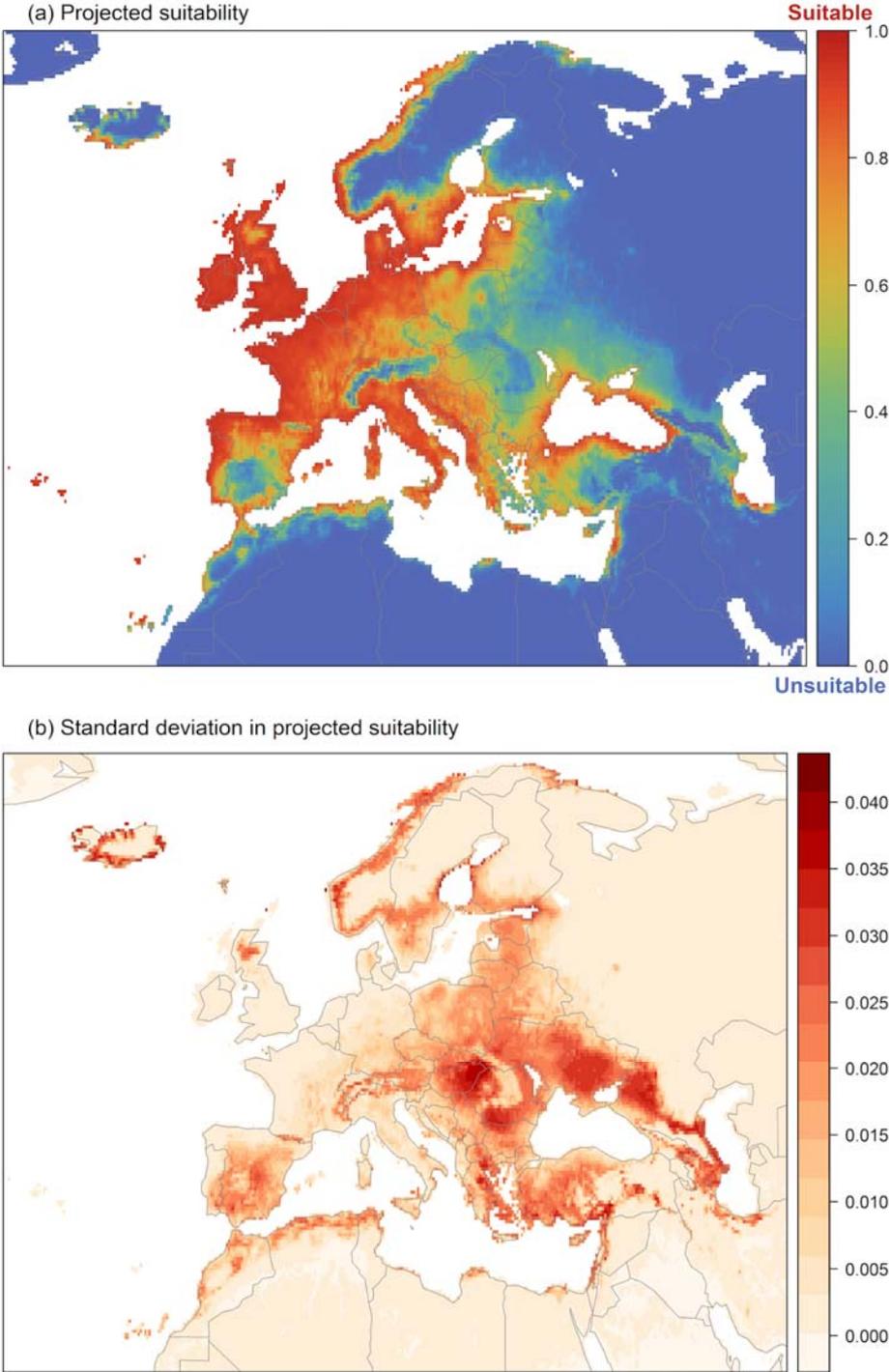


Figure 9. Variation in projected suitability for *Delairea odorata* establishment among Biogeographical Regions of Europe (<https://www.eea.europa.eu/data-and-maps/data/biogeographical-regions-europe-3>). The bar plots show the proportion of grid cells in each region classified as suitable (with values > 0.61) in the current climate and projected climate for the 2070s under two RCP emissions scenarios. Error bars indicate uncertainty due to both the choice of classification threshold (cf. p.5/6) and uncertainty in the projections themselves (cf. part (b) of Figs. 5,7,8). The location of each region is also shown. The Arctic and Macaronesian regions are not part of the study area, but are included for completeness.

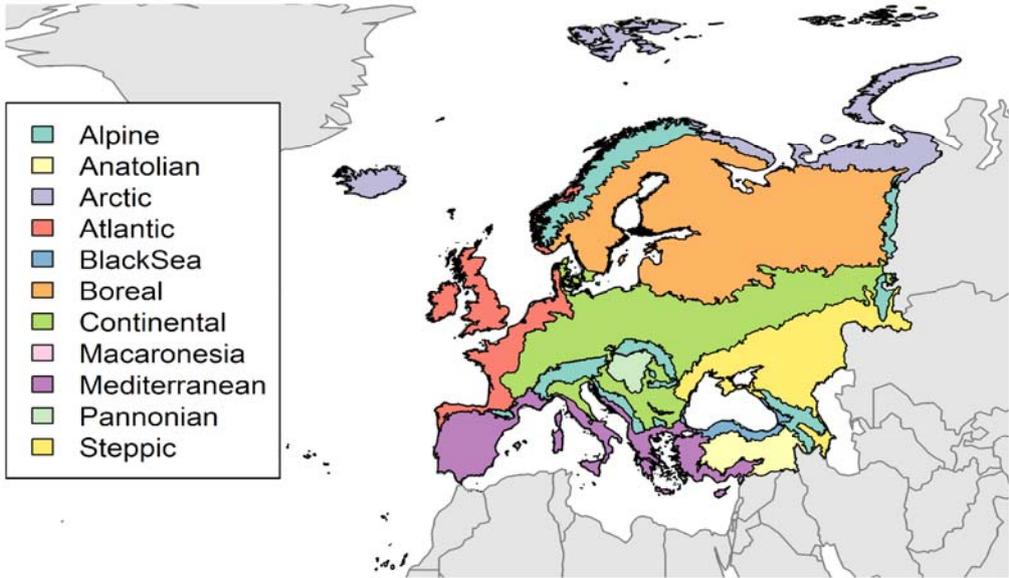
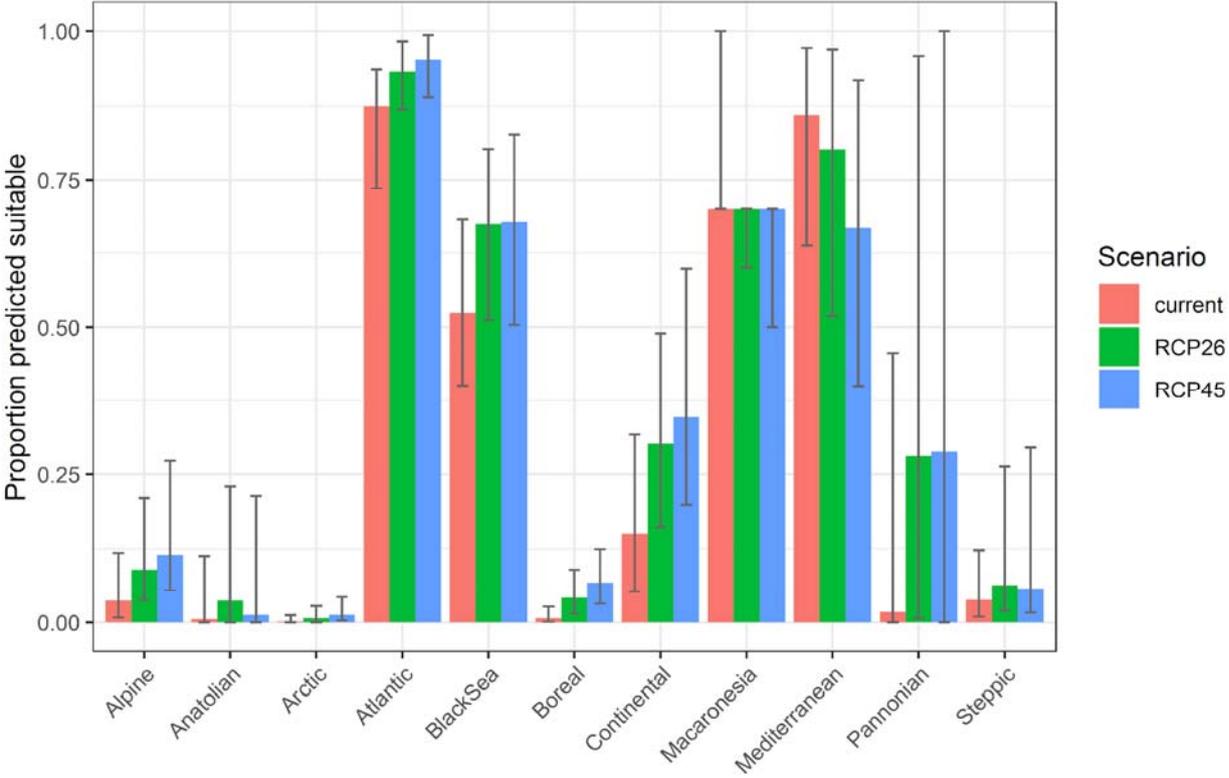


Table 2. Variation in projected suitability for *Delairea odorata* establishment among Biogeographical regions of Europe (numerical values of Figure 9 above). The numbers are the proportion of grid cells in each region classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. The Arctic and Macaronesian biogeographical regions are not part of the study area, but are included for completeness.

	current climate			2070s RCP2.6			2070s RCP4.5		
	lowe r	central estimat e	uppe r	lowe r	central estimat e	uppe r	lowe r	central estimat e	uppe r
Alpine	0.01	0.04	0.12	0.04	0.09	0.21	0.05	0.12	0.27
Anatolian	0.00	0.01	0.11	0.00	0.04	0.23	0.00	0.01	0.21
Arctic	0.00	0.00	0.01	0.00	0.01	0.03	0.00	0.01	0.04
Atlantic	0.73	0.87	0.94	0.87	0.93	0.98	0.89	0.95	0.99
Black Sea	0.40	0.52	0.68	0.51	0.67	0.80	0.50	0.68	0.83
Boreal	0.00	0.01	0.03	0.01	0.04	0.09	0.03	0.07	0.12
Continental	0.05	0.15	0.32	0.16	0.30	0.49	0.20	0.35	0.60
Macaronesia	0.70	0.70	1.00	0.60	0.70	0.70	0.50	0.70	0.70
Mediterranean	0.64	0.86	0.97	0.52	0.80	0.97	0.40	0.67	0.92
Pannonian	0.00	0.02	0.46	0.01	0.28	0.96	0.00	0.29	1.00
Steppic	0.01	0.04	0.12	0.02	0.06	0.26	0.02	0.06	0.30

Figure 10. Variation in projected suitability for *Delairea odorata* establishment among European Union countries and the UK. The bar plots show the proportion of grid cells in each country classified as suitable (with values > 0.61) in the current climate and projected climate for the 2070s under two RCP emissions scenarios. Error bars indicate uncertainty due to both the choice of classification threshold (cf. p.5/6) and uncertainty in the projections themselves (cf. part (b) of Figs. 5,7,8). Malta has been excluded because the Human Influence Index dataset lacks coverage for Malta.

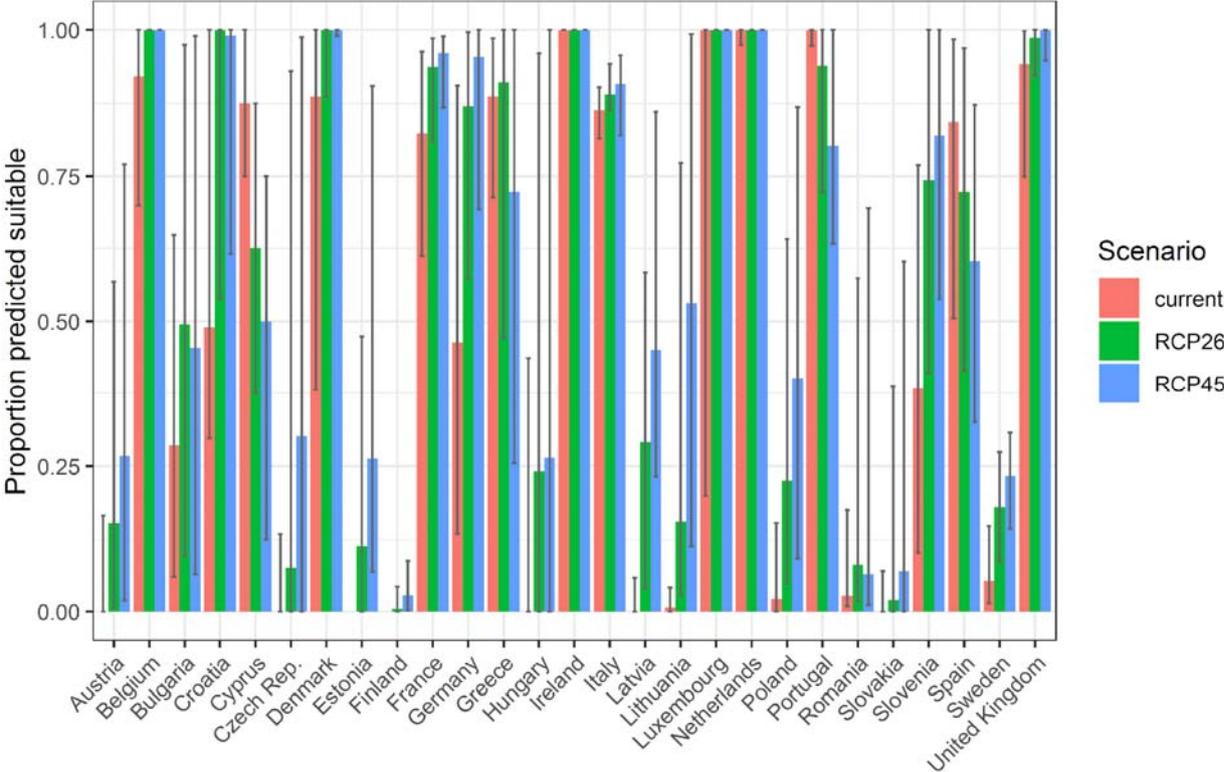


Table 3. Variation in projected suitability for *Delairea odorata* establishment among European Union countries and the UK (numerical values of Figure 10 above). The numbers are the proportion of grid cells in each country classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios.

	current climate			2070s RCP2.6			2070s RCP4.5		
	lower	central estimate	upper	lower	central estimate	upper	lower	central estimate	upper
Austria	0.00	0.00	0.17	0.01	0.15	0.57	0.02	0.27	0.77
Belgium	0.70	0.92	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Bulgaria	0.06	0.29	0.65	0.10	0.49	0.97	0.07	0.45	0.99
Croatia	0.30	0.49	1.00	0.54	1.00	1.00	0.62	0.99	1.00
Cyprus	0.75	0.88	1.00	0.38	0.62	0.88	0.12	0.50	0.75
Czech Rep.	0.00	0.00	0.13	0.00	0.08	0.93	0.00	0.30	0.99
Denmark	0.38	0.89	1.00	0.89	1.00	1.00	0.99	1.00	1.00
Estonia	0.00	0.00	0.00	0.00	0.11	0.47	0.07	0.26	0.90
Finland	0.00	0.00	0.00	0.00	0.01	0.04	0.00	0.03	0.09
France	0.61	0.82	0.96	0.81	0.94	0.99	0.87	0.96	0.99
Germany	0.14	0.46	0.90	0.57	0.87	1.00	0.69	0.95	1.00
Greece	0.71	0.89	0.99	0.47	0.91	1.00	0.25	0.72	1.00
Hungary	0.00	0.00	0.44	0.00	0.24	0.96	0.00	0.26	1.00
Ireland	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Italy	0.81	0.86	0.90	0.86	0.89	0.94	0.82	0.91	0.96
Latvia	0.00	0.00	0.06	0.04	0.29	0.58	0.23	0.45	0.86
Lithuania	0.00	0.01	0.04	0.03	0.16	0.77	0.11	0.53	0.99
Luxembourg	0.20	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Netherlands	0.97	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Poland	0.00	0.02	0.15	0.05	0.23	0.64	0.09	0.40	0.87
Portugal	0.97	1.00	1.00	0.72	0.94	1.00	0.63	0.80	1.00
Romania	0.01	0.03	0.18	0.02	0.08	0.57	0.01	0.07	0.69
Slovakia	0.00	0.00	0.07	0.00	0.02	0.39	0.00	0.07	0.60
Slovenia	0.10	0.38	0.77	0.41	0.74	1.00	0.54	0.82	1.00
Spain	0.51	0.84	0.98	0.41	0.72	0.97	0.33	0.60	0.87
Sweden	0.01	0.05	0.15	0.09	0.18	0.27	0.14	0.23	0.31
UK	0.75	0.94	1.00	0.92	0.99	1.00	0.95	1.00	1.00

Caveats to the modelling

To remove spatial recording biases, the selection of the background sample from the accessible background was weighted by the density of Tracheophyta records on the Global Biodiversity Information Facility (GBIF). While this is preferable to not accounting for recording bias at all, it may not provide the perfect measure of recording bias.

There was substantial variation among modelling algorithms in the partial response plots (Figure 3). In part this will reflect their different treatment of interactions among variables. Since partial plots are made with other variables held at their median, there may be values of a particular variable at which this does not provide a realistic combination of variables to predict from.

Other variables potentially affecting the distribution of the species, such as types of land cover other than trees were not included in the model.

Page Break

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Annex with evidence on measures and their implementation cost and cost-effectiveness

Species (scientific name)	<i>Delairea odorata</i> Lem.
Species (common name)	Cape ivy
Author(s)	Helmut Kudrnovsky, Richard Shaw, Rob Tanner
Reviewer	Johan van Valkenburg and Giuseppe Brundu
Date completed	15/10/2021

Summary¹

Highlight of measures that provide the most cost-effective options to prevent the introduction, achieve early detection, rapidly eradicate and manage the species, including significant gaps in information or knowledge to identify cost-effective measures.

Delairea odorata is already established within the risk assessment area. It is recorded in eight EU Member States where it is established in six. The species is recorded in the Alpine, Atlantic, Boreal, Continental and Mediterranean biogeographical regions and can be assessed as “established” in the Atlantic and Mediterranean biogeographical regions. The main introduction and entry pathways are *Horticulture (escape from confinement)*, *Ornamental purpose other than horticulture: (escape from confinement)* and *Landscape / flora / fauna (improvement in the wild) (cover for walls buildings etc.)*. The overall potential rate of spread under current conditions is assessed as moderate. Thus, to mitigate the impact of this species within the risk assessment area, measures should focus on:

- early detection and eradication of the species where it occurs in new outbreaks,
- limiting spread into new areas,
- suppressing populations where this is still feasible.

The best form of management is the prevention of the introduction of an invasive species into new areas of Member States. Cape ivy is traded within the risk assessment area. A ban from sale requires resources including financial resources, staff time and the development of communication material from several sectors, including governmental, regulators, horticulture and horticulture suppliers, the general public and environmental NGOs. Phytosanitary inspections can be implemented on commodities coming into the EU from outside, but the risk of *D. odorata* entering as a contaminant is low. Biosecurity campaigns have been shown to have a significant effect on awareness raising of negative impacts of invasive alien species. This method is expected to be generally acceptable to the public as it acts to engage stakeholders and put the responsibility into their hands. Early detection could be achieved by surveying the most likely areas of disturbed habitats in urban and peri-urban areas and natural and semi natural areas, especially in coastal regions. If prevention is no longer possible, it is best to treat the weed infestations when they are small to prevent them from establishing (early detection and rapid response). Alvarez (1997) suggested for the Golden Gate National Recreation Area (California) following containment strategy: (1) Achieve parkwide

containment; (2) Remove highest priority infestations, and remove all small or remote Cape-ivy patches; (3) Improve control methods to secure long-term control; (4) Expand community-based involvement in long-term control program. NPS (2021) reports of 80ha Cape ivy removal at the Golden Gate National Recreation Area in 2015. Control is generally best applied to the least infested areas before dense infestations are tackled. Consistent follow-ups are required for effective management. Cape ivy can be managed by traditional physical, mechanical and chemical control methods. However, the latter should be used with caution as chemical application is often non-selective and the plant often climbs over other non-target vegetation.

Management on several levels may be:

- Preventive: Do not plant Cape ivy. Educate homeowners and others on its invasive nature.
- Cultural: Recommend alternative native plants that can be planted after Cape ivy has been removed.
- Mechanical: hand-pulling of Cape ivy in small infestations; repeating as necessary to control any re-growth from sprouts.
- Biological: There are fully researched biocontrol agents in use against Cape ivy but these are not available or tested for the risk assessment area.
- Chemical: application of chemicals can reduce the population of Cape ivy.

CABI (2021) provides some indications related to management but these may not yet be suitable for application in the risk assessment area:

- Although very labour intensive, hand-pulling of *D. odorata* is usually the preferred control method. To prevent new infestations in other areas, the plant material that has been removed, must be disposed of carefully.
- Clopyralid has been used successfully in Australia (Fagg, 1989). In California, glyphosate alone provided only very temporary control, but when a mixture of glyphosate + triclopyr + silicone surfactant in water was used, it provided successful control after two applications (Bossard, 2000). Damage to non-target vegetation is likely, and care should be taken to minimize this.
- There are no approved biological control agents available to manage *D. odorata* in the risk assessment area. However, under a project to develop such agents from South Africa by USDA-ARS in 1998 (Balciunas and Archbald, 1999), a shoot tip-galling fly (*Parafreutreta regalis* Munro) has been released in California and is becoming established (ARS 2020) In addition, a moth, *Secusio extensa* (Lepidoptera: Erebidae), has been released in Hawaii and is recorded as causing heavy damage on the Cape ivy as well as its primary target *Senecio madagascariensis* in Hawaii (Krushelnyckya et al., 2018).
- Whatever control method is used, careful monitoring and removal of any resprouts and seedlings is essential, or within a matter of months the treated area will become indistinguishable from adjacent untreated areas.

Effective management should be supported by a dedicated Action Plan. There is a need to map the distribution, identify management priorities and areas and integrated control strategy (i.e. combination of different methods).

Detailed assessment			
	Description of measures ²	Assessment of implementation cost and cost-effectiveness (per measure) ³	Level of confidence ⁴
Methods to achieve prevention of intentional and unintentional introduction⁵	<p>Ban from sale</p> <p>One potential pathway for entry or spread of <i>Delairea odorata</i> into the EU, or between Member States, is through the purchase of material (plants and seeds) through the horticultural trade. A ban from sale would help to regulate this pathway for Cape ivy.</p>	<p><i>Delairea odorata</i> is already traded within the risk assessment area and sold as a garden ornamental, and a houseplant. A ban on the sale of the species would be a reasonably cost-effective measure to prevent the movement into and within the risk assessment area. A ban on sale requires resources including financial resources, staff time and the development of communication material from a number of sectors, including governmental, regulators, horticulture and horticultural suppliers, the general public, and environmental NGOs. A ban on sale may be relatively easy to achieve with limited opposition as the species is not popular or widely available via the horticulture industry or e-commerce sites and many alternative species exist. Costs may be saved by being part of a broader effort covering other invasive alien species. The method is likely to be effective if all stakeholders are convinced of the risk of the species to biodiversity and ecosystems in the risk assessment area. Stakeholder acceptance can be achieved if the risk assessment shows a high risk. However, where the species has low likelihood of establishment outdoors, Member States may question the need for a ban. The general public may find the approach acceptable if supporting information is provided to justify the risk. Health and safety considerations are not appropriate for this measure.</p>	High
	<p>Public awareness campaigns</p> <p>Communication material detailing the negative impacts of the species would be essential to educate the public and support a ban on sale.</p>	<p>Public awareness campaigns may highlight the risk of the species and prevent further spread of the species from existing populations. Public awareness campaigns should highlight that fragments of the species should not be collected and planted or dumped in waste piles or natural areas. Where possible campaigns should highlight alternative species that could be planted. It is estimated that the cost for an awareness raising campaign could be up to EUR 10,000 per year (which would include the cost to</p>	High

		<p>produce and disseminate information material along with associated staff costs) for each Member State which is already affected by the invasive species. An alternative approach may be an European wide awareness campaign with information material prepared by the European Commission and translated into Member States languages. Public awareness campaigns should highlight that following management, plant material should be disposed of according to national regulations. Where possible, campaigns should highlight alternative species that could be used as ornamentals. Health and safety considerations are not appropriate for this measure.</p>	
	<p>Phytosanitary inspections The transport of stem material as a contaminant of habitat material (soil and vegetation) may facilitate its entry and spread within the EU. Phytosanitary inspections along with associated phytosanitary measures can act to prevent the entry of the species into specific countries/regions. Preventing the spread of <i>D. odorata</i> should also be regarded as a priority for national phytosanitary inspection services</p>	<p>Phytosanitary inspections can be implemented on commodities coming into the EU from outside but the risk of <i>D. odorata</i> entering as a contaminant is, depending on the commodity, moderately low as the seed is often not viable and stem material would be visible. The authors could not find any examples where stem or root material has been intercepted as a containment. It is difficult to implement phytosanitary measures within the EU due to freedom of movement of commodities between countries. Therefore, this limits the effectiveness of inspections regarding the repeated introduction and spread of the species within the EU. At the Member State level, national phytosanitary authorities are responsible for raising awareness of such inspections. If measures are not implemented by all countries, they will not be effective since the species could enter a country or spread within a country or from one country to another. National measures should be combined with international measures, and international coordination of management of the species between countries is recommended. The cost of such a measure is likely to be relatively low in preparation but key identification information would need to be supplied to inspectors (e.g. 5-10,000 Euros). The cost for implementation may be higher as training for inspectors may be needed. Inspectors will be well trained in health and safety</p>	<p>Medium</p>

		procedures, although there are no specific concerns relating to this species.	
	Awareness raising material	To prevent the movement of contaminated material between EU Member States, management plans for habitat material, identification guides, factsheets, Codes of conduct (e.g. Queensland Government 2016) should be developed. These materials would support staff training. Costs to develop awareness raising material can vary depending on the types and format it is disseminated. Online factsheets and management documents can be developed at a low cost i.e. 500-1000 euros. Health and safety considerations are not appropriate for this measure.	Medium
Methods to achieve early detection⁶	Awareness raising campaigns combined with citizen science projects Communication material detailing the characteristics and negative impacts of the species would be essential to educate the public. Public awareness campaigns (combined with citizen science projects to detect new occurrences) may highlight the risk of the species and prevent further spread of the species from existing populations.	Citizen science and public participation have emerged as powerful techniques to engage the public in the content and practices of applied science. Citizen science programs need to attract and retain participants with clear goals and motivations. It is useful to identify and acknowledge the contributions that individual stakeholders can bring to project efforts. By doing so, the program can build on known strengths and target where additional capacity needs to be developed. Involving partners and other stakeholders early in the process of project design can increase investment and enhance outcomes. Where possible it is worth adopting standardized and vetted protocols used by others after adaptation for local or regional conditions. Plans should be made early on for the infrastructure necessary to capture, manage, store, and share project data, where appropriate. Many existing citizen science projects can provide the core infrastructure around which a regional or topical effort can be developed (e.g., iNaturalist). An increasing number of technology platforms and tools are also arising to facilitate custom citizen science project design (Rückert-John et al. 2017). There may be a spatial imbalance in data collection due to differences in participation in the different regions and countries.	Medium

		Costs: The costs could be minimal if technology (e.g. apps) and the framework (citizen scientists) are already in place. Resources would involve IT-costs, staff time. The staff involved could come from government agencies and/or citizen scientists. Some regional training workshops could be conducted to train stakeholders in identification; it is estimated that each training workshop may cost approx. EUR 3,000. Health and safety considerations are not appropriate for this measure.	
	<p>Surveillance by experts</p> <p>This type of surveillance includes involvement of experts e.g. in field surveys (e.g. habitat mapping or dedicated invasive alien species mapping).</p>	<p>Habitat mapping has become the standard technique of site related inventories for nature conservation and landscape planning (EEA 2014). Often, habitat mapping is not an area-wide but a selective survey of natural or semi-natural high value biotopes. Collection of invasive alien species data is a by-product in most cases. Dedicated invasive alien species surveillance is not yet a common tool. Field surveys, especially in larger areas, are cost intensive.</p> <p>Costs: It is estimated that the cost for an annual surveillance could be from about EUR 10,000 per year in smaller Member States to much larger figures in larger Member States. This could incorporate multiple species and widespread surveillance. Costs could significantly increase if novel technologies are added (drones, computer software). Health and safety considerations are not appropriate for this measure.</p>	Medium
<p>Methods to achieve eradication⁷</p> <p>There are a number of methods that can potentially achieve eradication of discrete populations. However, if the population occurs</p>	<p>Control using mechanical or manual removal</p> <p>Manual measures assisted by mechanical tools (cutting and excavating) and in combination with chemicals. Due to the vigorous regrowth from cut stumps, vegetative root regeneration and seed dispersal, control using traditional methods is both labour intensive and costly with repeated applications needed to achieve a</p>	<p>In many types of invaded sites mechanical and chemical control for Cape ivy are more effective when used in combination, therefore the two methods are often combined and are here treated as a single management measure. Cape ivy is very difficult or impossible to control by simple cutting or by other mechanical means alone.</p> <p>The resources required will be related to the extent of the invaded area, the land accessibility, the need to repeat the interventions. The resources needed for an integrated control strategy within a dedicated management plan will be dependent upon specific</p>	Medium

<p>over a large area, eradication attempts may be limited or will require investment over a number of years. It is also important to note that any eradication method may need to be used in combination, e.g. removal of the above-ground stem will not achieve eradication alone and will need to be used in combination with excavation of the below-ground roots or repeated chemical application to deplete the below-ground biomass. Eradication methods can be applied on a local scale. Methods would depend on the habitat where the species is invasive and the</p>	<p>restored habitat. Manual removal (uprooting) and cutting coupled with an application of systemic herbicides have been shown to be effective in controlling shrub forms of the plant (Stern 2011). Manual removal of plants including roots and rhizomes, before viable seed develops can help control infestations in areas where plants are accessible. Removing all plant materials from the site will help prevent re-rooting of rhizomes, stolons, or stem fragments. Follow-up removal of resprouts is essential. In some large patches, all stems can be cut at ground level and Cape ivy rolled up like a rug. Although the below-ground reproductive tissues will resprout, this strategy makes it possible to detect and spot-treat new sprouts avoiding contact with desirable vegetation. Because Cape ivy can resprout and establish from stem fragments, mowing is not recommended. Cutting Cape ivy before it flowers will reduce seed production and deplete the plant's energy reserves. Resprouts are common after treatment. Cutting should be combined with a herbicide treatment or with multiple cuttings over a period of years. All plant parts should be bagged and properly disposed of (DiTomaso et al. 2013). Small plants can be uprooted but it is important to remove the roots completely as <i>Delairea odorata</i> reproduces vegetatively from roots and stolons. Effective removal of</p>	<p>circumstances, but in general will include trained staff, access to necessary tools and equipment to undertake mechanical or manual control, the purchasing and application of plant protection products (PPP), and the disposal costs for the removed plants. Staff should be trained in health and safety aspects for applying chemicals. Costs will vary depending on the area infested and the type of habitat. If plant material is on a discrete piece of land (e.g. removing a single patch, legal disposal and recovering the soil), control costs could be as little as 2,000-5,000 EUR. However, attention to detail is needed as leaving just a little part of the plant may regenerate (Bossard, 2000). Stern (2011) indicates for areas in California with less than 50% Cape ivy cover an average annual hand removal cost about \$6,160 per acre per year (13500 euro per Ha per year).). These costs were based on volunteer crews; with appropriate training, these labor costs can be reduced. Costs can also be substantially reduced by using volunteers. Hiring a part-time volunteer coordinator for \$20 to \$25/hour to coordinate volunteer days if existing staff is not available is also an option. Using volunteers is an option not only for cost reasons, but to engage the community in stewardship, and provide education to prevent future introduction of invasive species. However, where the species invades a site with multiple landownerships and/or if the species is growing over native vegetation, costs may be increased. Costs also include the disposal of plant material in an authorized site and soil replacement. No monetary figures have been found on the manual control of larger populations. Depending on the size of the infestation and the habitat costs may be substantial to eradicate the species. For example, Golden Gate National Recreation Area near San Francisco, California, USA has spent over US\$600,000 over three years trying to eradicate this vine across an area of up to 162 acres</p>	
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extent of the infestation.	the above and belowground plant material is essential for the eradication of the species and this can be labour intensive.	(65.5 Ha) (Alvarez, 1997). More recent estimate of the invasion suggests 76 ha.	
	Excavation of the roots from the soil	Excavating soil contaminated with root material or seed material could potentially assist in eradication attempts but may be costly as heavy machinery may be needed and the contaminated soil may need to be removed to a licensed landfill which will incur additional costs. Health and safety precautions should be employed as this method can involve the use of machinery and equipment. Some disturbance to the environment is likely, though this is unlikely to be long-lived. Restoration activities can minimize environmental disturbance.	Medium
	Chemical application (herbicides) As a general disclaimer, plant protection products are subject to national restrictions and regulations of the EU Member States. Furthermore, there may be legal constraints as well as low public acceptance to use chemicals in larger or in protected areas. Chemical control methods should be used with caution as substances often are non-selective. In addition, time of the year/season, day time and especially weather conditions can make a difference.	Growth regulators: <i>Clopyralid</i> is a selective herbicide for broadleaf species. This compound has been shown to be successful in controlling Cape ivy in Australia (Fagg 1989). Timing: postemergence when plants are growing rapidly. <i>Triclopyr</i> is a selective herbicide for broadleaf species. In areas where desirable grasses are growing under or around Cape ivy, triclopyr can be used without non-target damage. Timing: postemergence when plants are growing rapidly. Aromatic amino-acid inhibitors: <i>Glyphosate</i> is a nonselective systemic herbicide. It gives good control with some resprouts. In many situations, it may be more appropriate to use a wiper application to achieve selectivity. Glyphosate can be combined with triclopyr for more effective control (and using a surfactant when applying this combination). Timing: postemergence when plants are growing rapidly. Best results when treated in late summer or early fall (DiTomaso et al. 2013; Weedbusters 2021). Health and safety precautions should be employed as this method involves the use of chemical application. Some disturbance to the environment is likely, when applying chemicals. These can be minimized with direct application on the target species.	Medium

		<p>Acceptability of chemical control in natural areas is generally low within the EU.</p> <p>Costs: Stern (2011) estimates the first-year costs of \$11,893 per acre per year (29 375 euros per hectare per year) for areas with greater than 50% Cape ivy cover and high native plant cover. This would probably need to be repeated over a number of years to achieve eradication.</p>	
<p>Methods to achieve management⁸</p> <p>Management methods can be applied on a local scale. Methods would depend on the habitat where the species is invasive and the extent of the infestation.</p>	<p>Manual removal</p> <p>Manual removal can be used to control the spread of the species, in particular of small populations. Further, manual removal of incremental spread from the edge of established stocks is worth for consideration.</p>	<p>Manual removal (uprooting) and cutting coupled with an application of systemic herbicides have been shown to be effective in controlling shrub forms of the plant (Stern 2011). Small plants can be uprooted but it is important to remove the roots completely as <i>Delairea odorata</i> reproduces vegetatively from the roots. Mechanical removal of <i>D. odorata</i> (using machinery or larger equipment) is difficult and time consuming since the vine can resprout from any node that is not disposed off properly. When using mechanical control, the species has to be dug out, a containment zone should be established to prevent further spread and all plant debris has to be removed according to national legislations (EPPO 2021). Small infestations of Cape Ivy can be removed completely. Climbing stems can be severed at the base and left to dry out in the canopy. Broken stem pieces must be collected and removed from the site to prevent them from taking root (Weeds Australia 2021). Health and safety precautions should be employed as this method can involve the use of equipment (e.g. secateurs, saws). Some disturbance to the environment is likely. Acceptability of this method may vary depending on the habitat quality (natural versus ruderal) and the level of disturbance, but is likely to be more acceptable than chemical application.</p> <p>This can be minimized with precaution and restoration activities. Costs: Stern (2011) indicates for areas in California with less than 50% Cape ivy cover an average annual hand removal cost about \$6,160 per acre per year (13500 euro per Ha per year).</p>	<p>Medium</p>

	<p>Excavation of the roots from the soil</p>	<p>Excavation of roots from the soil, and contaminated soil, may be cost effective over a long period of time but the initial outlay of costs can be high and include costs of heavy machinery and costs of disposal of contaminated soil. Further complications can arise depending on the habitat type invaded, where for example invasions into natural habitats may act to increase the costs associated with management. Removing a small number of plants from a limited area may be feasible and relatively inexpensive. However, at larger scales and in more complex environments, feasibility of eradication is likely to drop rapidly with significant increases in cost. However, little evidence is available on which to base further assessments. Health and safety precautions should be employed, as this method can involve the use of equipment.</p> <p>Acceptability of this method may vary depending on the habitat quality (natural versus ruderal) and the level of disturbance, but is likely to be more acceptable than chemical application.</p> <p>Costs: there are no specific costs estimates for the management with excavation of roots and seeds from the soil.</p>	<p>Medium</p>
	<p>Chemical control</p>	<p>Chemical control is probably not a cost-effective method for this species over a large area but it could be useful when controlling small populations of the species. Repeated and targeted applications may be needed to maintain suppression of the population. Staff should be trained in health and safety aspects for applying chemicals.</p> <p>Chemical control of <i>D. odorata</i> depends on location of the infestation, sensitivity of the associated species, and the surface area covered by the plant (EPPO 2021). Some disturbance to the environment is likely, when applying chemicals. These can be minimized with direct application on the target species.</p>	<p>Medium</p>

		<p>Acceptability of chemical control in natural areas is generally low within the EU.</p> <p>Costs: Stern (2011) estimates the first year costs with \$11,893 per acre per year (29 375 euros per hectare per year) for areas with greater than 50% Cape ivy cover and high native plant cover.</p>	
	Biological control	<p>There are no approved biological control agents available to manage <i>D. odorata</i> in the risk assessment area. However, under a project to develop such agents from South Africa by USDA-ARS in 1998 (Balciunas & Archbald 1999), a shoot tip-galling fly (<i>Parafreutreta regalis</i> Munro) has been released in California and is becoming established (ARS 2020). In addition, a moth, <i>Secusio extensa</i> (Lepidoptera: Erebidae) has been released in Hawaii and is recorded as causing heavy damage on the cape ivy as well as its primary target <i>Senecio madagascariensis</i> in Hawaii (Krushelnyckya et al. 2018; Cal-IPC 2021a/b).</p> <p>Acceptability of biological control is increasing within Europe as the acceptability of chemical applications is decreasing. With awareness raising and information dissemination, acceptability can be increased.</p> <p>Costs: there are no specific costs estimates for the management by biological control, but once releases have been successful there is no on-going cost to such control programs other than for monitoring. Initially, a biological control programme could cost in the region of 200 - 600 000 Euros to implement and costs are lower for those targets for which successful biocontrol has been demonstrated elsewhere as the R&D input is very much lower. Health and safety considerations are not appropriate for this measure.</p>	High
	Action plan	<p>Effective management should be supported by a dedicated Action Plan. There is a need to map the distribution, identify management priorities and areas and integrated control strategy (i.e.</p>	Medium

		<p>combination of different methods). Health and safety considerations are not appropriate for this measure.</p> <p>Costs: an action plan can vary in potential costs dependent on the coverage of the plan. A plan that includes several habitats for example may be greater than one that focuses on a specific area/habitat. An action plan can be produced at a cost of 500 -2 000 euros.</p>	
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Guidelines for Completing the Annex

1 Provide a brief summary description of the most cost-effective methods drawing on the reviews in the detailed assessments

2 Provide a description of the potential method. This should be based on the available key scientific evidence which should be gathered from sources including articles and reviews in technical and scientific journals, internet searches, online databases, grey literature and relevant books and personal communications from scientists, stakeholders, conservation practitioners and governmental bodies. This information should include a full bibliographic list detailing the literature and sources considered.

3 Provide an assessment of the likely cost and effectiveness of the method. Where information is available, consider the following range of questions, accepting that not all questions will be appropriate in all circumstances.

- How effective has this approach proven to be in the past or in an analogous situation?
- How publically acceptable is the approach likely to be?
- Over what period of time would this approach need to be applied to be effective?
- What is the direct cost of implementing this approach?
- How likely are the methods used in the approach to be available?
- How likely is it that relevant licences or other approvals to undertake the approach would be difficult to obtain?
- How likely is it that health and safety issues would prevent the use of this approach?
- How significant is the environmental harm caused by this approach?
- How significant is the economic harm caused by this approach?
- How significant is the social harm caused by this approach?
- How likely is it that the approach will be criticised on welfare grounds?
- How likely is it that the approach will be acceptable to other stakeholders?

Where available, factual information on the costs of specialist equipment, or case studies of management costs from across the Union or third countries should be provided. When describing case studies, if the information is available then provide both total cost and the area over which control was undertaken so that a cost per unit area might be derived. Where such quantitative information is not available, then any qualitative information from the literature is acceptable to help guide decision making. It is accepted that in the majority of cases the information required to assess the potential total cost of management at a member state level is unlikely to be available. This would normally require information on the extent and abundance of the species which is beyond the scope of this assessment. Assessors are not expected to extrapolate the potential total costs of management at a member state level, only to report on the information provided within the literature.

4 Provide an overall assessment of the confidence that can be applied to the information provided for this method. This confidence should relate to the quality of the available information using the guidance below. It should NOT relate to the confidence in the effectiveness of the method

The confidence scores are:

- **High:** Information comes from published material, or current practices based on expert experience applied in one of the EU countries or third country with similar environmental, economic and social conditions.
- **Medium:** Information comes from published data or expert opinion, but it is not commonly applied, or it is applied in regions that may be too different from Europe (e.g. tropical regions) to guarantee that the results will be transposable.
- **Low:** data are not published in reliable information sources and methods are not commonly practiced or are based solely on opinion; This is for example the case of a novel situation where there is little evidence on which to base an assessment.

If there are further factors beyond these that have determined the chosen level of confidence, then provide a brief written description to support the choice of the level of confidence.

5 Measures for preventing the species being introduced intentionally or unintentionally into the territory of a Member State (cf. Articles 7(1)(a), 7(2), 13, 15), i.e. preventing a species entering by blocking its pathways, including pre-border prevention and prevention of escape or release into the environment and secondary spread whenever relevant. Measures for preventing the species reproducing while in containment should be included, if appropriate. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

6 Measures to run an effective surveillance system for achieving an early detection of a new occurrence (cf. Article 16). This section assumes that the species is not currently present in a Member State, or part of a Member State's territory.

7 Measures to achieve eradication. Preferably, but not exclusively, used at an early stage of invasion, after an early detection of a new occurrence (cf. Article 17). Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

8 Measures to achieve management of the species once it has become widely spread within a Member State territory (cf. Article 19). These measures can be aimed at eradication, population control or containment of a population of the species. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

The development and completion of this template forms part of the EU project **No 07.0202/2020/834529/ETU/ENV.D.2** Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention.

**Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention"
Contract No 07.0202/2020/834529/ETU/ENV.D.2¹**

Name of organism: *Marisa cornuarietis* (Linnaeus 1758)

Author(s) of the assessment:

Frances Lucy, IT Sligo, Sligo, Ireland and Darren Garland, IT Sligo, Sligo, Ireland

Risk Assessment Area: The risk assessment area is the territory of the European Union 27 and the United Kingdom, excluding the EU-outermost regions.

Peer review 1: *Elena Tricarico, Department of Biology, University of Florence, Sesto Fiorentino (FI), Italy*

Peer review 2 *Hugo Verreycken, INBO, Brussels, Belgium*

Date of completion: 15/10/2021

¹ This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA). A number of amendments have been introduced to ensure compliance with Regulation (EU) 1143/2014 on IAS and relevant legislation, including the Delegated Regulation (EU) 2018/968 of 30 April 2018, supplementing Regulation (EU) No 1143/2014 of the European Parliament and of the Council with regard to risk assessments in relation to invasive alien species (see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>).

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SECTION A – Organism Information and Screening

A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?

including the following elements:

- the taxonomic family, order and class to which the species belongs;
- the scientific name and author of the species, as well as a list of the most common synonym names;
- names used in commerce (if any)
- a list of the most common subspecies, lower taxa, varieties, breeds or hybrids

As a general rule, one risk assessment should be developed for a single species. However, there may be cases where it may be justified to develop one risk assessment covering more than one species (e.g. species belonging to the same genus with comparable or identical features and impact). It shall be clearly stated if the risk assessment covers more than one species, or if it excludes or only includes certain subspecies, lower taxa, hybrids, varieties or breeds (and if so, which subspecies, lower taxa, hybrids, varieties or breeds). Any such choice must be properly justified.

Response: *Marisa cornuarietis* (Linnaeus 1758) is the species for which this risk assessment has been conducted, it is a single species (no lower taxa or hybrids) belonging to the genus *Marisa* which is part of the Ampullariidae family, commonly known as the apple snails (Barker 2019).

Domain: Eukaryota

Kingdom: Metazoa

Phylum: Mollusca

Class: Gastropoda

Subclass: Caenogastropoda

Order: Architaenioglossa

Family: Ampullariidae

Genus: *Marisa*

Species: *Marisa cornuarietis*

The Ampullariidae family consists of 9 genera and 120 species (Arias & Torralba-Burrial 2014). The genus *Marisa* consists of two species: *M. cornuarietis* and *M. planogyra* (Pilsbry 1933). According to the Integrated Taxonomic Information System (www.itis.org) *Marisa cornuarietis* is commonly referred to as the giant ramshorn snail or golden horn marisa. In the RA area, the common English name for *M. cornuarietis* is giant ramshorn snail (Arias & Torralba-Burrial 2014). Other common names are: Paradiesschnecke, Brasilianische Streifenschnecke (DE).

A2. Provide information on the existence of other species that look very similar [that may be detected in the risk assessment area, either in the environment, in confinement or associated with a pathway of introduction]

Include both native and non-native species that could be confused with the species being assessed, including the following elements:

- other alien species with similar invasive characteristics, to be avoided as substitute species (in this case preparing a risk assessment for more than one species together may be considered);
- other alien species without similar invasive characteristics, potential substitute species;
- native species, potential misidentification and mis-targeting

Response: Barker (2019) details two species similar in appearance to *M. cornuarietis*, these include *M. planogyra* and *Planorbarius corneus*. *M. planogyra* is nonnative to the EU while *Planorbarius corneus* occurs throughout Europe. The general morphological features of adult *Marisa cornuarietis* according to Barker (2019) include: a dextrally coiled shell, shell in adults 18-22 mm in height, 48-56 mm in diameter, more-or-less glossy but with growth lines that are most prominent near the aperture *M. cornuarietis* is differentiated from *M. planogyra* as the latter has a smaller shell size which is about 30mm, is more strongly planispiral, with both dorsal and ventral (umbilical) aspects strongly concave and openly perspective. *Planorbarius corneus* is distinguished from *M. cornuarietis* as it only grows to a shell size of 35-40 mm, the shell is sinistral lacking spiral colour bands and the mantle cavity is sealed except for a small contractile opening (*pneumostome*). Furthermore, juveniles of *P. corneus* lack an elevated spire.

A3. Does a relevant earlier risk assessment exist? Give details of any previous risk assessment, including the final scores and its validity in relation to the risk assessment area.

Response: No risk assessment specific only to *M. cornuarietis* has previously been carried out for the RA area. However, a risk assessment of incidental molluscs associated with aquarium trade was carried out for the EU region in 2017 (Patoka *et al.* 2017). This risk assessment included *M. cornuarietis* and utilised the Freshwater Invertebrate Invasiveness Scoring Kit (FI-ISK). A scoring kit based on 49 questions focused on zoogeography, biology, and ecology (e.g. has the species established beyond its native range? Is the species' reproductive tolerance suited to climates in the risk assessment area? Is the species unpalatable to predators? Does the species tolerate a wide range of salinity regimes?). After processing, the FI-ISK distinguishes potentially invasive and non-invasive species by invasiveness score.

Patoka *et al.* (2017) concluded that *M. cornuarietis* was established in the European Union, its temperature suitability was 25.25% (based on proportion of all meteorological stations monitored within the EU), its potential invasiveness score was 15, which deemed to be of medium invasiveness in the EU (low-risk: score <1, medium-risk: score >1 and <16, and high-risk: score >16). This risk assessment is valid for the RA area of this current risk assessment for *M. cornuarietis*.

A4. Where is the organism native?

including the following elements:

- an indication of the continent or part of a continent, climatic zone and habitat where the species is naturally occurring
- if applicable, indicate whether the species could naturally spread into the risk assessment area

Response: The species native range extends from the northern part of South America (from Brazil and Bolivia to Venezuela, Colombia) to Central America (Panama, Costa Rica) (Arias & Torralba-Burrial 2014). It must be noted that its native range may only encompass Venezuela and Columbia but it is unknown if its wide ranging occurrence in the other aforementioned areas is natural or a result of introductions (Cowie *et al.* 2017). It primarily inhabits freshwater lotic and lentic systems including lakes, river, ponds, irrigation channels, swamps and wetlands where it occurs at depths of less than 1 metre (Arias & Torralba-Burrial 2014). *Marisa cornuarietis* is an omnivore renowned for its voracious appetite feeding on live or decaying plants, fish, snail eggs and juveniles (Benson *et al.* 2021). Climatic conditions have been cited as a factor limiting the establishment of this snail species as per its temperature requirements. Another limiting factor in its ability to establish new areas are physiochemical parameters of potential water bodies it could inhabit (Arias & Torralba-Burrial 2014).

The main limiting factors for survival are thermal requirements. The species thrives in water temperatures ranging from 18 to 30 °C with an ideal range of 20-26 °C (Frisóczki *et al.* 2016). At temperatures below 18 °C the snail becomes inactive, and it cannot thrive for extended periods of time below 12 °C (Frisóczki *et al.* 2016). Temperatures of 8 °C have been proven to be lethal to *M. cornuarietis* after 8 hours of exposure. Another limiting factor impeding this alien species successful establishment is calcium concentration in the water body as this organism utilises this mineral for its shell (Arias & Torralba-Burrial 2014). These factors limit the species successful establishment in the environment to areas where calcium concentrations are sufficient and year-round temperatures meet their biological requirements. The species could not naturally spread to the risk assessment area due to the sea barriers between continents.

A5. What is the global non-native distribution of the organism outside the risk assessment area?

Response: *Marisa cornuarietis* has been introduced in North America, some Caribbean islands (Cuba, Guadeloupe, Martinique, Puerto Rico) and Africa (Egypt, Sudan, Tanzania) (Arias & Torralba-Burrial 2014).

A6. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species been recorded and where is it established? The information needs be given separately for recorded (including casual or transient occurrences) and established occurrences. “Established” means the process of an alien species successfully producing viable offspring with the likelihood of continued survival².

² Convention on Biological Diversity, Decision VI/23

A6a. Recorded: List regions

A6b. Established: List regions

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

- Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea.

Comment on the sources of information on which the response is based and discuss any uncertainty in the response.

For delimitation of EU biogeographical regions please refer to <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2> (see also Annex VI).

For delimitation of EU marine regions and subregions consider the Marine Strategy Framework Directive areas; please refer to <https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions/technical-document/pdf> (see also Annex VI).

Response (6a): The species was first recorded in the risk assessment area in 2014 in the Mediterranean biogeographic region (northern Spain, Arias & Torralba-Burrial 2014) and has subsequently been found in the Pannonian biogeographic region (Hungary) at the outflow of a thermal spa (Cowie *et al.* 2017).

Response (6b): Due to its viable reproduction in the risk assessment area since 2014, it is deemed to be established in both the Mediterranean and in the Pannonian biogeographical regions (Arias & Torralba-Burrial 2014, Cowie *et al.* 2017)

A7. In which biogeographic region(s) or marine subregion (s) in the risk assessment area could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A7a. Current climate: List regions

A7b. Future climate: List regions

With regard to EU biogeographic and marine (sub) regions, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)

- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (7a): Under current climate conditions, according to a Species Distribution model (Annex VIII), *M. cornuarietis* could potentially establish in several biogeographical regions of the risk assessment area including the Continental, Alpine, Atlantic and Black Sea regions. This is in addition to expansion in the Mediterranean and the Pannonian regions. Figure 5 illustrates the projected the current suitability of regions in the RA area. Note that the current model suggests that it should not be present in the Pannonian region, although present there in water associated with a thermal spring.

Response (7b): Predicted future climate conditions for the 2070s under both scenarios (RCP2.6, RCP4.5) indicate a low probability of *M. cornuarietis* establishing in the Alpine, Pannonian and Steppic regions, and also extending within the Atlantic, Black Sea, Continental and Mediterranean regions, compared to the present climatic conditions within the RA area. Increase in average winter temperature is the most likely factor to increase probability of projected suitability for this species. Figure 8 illustrates projected future suitability (2070s) of regions in the RA area under climate change scenario RCP4.5.

A8. In which EU Member States has the species been recorded and in which EU Member States has it established? List them with an indication of the timeline of observations. The information needs be given separately for recorded and established occurrences.

A8a. Recorded: List Member States

A8b. Established: List Member States

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden.

The description of the invasion history of the species shall include information on countries invaded and an indication of the timeline of the first observations, establishment and spread.

Response (8a): The species was first recorded in the risk assessment area in 2014 in northern Spain (Arias & Torralba-Burrial 2014) and has been found in one area in Hungary close to the outflow of a thermal spa in 2015-2016 (Cowie *et al.* 2017). In Spain, Arias & Torralba-Burrial (2014) state that several specimens of *M. cornuarietis* were reported from multiple searches of the Nora River with adults and juveniles found indicating a thriving and reproducing population. In Hungary, the

population occurring downstream of a thermal spa in the Eger stream had a mixture of both adult and juvenile individuals indicating a reproducing population in the locality (Cowie *et al.* 2017).

Response (8b): The information from both the Spanish and Hungarian populations indicate that populations are established there within at least the last ten years (Arias & Torralba-Burrial 2014, Cowie *et al.* 2017).

A9. In which EU Member States could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A9a. Current climate: List Member States

A9b. Future climate: List Member States

With regard to EU Member States, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (9a): Under current climate conditions, the SDM (Annex VIII) indicates that *M. cornuarietis* could establish in the UK, France, Spain, Italy, Slovenia, Greece, Portugal and Croatia. There is a degree of uncertainty, as noted in Figure 5b and Table 1.

Response (9b): In the 2070s, under climate change scenario RCP 4.5, *M. cornuarietis* could establish in many EU Member States. In particular, there seems to be a high establishment potential for the majority of Member States with increased potential (compared to current conditions) for Austria, Belgium, Bulgaria, Czech Republic, Denmark, Finland, Germany, Ireland, Luxembourg, the Netherlands and Sweden (Figure 7a) (Annex VIII). There is however considerable uncertainty associated with the model. Increase in average winter temperature is the most likely climate change factor to increase probability of establishment.

A10. Is the organism known to be invasive (i.e. to threaten or adversely impact upon biodiversity and related ecosystem services) anywhere outside the risk assessment area?

Response: The species is currently considered invasive in the USA and in Egypt, Tanzania and the Caribbean. *M. cornuarietis* impact on biodiversity and ecosystem services where introduced is directly related to density, with few impacts observed where densities remain low (Arias & Torralba-Burrial 2014). For example, in the USA alien populations of *M. cornuarietis* have shown to negatively impact macrophytes when the snail density becomes high with reduced macrophyte diversity and species elimination potentially occurring (Arias & Torralba-Burrial 2014). For this reason, in Puerto Rico and Florida, the species has been introduced for the purposes of aquatic weed control (Barker 2019).

This species is a voracious omnivore which can directly impact native snail populations through direct competition and predation (Arias & Torralba-Burrial 2014). The food habits of *M. cornuarietis* has previously led this species to be utilised as a biological control agent to control the snail vectors of *Schistosoma* spp., the cause of human schistosomiasis, through competition and predation, in Egypt, Tanzania and the Caribbean (Cowie *et al.* 2017). However, due to its invasive features, the introduction of the species for the purposes of controlling other organisms is no longer considered environmentally acceptable (Barker 2019).

A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea

Response: The species has only been recorded in the wild in two biogeographical regions of the risk assessment area (Arias & Torralba-Burrial 2014, Cowie *et al.* 2017): Pannonian and Mediterranean. In these areas the species has become established, but no impact is currently known, although it is expected they will show signs of invasiveness in the future when they occur in high densities..

A12. In which EU Member States has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden

Response: Reported occurrences of the species do not report signs of invasive characteristics in the risk assessment area. However, the species has been reported as alien and established in Spain and it is expected that indicators of invasiveness will increase in the future with increased population densities. Particularly in Spain where the climate facilitates the widespread occurrence of the species. In Hungary, the negative impacts of the species may be localised to hydrothermal waters.

A13. Describe any known socio-economic benefits of the organism.

including the following elements:

- Description of known uses for the species, including a list and description of known uses in the risk assessment area and third countries, if relevant.
- Description of social and economic benefits deriving from those uses, including a description of the environmental, social and economic relevance of each of those uses and an indication of associated beneficiaries, quantitatively and/or qualitatively depending on what information is available.

If the information available is not sufficient to provide a description of those benefits for the entire risk assessment area, qualitative data or different case studies from across the risk assessment area or third countries shall be used, if available.

Response: *M. cornuarietis* is a known biological control agent beneficial to humans under certain circumstances outside the RA area. It has been used to control snail vectors of *Schistosoma* spp., the cause of human schistosomiasis (Cowie *et al.* 2017). The control is through predation and competition with the potential to eradicate other snail species which are vectors of this parasitic disease (Pointier & David 2004). In addition, this species has previously been used to control aquatic weeds due to its voracious appetite in multiple weed control programs (Barker 2019). *M. cornuarietis* has been introduced to multiple territories outside of the risk assessment area for the control of both weeds and schistosomiasis (Arias & Torralba-Burrial 2014).

M. cornuarietis is also widely utilised as an aquatic pet and is traded worldwide in aquatic retail outlets but it is not possible to quantify the economic benefits of the trade of this species. Furthermore, the species is routinely used in laboratory and education settings due to its availability via aquatic retail (Barker 2019).

SECTION B – Detailed assessment

Important instructions:

- In the case of lack of information the assessors are requested to use a standardized answer: “No information has been found.” In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable).
- With regard to the scoring of the likelihood of events or the magnitude of impacts see Annexes I and II.
- With regard to the confidence levels, see Annex III.
- Highlight the selected response score and confidence level in **bold** but keep the other scores in normal text (so that the selected score is evident in the final document).

1 PROBABILITY OF INTRODUCTION AND ENTRY

Important instructions:

- **Introduction** is the movement of the species into the risk assessment area (it may be either in captive conditions and/or in the environment, depending on the relevant pathways).
- **Entry** is the release/escape/arrival in the environment, i.e. occurrence in the wild
- Introduction and entry may coincide for species entering through pathways such as “corridor” or “unaided”, but it also may differ. If different, please consider all relevant pathways, both for the introduction into the risk assessment area and the entry in the environment.
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used (see Annex IV). For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document³ and the provided key to pathways⁴.
- For organisms which are already present (recorded or established) in the risk assessment area, the likelihood of introduction and entry should be scored as “very likely” by default.
- Repeated (independent) introductions and entries at separate locations in the risk assessment area should be considered here (see Qu. 1.7).

Qu. 1.1. List relevant pathways through which the organism could be introduced into the risk assessment area and/or enter into the environment. Where possible give details about the specific origins and end points of the pathways as well as a description of any associated commodities.

For each pathway answer questions 1.2 to 1.8 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 1.2a, 1.3a, etc. and then 1.2b, 1.3b etc. for the next pathway.

In this context a pathway is the route or mechanism of introduction and/or entry of the species.

³ <https://circabc.europa.eu/sd/a/7e5f0bd4-34e8-4719-a2f7-c0cd7ec6a86e/2020-CBD-pathways-interpretation.pdf>

⁴ <https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf>

The description of commodities with which the introduction of the species is generally associated shall include a list and description of commodities with an indication of associated risks (e.g. the volume of trade; the likelihood of a commodity being contaminated or acting as vector).

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 1.2-1.9.

Pathway name: Two main pathways of introduction for the species have been defined:

Escape from confinement – Pet/Aquarium/Terrarium species (including live food for such species)

Marisa cornuarietis is utilised for aquarium trade, laboratory studies (ecotoxicological studies) and educational purposes (primarily due to its availability via the aquatic retail).

It has previously been introduced to non-native regions through the aquarium trade (Pointier & David 2004). Establishment of the species in the wild has been attributed to the deliberate dumping of unwanted aquarium contents into natural water bodies (Barkler. 2019). The species have been utilised in the aquarium trade since the 1930s and contemporarily, the species is available to purchase both online and in aquatic retail outlets (Benson *et al.* 2021).

Contaminant on plants (excluding parasites and species transported by host and vector)

Another pathway for introduction is contamination on aquatic plants. *Marisa cornuarietis* has been noted to be introduced via attaching to floating macrophytes in the wild (Benson *et al.* 2021).

Individual specimens can be introduced on aquatic plants utilised for the aquatic plant trade and pond gardening and landscaping. This pathway is believed to have contributed to the introduction of this apple snail species to new regions (Barker 2019).

Escape from confinement – Pet/Aquarium/Terrarium species (including live food for such species)

Qu. 1.2a. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: *M. cornuarietis* introduction to its non-native regions through this pathway can be both intentional and unintentional. The species has previously been introduced to the wild through the intentional disposal of aquarium contents in areas outside of the RA such as Florida and the Caribbean islands (Pointier & David 2004), which is considered being an active pathway of introduction and entry also for the risk assessment area. From August 2013, all species of Ampullariidae have been included in the Spanish legislation (Royal Decree 630/2013) as invasive species and listed in the Catálogo Español de Especies Exóticas Invasoras. Listing demands that procedures are in place to prevent introduction and establishment in Spain and its European territories (CABI.org). Since 2013 the

intentional release of *Marisa cornuarietis* to the wild is prohibited within the European Union (Frisóczki *et al.* 2016).

Unintentional escape from confinement, e.g. aquaria, can also occur (Arias & Torralba-Burrial 2014). It is believed that this is how the species first entered the wild in the RA area (Arias & Torralba-Burrial 2014, Frisóczki *et al.* 2016).

Qu. 1.3a. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

Including the following elements:

- Discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- If relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: It is envisaged that the introduction of non- native species is likely to increase with increased global trade (Patoka *et al.* 2018). Contemporarily, it is likely a significant number of specimens of *Marisa cornuarietis* can potentially be introduced via this pathway as the species is commonly traded in aquatic retail outlets inside the RA region. Published literature only provides broad generalisations with regard to the number of individuals coming into the RA area for the pet trade, but the frequency is believed to be continuous as the target species is available for purchase online year round. The introductions and entry into the wild of *Marisa cornuarietis* in two separated biogeographical regions in the RA area are both suspected to be a result of the discarding of aquarium contents (Arias & Torralba-Burrial 2014, Frisóczki *et al.* 2016). Both introductions occurred within a short timeframe believed to be in the 2010s with both regions having regulations prohibiting the introduction of *Marisa cornuarietis* to the wild. Furthermore, both entries into the wild resulted in established populations. Due to the widespread availability of the species for the pet trade, reinvasion is likely to occur in the RA area if the species has been eradicated in the wild due to the possibility of further intentional or unintentional releases.

Qu. 1.4a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *M.cornuarietis* is regularly imported into the RA area for the purposes of aquatic ornamental trade. The cited primary risk of spread for this species is aquatic trade (Barker. 2019) It is highly likely it will survive transport and subsequent storage in aquariums because the retailers create optimal conditions for transport. Furthermore, the species commonly reproduces in aquarium settings where its numbers are likely to increase.

Qu. 1.5a. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The management practices currently in place to prevent the introduction of this organism via this pathway are limited to legislation prohibiting the introduction of the species to the wild (Arias & Torralba-Burrial . 2014). Since 2013 the intentional release of *Marisa cornuarietis* to the wild is prohibited within the European Union (Frisóczki *et al.* 2016). The species is sold via the aquarium trade throughout the RA area and they breed in captivity.

Qu. 1.6a. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely	CONFIDENCE	low medium
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	moderately likely likely very likely		high
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Response: The organism is very likely to be introduced into the RA area via the aquarium trade and to enter the environment via this pathway as eggs of individual specimens. These could unintentionally be introduced to the environment through either discarding contents of an aquarium or during routine tank maintenance (Frisoczki *et al.* 2016). Furthermore, adult specimens could be introduced unintentionally via this pathway and go undetected in the natural environment for a significant period of time.

Qu. 1.7a. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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Response: The introduction and potential release of this non-native species through this pathway is widespread throughout the RA area due to its widespread trade for aquaria as an aquarium pet (CABI.org; www.aquariumnexus.com). and widespread proximity to both lotic and lentic freshwater environments.

Qu. 1.8a. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: In general, the aquarium trade is one of the primary pathways of freshwater snails being translocated around the world (Appleton & Miranda 2015). *M. cornuarietis* is continually being utilised in the RA area for aquaria, making it currently a very likely pathway for introduction. Evidence based on the currently recorded occurrences of the species to the wild in the RA area were all associated with the aquarium pet trade making it highly plausible that the likelihood of further introductions of this species are inevitable under current management strategies.

Pathway Name: Contaminant on plants (excluding parasites and species transported by host and vector)

Qu. 1.2b. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: Introduction via this pathway is unintentional as *Marisa cornuarietis* is a contaminant on aquatic plants sold for trade purposes (Barker 2019).

Qu. 1.3b. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

Including the following elements:

- Discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The transport and sale of aquatic plants for trade purposes is a significant economic activity which occurs throughout the year in the RA area. Given that *M. cornuarietis* can be a contaminant on plants it is very likely that a large number of specimens enter the RA area. In terms of the number of specimens which reach the natural environment in the RA area via this pathway, no data are available. One fertilized female which spreads through this pathway has the potential to produce hundreds of eggs or more as they can store sperm needed in their genital tract. It is suspected that the species was

introduced to the environment via the disposal of aquarium contents in other regions (Barker 2019), suggesting it is highly plausible that the species is and will continue to be introduced unintentionally to the environment.

Qu. 1.4b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Given that the species is transported and sold via aquatic trade (Barker 2019), it is highly likely that both its adults, eggs and juveniles will survive via this pathway as aquatic plants will come from regions inhabited by *M. cornuarietis* and have similar thermal requirements to the target species facilitating its survival and reproduction from the transport and storage of the plant matter.

Qu. 1.5b. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Plants transported for aquarium trade that the species could potentially hitchhike on would more than likely have thermal requirements that would facilitate the survival of the species. Current management practices permit its safe passage in the RA area from this pathway as the species is not on the EPPO lists and no specific biosecurity measures are apparently currently in place during inspections at EU borders to prevent the species from using this pathway.

Qu. 1.6b. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species has previously been found in the wild in the RA area both in Hungary and Spain. On both occasions the origin for entry was suspected to have been via the disposal of aquarium contents and/or aquatic plant trade or direct trade of the species (Arias & Torralba-Burrial 2014, Frisóczki *et al.* 2016). There is a very low chance of noticing anyone disposing of aquatic plants that are contaminated with this species. Given the species large size, it may be found in the wild via searches by experienced personnel and citizen scientists trained to identify this species (Barker 2019). However, populations in the wild may not be identified until they are established in a region.

Qu. 1.7b. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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Response: Aquatic plant trade and aquarium pet trade are widespread occurring year round in the RA area. This indicates that *M. cornuarietis* can be introduced or enter the natural environment via this pathway in a significant proportion of the RA area.

Qu. 1.8b. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Given that the two recorded establishments in the RA area were suspected of coming from this pathway, it is highly indicative that the introduction and entry of this species has previously occurred and will likely occur again under current management strategies as no biosecurity protocols are in place to prevent its transport via this pathway.

Qu. 1.9. Estimate the overall likelihood of introduction into the risk assessment area or entry into the environment based on all pathways and specify if different in relevant biogeographical regions in current conditions.

Provide a thorough assessment of the risk of introduction in relevant biogeographical regions in current conditions: providing insight into the risk of introduction into the risk assessment area.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species is already present in the RA area. The Aquarium trade specifically aimed at the sale of this species, and the aquarium plant trade through which *M. cornuarietis* may be introduced into the risk assessment area as a contaminant, are the two most likely pathways. The two known introductions in the RA area are associated with these activities in both the Mediterranean and Pannonian biogeographic region (Arias & Torralba-Burrial 2014, Cowie *et al.* 2017).

Qu. 1.10. Estimate the overall likelihood of introduction into the risk assessment area or entry into the environment based on all pathways in foreseeable climate change conditions?

Thorough assessment of the risk of introduction in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of introduction (e.g. change in trade or user preferences)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely introduction within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely	CONFIDENCE	low medium
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	moderately likely likely very likely		high
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Response: Although the number of introductions is unlikely to change under future climate conditions, this warm water species is likely to be introduced to more biogeographical regions where the potential for establishment is high under predicted climate models (Annex VIII) (Beckman *et al.* 2021). Predicted increases in annual precipitation and temperature will make these biogeographical regions susceptible to introductions and facilitate the establishment of the species in these biogeographical regions of the RA area.

2 PROBABILITY OF ESTABLISHMENT

Important instructions:

- For organisms which are already established in parts of the risk assessment area or have previously been eradicated, the likelihood of establishment should be scored as “very likely” by default.
- Discuss the risk also for those parts of the risk assessment area, where the species is not yet established.

Qu. 2.1. How likely is it that the organism will be able to establish in the risk assessment area based on similarity of climatic and abiotic conditions in its distribution elsewhere in the world?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species is already established in both Hungary and Spain (Mediterranean and Pannonian biogeographic regions), although their occurrence in Hungary is limited to the outflow of a thermal spa as natural climatic conditions in Hungary do not support the species year round, which impedes their further spread in Hungary (see section A4 and Annex VIII). Other biogeographical regions which have a climate that can support the species requirements in which there are water bodies that have sufficient calcium concentrations (below 25 mg/l deemed suboptimal according to Arias & Torralba-Burrial 2014) are susceptible to introduced populations becoming established (Annex VIII). No information is available in regard to the extent of water bodies in which abiotic conditions are currently suitable for *M. cornuarietis* in the risk assessment area. However, information has been published on this species relating to the extent of areas where climatic conditions are currently suitable in the EU, a region encompassing the majority of the RA area (Patoka *et al.* 2017). Temperature suitability predictions based on all EU meteorological stations estimate 25.25% of the regions with currently climatic conditions suitable for the establishment of *M. cornuarietis*.

Qu. 2.2. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area? Consider if the organism specifically requires another species to complete its life cycle.

RESPONSE	very isolated isolated moderately widespread widespread ubiquitous	CONFIDENCE	low medium high
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Response: *M. cornuarietis* can inhabit a multitude of lotic and lentic habitats such as lakes, river, ponds, irrigation channels, swamps and wetlands where it occurs at depths of less than 1 metre (Arias & Torralba-Burrial 2014). These habitats are widespread in the RA area suggesting that where climatic and abiotic factors are favourable these habitats are suitable for the establishment of populations of *M. cornuarietis*.

In terms of other organisms needed for its establishment, *Marisa cornuarietis* is an omnivore renowned for its voracious appetite feeding on live or decaying plants, fish, snail eggs, and juvenile snails (Benson *et al.* 2021). This generalist feeding behavior suggests that where the species is introduced to natural waters and where abiotic and climatic factors are favourable, it will thrive. Furthermore, as the species is already established in two, separate biogeographical regions of the RA area, habitats and the species required as food for its establishment are available in the RA region.

Qu. 2.3. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: There is limited information available in regard to species that will compete with *M. cornuarietis* in the RA area. Furthermore, there is limited information from other regions of the world indicating species that compete with *M. cornuarietis*. On the contrary, in many published works, the ability of *M. cornuarietis* to outcompete native snails and significantly alter macrophyte community structure (Arias & Torralba-Burrial 2014; Seaman & Porterfield 1964; Benson *et al.* 2021) in their newly established habitats indicates they are an ecosystem engineer. In any case, the establishment of this species in two separate biogeographical region of the RA signifies that it is able to establish despite the presence of other species with which it may compete.

Qu. 2.4. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Similar to competition, there is limited information on predators, pathogens or parasites which will impede the establishment of the species in areas with suitable climatic conditions (Seaman and Porterfield 1964; Snyder and Kale 1983; Howells et al. 2006). However, the establishment of the species in two separate biogeographical regions of the RA indicate that establishment has occurred without the influence of avian or mammal predators, molluscan parasites or pathogens impeding the process.

Qu. 2.5. How likely is the organism to establish despite existing management practices in the risk assessment area? Explain if existing management practices could facilitate establishment.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: In certain regions, e.g. Belgium (Species Act 2009), and Spain the introduction of *Marisa cornuarietis* is prohibited. From August 2013, all species of Ampullariidae have been included in the Spanish legislation (Royal Decree 630/2013) as invasive species and listed in the Catálogo Español de Especies Exóticas Invasoras. However, the species has been introduced and entered into the wild and established populations in regions where such introductions are prohibited (Arias & Torralba-Burrial 2014, Frisóczki *et al.* 2016). As the species is commonly traded for aquatic retail, it is difficult to control incidences of entry into the wild and establishment in the RA either from intentional or unintentional introductions.

Qu. 2.6. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: No information was found in relation to eradication techniques of *Marissa cornuarietis*. However, information in relation to the control of apple snails, the family to which *Marisa cornuarietis* belongs, was available. Currently no management practices to control introduced populations in the RA area have been implemented.

Multiple control techniques for apple snails have been developed which include mechanical, chemical and biological control methods. Mechanical, control methods commonly used include snail traps, hand collection and mechanical collection (Gilioli *et al.* 2017). Biological controls methods include the use of molluscivorous fish and ducks (Teo 2001, Ip *et al.* 2014). Chemical control for apple snails routinely used molluscicides (Schnorbach *et al.* 2006). It is plausible that control methods listed above could be utilised for *M. cornuarietis* given the similarities between species upon which the control mechanisms were applied. However, *M. cornuarietis* may survive control techniques where the established population of the species in the RA is not isolated. In interconnected water courses where the species has spread sufficiently the species may avoid these control mechanisms.

Qu. 2.7. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?

including the following elements:

- a list and description of the reproduction mechanisms of the species in relation to the environmental conditions in the risk assessment area
- an indication of the propagule pressure of the species (e.g. number of gametes, seeds, eggs or propagules, number of reproductive cycles per year) of each of those reproduction mechanisms in relation to the environmental conditions in the risk assessment area.
- If relevant, comment on the likelihood of establishment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in establishment whereas for others high propagule pressure (many thousands of individuals) may not.
- If relevant, comment on the adaptability of the organism to facilitate its establishment and if low genetic diversity in the founder population would have an influence on establishment.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: 25.25% of the RA area is suitable in terms of temperature for this invasive species (Patoka *et al.* 2017). Furthermore, these areas support habitats with suitable water quality parameters for the species and it has already established reproductive populations in two biogeographical regions of the RA area indicating its biological characteristics are suitable for establishment in the RA area. *Marisa cornuarietis* reproduces sexually with breeding occurring in groups. Females are estimated to produce 1700 eggs per year and they have the ability to store sperm in their genital tract for months which facilitates spawning to coincide with favorable environmental conditions (Barker 2019). This indicates

that even very few introduced specimens could facilitate the establishment of new populations within the RA area.

Qu. 2.8. If the organism does not establish, then how likely is it that casual populations will continue to occur?

Consider, for example, a species which cannot reproduce in the risk assessment area, because of unsuitable climatic conditions or host plants, but is present because of recurring introduction, entry and release events. This may also apply for long-living organisms.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: This species has established two populations in the RA area in the last decade. Considering its availability in the ornamental trade, casual populations may occur in areas which are subject to continuing introductions even if their climatic conditions are not suitable to for the survival of the species throughout the year (for example, *Marisa cornuarietis* is not well adapted to surviving for extended periods of time below 12 °C, as reported by Frisóczki *et al.* 2016).

Qu. 2.9. Estimate the overall likelihood of establishment in the risk assessment area under current climatic conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under current climatic conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in current conditions: providing insight in the risk of establishment in (new areas in) the risk assessment area.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species is already established in two biogeographical regions of the RA area (Arias & Torralba-Burrial 2014, Frisóczki *et al.* 2016). A previous risk assessment for the EU that was completed in 2017 detailed 25.25% of the EU was suitable for the establishment of *M. cornuarietis* under current climatic conditions (Patoka *et al.* 2017). This information indicates that the species can establish in multiple biogeographical regions of the RA area (Annex VIII).

Qu. 2.10. Estimate the overall likelihood of establishment in the risk assessment area under foreseeable climate change conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under foreseeable climate change conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of establishment (e.g. increase in average winter temperature, increase in drought periods)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely establishment within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Under future predicted climate models for the 2070s (both RCP 2.6 and RCP 4.5) the species is likely to establish in more biogeographical regions (Annex VIII) in comparison to current conditions. Annual precipitation and annual minimum winter temperatures have been identified as drivers in predicting the suitability of an area to *M. cornuarietis* establishment in the future with increases in both cited as a driver of establishment in new areas. Individual biogeographical suitability under predicted climate conditions for *M. cornuarietis* are illustrated in Figures 7 and 8 in Annex VIII.

3 PROBABILITY OF SPREAD

Important instructions:

- Spread is defined as the expansion of the geographical distribution of an alien species within the risk assessment area.
- Repeated releases at separate locations do not represent continuous spread and should be considered in the probability of introduction and entry section (Qu. 1.7).

Qu. 3.1. How important is the expected spread of this organism within the risk assessment area by natural means? (List and comment on each of the mechanisms for natural spread.)

including the following elements:

- a list and description of the natural spread mechanisms of the species in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of spread discussed in relation to the species biology and the environmental conditions in the risk assessment area.

The description of spread patterns here refers to the CBD pathway category “Unaided (Natural Spread)”. It should include elements of the species life history and behavioural traits able to explain its ability to spread, including: reproduction or growth strategy, dispersal capacity, longevity, dietary requirements, environmental and climatic requirements, specialist or generalist characteristics.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: In the two regions where *M. cornuarietis* has established, it is expected to spread in Spain only due to the availability of habitat and suitable climatic conditions. The population downstream of a thermal spa in Hungary is expected not to spread as the thermal requirements of the species will not be supported year-round in the region.

M. cornuarietis has been noted to be able to spread naturally in localised areas (Barker 2019) often thriving due to generalist feeding behavior. The species is largely immobile and its spread is limited by water parameters, hydrological characteristics of the catchment and the species thermal requirement (Barker 2019). Spread has also been noted following stream flooding during storms (Hunt 1958). Its life cycle is dependent on water temperature with specimens surviving for a maximum of two year (Benson *et al.* 2021). Furthermore, the species has been noted to spread downstream of localised sites on floating vegetation (Barker 2019) but no rate of spread has been found in the literature.. Females which spread to new areas may be able to spawn due to their ability to store sperm for months at a time which facilitates the establishment of new populations in new areas. Furthermore, it can produce up to 1700 eggs per year and spawn year-round under favourable conditions (Barker 2019, Benson *et al.* 2021).

Qu. 3.2a. List and describe relevant pathways of spread other than "unaided". For each pathway answer questions 3.3 to 3.9 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 3.3a, 3.4a, etc. and then 3.3b, 3.4b etc. for the next pathway.

including the following elements:

- a list and description of pathways of spread with an indication of their importance and associated risks (e.g. the likelihood of spread in the risk assessment area, based on these pathways; likelihood of survival, or reproduction, or increase during transport and storage; ability and likelihood of transfer from the pathway to a suitable habitat or host) in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of spread for each pathway discussed in relation to the species biology and the environmental conditions in the risk assessment area.
- All relevant pathways of spread (except “Unaided (Natural Spread)”, which is assessed in Qu. 3.1) should be considered. The classification of pathways developed by the Convention of Biological Diversity shall be used (see Annex IV).

Pathway names: Three pathways of spread have been identified: **Corridor – Interconnected waterways / basins / seas (A)**, **Unintentional – Transport stowaway Angling equipment/ boat hull fouling (B)** and **Unintentional- Transport –Contamination on plants(C)**. These pathways importance for the spread of *M. cornuarietis* is currently undefined in the RA area.

Qu. 3.3a. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: In the RA area, the majority of water bodies are interconnected in some way which provides corridors of spread. allowing passive drift with water currents to facilitate settlement and establishment. For example, waterways and reservoirs connecting the Danube and Rhine basins have facilitated the establishment of the quagga mussel and later spread between previously isolated catchments (Bij de Vaate et al., 2013)

Qu. 3.4a. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway

- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: It is very likely that a sufficient number of individuals may spread along this pathway to originate a viable population. One fertilized female which spreads through this pathway has the potential to produce hundreds of eggs or more as they can store sperm needed in their genital tract (Howells et al. 2006). Reinvasion is highly likely if the waterbody is interconnected to a waterbody with an established population of *M. cornuarietis*. No data is available on numbers spread along this pathway.

Qu. 3.5a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE		CONFIDENCE	
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Response: The question does not apply for this pathway

Qu. 3.6a. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Currently, there are no known legislated management practices for this species in place in the RA area to limit their spread once established. In regions outside the RA area there is no published literature on management techniques for stopping the spread of the species in waterways. The species is likely to survive any engineering of waterways, e.g. development of reservoirs or diversion of river sections.

Qu. 3.7a. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: No information has been found in relation to monitoring of the species in the RA area. As this species is aquatic and its established populations are not routinely monitored under EU regulations e.g. Habitats and Water Framework Directive, there will probably be a lag between when a species invades a new area and when it is detected. However, monitoring for other snails or invertebrates may coincide with the early detection of this species if personnel are aware of the taxonomic characteristics of the species in order to identify their presence in non-native regions.

Qu. 3.8a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: 25.25% of the RA area is suitable for *M. cornuarietis* in terms of suitable water temperature (Patoka *et al.* 2017). Furthermore, these thermally suitable areas support habitats with suitable physiochemical parameters (calcium concentrations) for *M. cornuarietis*. The species is also a generalist omnivore (Arias & Torralba-Burrial 2014). These aquatic corridors which occur throughout the RA area facilitate the species spread to new habitats suitable for its establishment. Additionally, the climate modelling provided in the Annex indicated several regions are currently suitable for *M. cornuarietis* in the RA area.

Qu. 3.9a. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area. (please provide quantitative data where possible).

RESPONSE	very slowly slowly	CONFIDENCE	low medium
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	moderately rapidly very rapidly		high
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Response: No information is available in relation to the rate of spread using this pathway. Given that there are still relatively low numbers of established populations in the RA area (one of which is isolated and not spreading), the rate of spread is considered moderate. The environmental conditions facilitating spread through this pathway are restricted in terms of temperature and physiochemical parameters of interconnected water bodies.

Qu. 3.3b. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Angling forms a significant proportion of recreational activities in freshwater sites in the RA area, However, spread via this pathway is generally unintentional as eggs, juveniles and adult specimens of *Marisa cornuarietis* can attach to nets, boats, waders etc and be transported to other water bodies. This is aided by the species capacity to aeriually respirate via a lung (Barker 2019),

Qu. 3.4b. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Due to the species high fecundity rate in conjunction with the potential for eggs to be transported via this pathway, it is highly likely a sufficient number of individuals could be transported to originate a viable population over the course of one year. A single female is estimated to produce 1700 eggs per year and they have the ability to store sperm in their genital tract for months which facilitates spawning to coincide with favorable environmental conditions (Barker 2019).

Qu. 3.5b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Local spread due to anglers moving between water bodies, it is highly likely as the species will survive as in egg masses and in adults due to their capacity to breathe terrestrially via a lung. Reproduction is unlikely during transport. It must be noted that eggs could be attached to angling equipment and the number of specimens transported may be increased

Qu. 3.6b. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Indirect biosecurity measures applied by recreational users of water bodies in the RA area may impede the spread of this invasive. Disinfectants commonly used for cleaning angling equipment for biosecurity purposes may potentially be useful against the spread of this invasive (De Stasio *et al.* 2019). However, it must be noted that these measures are usually the responsibility of individuals to disinfect their equipment before using water bodies and disinterest or lack of public awareness in recreational anglers may facilitate the spread of *Marisa cornuarietis*. Due to this it is likely a certain proportion of stowaways on angling equipment. In terms of boat hulls, eggs and snails can attach facilitating the spread of the species to new water bodies if biosecurity measures are not applied,

Qu. 3.7b. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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As no information is available regarding the monitoring of this species in the RA area, it is plausible that the species may spread from angling activities from one water body to another. This is especially the case with the eggs which could easily go undetected in apparatus containing water or moist keep nets.

Qu. 3.8b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low <i>medium</i> high
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Stowaways on angling equipment generally facilitate local spread. Due to this, the climatic conditions of areas where established populations are being transported from are generally similar thus allowing organisms to establish in new waterways where suitable physico-chemical parameters facilitate.

Qu. 3.9b. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area. (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Given that there are still relatively low numbers of established populations in the RA area (one of which is isolated and not spreading), the rate of spread is considered moderate. Environmental conditions facilitating spread through this pathway are restricted in terms of temperature and physiochemical parameters and spread is considered local/regional.

Qu. 3.3c. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Spread along this pathway is unintentional as *Marisa cornuarietis* specimens and eggs can be unintentionally transported to new water bodies through commercial trade of plants for gardening and landscaping and aquatic plant trade (Barker. 2019).

Qu. 3.4c. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Due to the reproductive capacity of the species and the capacity for eggs to be transported attached to plants (Barker 2019), it is very likely that this method of spread can facilitate populations spread to create independent new populations of the species over the course of one year.

Qu. 3.5c. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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It is highly likely that species can survive and reproduce during transport and storage along this pathway as transported plants, especially those selected for the aquatic ornamental trade will likely have similar temperature and physicochemical requirement similar to the invasive (Barker 2019). Traders will create ideal conditions for transport of the plants and therefore conditions will also be ideal for *M. cornuarietis*.

Qu. 3.6c. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely	CONFIDENCE	low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

No specific management practices exist and therefore the species is likely to survive.

Qu. 3.7c. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely	CONFIDENCE	low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

The species is likely to spread undetected in the RA area. Response: No information has been found in relation to monitoring of the species in the RA area. As this species is aquatic and its established populations are not routinely monitored under EU regulations e.g. Habitats and Water Framework Directive, there will probably be a lag between when a species invades a new area and when it is detected. However, monitoring for other snails or invertebrates may coincide with the early detection of this species if personnel are aware of the taxonomic characteristics of the species in order to identify their presence in non-native regions.

Qu. 3.8c. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely	CONFIDENCE	low
	unlikely		medium
	moderately likely		high
	likely		

	very likely		
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This is considered highly likely as plants from which *M.cornuarietis* are hitchhikers generally have similar environmental requirements as the invasive, furthermore, it has been highlighted that introductions of this invasive has occurred in other regions due to the hitchhiking on aquatic plants (Barker 2019).

Qu. 3.9c. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area. (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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25.25% of the RA area is deemed suitable for *Marisa cornuarietis* with regard to temperature (Patoka et al. 2017), these thermally suitable areas support habitats which are optimal in terms of their physicochemical attributes for this invasive. This in conjunction with the widespread transport of plant material, for horticulture, gardening and aquaria, further exacerbates the rate of spread through this pathway in the RA area.

Qu. 3.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?

RESPONSE	very easy easy with some difficulty difficult very difficult	CONFIDENCE	low medium high
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Response: In the RA area, containment of populations who utilise these pathways of spread is very difficult. In ponds, which are not interconnected with other water bodies, containment may be possible (Pointier & David 2004). However, in interconnected and large waterbodies containment will almost be impossible. The high potential of *M. cornuarietis* specimens and eggs to attach on floating vegetation and being carried with water currents to new areas and establish at considerable distances from its original sites is the main factor for the difficulty of containment. Additionally, specimens may also be swept away in strong currents. The species will also potentially spread to new areas unnoticed due to monitoring programs not being in place to identify this species. Round goby, zebra mussel and

quagga mussel are examples of aquatic invasive species that have spread throughout interconnected waterways in the RA area (Bij de Waate et al. 2002, 2013)

Qu. 3.11. Estimate the overall potential rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (indicate any key issues and provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in current conditions, providing insight in the risk of spread into (new areas in) the risk assessment area.

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: A previous risk assessment conducted by Patoka *et al* (2017) determined that climatic conditions were currently suitable for *Marisa cornuarietis* in 25.25% of the RA area. As the species thrive in water temperatures from 18 to 30 °C with an ideal range of 20- 26 °C (Frisóczyki *et al.* 2016) the areas to which it can spread to is limited but still significant in the RA area. Their spread is further restricted by their calcium requirements for shell building (Barker 2019).

Qu. 3.12. Estimate the overall potential rate of spread in relevant biogeographical regions in foreseeable climate change conditions (provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if rates of spread are likely slowed down or accelerated.

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: The rate of spread is not expected to change, however both, the establishment rate and the biogeographical regions in which the species can potentially spread to are predicted to increase in the future (Annex VIII).

4 MAGNITUDE OF IMPACT

Important instructions:

- Questions 4.1-4.5 relate to biodiversity and ecosystem impacts, 4.6-4.8 to impacts on ecosystem services, 4.9-4.13 to economic impact, 4.14-4.15 to social and human health impact, and 4.16-4.18 to other impacts. These impacts can be interlinked, for example, a disease may cause impacts on biodiversity and/or ecosystem functioning that leads to impacts on ecosystem services and finally economic impacts. In such cases the assessor should try to note the different impacts where most appropriate, cross-referencing between questions when needed.
- Each set of questions starts with the impact elsewhere in the world, then considers impacts in the risk assessment area (=EU excluding outermost regions) separating known impacts to date (i.e. past and current impacts) from potential future impacts (including foreseeable climate change).
- Only negative impacts are considered in this section (socio-economic benefits are considered in Qu. A.7)
- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable).

Biodiversity and ecosystem impacts

Qu. 4.1. How important is the impact of the organism on biodiversity at all levels of organisation caused by the organism in its non-native range excluding the risk assessment area?

including the following elements:

- Biodiversity means the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems
- impacted chemical, physical or structural characteristics and functioning of ecosystems

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: The impact of *M. cornuarietis* is believed to be density dependent with low densities coinciding with minimal impacts in the USA (Arias & Torralba-Burrial 2014). The densities of *M. cornuarietis* in its currently introduced range can reach 50-175 individuals per m². Under these densities the most severe impacts of this species are observed.

The species is known as a voracious omnivore which can potentially significantly impact macrophyte flora (Pointier & David 2004). In the USA non-native populations of *Marissa cornuarietis* have shown

to negatively impact macrophytes when the snail density becomes high with reduced macrophyte diversity and species elimination potentially occurring (Arias & Torralba-Burrial 2014). Their ability to influence macrophyte composition and biomass in waterbodies is believed to be due to their dietary habits, high reproductive output and capacity to form dense populations (Barker 2019). This impact is linked to indirect impacts to the affected nutrient balance, turbidity and trophic structure in the ecosystem. Furthermore, their herbivory can have a significant effect on native aquatic flora (Barker 2019). This species is a voracious omnivore which can directly impact native snail populations through direct competition and predation (Arias & Torralba-Burrial .2014). It is a competitive feeder which actively feed on eggs and juveniles of certain snail species (Barker 2019, Benson *et al.* 2021). Furthermore, its appetite can potentially outcompete native snails (Pointier & David 2004).

Qu. 4.2. How important is the current known impact of the organism on biodiversity at all levels of organisation (e.g. decline in native species, changes in native species communities, hybridisation) in the risk assessment area (include any past impact in your response)?

Discuss impacts that are currently occurring or are likely occurring or have occurred in the past in the risk assessment area. Where there is no direct evidence of impact in the risk assessment area (for example no studies have been conducted), evidence from outside of the risk assessment area can be used to infer impacts within the risk assessment area.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No published literature states the impacts of the established populations of *Marisa cornuarietis* in the RA area. It can be assumed that the same biodiversity impacts will occur in the RA which were discussed in Qu 4.1 where high densities occur. Arias & Torralba-Burrial (2014) reiterated these remarks highlighting the potential for Red List snails to be impacted by *M. cornuarietis*.

Qu. 4.3. How important is the potential future impact of the organism on biodiversity at all levels of organisation likely to be in the risk assessment area?

See comment above. The potential future impact shall be assessed only for the risk assessment area. A potential increase in the distribution range due to climate change does not *per se* justify a higher impact score.

RESPONSE	minimal minor moderate	CONFIDENCE	low medium high
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	major massive		
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Response: There is limited published literature in relation to the expected impacts of established *Marisa cornuarietis* populations in the RA area. However, given that it can be assumed that similar impacts will be observed as to what has occurred in other regions, loss of macrophyte diversity and indigenous snail populations will occur where its population density reaches high levels in affected water bodies. This in turn will cause the biotope wide changes discussed in Qu 4.1 but not in the near future. The two established populations are not currently situated in ideal conditions, however it must be noted they are still established populations. The Hungarian population is isolated while the population inhabiting the Nora river in Spain is subject to less than ideal calcium concentrations in the water (Frisóczki *et al.* 2016, Arias & Torralba-Burrial 2014). Arias & Torralba-Burrial (2014) have noted the widespread use of the species in the aquarium trade and, given the environmental plasticity and natural spread of the established population in Spain, a further expansion is expected.

Qu. 4.4. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism currently in the risk assessment area?

including the following elements:

- native species impacted, including red list species, endemic species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No published information in relation to impacts on species or habitats protected under European or National conservation legislation in the RA area is available. Given that the species is expected to spread naturally and continued introductions will occur (Arias & Torralba-Burrial 2014), impacts to protected and/or endangered species or habitats, particularly aquatic snails, macrophytes, and their associated protected habitats could potentially occur where snail densities reach significant levels similar to what has occurred in regions outside of the RA area (50-175 individuals per m²) (Barker 2019). For example, Arias & Torralba-Burrial (2014) highlighted the potential for Red List organisms to be impacted by *M. cornuarietis*. In Europe, almost half of aquatic snails are threatened with extinction with 20% of species considered endemic (Cuttlod *et al.* 2011). Due to this, it is likely that *M. cornuarietis* may cause a severe impact to threatened European snails in the future. Aquatic freshwater habitats are also at risk due to the colonisation of the species and its ability to be an

ecosystem engineer. The Habitats Directive Annex 1 Lake Habitats Mixed *Najas flexilis* lake habitat (3130) and Hard-water lake habitat (3140) may be impacted by the invasion of this species.

Qu. 4.5. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.3. and 4.4.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: In the near future, no major changes are expected to occur to the conservation value of protected habitats and species in the RA area. This is primarily due to the species occupation of less than ideal habitats in the RA area. The population in Hungary is expected not to spread via natural means due to their thermal requirements. The established population in Spain is currently spreading, but exists under less than ideal physicochemical parameters. However, given the potential for future introductions and the species ability to spread via natural and interconnected waterways it is plausible that the full impacts of this species will develop in water bodies in the RA in the future.

Ecosystem Services impacts

Qu. 4.6. How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?

- For a list of services use the CICES classification V5.1 provided in Annex V.
- Impacts on ecosystem services build on the observed impacts on biodiversity (habitat, species, genetic, functional) but focus exclusively on reflecting these changes in relation to their links with socio-economic well-being.
- Quantitative data should be provided whenever available and references duly reported.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	Low medium high
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Response: Under the CICES classification V5.1 provided in Annex V for the provisioning, regulating, and cultural services *Marisa cornuarietis* can potentially have negative impacts in several services. It

must be noted however that no information directly addressing impacts on ecosystem services has been found.

The species can impact

(1) Provisioning/Genetic material from plant/animals. *M. cornuarietis* is known to cause species decline and loss of certain macrophytes species under certain circumstances resulting in the loss of genetic diversity (Arias & Torralba-Burrial 2014). Additionally, the species' voracious appetite can cause the elimination of indigenous snails under certain circumstances (Pointier & David 2004) thus reducing genetic diversity.

(2) Provisioning/ Wild plants. This is due to the species generalist herbivory feeding habits (Barker 2019) which can potentially eliminate wild aquatic plants used for nutritional purposes.

(3) Regulation & Maintenance. *M. cornuarietis* can impact Transformation/mediation of wastes and water conditions services. This is due to the species feeding habits which can consume significant quantities of macrophyte biomass which in turn can potentially impact bioremediation services of aquatic macrophytes in controlled and natural environments. Due to changes in macrophyte community structure there can be changes in nutrient balance, turbidity, benthos and trophic structure of water bodies (Horgan et al. 2014).

(4) Cultural services, this species can potentially impact protected species and habitats which are classed as biotic characteristics of non-use value.

Qu. 4.7. How important is the impact of the organism on provisioning, regulating, and cultural services currently in the different biogeographic regions or marine sub-regions where the species has established in the risk assessment area (include any past impact in your response)?

- See guidance to Qu. 4.6.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: As the species is a relatively new arrival to the RA area, no information has been published in relation to its impact on provisioning, regulating and cultural services. However, multiple potential impacts have been stated by Arias & Torralba-Burrial (2014). It is expected that impacts observed in the species non-native range outside the RA area could potentially also occur in the RA, including high impact on other provisioning services (e.g. rice and other wetland crops).

Qu. 4.8. How important is the impact of the organism on provisioning, regulating, and cultural services likely to be in the different biogeographic regions or marine sub-regions where the species can establish in the risk assessment area in the future?

- See guidance to Qu. 4.6.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No information has been found in relation to this issue but the impacts stated in 4.6 could potentially occur in suitable habitats located in the 25.25 % of the RA regions thermally suitable for the species to become established. In the predicted future models (Annex VIII) when the potential area for the species to become established in the RA increases, the impacts will entail other biogeographical areas not currently prone to the negative impacts of *Marisa cornuarietis*. At present these impacts and their extent is unknown.

Economic impacts

Qu. 4.9. How great is the overall economic cost caused by the organism within its current area of distribution (excluding the risk assessment area), including both costs of / loss due to damage and the cost of current management.

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the world these should be reported here. The assessment of the potential costs of / loss due to damage shall describe those costs quantitatively and/or qualitatively depending on what information is available. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage. As far as possible, it would be useful to separate costs of / loss due to the organism from costs of current management.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: The economic impacts associated with *M. cornuarietis* are dependent on the density of infestation (Arias & Torralba-Burrial 2014). No information providing estimates of the economic cost associated with the species was found. Limited evidence of active control programs of the species is available. The economic implication of introductions of the species are currently unknown but they are known to incur costs in relation to multiple ecosystem services e.g. agriculture (Howells *et al.* 2006, Horgan *et al.* 2014).

Qu. 4.10. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism currently in the risk assessment area (include any past costs in your response)?

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the EU these should be reported here. Assessment of the potential costs of damage on human health, safety, and the economy, including the cost of non-action. A full economic assessment at EU scale might not be possible, but qualitative data or different case studies from across the EU (or third countries if relevant) may provide useful information to inform decision making. In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable). Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	N/A	CONFIDENCE	
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Response: No information has been found on the issue.

Qu. 4.11. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.10.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No economic projections regarding this species are known for the current or future impacts in the RA area. Established populations are currently isolated to small regions but with the expected potential for continued introductions and natural spread, it is plausible that moderate costs may be incurred in the future from the impacts imparted in the RA region by this species.

A negative economic implication of this species is due to its voracious herbivorous dietary intake. The species is a known pest to crops in certain regions incurring costs in relation to lost production and control measures (Barker 2019). It is currently unknown if the species will negatively influence agriculture inside the RA area.

Qu. 4.12. How great are the economic costs / losses associated with managing this organism currently in the risk assessment area (include any past costs in your response)?

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable).

RESPONSE	N/A	CONFIDENCE	
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Response No information has been found on the issue.

Qu. 4.13. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.12.

RESPONSE	N/A	CONFIDENCE	
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Response: No information has been found on the issue.

Social and human health impacts

Qu. 4.14. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism for the risk assessment area and for third countries, if relevant (e.g. with similar eco-climatic conditions).

The description of the known impact and the assessment of potential future impact on human health, safety and the economy, shall, if relevant, include information on

- illnesses, allergies or other affections to humans that may derive directly or indirectly from a species;
- damages provoked directly or indirectly by a species with consequences for the safety of people, property or infrastructure;
- direct or indirect disruption of, or other consequences for, an economic or social activity due to the presence of a species.

Social and human health impacts can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	N/A	CONFIDENCE	
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Response: No information has been found on the issue.

Qu. 4.15. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism in the future for the risk assessment area.

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable).

RESPONSE	N/A	CONFIDENCE	
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Response: No information has been found on the issue.

Other impacts

Qu. 4.16. How important is the organism in facilitating other damaging organisms (e.g. diseases) as food source, a host, a symbiont or a vector etc.?

RESPONSE	N/A	CONFIDENCE	
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Response: No information has been found on the issue.

Qu. 4.17. How important might other impacts not already covered by previous questions be resulting from introduction of the organism?

RESPONSE	N/A	CONFIDENCE	
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Response: No information has been found on the issue.

Qu. 4.18. How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area?

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: *Marisa cornuarietis* has been noted to be predated on by fish and birds (Barker 2019). Hypothetically, this may provide some natural control in the RA area, however, at present natural control mechanisms in the RA area are unknown.

Qu. 4.19. Estimate the overall impact in the risk assessment area under current climate conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, in current conditions.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: As the RA conducted by Patoka *et al.* (2017) concluded that 25.25% of the RA area is subject to the establishment of *Marisa cornuarietis* where suitable habitat is occurring, it is expected that under current climate conditions and the potential for further introductions throughout the RA, thermally suitable areas in relevant biogeographical regions will be prone to the aforementioned impacts of this species introduction and subsequent establishment.

Qu. 4.20. Estimate the overall impact in the risk assessment area in foreseeable climate change conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, under future conditions.

- See also guidance to Qu. 4.3.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: The significance of the impacts is not expected to change under future climate conditions although some biogeographical regions (see Q. A7) (Annex VIII) may have a higher suitability of establishment in the future leading to a potential increase in the distribution of the impacts imparted by this species.

RISK SUMMARIES			
	RESPONSE	CONFIDENCE	COMMENT
Summarise Introduction and Entry*	very unlikely unlikely moderately likely likely very likely	low medium high	Direct trade of <i>Marisa cornuarietis</i> through aquaria or the species being a contaminant on traded plants is the most likely cause of introduction. The species is already introduced and has entered into the wild in the Mediterranean and the Pannonian biogeographical regions in the RA area. It is very likely that the species will be repeatedly introduced and entered into the wild through these pathways
Summarise Establishment*	very unlikely unlikely moderately likely likely very likely	low medium high	Currently, the species is established in two EU Member States (Spain and Hungary). Establishment is expected in 25% of the RA area (Patoka <i>et al.</i> 2017), depending on physico-chemical parameters such as calcium concentrations and suitable habitats such as lakes, rivers, ponds, streams. Further establishment of new populations is very likely. Under predicted future climate conditions, the species area within the RA where it could establish will increase
Summarise Spread*	very slowly slowly moderately rapidly very rapidly	low medium high	Spread is restricted by environmental conditions. Unaided spread and spread via interconnected corridors, as a transport stowaway on angling equipment and via –contamination on plants are deemed important for the species. Under predicted future climate conditions these pathways will still be the primary ways the species will spread.
Summarise Impact*	minimal minor moderate major massive	low medium high	The impacts of <i>M. cornuarietis</i> are currently unknown in the RA area due to its relatively recent arrival but impacts are expected to be similar to other regions of the world where the species has established in its non-native range. Impacts on indigenous flora and fauna and some ecosystem

			services (e.g. provisioning) have been identified in areas outside the RA and severity is dependent on population density. Economic costs (on agriculture) can also occur.
Conclusion of the risk assessment (overall risk)	low moderate high	low medium high	The species is established in two regions of the RA area and has the potential to spread. It is known to be invasive in regions outside the native range due to their ability to outcompete and displace native flora and fauna and impacts on the economy are possible.

*in current climate conditions and in foreseeable future climate conditions

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Distribution Summary

Please answer as follows:

- Yes if recorded, established or invasive
- if not recorded, established or invasive
- ? Unknown; data deficient

The columns refer to the answers to Questions A5 to A12 under Section A.

For data on marine species at the Member State level, delete Member States that have no marine borders.

In all other cases, provide answers for all columns.

Member States, and the United Kingdom

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Austria	–	–	–	Yes	–
Belgium	–	–	–	Yes	–
Bulgaria	–	–	–	Yes	–
Croatia	–	–	Yes	Yes	–
Cyprus	–	–	–	–	–
Czech Republic	–	–	–	–Yes	–
Denmark	–	–	–	–Yes	–
Estonia	–	–	–	–	–
Finland	–	–	–	–Yes	–
France	–	–	Yes	–Yes	–
Germany	–	–	–	–Yes	–
Greece	–	–	Yes	–Yes	–
Hungary	Yes	Yes	–	–	–
Ireland	–	–	–	–Yes	–
Italy	–	–	Yes	–Yes	–
Latvia	–	–	–	–	–
Lithuania	–	–	–	–Yes	–
Luxembourg	–	–	–	Yes	–
Malta	–	–	–	–	–
Netherlands	–	–	–	Yes	–
Poland	–	–	–	–	–
Portugal	–	–	Yes	–Yes	–
Romania	–	–	–	–	–
Slovakia	–	–	–	–	–
Slovenia	–	–	–	–Yes	–
Spain	Yes	Yes	Yes	–Yes	–
Sweden	–	–	–	–Yes	–
United Kingdom	–	–	–Yes	–Yes	–

Biogeographical regions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Alpine	–	–	Yes	Yes	–
Atlantic	–	–	Yes	Yes	–
Black Sea	–	–	Yes	Yes	–
Boreal	–	–	–	–	–
Continental			Yes	Yes	–
Mediterranean	Yes	Yes	Yes	Yes	–
Pannonian	Yes	Yes	–	–	–
Steppic	–	–	–	–Yes	–

ANNEX I Scoring of Likelihoods of Events

(taken from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Description	Frequency
Very unlikely	This sort of event is theoretically possible, but is never known to have occurred and is not expected to occur	1 in 10,000 years
Unlikely	This sort of event has occurred somewhere at least once in the last millenium	1 in 1,000 years
Moderately likely	This sort of event has occurred somewhere at least once in the last century	1 in 100 years
Likely	This sort of event has happened on several occasions elsewhere, or on at least once in the last decade	1 in 10 years
Very likely	This sort of event happens continually and would be expected to occur	Once a year

ANNEX II Scoring of Magnitude of Impacts

(modified from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Biodiversity and ecosystem impact	Ecosystem Services impact	Economic impact (Monetary loss and response costs per year)	Social and human health impact, and other impacts
	<i>Question 5.1-5</i>	<i>Question 5.6-8</i>	<i>Question 5.9-13</i>	<i>Question 5.14-18</i>
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected ⁵	Up to 10,000 Euro	No social disruption. Local, mild, short-term reversible effects to individuals.
Minor	Some ecosystem impact, reversible changes, localised	Local and temporary, reversible effects to one or few services	10,000-100,000 Euro	Significant concern expressed at local level. Mild short-term reversible effects to identifiable groups, localised.
Moderate	Measureable long-term damage to populations and ecosystem, but reversible; little spread, no extinction	Measureable, temporary, local and reversible effects on one or several services	100,000-1,000,000 Euro	Temporary changes to normal activities at local level. Minor irreversible effects and/or larger numbers covered by reversible effects, localised.
Major	Long-term irreversible ecosystem change, spreading beyond local area	Local and irreversible or widespread and reversible effects on one / several services	1,000,000-10,000,000 Euro	Some permanent change of activity locally, concern expressed over wider area. Significant irreversible effects locally or reversible effects over large area.
Massive	Widespread, long-term population loss or extinction, affecting several species with serious ecosystem effects	Widespread and irreversible effects on one / several services	Above 10,000,000 Euro	Long-term social change, significant loss of employment, migration from affected area. Widespread, severe, long-term, irreversible health effects.

⁵ Not to be confused with “no impact”.

ANNEX III Scoring of Confidence Levels

(modified from Bacher *et al.* 2017)

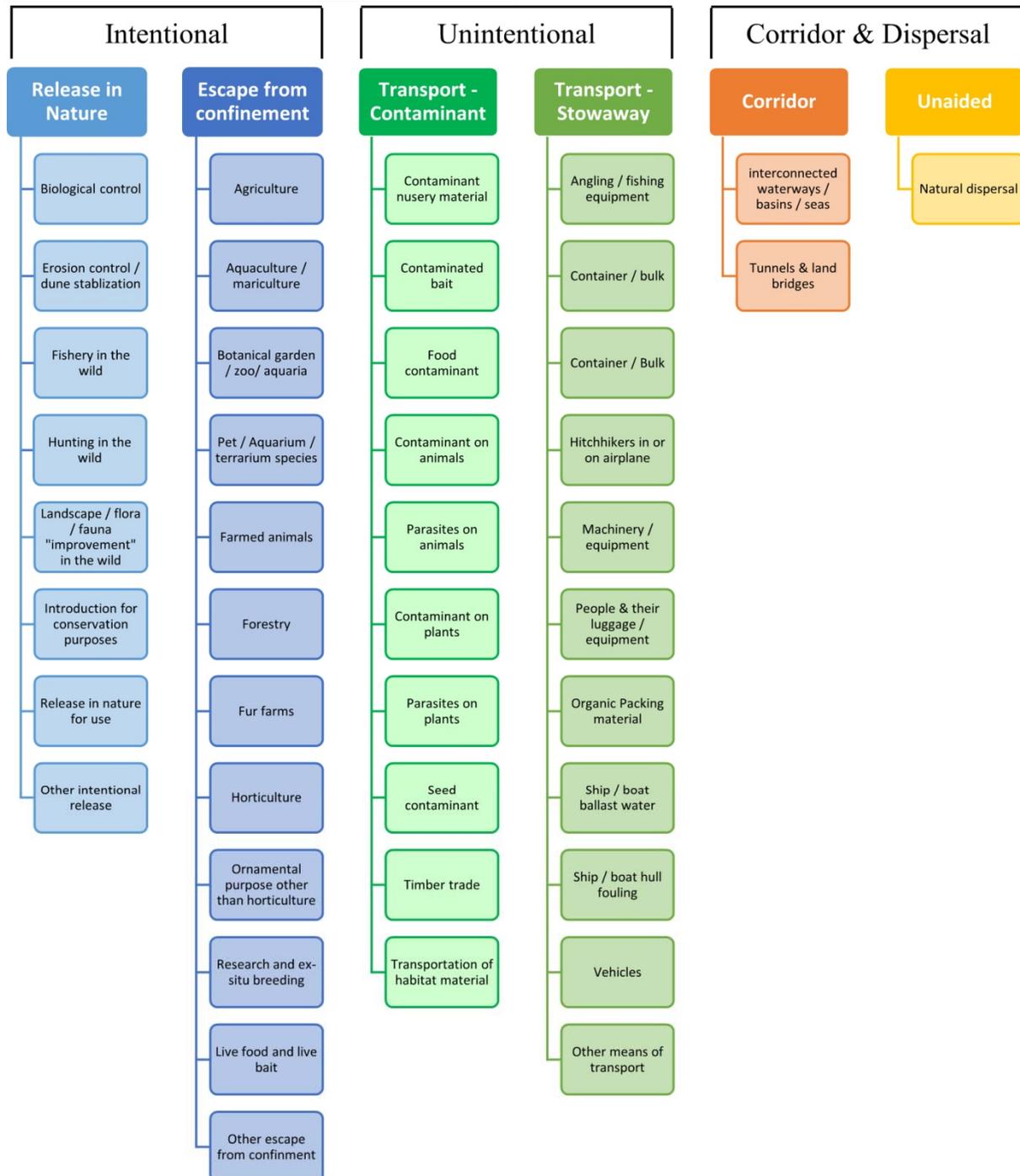
Each answer provided in the risk assessment must include an assessment of the level of confidence attached to that answer, reflecting the possibility that information needed for the answer is not available or is insufficient or available but conflicting.

The responses in the risk assessment should clearly support the choice of the confidence level.

Confidence level	Description
Low	There is no direct observational evidence to support the assessment, e.g. only inferred data have been used as supporting evidence <i>and/or</i> Impacts are recorded at a spatial scale which is unlikely to be relevant to the assessment area <i>and/or</i> Evidence is poor and difficult to interpret, e.g. because it is strongly ambiguous <i>and/or</i> The information sources are considered to be of low quality or contain information that is unreliable.
Medium	There is some direct observational evidence to support the assessment, but some information is inferred <i>and/or</i> Impacts are recorded at a small spatial scale, but rescaling of the data to relevant scales of the assessment area is considered reliable, or to embrace little uncertainty <i>and/or</i> The interpretation of the data is to some extent ambiguous or contradictory.
High	There is direct relevant observational evidence to support the assessment (including causality) <i>and</i> Impacts are recorded at a comparable scale <i>and/or</i> There are reliable/good quality data sources on impacts of the taxa <i>and</i> The interpretation of data/information is straightforward <i>and/or</i> Data/information are not controversial or contradictory.

ANNEX IV CBD pathway categorisation scheme

Overview of CBD pathway categorisation scheme showing how the 44 pathways relate to the six main pathway categories. All of the pathways can be broadly classified into 1) those that involve intentional transport (blue), 2) those in which the taxa are unintentionally transported (green) and 3) those where taxa moved between regions without direct transportation by humans and/or via artificial corridors (orange and yellow). **Note that the pathways in the category “Escape from confinement” can be considered intentional for the introduction into the risk assessment area and unintentional for the entry into the environment.**



ANNEX V Ecosystem services classification (CICES V5.1, simplified) and examples

For the purposes of this risk assessment, please feel free to use what seems as the most appropriate category / level / combination of impact (Section – Division – Group), reflecting information available.

Section	Division	Group	Examples (i.e. relevant CICES “classes”)
Provisioning	Biomass	Cultivated <i>terrestrial</i> plants	<p>Cultivated terrestrial plants (including fungi, algae) grown for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from cultivated plants, fungi, algae and bacteria for direct use or processing (excluding genetic materials); Cultivated plants (including fungi, algae) grown as a <u>source of energy</u></p> <p><i>Example: negative impacts of non-native organisms to crops, orchards, timber etc.</i></p>
		Cultivated <i>aquatic</i> plants	<p>Plants cultivated by in- situ aquaculture grown for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from in-situ aquaculture for direct use or processing (excluding genetic materials); Plants cultivated by in- situ aquaculture grown as an <u>energy source</u>.</p> <p><i>Example: negative impacts of non-native organisms to aquatic plants cultivated for nutrition, gardening etc. purposes.</i></p>
		Reared animals	<p>Animals reared for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from reared animals for direct use or processing (excluding genetic materials); Animals reared to provide <u>energy</u> (including mechanical)</p> <p><i>Example: negative impacts of non-native organisms to livestock</i></p>
		Reared <i>aquatic</i> animals	<p>Animals reared by in-situ aquaculture for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from animals grown by in-situ aquaculture for direct use or processing (excluding genetic materials); Animals reared by in-situ aquaculture as an <u>energy source</u></p> <p><i>Example: negative impacts of non-native organisms to fish farming</i></p>
		Wild plants (terrestrial and aquatic)	<p>Wild plants (terrestrial and aquatic, including fungi, algae) used for <u>nutrition</u>; <u>Fibres and other materials</u> from wild plants for direct use or processing (excluding genetic materials); Wild plants (terrestrial and aquatic, including fungi, algae) used as a <u>source of energy</u></p> <p><i>Example: reduction in the availability of wild plants (e.g. wild berries, ornamentals) due to non-native organisms (competition, spread of disease etc.)</i></p>
		Wild animals (terrestrial and aquatic)	<p>Wild animals (terrestrial and aquatic) used for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from wild animals for direct use or processing (excluding genetic materials); Wild animals (terrestrial and aquatic) used as a <u>source of energy</u></p>

			<i>Example: reduction in the availability of wild animals (e.g. fish stocks, game) due to non-native organisms (competition, predations, spread of disease etc.)</i>
	Genetic material from all biota	Genetic material from plants, algae or fungi	<p><u>Seeds, spores and other plant materials</u> collected for maintaining or establishing a population; Higher and lower plants (whole organisms) used to <u>breed new strains or varieties</u>; Individual genes extracted from higher and lower plants for the <u>design and construction of new biological entities</u></p> <p><i>Example: negative impacts of non-native organisms due to interbreeding</i></p>
		Genetic material from animals	<p>Animal material collected for the purposes of maintaining or establishing a population; Wild animals (whole organisms) used to breed new strains or varieties; Individual genes extracted from organisms for the design and construction of new biological entities</p> <p><i>Example: negative impacts of non-native organisms due to interbreeding</i></p>
	Water ⁶	Surface water used for nutrition, materials or energy	<p>Surface water for <u>drinking</u>; Surface water used as a material (<u>non-drinking purposes</u>); Freshwater surface water, coastal and marine water used as an <u>energy source</u></p> <p><i>Example: loss of access to surface water due to spread of non-native organisms</i></p>
		Ground water for used for nutrition, materials or energy	<p>Ground (and subsurface) water for <u>drinking</u>; Ground water (and subsurface) used as a material (<u>non-drinking purposes</u>); Ground water (and subsurface) used as an <u>energy source</u></p> <p><i>Example: reduced availability of ground water due to spread of non-native organisms and associated increase of ground water consumption by vegetation.</i></p>
Regulation & Maintenance	Transformation of biochemical or physical inputs to ecosystems	Mediation of wastes or toxic substances of anthropogenic origin by living processes	<p><u>Bio-remediation</u> by micro-organisms, algae, plants, and animals; <u>Filtration/sequestration/storage/accumulation</u> by micro-organisms, algae, plants, and animals</p> <p><i>Example: changes caused by non-native organisms to ecosystem functioning and ability to filtrate etc. waste or toxics</i></p>
		Mediation of nuisances of anthropogenic origin	<p><u>Smell reduction</u>; <u>noise attenuation</u>; <u>visual screening</u> (e.g. by means of green infrastructure)</p> <p><i>Example: changes caused by non-native organisms to ecosystem structure, leading to reduced ability to mediate nuisances.</i></p>
	Regulation of physical, chemical, biological conditions	Baseline flows and extreme event regulation	<p>Control of <u>erosion</u> rates; Buffering and attenuation of <u>mass movement</u>; <u>Hydrological cycle and water flow regulation</u> (Including flood control, and coastal protection); <u>Wind</u> protection; <u>Fire</u> protection</p>

⁶ Note: in the CICES classification provisioning of water is considered as an abiotic service whereas the rest of ecosystem services listed here are considered biotic.

			<p><i>Example: changes caused by non-native organisms to ecosystem functioning or structure leading to, for example, destabilisation of soil, increased risk or intensity of wild fires etc.</i></p>
		<p>Lifecycle maintenance, habitat and gene pool protection</p>	<p>Pollination (or 'gamete' dispersal in a marine context); <u>Seed dispersal</u>; Maintaining <u>nursery populations and habitats</u> (Including gene pool protection)</p> <p><i>Example: changes caused by non-native organisms to the abundance and/or distribution of wild pollinators; changes to the availability / quality of nursery habitats for fisheries</i></p>
		<p>Pest and disease control</p>	<p>Pest control; Disease control</p> <p><i>Example: changes caused by non-native organisms to the abundance and/or distribution of pests</i></p>
		<p>Soil quality regulation</p>	<p><u>Weathering processes</u> and their effect on soil quality; <u>Decomposition and fixing processes</u> and their effect on soil quality</p> <p><i>Example: changes caused by non-native organisms to vegetation structure and/or soil fauna leading to reduced soil quality</i></p>
		<p>Water conditions</p>	<p>Regulation of the <u>chemical condition</u> of freshwaters by living processes; Regulation of the chemical condition of salt waters by living processes</p> <p><i>Example: changes caused by non-native organisms to buffer strips along water courses that remove nutrients in runoff and/or fish communities that regulate the resilience and resistance of water bodies to eutrophication</i></p>
		<p>Atmospheric composition and conditions</p>	<p>Regulation of <u>chemical composition</u> of atmosphere and oceans; Regulation of <u>temperature and humidity</u>, including ventilation and transpiration</p> <p><i>Example: changes caused by non-native organisms to ecosystems' ability to sequester carbon and/or evaporative cooling (e.g. by urban trees)</i></p>
Cultural	Direct, in-situ and outdoor interactions with living systems that depend on presence in the environmental setting	<p>Physical and experiential interactions with natural environment</p>	<p>Characteristics of living systems that that enable activities promoting health, recuperation or enjoyment through <u>active or immersive interactions</u>;</p> <p>Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through <u>passive or observational interactions</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that make it attractive for recreation, wild life watching etc.</i></p>
		<p>Intellectual and representative interactions with natural environment</p>	<p>Characteristics of living systems that enable <u>scientific investigation</u> or the creation of traditional ecological knowledge;</p> <p>Characteristics of living systems that enable <u>education and training</u>;</p> <p>Characteristics of living systems that are resonant in terms of <u>culture or heritage</u>;</p> <p>Characteristics of living systems that enable <u>aesthetic experiences</u></p>

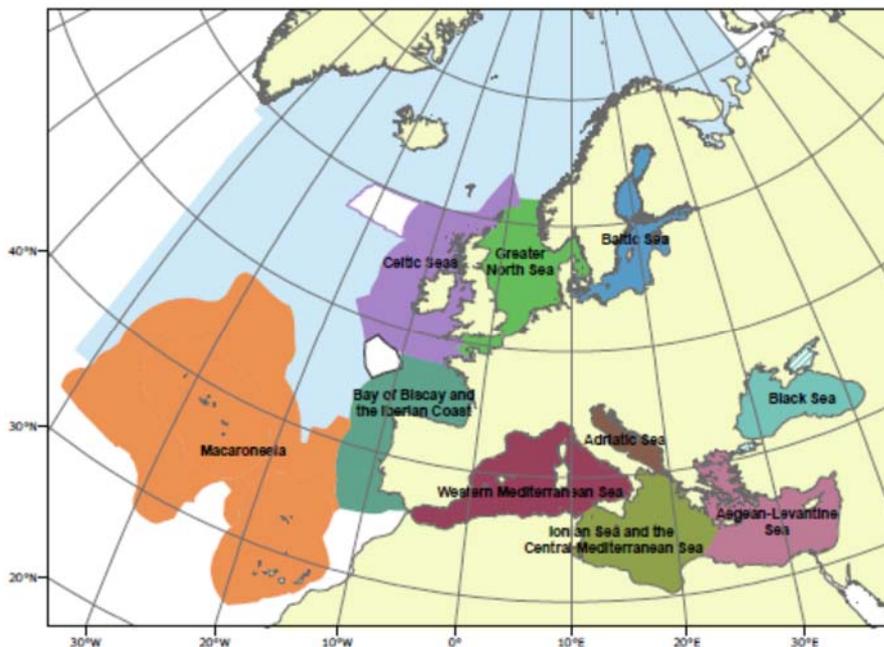
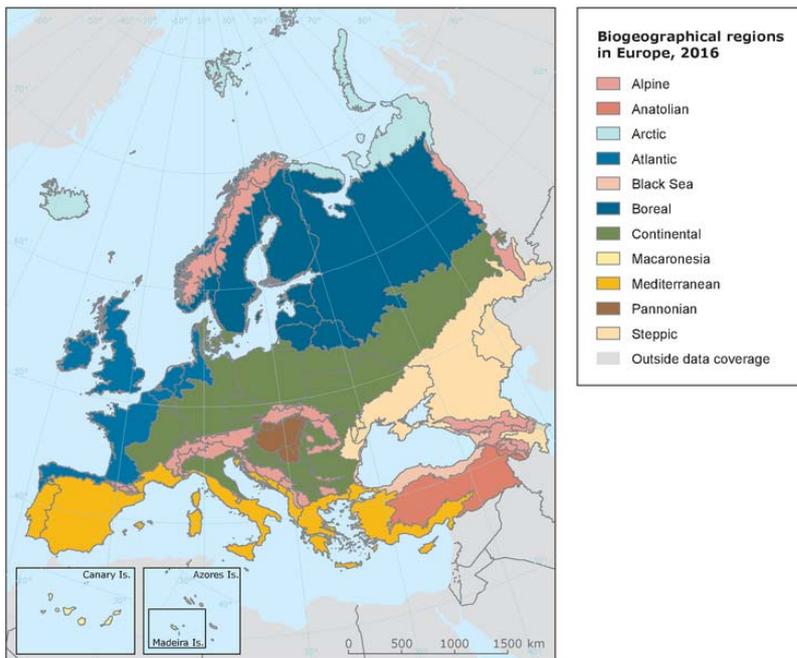
			<i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have cultural importance</i>
	Indirect, remote, often indoor interactions with living systems that do not require presence in the environmental setting	Spiritual, symbolic and other interactions with natural environment	<p>Elements of living systems that have <u>symbolic meaning</u>;</p> <p>Elements of living systems that have <u>sacred or religious meaning</u>;</p> <p>Elements of living systems used for <u>entertainment or representation</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have sacred or religious meaning</i></p>
		Other biotic characteristics that have a non-use value	<p>Characteristics or features of living systems that have an <u>existence value</u>;</p> <p>Characteristics or features of living systems that have an <u>option or bequest value</u></p> <p><i>Example: changes caused by non-native organisms to ecosystems designated as wilderness areas, habitats of endangered species etc.</i></p>

ANNEX VI EU Biogeographic Regions and MSFD Subregions

See <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2> ,
http://ec.europa.eu/environment/nature/natura2000/biogeog_regions/

and

<https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions-1/technical-document/pdf>



ANNEX VII Delegated Regulation (EU) 2018/968 of 30 April 2018

see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>

ANNEX VIII Projection of environmental suitability for *Marisa cornuarietis* establishment in Europe

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27 September 2021

Aim

To project the suitability for potential establishment of *Marisa cornuarietis* in Europe, under current and predicted future climatic conditions.

Data for modelling

Species occurrence data were obtained from the Global Biodiversity Information Facility (GBIF) (228 records), iNaturalist (111 records), the Integrated Digitized Biocollections (iDigBio) (70 records), the Biodiversity Information Serving Our Nation database (BISON) (38 records), and additional records from the risk assessment team. We scrutinised occurrence records from regions where the species is not known to be established and removed any dubious records or where the georeferencing was too imprecise (e.g. records referenced to a country or island centroid) or outside of the coverage of the predictor layers (e.g. small island or coastal occurrences). We excluded a record from Hungary at the outflow of a thermal spa (Frisoczki et al. 2016), because even though this is an established outdoor population, the artificially warm and constant conditions here would not be reflected in the predictor layers. The remaining records were gridded at a 0.25 x 0.25 degree resolution for modelling, yielding 118 grid cells with occurrences (Figure 1a). As a proxy for recording effort, the density of Ampullariidae records held by GBIF was also compiled on the same grid (Figure 1b).

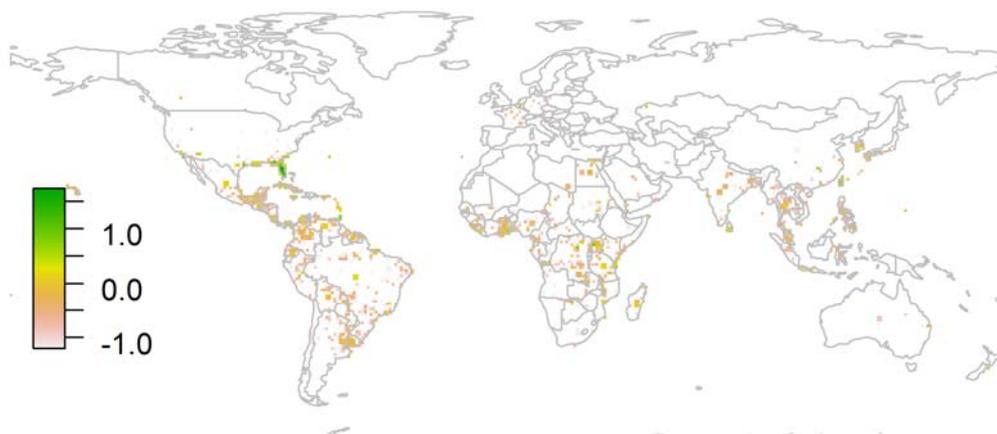
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Figure 1. (a) Occurrence records obtained for *Marisa cornuarietis* and used in the modelling, showing native and invaded distributions. (b) The recording density of Ampullariidae on GBIF, which was used as a proxy for recording effort.

(a) Species distribution used in modelling



(b) Estimated recording effort (log10-scaled)



Climate data were selected from the 'Biodim' variables contained within the WorldClim database (Hijmans et al., 2005), originally at 5 arcminute resolution (0.083 x 0.083 degrees of longitude/latitude) and aggregated to a 0.25 x 0.25 degree grid for use in the model.

Based on the biology of *Marisa cornuarietis*, the following climate variables were used in the modelling:

- Mean temperature of the warmest quarter (Bio10)
- Mean temperature of the coldest quarter (Bio11)
- Annual precipitation (Bio12)
- Precipitation seasonality (Bio15)

To estimate the effect of climate change on the potential distribution, equivalent modelled future climate conditions for the 2070s under the Representative Concentration Pathways (RCP) 2.6 and 4.5 were also obtained. These represent low and medium emissions scenarios, respectively. The above variables were obtained as averages of outputs of eight Global Climate Models (BCC-CSM1-1, CCSM4, GISS-E2-R, HadGEM2-AO, IPSL-CM5A-LR, MIROC-ESM, MRI-CGCM3, NorESM1-M), downscaled and calibrated against the WorldClim baseline (see http://www.worldclim.org/cmip5_5m).

Species distribution model

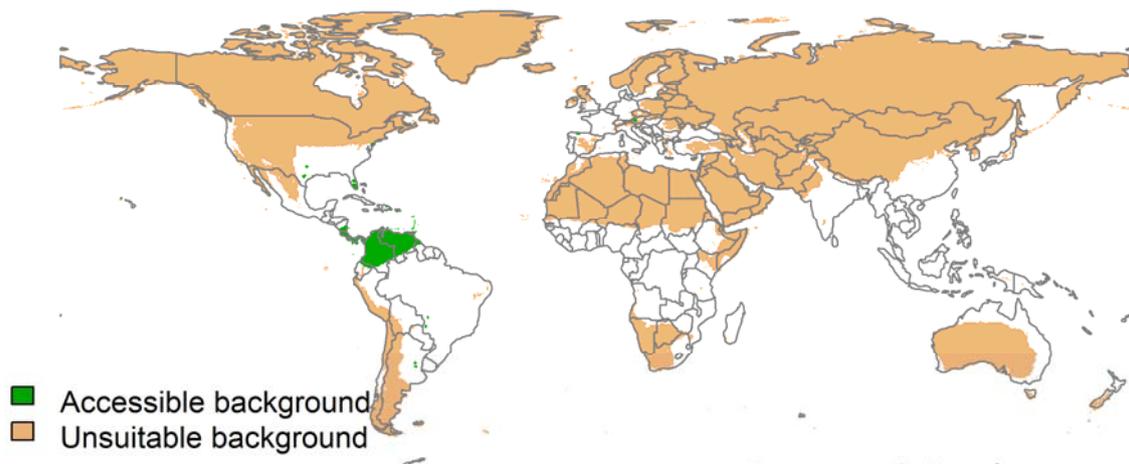
A presence-background (presence-only) ensemble modelling strategy was employed using the BIOMOD2 R package version 3.4.6 (Thuiller et al., 2020, Thuiller et al., 2009). These models contrast the environment at the species' occurrence locations against a random sample of the global background environmental conditions (often termed 'pseudo-absences') in order to characterise and project suitability for occurrence. This approach has been developed for distributions that are in equilibrium with the environment. Because invasive species' distributions are not at equilibrium and subject to dispersal constraints at a global scale, we took care to minimise the inclusion of locations suitable for the species but where it has not been able to disperse to (Chapman et al. 2019). Therefore the background sampling region included:

- The area accessible by native *Marisa cornuarietis* populations, in which the species is likely to have had sufficient time to disperse to all locations. Based on presumed maximum dispersal distances, the accessible region was defined as a 400km buffer around the native range occurrences; AND
- A 50km buffer around the non-native occurrences, encompassing regions likely to have had high propagule pressure for introduction by humans and/or dispersal of the species; AND
- Regions where we have an *a priori* expectation of high unsuitability for the species so that absence is assumed irrespective of dispersal constraints (see Figure 2). The following rules were applied to define a region expected to be highly unsuitable for *Marisa cornuarietis* at the spatial scale of the model:
 - Mean temperature of the warmest quarter (Bio10) < 14°C
 - Mean temperature of the coldest quarter (Bio11) < -1.3°C
 - Annual precipitation (Bio12) < 500mm

Altogether, 2.5% of occurrence grid cells were located in the unsuitable background region.

Within the unsuitable background region, 10 samples of 5000 randomly sampled grid cells were obtained. In the accessible background (comprising the accessible areas around native and non-native occurrences as detailed above), the same number of pseudo-absence samples were drawn as there were presence records (118), weighting the sampling by a proxy for recording effort (Figure 1(b)).

Figure 2. The background from which pseudo-absence samples were taken in the modelling of *Marisa cornuarietis*. Samples were taken from a 400km buffer around the native range and a 50km buffer around non-native occurrences (together forming the accessible background), and from areas expected to be highly unsuitable for the species (the unsuitable background region). Samples from the accessible background were weighted by a proxy for recording effort (Figure 1(b)).



Each dataset (i.e. combination of the presences and the individual background samples) was randomly split into 80% for model training and 20% for model evaluation. With each training dataset, seven statistical algorithms were fitted with the default BIOMOD2 settings and rescaled using logistic regression, except where specified below:

- Generalised linear model (GLM)
- Generalised boosting model (GBM)
- Generalised additive model (GAM) with a maximum of four degrees of freedom per smoothing spline
- Artificial neural network (ANN)
- Multivariate adaptive regression splines (MARS)
- Random forest (RF)
- Maxent

Since the total background sample was larger than the number of occurrences, prevalence fitting weights were applied to give equal overall importance to the occurrences and the background. Normalised variable importance was assessed and variable response functions were produced using BIOMOD2's default procedure.

Model predictive performance was assessed by the following three measures:

- AUC, the area under the receiver operating characteristic curve (Fielding & Bell 1997). Predictions of presence-absence models can be compared with a subset of records set aside for model evaluation (here 20%) by constructing a confusion matrix with the number of true positive, false positive, false negative and true negative cases. For models generating non-dichotomous scores (as here) a threshold can be applied to transform the scores into a dichotomous set of presence-absence predictions. Two measures that can be derived from the confusion matrix are sensitivity (the proportion of observed presences that are predicted as such, quantifying omission errors), and specificity (the proportion of observed absences that are predicted as such, quantifying commission errors). A receiver operating characteristic (ROC)

curve can be constructed by using all possible thresholds to classify the scores into confusion matrices, obtaining sensitivity and specificity for each matrix, and plotting sensitivity against the corresponding proportion of false positives (equal to $1 - \text{specificity}$). The use of all possible thresholds avoids the need for a selection of a single threshold, which is often arbitrary, and allows appreciation of the trade-off between sensitivity and specificity. The area under the ROC curve (AUC) is often used as a single threshold-independent measure for model performance (Manel et al. 2001). AUC is the probability that a randomly selected presence has a higher model-predicted suitability than a randomly selected absence (Allouche et al. 2006).

- Cohen's Kappa (Cohen 1960). This measure corrects the overall accuracy of model predictions (ratio of the sum of true presences plus true absences to the total number of records) by the accuracy expected to occur by chance. The Kappa statistic ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random. Advantages of Kappa are its simplicity, the fact that both commission and omission errors are accounted for in one parameter, and its relative tolerance to zero values in the confusion matrix (Manel et al. 2001). However, Kappa has been criticised for being sensitive to prevalence (the proportion of sites in which the species was recorded as present) and may therefore be inappropriate for comparisons of model accuracy between species or regions (McPherson et al. 2004, Allouche et al. 2006).
- TSS, the true skill statistic (Allouche et al. 2006). TSS is defined as sensitivity + specificity - 1, and corrects for Kappa's dependency on prevalence. TSS compares the number of correct forecasts, minus those attributable to random guessing, to that of a hypothetical set of perfect forecasts. Like Kappa, TSS takes into account both omission and commission errors, and success as a result of random guessing, and ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random (Allouche et al. 2006).

An ensemble model was created by first rejecting poorly performing algorithms with relatively extreme low AUC values and then averaging the predictions of the remaining algorithms, weighted by their AUC. To identify poorly performing algorithms, AUC values were converted into modified z-scores based on their difference to the median and the median absolute deviation across all algorithms (Iglewicz & Hoaglin 1993). Algorithms with $z < -2$ were rejected. In this way, ensemble projections were made for each dataset and then averaged to give an overall suitability, as well as its standard deviation.

Projections were classified into suitable and unsuitable regions using a "lowest presence threshold" (Pearson et al. 2007), setting the cut-off as the lowest value at which 98% of all presence records are classified correctly under the current climate (here 0.69). In order to express the sensitivity of classifications to the choice of this threshold, thresholds at which 95% and 99% of records are classified correctly (here 0.86 and 0.65 respectively) were used in the calculation of error bars in Figures 9 and 10 below in addition to taking account of uncertainty in the projections themselves.

We also produced a limiting factor map for Europe following Elith et al. (2010). For this, projections were made separately with each individual variable fixed at a near-optimal value. These were chosen as the median values at the occurrence grid cells. Then, the most strongly limiting factors were identified as the ones resulting in the highest increase in suitability in each grid cell.

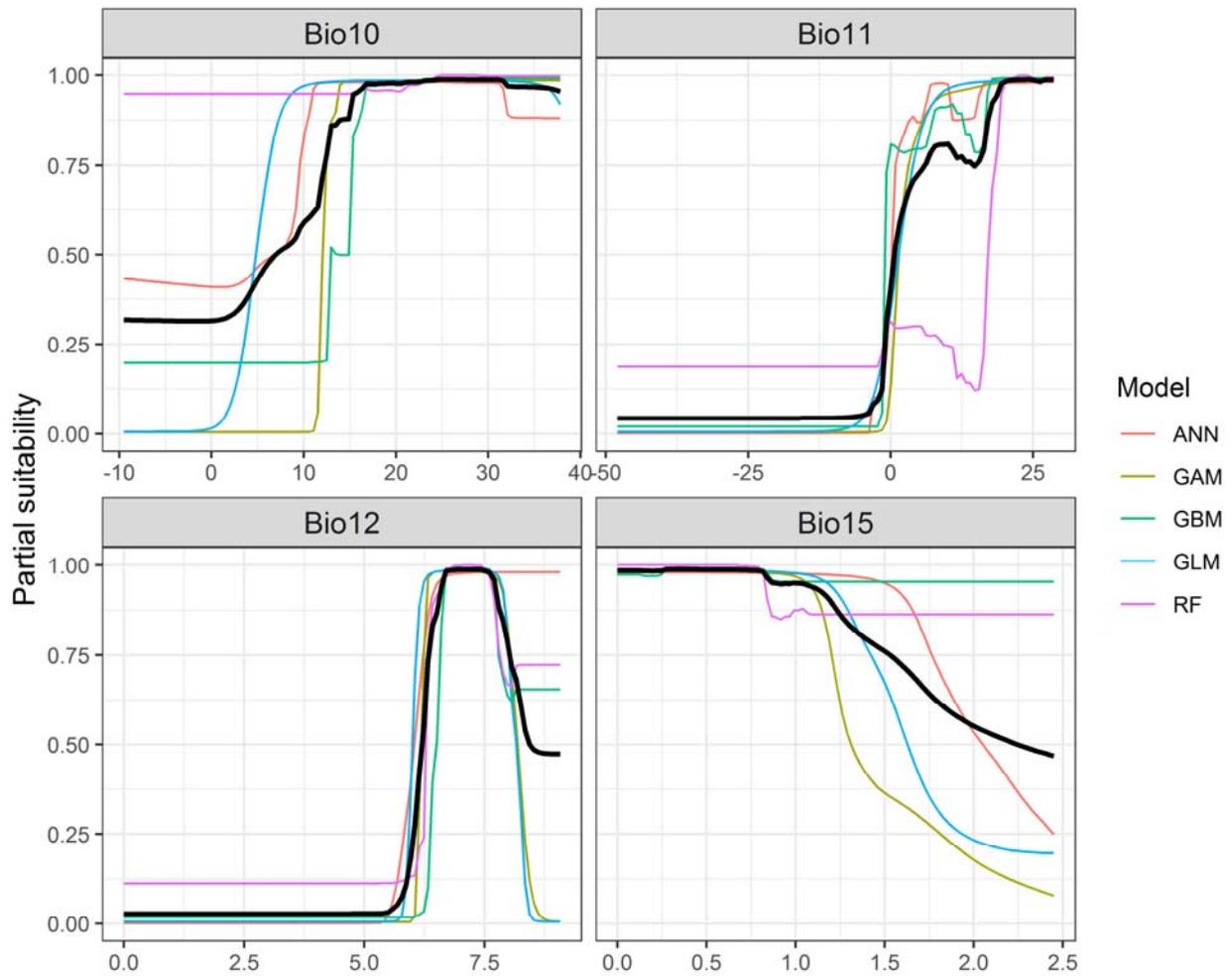
Results

The ensemble model suggested that suitability for *Marisa cornuarietis* was most strongly determined by Annual precipitation (Bio12), accounting for 42.3% of variation explained, followed by Mean temperature of the coldest quarter (Bio11) (41.2%), Mean temperature of the warmest quarter (Bio10) (13.5%) and Precipitation seasonality (Bio15) (2.9%) (Table 1, Figure 3).

Table 1. Summary of the cross-validation predictive performance (AUC, Kappa, TSS) and variable importance of the fitted model algorithms and the ensemble (AUC-weighted average of the best performing algorithms). Results are the average from models fitted to 10 different background samples of the data.

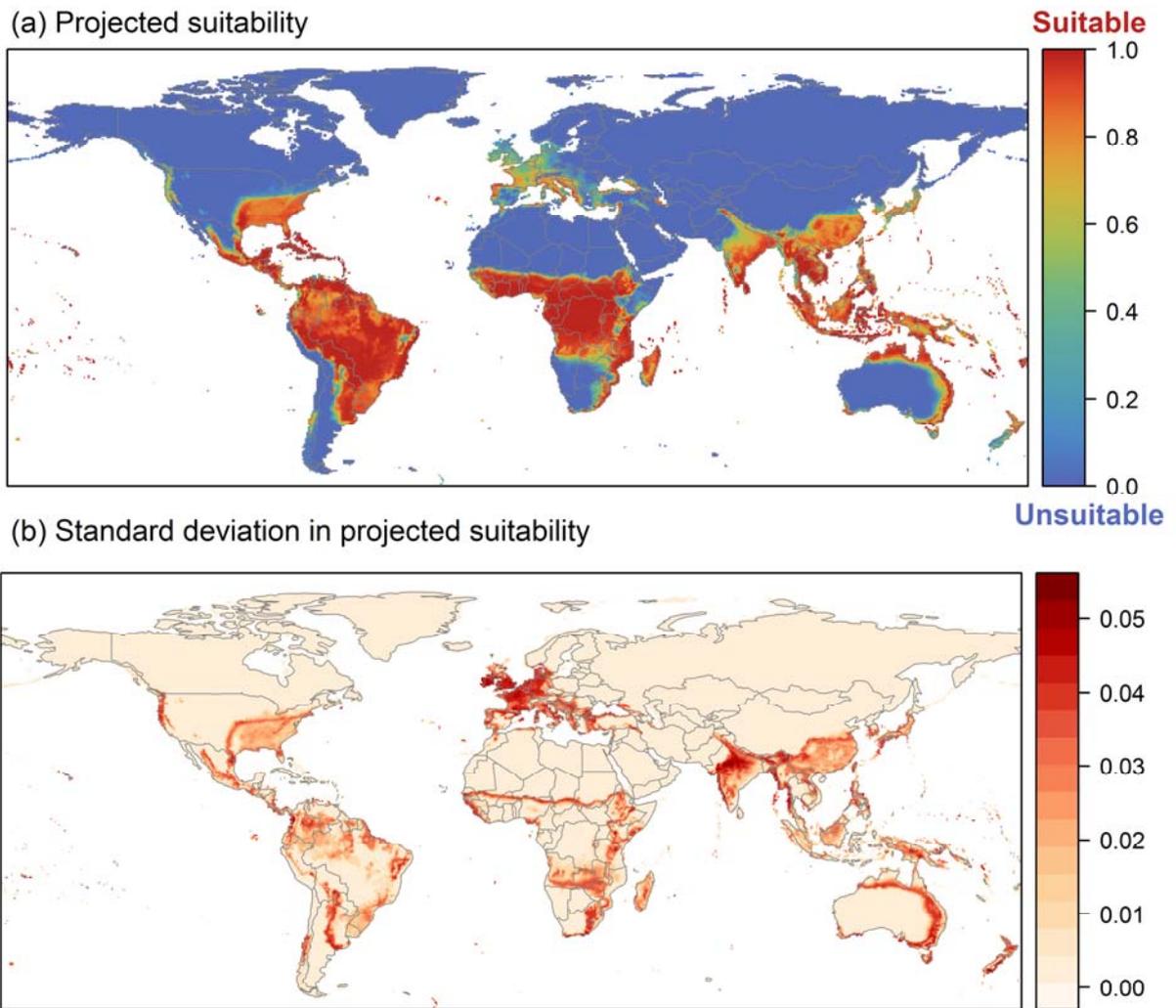
Algorithm	AUC	Kappa	TSS	Used in the ensemble	Variable importance (%)			
					Annual precipitation (Bio12)	Mean temperature of the coldest quarter (Bio11)	Mean temperature of the warmest quarter (Bio10)	Precipitation seasonality (Bio15)
GLM	0.989	0.643	0.955	yes	40	47	8	5
GAM	0.988	0.650	0.959	yes	40	41	16	3
GBM	0.986	0.652	0.934	yes	47	38	15	0
ANN	0.987	0.634	0.973	yes	37	47	11	4
MARS	0.985	0.643	0.957	no	38	47	8	7
RF	0.986	0.640	0.940	yes	47	33	18	2
Maxent	0.969	0.648	0.911	no	39	38	16	6
Ensemble	0.988	0.647	0.975		42	41	13	3

Figure 3. Partial response plots from the fitted models. Thin coloured lines show responses from the algorithms in the ensemble, while the thick black line is their ensemble. In each plot, other model variables are held at their median value in the training data. Some of the divergence among algorithms is because of their different treatment of interactions among variables.



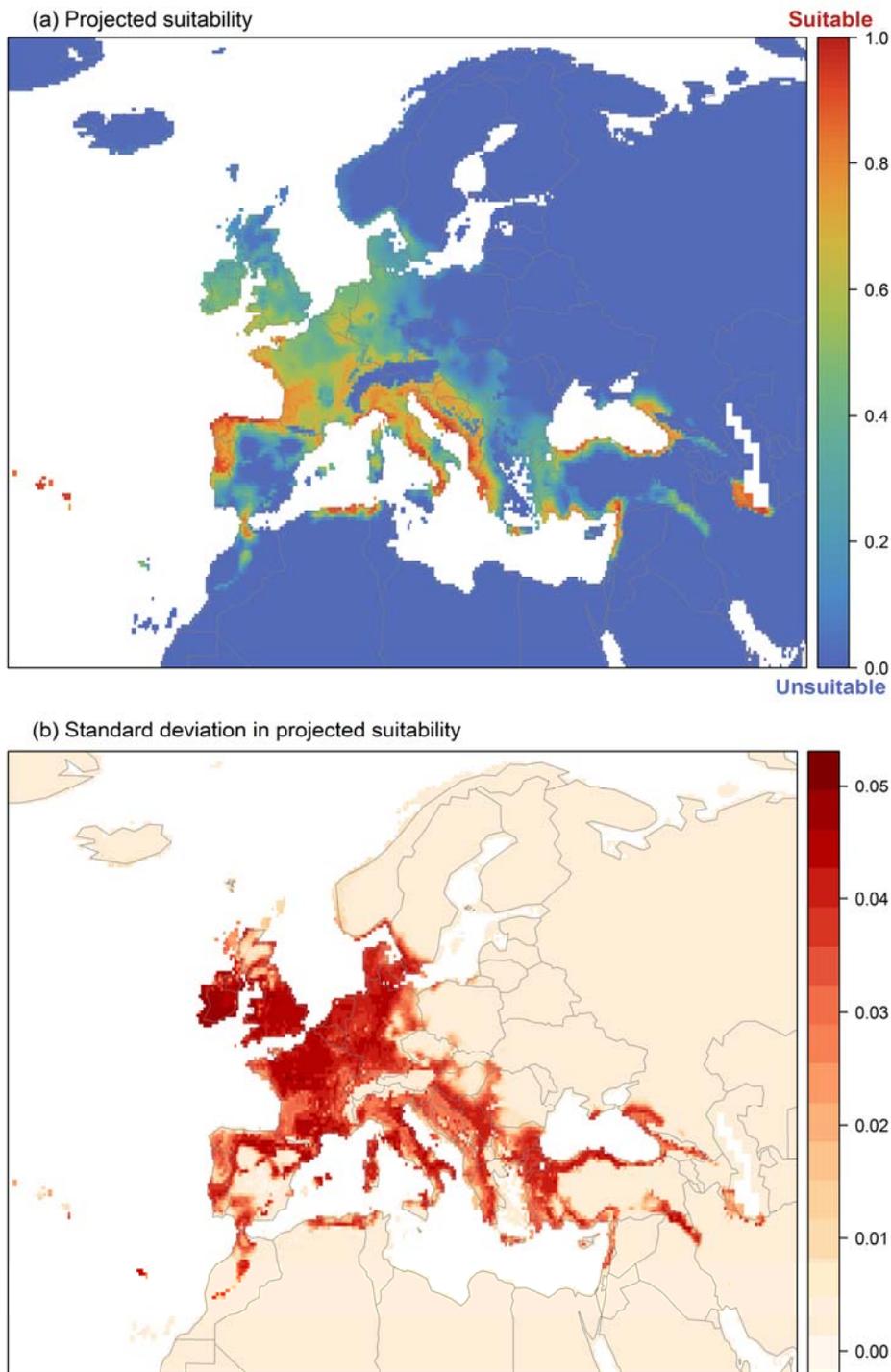
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Figure 4. (a) Projected global suitability for *Marisa cornuarietis* establishment in the current climate. For visualisation, the projection has been aggregated to a 0.5 x 0.5 degree resolution, by taking the maximum suitability of constituent higher resolution grid cells. Values > 0.69 are suitable for the species, with 98% of global presence records above this threshold. Values below 0.69 indicate lower relative suitability. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.



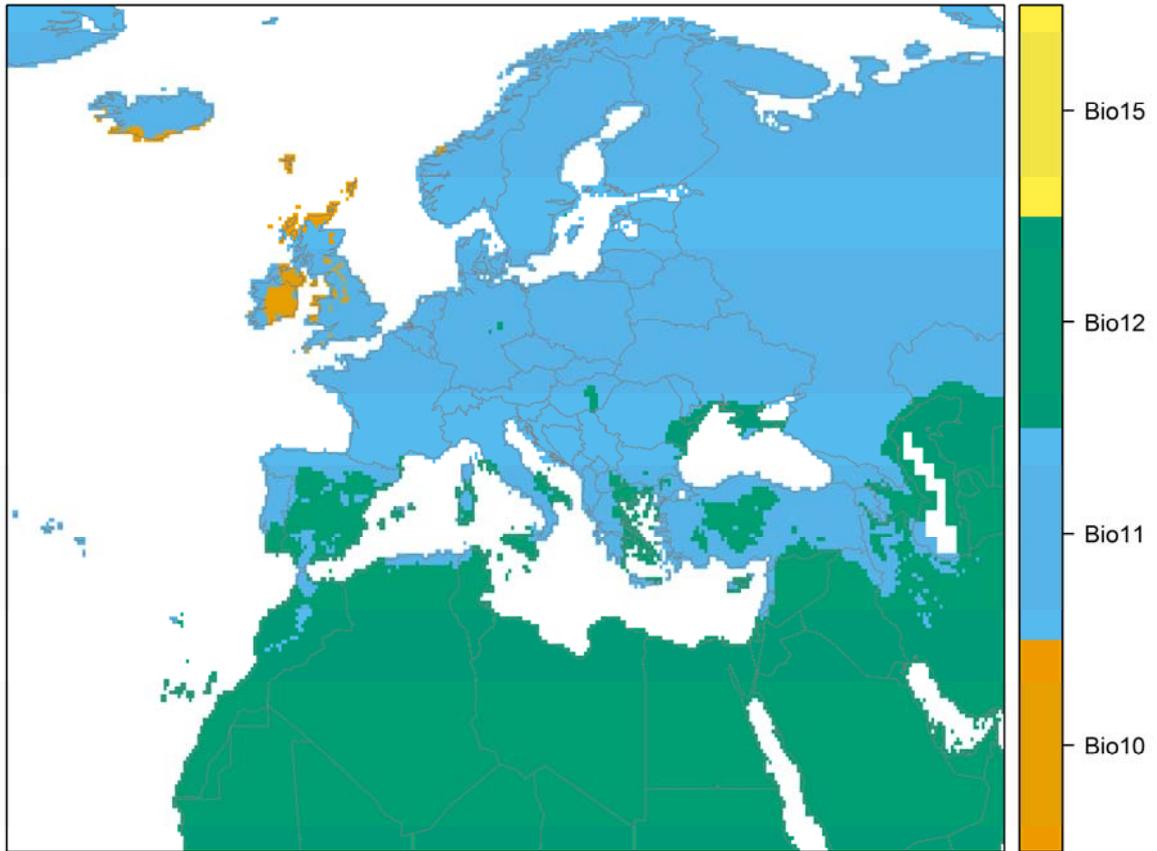
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Figure 5. (a) Projected current suitability for *Marisa cornuarietis* establishment in Europe and the Mediterranean region. Values > 0.69 are suitable for the species, with 98% of global presence records above this threshold. Values below 0.69 indicate lower relative suitability. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.



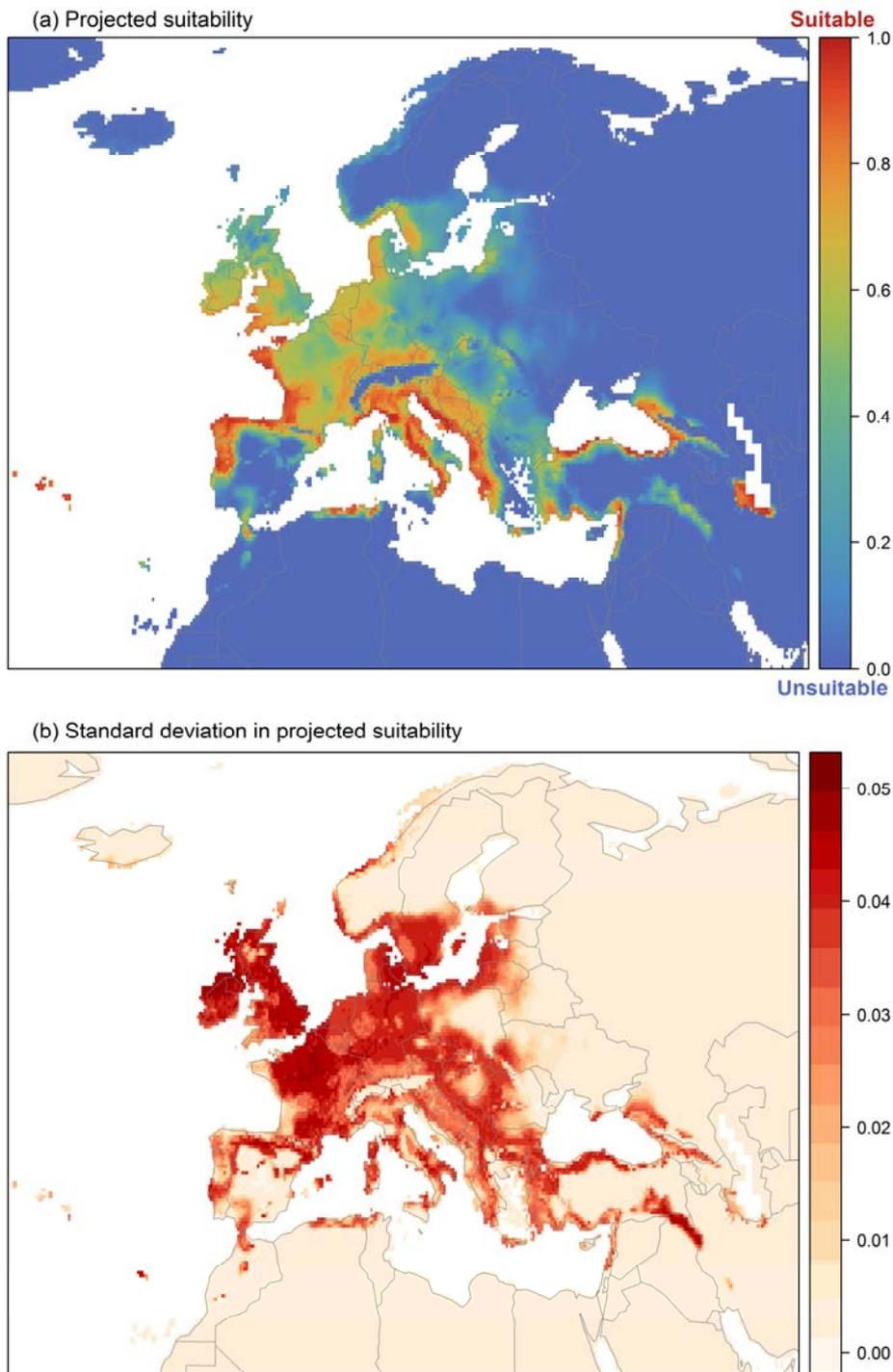
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Figure 6. The most strongly limiting factors for *Marisa cornuarietis* establishment estimated by the model in Europe and the Mediterranean region in current climatic conditions.



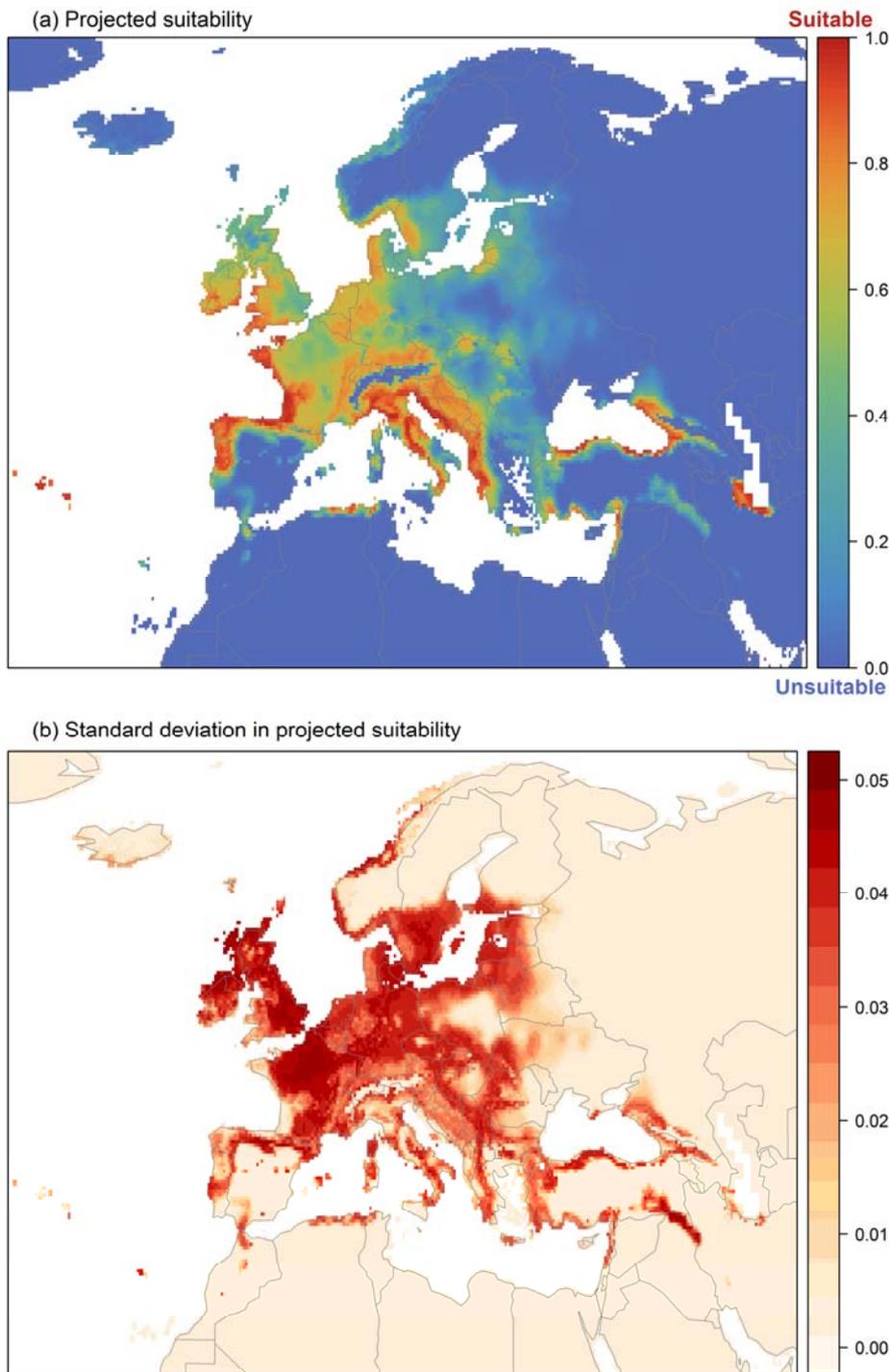
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Figure 7. (a) Projected suitability for *Marisa cornuarietis* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP2.6. Values > 0.69 are suitable for the species, with 98% of global presence records above this threshold under current climate. Values below 0.69 indicate lower relative suitability. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.



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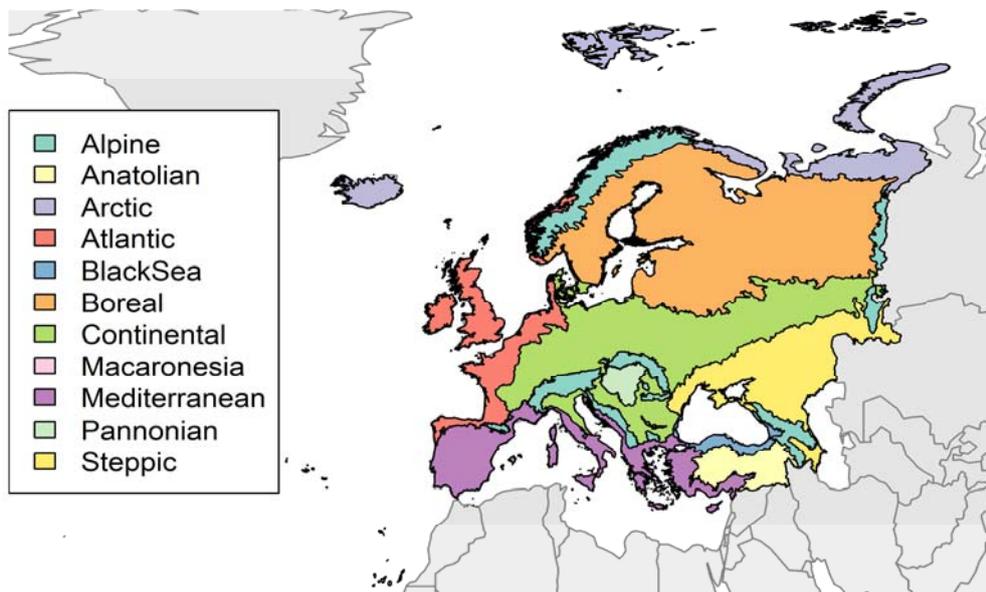
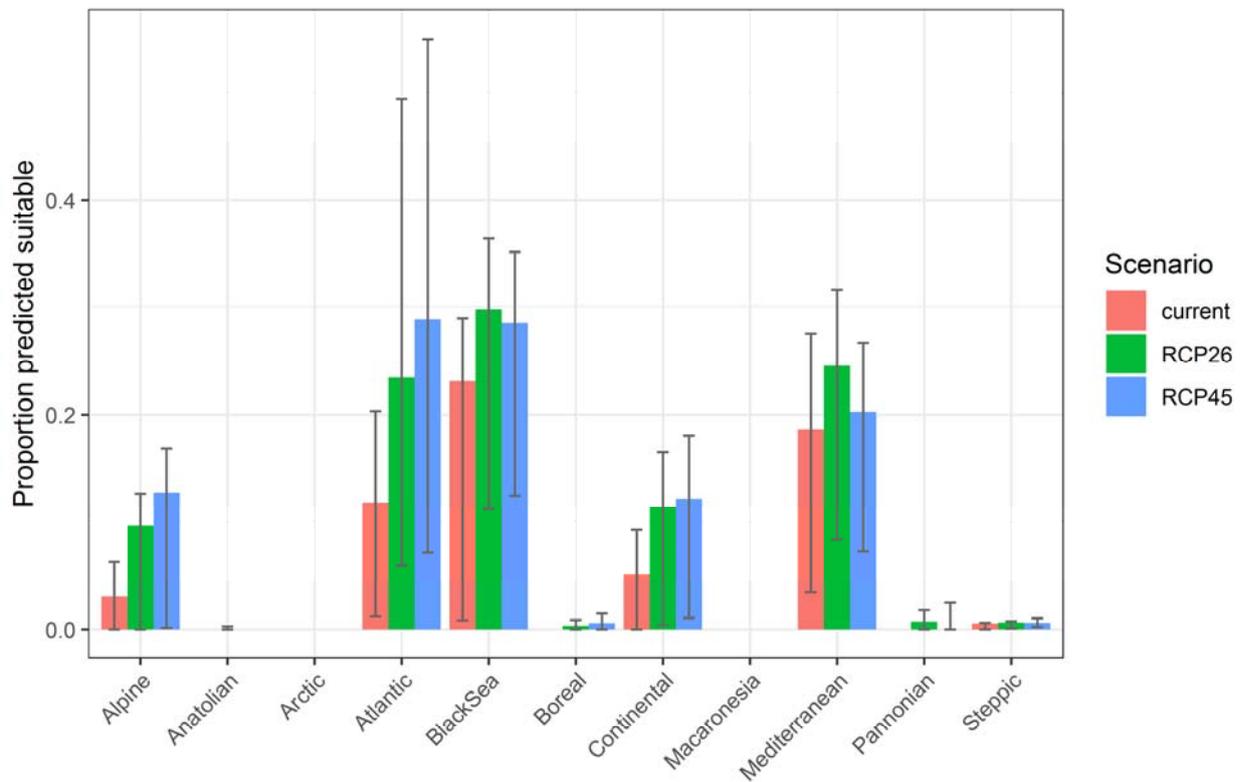
Figure 8. (a) Projected suitability for *Marisa cornuarietis* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP4.5. Values > 0.69 are suitable for the species, with 98% of global presence records above this threshold under current climate. Values below 0.69 indicate lower relative suitability. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.



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Figure 9. Variation in projected suitability for *Marisa cornuarietis* establishment among Biogeographical Regions of Europe (<https://www.eea.europa.eu/data-and-maps/data/biogeographical-regions-europe-3>). The bar plots show the proportion of grid cells in each region classified as suitable (with values > 0.69) in the current climate and projected climate for the 2070s under two RCP emissions scenarios. Error bars indicate uncertainty due to both the choice of classification threshold (cf. p.5/6) and uncertainty in the projections themselves (cf. part (b) of

Figures 5, 7 and 8). The location of each region is also shown. The Arctic and Macaronesian regions are not part of the study area, but are included for completeness.



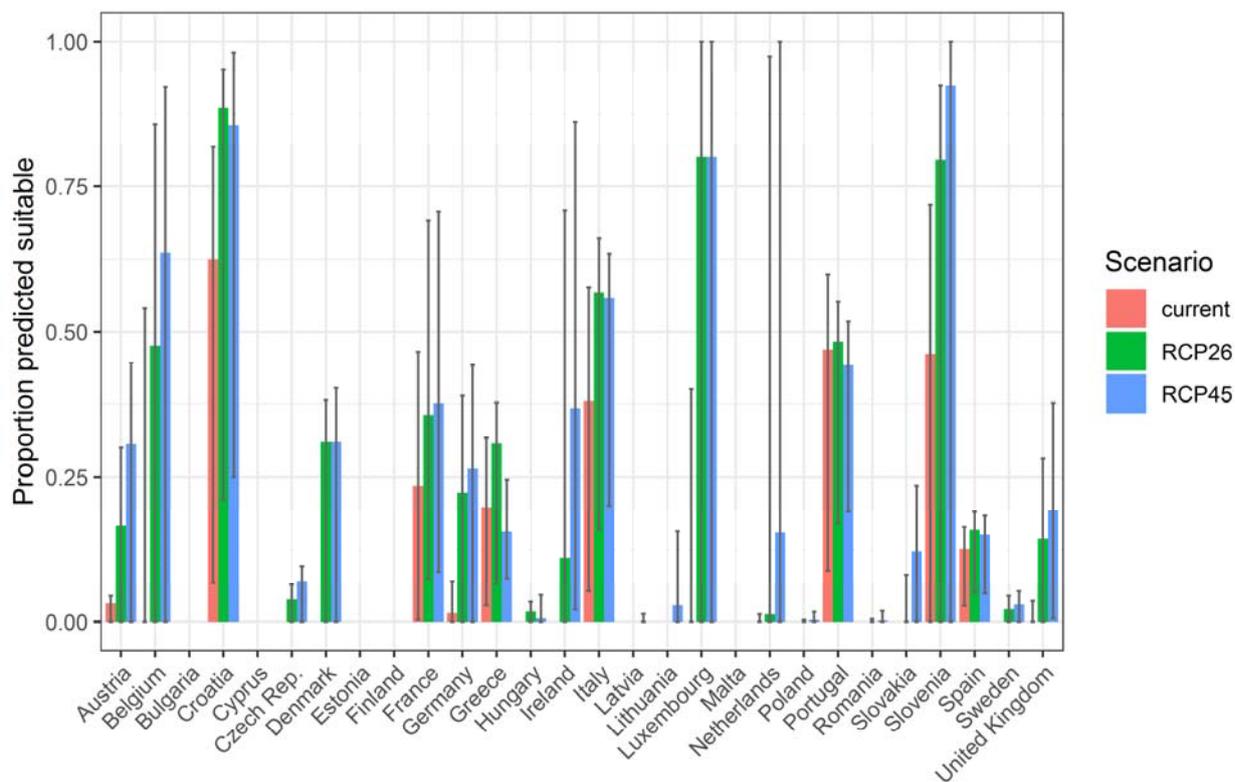
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Table 2. Variation in projected suitability for *Marisa cornuarietis* establishment among Biogeographical regions of Europe (numerical values of Figure 9 above). The numbers are the proportion of grid cells in each region classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. The Arctic and Macaronesian biogeographical regions are not part of the study area, but are included for completeness.

	current climate			2070s RCP2.6			2070s RCP4.5		
	lower	central estimate	upper	lower	central estimate	upper	lower	central estimate	upper
Alpine	0.00	0.03	0.06	0.00	0.10	0.13	0.00	0.13	0.17
Anatolian	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Arctic	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Atlantic	0.01	0.12	0.20	0.06	0.23	0.49	0.07	0.29	0.55
Black Sea	0.01	0.23	0.29	0.11	0.30	0.36	0.12	0.29	0.35
Boreal	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.01
Continental	0.00	0.05	0.09	0.00	0.11	0.16	0.01	0.12	0.18
Macaronesia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Mediterranean	0.03	0.19	0.28	0.08	0.25	0.32	0.07	0.20	0.27
Pannonian	0.00	0.00	0.00	0.00	0.01	0.02	0.00	0.00	0.02
Steppic	0.00	0.01	0.01	0.00	0.01	0.01	0.00	0.01	0.01

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Figure 10. Variation in projected suitability for *Marisa cornuarietis* establishment among European Union countries and the UK. The bar plots show the proportion of grid cells in each country classified as suitable (with values > 0.69) in the current climate and projected climate for the 2070s under two RCP emissions scenarios. Error bars indicate uncertainty due to both the choice of classification threshold (cf. p.5/6) and uncertainty in the projections themselves (cf. part (b) of Figures 5, 7 and 8).



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Table 3. Variation in projected suitability for *Marisa cornuarietis* establishment among European Union countries and the UK (numerical values of Figure 10 above). The numbers are the proportion of grid cells in each country classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios.

	current climate			2070s RCP2.6			2070s RCP4.5		
	lower	central estimate	upper	lower	central estimate	upper	lower	central estimate	upper
Austria	0.00	0.03	0.04	0.00	0.17	0.30	0.00	0.31	0.45
Belgium	0.00	0.00	0.54	0.00	0.48	0.86	0.00	0.63	0.92
Bulgaria	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Croatia	0.07	0.62	0.82	0.21	0.88	0.95	0.25	0.86	0.98
Cyprus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Czech Rep.	0.00	0.00	0.00	0.00	0.04	0.06	0.00	0.07	0.10
Denmark	0.00	0.00	0.00	0.00	0.31	0.38	0.00	0.31	0.40
Estonia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Finland	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
France	0.00	0.24	0.47	0.08	0.36	0.69	0.09	0.38	0.71
Germany	0.00	0.01	0.07	0.00	0.22	0.39	0.00	0.26	0.44
Greece	0.03	0.20	0.32	0.07	0.31	0.38	0.08	0.16	0.25
Hungary	0.00	0.00	0.00	0.00	0.02	0.03	0.00	0.01	0.05

Ireland	0.00	0.00	0.00	0.00	0.11	0.71	0.02	0.37	0.86
Italy	0.05	0.38	0.58	0.16	0.57	0.66	0.20	0.56	0.63
Latvia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
Lithuania	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.16
Luxembourg	0.00	0.00	0.40	0.00	0.80	1.00	0.00	0.80	1.00
Malta	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Netherlands	0.00	0.00	0.01	0.00	0.01	0.97	0.00	0.15	1.00
Poland	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02
Portugal	0.09	0.47	0.60	0.17	0.48	0.55	0.19	0.44	0.52
Romania	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02
Slovakia	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.12	0.23
Slovenia	0.00	0.46	0.72	0.00	0.79	0.92	0.00	0.92	1.00
Spain	0.03	0.13	0.16	0.05	0.16	0.19	0.05	0.15	0.18
Sweden	0.00	0.00	0.00	0.00	0.02	0.04	0.00	0.03	0.05
UK	0.00	0.00	0.04	0.00	0.14	0.28	0.01	0.19	0.38

Caveats to the modelling

The number of presence records available for modelling (118 at the model resolution) was low, and therefore the indication they provide of the species' environmental preferences may not be very comprehensive.

To remove spatial recording biases, the selection of the background sample from the accessible background was weighted by the density of Ampullariidae records on the Global Biodiversity Information Facility (GBIF). While this is preferable to not accounting for recording bias at all, it may not provide the perfect measure of recording bias.

There was substantial variation among modelling algorithms in the partial response plots (Figure 3). In part this will reflect their different treatment of interactions among variables. Since partial plots are made with other variables held at their median, there may be values of a particular variable at which this does not provide a realistic combination of variables to predict from.

Other variables potentially affecting the distribution of the species, such as types of land cover were not included in the model.

[Page Break](#)

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Annex with evidence on measures and their implementation cost and cost-effectiveness

Species (scientific name)	<i>Marisa cornuarietis</i>
Species (common name)	Giant Ramshorn Snail
Author(s)	Frances Lucy and Darren Garland
Reviewer	Elena Tricarico, Hugo Verreycken
Date Completed	15/10/2021

Summary¹

Highlight of measures that provide the most cost-effective options to prevent the introduction, achieve early detection, rapidly eradicate and manage the species, including significant gaps in information or knowledge to identify cost-effective measures.

Marisa cornuarietis is a freshwater snail native to South America, inhabiting both open and closed water systems, is quite large in size with shell size in adults typically reaching 18-22 mm in height and 48-56 mm in diameter (Barkley 2019). In the RA area, *M. cornuarietis* has established in both Spain and Hungary (Frisóczki *et al.* 2016) with the Hungarian population isolated to an outflow downstream of a thermal spa. A significant proportion of the RA area is prone to the introduction of *M. cornuarietis* due to its ongoing use in the ornamental aquatic trade where introductions to both open and closed water bodies are likely from people discarding contents of aquaria (Arias & Torralba-Burrial 2014, Frisóczki *et al.* 2016, Pointier & David 2004). Legislation which aims to ban or restrict the importation and trade of this species and associated ornamental aquatic flora is one proposed measure to prevent the introduction of this invasive to natural water bodies (open and closed systems) in the RA. However, control of the internet trade for this aquarium pet indicates a low confidence in terms of managing the pathway.

Provision of information in a range of member state languages on *M. cornuarietis* could be provided for a range of stakeholders, initially targeting those involved in the aquatic ornamental trade and customer base. This is given a medium level of confidence.

Due to the widespread occurrence of the species in the pet trade, early detection of populations entered into the wild is important and can be achieved through regular monitoring and surveillance of watercourses where climatic and physiochemical parameters are suitable for its establishment. This early detection can be achieved in a cost-effective way, through incorporating surveillance of the species into already developed monitoring programmes such as the Water Framework Directive (2000/60/EC) and by other surveys carried out by academic researchers, MS agencies, NGOs and citizen scientists. This would provide a method of routinely monitoring open and closed water bodies to potentially detect recently introduced populations of *Marisa cornuarietis* and is considered medium in terms of effectiveness.

Like many freshwater species, eradication is only potentially feasible if the species is confined to a small, closed system such as a small lake or pond. If the species is widely established or present in an open water system then eradication is very unlikely to be achieved. There are no studies that describe the successful eradication of this species, or the similar *Pomacea canaliculata*. In terms of potential methods, larger specimens can be physically

removed. It is plausible that chemical control methods listed for *Pomacea canaliculata* could be utilised for *M. cornuarietis* given the similarities between species upon which the control mechanisms were applied. However, it is not known whether these would be effective, they would require permits/derogations and could only be utilized for small closed systems. Biological control measures to suppress numbers have been successful for the similar apple snail *Pomacea canaliculata*, using different ducks, both wild and domestic breeds, in rice fields (Vega et al. 1992; Teo. 2001) and this could be successful in controlling *M. cornuarietis* in closed and open water systems. Control in open water systems is considered unmanageable unless populations were restricted to a very small area. Confidence in all control methods is low as no research has been found on specific control and management of *M. cornuarietis*. No costings were available for treatment methods on *P. canaliculata*. Further research trialling control options on this species, either where it occurs in the RA area or in other invaded regions would be beneficial in developing tools which could then be utilised where established populations are occurring.

Detailed assessment			
	Description of measures ²	Assessment of implementation cost and cost-effectiveness (per measure) ³	Level of confidence ⁴
Methods to achieve prevention of intentional and unintentional introduction⁵	<p>Managing the pathways</p> <p><i>M. cornuarietis</i> is already present in the RA area, but we cannot discount the risk of further intentional and unintentional release of this species to the wild. This could be achieved through a ban of importation and trade of <i>M. cornuarietis</i> for the aquatic ornamental trade. Further action could involve banning the import and trade of aquatic plants, coming from areas where <i>M. cornuarietis</i> is established, as the species is known to hitchhike on aquatic plants, which can potentially result in their unintentional release to natural watercourses. Although the EU legislation for apple snails (2012/697/EU) provides legal</p>	<p>This species is popular as a pet snail for the aquatic ornamental trade, so a ban on importation could create some opposition from the public. Importation can still continue illegally via the internet trade even if a legal ban was imposed and therefore the supply to customers could continue.</p> <p>However, as there are other snail species that pose no or little risk available to the aquarium and live food trades, the ban of live sale of <i>M. cornuarietis</i> would have little or no economic impact. Costs of implementation could be kept to a minimum by utilising existing enforcement processes for other IAS species particularly for aquatic plants on which they hitchhike. Specific training in snail recognition should be provided for the aquatic ornamental trade so they can recognise this species. Implementation of legislation should be coupled with public awareness campaigns and spread of codes of conduct to be more effective. No literature was found regarding effectiveness of implementing the 2012 ban on <i>Pomacea</i> apple snail species in the RA area and in fact it is still for sale on some websites.</p>	Low.

	<p>measures to prevent the introduction into and the spread within the Union of the genus <i>Pomacea</i> (Perry) it does not include this apple snail (<i>M. cornuarietis</i>). Release of all apple snails to the wild has been prohibited in some member states in the RA area such as Spain (Arrias and Toralba-Burrial 2014)</p>		
	<p>Increasing public awareness Provision of information in a range of member state languages on <i>M. cornuarietis</i> could be provided for a range of stakeholders, initially targeting those involved in the aquatic ornamental trade and customer base.</p>	<p>Implementation of legislation should be coupled with targeted public awareness campaigns, e.g. codes of conduct for aquatic ornamental retailers and for purchasers. Effective communication of clear messages is essential to raise awareness of the real threat posed by IAS. Such communication should be centred on species, habitats and invasion biology (Caffrey et al. 2015) Campaigns to educate and increase awareness on IAS are an effective way to curb illegal introductions, especially those targeted at specific sectors. Public awareness campaigns, however, do need to be maintained so they do not drop out of the collective consciousness, but are also renewed periodically to avoid fatigue. Engagement with stakeholders involved in waterbody management and citizens to promote awareness of the potential impacts posed by the establishment of this species would potentially limit the extent of introductions. Cost for outreach such as seminars, codes of practice, and other awareness campaigns can be expensive particularly when translations into different Member State languages are considered. Design, translation and provision of leaflets and pdfs is estimated at 1,000 euro per language and can be produced approximately within three months.</p>	<p>Medium</p>

		There is no information available on the impact this measure would have in reducing introductions of <i>M. cornuarietis</i> .	
Methods to achieve early detection⁶	<p>Effective surveillance and regulations</p> <p>In the RA area, molluscs are indirectly routinely monitored in some open and closed waters as part of the requirement of the Water Framework Directive. They may also be found during field studies by academics, NGOs or by citizen scientists. The species can be detected via direct searches (Barkley 2019) or through indirect sampling via kick sampling. Environmental DNA approaches could be also considered.</p>	<p>Routine monitoring personnel should be trained in identification, to increase potential for early detection and rapid response</p> <p>Identification training in freshwater IAS for citizen scientists and stakeholders involved in waterbody management that includes this species will also aid the early detection. Currently, there is no information available for costs of detecting <i>M. cornuarietis</i> in the RA area but if incorporated into existing water monitoring programmes or included with other IAS training for citizens and other stakeholders then costs would be significantly reduced in comparison to setting up individual monitoring programs.</p> <p>Monitoring is a low risk activity. Public opinion is likely to be supportive of early detection measures particularly if there is involvement via citizen science initiatives, for example bioblitzes.</p> <p>The traditional sampling methods may not be effective where low densities of the species are present, while environmental DNA approaches may be a suitable alternative for detecting if the species is present in a waterbody when low densities occur (Darling and Mahon 2011). Costings for eDNA approaches are approximately between 30 and 50 euro per sample, including eDNA extraction, inhibition test, and qPCR of one target species. The above includes laboratory labour (but not field work) and it assumes that a species specific eDNA (qPCR) assay has already been developed for the target organism/environment/geographic area. There is no information in relation to specific monitoring programs for the detection of this invasive in the RA area but this could relate to limited spread and in fact successful detection has been undertaken for this species in the USA where spread has been across a number of states (Howells et al. 2006) Citizen science campaigns have been found effective in detecting new</p>	Medium.

		<p>aquatic IAS in the RA area. Those involved in detection should ensure boots and equipment are disinfected to assure biosecurity and prevent spread of any aquatic IAS between waterbodies. Once this is in place, there is no perceived environmental harm or environmental cost involved in these methods.</p>	
<p>Methods to achieve eradication⁷</p>	<p>Chemical removal</p>	<p>Like many freshwater species, eradication is only potentially feasible if the species is confined to a small, closed system such as a small lake or pond. If the species is widely established or present in an open water system then eradication is very unlikely to be achieved. There are no studies that describe the successful eradication of this species, or of the similar <i>Pomacea canaliculata</i>.</p> <p>Chemical controls utilising molluscicides over several years can potentially eradicate the species, but 100% mortality has been noted to be difficult due to differential susceptibility of individuals in populations (Barkley 2019).</p> <p>One old study suggests that a concentration of 0-03 Frescon (N-tritylmorpholine) is applied for at least four days to a closed water body to control this species (Meier-Brook and Tjhen 1977) however this product appears to be no longer on the market.</p> <p>Tributyltin (TBT) has previously been shown to be effective as a control agent (Lyssimachou et al 2009) but this is now a banned substance in the RA and more widely.</p> <p>In South East Asia, chemical control for <i>Pomacea canaliculata</i> has been achieved with the use of two approved synthetic molluscicides, niclosamide and metaldehyde (Schnorbach et al. 2006). These compounds are likely to have negative impacts on the environment. It is not known if these compounds could be used for management of <i>M. cornuarietis</i> in the RA area and their use would likely be restricted to closed systems only and require permits from relevant agencies in MS. Public acceptance of chemical methods could be low. Health and Safety measures are required for safe usage of chemicals.</p>	<p>Low</p>

		<p>significant reduction in pest density from 5 snails m⁻¹ to less than 1 snail m⁻¹ consuming equally well under low and high pest population densities. Therefore, potential exists to use ducks to control <i>M. cornuarietis</i> in closed and possibly open waters. Implementation cost would be low as existing duck populations could be utilised. Acceptance by stakeholders is perceived to be high, and environmental harm low as ducks are a normal feature of wetland systems. Risk of harm to humans would be extremely low.</p>	
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Guidelines for Completing the Annex

1 Provide a brief summary description of the most cost-effective methods drawing on the reviews in the detailed assessments

2 Provide a description of the potential method. This should be based on the available key scientific evidence which should be gathered from sources including articles and reviews in technical and scientific journals, internet searches, online databases, grey literature and relevant books and personal communications from scientists, stakeholders, conservation practitioners and governmental bodies. This information should include a full bibliographic list detailing the literature and sources considered.

3 Provide an assessment of the likely cost and effectiveness of the method. Where information is available, consider the following range of questions, accepting that not all questions will be appropriate in all circumstances.

- How effective has this approach proven to be in the past or in an analogous situation?
- How publically acceptable is the approach likely to be?
- Over what period of time would this approach need to be applied to be effective?
- What is the direct cost of implementing this approach?
- How likely are the methods used in the approach to be available?
- How likely is it that relevant licences or other approvals to undertake the approach would be difficult to obtain?
- How likely is it that health and safety issues would prevent the use of this approach?
- How significant is the environmental harm caused by this approach?
- How significant is the economic harm caused by this approach?
- How significant is the social harm caused by this approach?
- How likely is it that the approach will be criticised on welfare grounds?
- How likely is it that the approach will be acceptable to other stakeholders?

Where available, factual information on the costs of specialist equipment, or case studies of management costs from across the Union or third countries should be provided. When describing case studies, if the information is available then provide both total cost and the area over which control was undertaken so that a cost per unit area might be derived. Where such quantitative information is not available, then any qualitative information from the literature is acceptable to help guide decision making. It is accepted that in the majority of cases the information required to assess the potential total cost of management at a member state level is unlikely to be available. This would normally require information on the extent and abundance of the species which is beyond the scope of this assessment. Assessors are not expected to extrapolate the potential total costs of management at a member state level, only to report on the information provided within the literature.

4 Provide an overall assessment of the confidence that can be applied to the information provided for this method. This confidence should relate to the quality of the available information using the guidance below. It should NOT relate to the confidence in the effectiveness of the method

The confidence scores are:

- **High:** Information comes from published material, or current practices based on expert experience applied in one of the EU countries or third country with similar environmental, economic and social conditions.
- **Medium:** Information comes from published data or expert opinion, but it is not commonly applied, or it is applied in regions that may be too different from Europe (e.g. tropical regions) to guarantee that the results will be transposable.
- **Low:** data are not published in reliable information sources and methods are not commonly practiced or are based solely on opinion; This is for example the case of a novel situation where there is little evidence on which to base an assessment.

If there are further factors beyond these that have determined the chosen level of confidence, then provide a brief written description to support the choice of the level of confidence.

5 Measures for preventing the species being introduced intentionally or unintentionally into the territory of a Member State (cf. Articles 7(1)(a), 7(2), 13, 15), i.e. preventing a species entering by blocking its pathways, including pre-border prevention and prevention of escape or release into the environment and secondary spread whenever relevant. Measures for preventing the species reproducing while in containment should be included, if appropriate. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

6 Measures to run an effective surveillance system for achieving an early detection of a new occurrence (cf. Article 16). This section assumes that the species is not currently present in a Member State, or part of a Member State's territory.

7 Measures to achieve eradication. Preferably, but not exclusively, used at an early stage of invasion, after an early detection of a new occurrence (cf. Article 17). Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

8 Measures to achieve management of the species once it has become widely spread within a Member State territory (cf. Article 19). These measures can be aimed at eradication, population control or containment of a population of the species. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

The development and completion of this template forms part of the EU project **No 07.0202/2020/834529/ETU/ENV.D.2** Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention.

**Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention"
Contract No 07.0202/2020/834529/ETU/ENV.D.2¹**

Name of organism: *Mulinia lateralis* (Say, 1822)

Author(s) of the assessment:

Christine A. Wood, The Marine Biological Association, Plymouth, UK

Marika Galanidi, Dr, Ustun Energy Engineering Ltd, Izmir, Turkey

Björn Beckmann, Dr, Centre for Ecology & Hydrology, Edinburgh, UK

Risk Assessment Area: The risk assessment area is the territory of the European Union 27 and the United Kingdom, excluding the EU-outermost regions.

Peer review 1: Argyro Zenetos, Hellenic Centre for Marine Reseach, Anavyssos, Greece

Peer review 2: Francis Kerckhof, Royal Belgian Institute of Natural Sciences (RBINS), Oostende, Belgium

Date of completion: 15/10/2021

¹ This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA). A number of amendments have been introduced to ensure compliance with Regulation (EU) 1143/2014 on IAS and relevant legislation, including the Delegated Regulation (EU) 2018/968 of 30 April 2018, supplementing Regulation (EU) No 1143/2014 of the European Parliament and of the Council with regard to risk assessments in relation to invasive alien species (see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>).

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SECTION A – Organism Information and Screening

A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?

including the following elements:

- the taxonomic family, order and class to which the species belongs;
- the scientific name and author of the species, as well as a list of the most common synonym names;
- names used in commerce (if any)
- a list of the most common subspecies, lower taxa, varieties, breeds or hybrids

As a general rule, one risk assessment should be developed for a single species. However, there may be cases where it may be justified to develop one risk assessment covering more than one species (e.g. species belonging to the same genus with comparable or identical features and impact). It shall be clearly stated if the risk assessment covers more than one species, or if it excludes or only includes certain subspecies, lower taxa, hybrids, varieties or breeds (and if so, which subspecies, lower taxa, hybrids, varieties or breeds). Any such choice must be properly justified.

Response: This risk assessment covers one species: *Mulinia lateralis* (Say, 1822)

Phylum: Mollusca, **Class:** Bivalvia, **Order:** Venerida, **Family:** Mactridae

Synonyms: *Mactra lateralis* Say, 1822
Mactra rostrata Philippi, 1849

Common names: Dwarf surf clam (EN), Mactre naine (FR), Amerikaanse strandschelp (NE), Amerikanische Trogmuschel, Zwergbrandungsmuschel (DE), Coot clam (US), Little surf clam (US).



Figure 1: Key morphological identification characteristics of *Mulinia lateralis*: (A) the outside of the valves with the radial ridge and (B) the anterior cardinal tooth, which is parallel to the edge of the shell, and the non-serrated lateral teeth. Photos by Koen Lock, and published on Waarnemingen.be



Figure 2: Photo by Vlaams Instituut voor de Zee (VLIZ).

A2. Provide information on the existence of other species that look very similar [that may be detected in the risk assessment area, either in the environment, in confinement or associated with a pathway of introduction]

Include both native and non-native species that could be confused with the species being assessed, including the following elements:

- other alien species with similar invasive characteristics, to be avoided as substitute species (in this case preparing a risk assessment for more than one species together may be considered);
- other alien species without similar invasive characteristics, potential substitute species;
- native species, potential misidentification and mis-targeting

Response: *Mulinia lateralis* is a small bivalve in the family Mactridae. The shells are triangular, distinctly convex, thin, and smooth with very fine concentric lines. The exterior of the shell is white to cream with a thin yellowish-orange periostracum, while the interior is bright white. It has a radial ridge along the posterior end of the valves, and a distinctly broad cardinal area between the beaks in larger specimens. It does not have an externally visible ligament. Adults are approximately 15 to 20 mm in length (but note – in The Netherlands shell lengths ranged from less than 7 mm to 21.2 mm), while newly metamorphosed juvenile shell lengths are 200 to 700 µm.

Similar species: *Mulinia lateralis* is most likely to be confused with native juvenile *Spisula subtruncata*, juvenile *Macra stultorum* or non-native juvenile *Rangia cuneata* (Vermeen et al., 2006; Kerckhof, 2019; Craeymeersch et al., 2019). *Macra stultorum* has an oval shell, no radial ridge, a thin visible external ligament, and no fine concentric growth lines. *Spisula subtruncata* has no radial ridge, a thin visible external ligament, and a brown periostracum. *Rangia cuneata* has distinctly inequilateral shells, an umbo that curves markedly towards the anterior, and a pale brown periostracum, in adults the shell is thick and heavy. Craeymeersch et al. (2019) provides more detail of the main morphological differences between these species, and a key to all species of the subfamily Mactrinae of the NE Atlantic, including the non-native *Rangia cuneata*.

Other possible native confusion species could be *Spisula solida* (F. Kerckhof, pers. comm., 17th August 2021), *S. elliptica* (M. Willing, Conchological Society of Great Britain and Ireland, pers. comm. 25th February 2021), although this species lives further offshore, and juvenile *Mercenaria mercenaria* (Lippson & Lippson, 2006).

DNA based identification is also possible (Craeymeersch et al., 2019; Klunder et al., 2019), and the complete mitochondrial genome is available (Liu et al., 2020).

A3. Does a relevant earlier risk assessment exist? Give details of any previous risk assessment, including the final scores and its validity in relation to the risk assessment area.

Response: No formal risk assessment has been carried out.

In the UK, *Mulinia lateralis* was included in the latest Horizon Scanning exercise for invasive alien species carried out in 2019 (Roy et al., 2019). It was rated in the top four marine species when combining likelihood of arrival, establishment, and impact on biodiversity. It is included in the MSFD UK priority surveillance list (MSFD UK, 2021). A risk assessment for Great Britain is in development (C. A. Wood, in prep.).

A4. Where is the organism native?

including the following elements:

- an indication of the continent or part of a continent, climatic zone and habitat where the species is naturally occurring
- if applicable, indicate whether the species could naturally spread into the risk assessment area

Response: *Mulinia lateralis* is native to the Western Atlantic, encompassing the Temperate North West Atlantic to the Tropical Western Atlantic (Spalding et al., 2007). Its range extends continuously from the Gulf of St Lawrence, Canada; along the eastern coast of the United States (US); and Mexico as far as the Yucatan peninsula, at the southeastern edge of the Gulf of Mexico (Verrill, 1879; Calabrese et al., 1970; Turgeon et al., 2009; GBIF, 2021; OBIS, 2021).

It is widely reported from bays along the Atlantic coast of the US, in a wide range of salinities, from 5 to 80 psu; where it appears to be a mainly estuarine species (Craeymeersch et al., 2019 and references therein). In the Gulf of Mexico, *M. lateralis* is reported from coastal lagoons, which can experience hypersaline conditions during droughts (González-Solis et al., 2018) and offshore banks (Turgeon et al., 2009).

This species prefers soft sediments, such as mud and sand, within the low intertidal and shallow subtidal zones (Snelgrove et al., 1993; Klunder et al., 2019). Although it is also found on offshore banks and shoals (Aller, 1912; Turgeon et al., 2009); Turgeon et al. (2009) report it occurring at depths of up to 134 m in the Gulf of Mexico.



Figure 3: Occurrence records of *M. lateralis* in the native (red) and invaded (blue) range. Data points retrieved from the literature, biodiversity databases and screened as explained in Qu. A4-A6 and the modelling Annex (Annex IX). Map created by Bjorn Beckmann for the purposes of this RA.

A5. What is the global non-native distribution of the organism outside the risk assessment area?

Response: None. *Mulinia lateralis* has only been reported as non-native from within the risk assessment area. GBIF (2021) shows a record from the west coast of the US, but the geographical coordinates do not match with the location described as being in Campeche, in the Gulf of Mexico.

A6. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species been recorded and where is it established? The information needs to be given separately for recorded (including casual or transient occurrences) and established occurrences. “Established” means the process of an alien species successfully producing viable offspring with the likelihood of continued survival².

A6a. Recorded: List regions

A6b. Established: List regions

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

- Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea.

Comment on the sources of information on which the response is based and discuss any uncertainty in the response.

For delimitation of EU biogeographical regions please refer to <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2> (see also Annex VI).

For delimitation of EU marine regions and subregions consider the Marine Strategy Framework Directive areas; please refer to <https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions/technical-document/pdf> (see also Annex VI).

Response (6a): Greater North Sea, incl. the Kattegat and the English Channel.

Mulinia lateralis was first discovered in this subregion in 2017 (Craeymeersch et al., 2019). It has been recorded from the southeastern part of the subregion, from The Netherlands, Germany and Belgium (Craeymeersch et al., 2019; Klunder et al., 2019; Kerckhof, 2019). The identity of specimens from The

² Convention on Biological Diversity, Decision VI/23

Netherlands and Germany have been confirmed by DNA analysis (Craeymeersch et al., 2019; Klunder et al., 2019). For more details on the locations within each country, see response to Qu. A8a.

Response (6b): Greater North Sea, incl. the Kattegat and the English Channel.

Mulinia lateralis is considered established in The Netherlands (Craeymeersch et al., 2019; Klunder et al., 2019), Germany and Belgium, see response to Qu. A8b for more details. It has been found at multiple locations within The Netherlands, sometimes at high densities, and it has been recorded every year from 2017 to 2021. As *M. lateralis* has a maximum life-span of only two years this indicates that reproduction is occurring (Calabrese, 1969b). In 2017, dense populations of up to almost 6000 ind. m² were recorded from the Voordelta (southwestern Dutch coastal waters) and again in 2018 at densities of 1000 ind. m² (Craeymeersch et al., 2019). In 2017, *M. lateralis* was reported from sites in the Eems-Dollard estuary spanning the Dutch and German parts of the Wadden Sea (Klunder et al., 2019). It was recorded there again in 2018 (Craeymeersch et al., 2019; Klunder et al., 2019), and 2019 (GBIF, 2021). In 2018, it was found in the western part of the Dutch Wadden Sea (Craeymeersch et al., 2019; GBIF, 2021). In 2018, *M. lateralis* was recorded at high density (820 ind. m²) in the Westerschelde (Craeymeersch et al., 2019). Subsequently, in 2019, it was recorded there at 18/44 sites sampled, at an average of five ind. m² (Wallès et al., 2020). *M. lateralis* has been reported from multiple sites, all along the Belgian coast.

A7. In which biogeographic region(s) or marine subregion(s) in the risk assessment area could the species establish in the future under current climate and under foreseeable climate change? The information needs to be given separately for current climate and under foreseeable climate change conditions.

A7a. Current climate: List regions

A7b. Future climate: List regions

With regard to EU biogeographic and marine (sub)regions, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (7a): The response is based on combining physiological tolerances and the results from the distribution modeling (see Qu. 2.1, Qu. 2.9 and Annexes VIII & IX for details). For purposes of mapping and assessing the risk of establishment, the following tolerance limits were defined:

- Average surface temperature of the warmest month > 15 °C
- Average surface temperature of the warmest month < 32.5 °C
- Minimum surface salinity > 12.5 psu

Baltic Sea: moderately likely, low confidence (western part)

Greater North Sea: very likely, high confidence

Celtic Seas: moderately likely, medium confidence

Bay of Biscay and the Iberian coast: likely, medium confidence

Mediterranean Sea: likely, medium confidence

Black Sea: moderately likely, medium confidence

Mulinia lateralis is expected to exhibit boom and bust dynamics in Atlantic Europe, where conditions will limit spawning and recruitment to the summer months. An abundance of suitable habitats (estuaries, extensive intertidal and subtidal seabed areas) will make widespread establishment likely. In the Mediterranean Sea, should the species be introduced there, wide establishment is more likely to take place in the western part of the basin which receives higher freshwater discharges, while the more arid eastern Mediterranean contains a lower number of highly suitable habitats, which are mostly concentrated in the northern Aegean. Nevertheless, because climatic conditions there are favourable for larval development throughout most of the year, it is more likely that populations will become more stable and dominant members of the macrobenthos. The Baltic Sea only offers salinity conditions suitable for establishment in its westernmost part. Establishment is also considered possible in the Black Sea, although low winter temperatures are likely to limit reproduction to the warmer months of the year.

Response (7b): The response is based on combining physiological tolerances and the results from the distribution modeling (see Qu. 2.1, Qu. 2.9 and Annexes VIII & IX for details). Aspects of climate change most likely to affect future distribution were considered as an increase in minimum and maximum Sea Surface Temperatures (SST). The methodology for the developed models is described in Annex IX and considers scenarios RCP 2.6 and RCP 4.5 by 2070. See also Qu. 2.10.

Baltic Sea: moderately likely, low confidence (western part)

Greater North Sea: very likely, high confidence

Celtic Seas: likely, medium confidence

Bay of Biscay and the Iberian coast: likely, medium confidence

Mediterranean Sea: likely, medium confidence

Black Sea: moderately likely, medium confidence

The species distribution model (SDM) predicted a small reduction in projected suitability for *M. lateralis* for the Mediterranean Sea and a corresponding increase for the North-East Atlantic marine subregions under future climate change and, consequently, a small northward shift of the overall suitable area for the species. An increase in sea surface temperature will offer suitable conditions for spawning and larval development for prolonged periods throughout the whole of Atlantic Europe and is predicted to extend its potential range further north along the coast of northern England, Scotland and Ireland. Suitable conditions in the Mediterranean are likely to become even more restricted spatially and temporally, rendering establishment in this marine subregion more localized, especially in the east and south.

Note: Even though the SDM takes into account salinity variability in the form of distance from river mouths, a future scenario for river discharge was not taken into account and would take very elaborate

data processing to bring into the model. It is anticipated that extreme weather phenomena, like droughts and storms/flooding, will be more frequent and intense under future climate conditions and this will increase the uncertainty of predictions as well as the population fluctuations of *M. lateralis*.

A8. In which EU Member States has the species been recorded and in which EU Member States has it established? List them with an indication of the timeline of observations. The information needs to be given separately for recorded and established occurrences.

A8a. Recorded: List Member States

A8b. Established: List Member States

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden.

The description of the invasion history of the species shall include information on countries invaded and an indication of the timeline of the first observations, establishment and spread.

Response (8a):

Belgium: *Mulinia lateralis* was first identified in January 2019 from washed up shells from a beach between Oostende and De Haan (Kerckhof, 2019). In February 2019, sixty-four live specimens were found offshore of De Haan (Waarnemingen, 2021), with subsequent reports of live clams from Heist, De Haan and Zeebrugge. There is a large population present in the port of Zeebrugge (F. Kerckhof, pers. comm. 17th August 2021).

Germany: This species was first reported from the German area of the Eems-Dollard estuary within the Wadden sea in 2017 and again in 2018 (Klunder et al., 2019). It has not been recorded from elsewhere in Germany.

The Netherlands: In 2017, *M. lateralis* was first discovered in the Voordelta (Dutch coastal waters) (Craeymeersch et al., 2019). Also in 2017, it was reported from sites in the Eems-Dollard estuary within the Dutch Wadden Sea (Craeymeersch et al., 2019; Klunder et al., 2019). Additionally in 2018, it was found in the western part of the Dutch Wadden Sea (Craeymeersch et al., 2019; GBIF, 2021) and in the Westerschelde (Craeymeersch et al., 2019).

Response (8b):

Belgium: All the records of live *Mulinia lateralis* from Belgium are from citizen science records, however many have been verified and are accompanied by high quality images. Although only recorded from Belgium over the last two years, it has been reported from multiple sites, all along the Belgian coast. It was first identified in Belgium in January 2019 from washed up shells from a beach between Oostende and De Haan (Kerckhof, 2019). In February 2019, sixty-four live specimens were found offshore of De Haan (Waarnemingen, 2021), with subsequent reports of live clams from Heist, De Haan and Zeebrugge. In 2020, there are records from multiple sites along the whole of the Belgian coast from Knokke to De Panne (on the border with France) (Waarnemingen, 2021).

Germany: *M. lateralis* was first reported from two sites, referred to as Ems-Krummhörn, in the German part of the Eems-Dollard estuary within the Wadden sea in 2017, and from one of the same sites again in 2018 (Klunder et al., 2019). It has not been recorded from elsewhere in Germany. However, its continued presence throughout the Dutch part of the Eems-Dollard estuary has been recorded from 2017 to 2019 (Craeymeersch et al., 2019; Klunder et al., 2019; GBIF, 2021), longer than the species' maximum life-span of two years (Calabrese, 1969b), so it can be presumed that it is also established in the German part.

The Netherlands: *M. lateralis* is considered established in The Netherlands (Craeymeersch et al., 2019; Klunder et al., 2019). It has been found at multiple locations, in high densities at some localities, and recorded every year from 2017 to 2021. As *M. lateralis* has a maximum life-span of only two years this indicates that reproduction is occurring (Calabrese, 1969b). In 2017, dense populations of up to almost 6000 ind. m² were recorded from the Voordelta (Dutch coastal waters) (Craeymeersch et al., 2019). It was found there again in 2018 at densities of 1000 ind. m² (Craeymeersch et al., 2019). In 2017, it was reported from sites in the Eems-Dollard estuary in the Dutch part of the Wadden Sea (Klunder et al., 2019). It was recorded from multiple sites again in 2018 (Craeymeersch et al., 2019; Klunder et al., 2019), and 2019 (GBIF, 2021). In 2018, it was found in the western part of the Dutch Wadden Sea (Craeymeersch et al., 2019, GBIF, 2021). In 2018 it was recorded at high density (820 ind. m²) in the Westerschelde (Craeymeersch et al., 2019). Subsequently, in 2019, it was recorded at 18/44 sites sampled in the estuary, at an average density of five ind. m² (Walles et al., 2020). In 2020 it was recorded at multiple sites along the Dutch coastal zone and estuaries as well as the Dutch part of the Wadden Sea (Troost et al., 2021).

A9. In which EU Member States could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A9a. Current climate: List Member States

A9b. Future climate: List Member States

With regard to EU Member States, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (9a): The Species Distribution Model outlined in Annex IX provides a projection of environmental suitability for *Mulinia lateralis* establishment in Europe. According to the model the species could establish in Belgium, Bulgaria, Croatia, Denmark, France, Germany, Greece, Italy, Netherlands, Slovenia, and Spain, and also in the non-member state the United Kingdom.

Along the Portuguese coast seasonal upwellings result in relatively low summer temperatures (Vidal et al., 2007) and cause a reduction in the overall GDD (Growing Degree Days, see Annex IX for details), which emerges as the limiting factor for establishment in the model. This effect however is less pronounced towards the south of the country, where late summer temperatures are well above the minimum needed for establishment (Silva et al., 2009). Furthermore, we expect that at a finer scale the lagoons and estuaries will be warmer and shallower than indicated by the model, and thus Portugal would also be suitable for establishment.

Response (9b): Belgium, Bulgaria, Croatia, Denmark, France, Germany, Greece, Italy, Netherlands, Portugal, Romania, Slovenia, Spain, and Sweden, and also in the non-member state the United Kingdom.

The response to 9b is based on the RCP 4.5 scenario for the period 2050/2070. The aspect of climate change most likely to affect the organism's ability to establish is an increase in winter sea surface temperatures. Higher winter temperatures in Atlantic Europe will favour larvae for longer periods and at higher latitudes (see Annexes VIII & IX for details on modelling and future climate conditions in the RA area).

A10. Is the organism known to be invasive (i.e. to threaten or adversely impact upon biodiversity and related ecosystem services) anywhere outside the risk assessment area?

Response: No. *Mulinia lateralis* has not been reported as present anywhere other than within its native range, or the risk assessment area.

A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea

Response: Greater North Sea, incl. the Kattegat and the English Channel.

Mulinia lateralis was only first recorded as an introduced species in The Netherlands and Germany in 2017 (Craeymeersch et al., 2019; Klunder et al., 2019), although Klunder et al. (2019) suggest that it probably arrived in 2016. With such a recent arrival, it is not yet possible to determine if it will be invasive in the long term. However, Craeymeersch et al. (2019) considered it potentially invasive due to its ability to rapidly colonize defaunated areas, its high fecundity, short generation time, planktonic larvae, and its tolerance for anoxia and temperature extremes. In the Voordelta, dense settlements of up to 6000 ind. m² have been recorded. In its native range it can become dominant when conditions are optimal, achieving average densities as high as 21,000 ind. m² (Santos & Simon, 1980; Chalermwat et al., 1991). Klunder et al. (2019) state that *M. lateralis* seems to have become a successful invader in the Dutch Wadden Sea, with fast spread to all suitable areas there to be expected, given the current rate of spread since its first detection. This species has now, within just four years of its arrival, spread to over 450 km of coast from the Eems-Dollard estuary on the German/Dutch border, to De Panne on the Belgian/French border. In the Westerschelde, in 2019, it was recorded at 18/44 sites sampled (Wallis et al., 2020). Potential environmental impacts of this species are discussed by Craeymeersch et al. (2019), including competition for space with native benthic species, and food with other filter-feeding organisms. Other potential impacts include the introduction and/or spread of parasites e.g. *Perkinsus* spp.

In its native range, *M. lateralis* frequently undergoes population crashes due to a variety of causes including starvation, predation, hypoxia, very low salinities events, or physical displacement from trawls or storm disturbance (Santos & Simon, 1980; Shumway & Newell, 1984; Powell et al., 1986; Cleveland, et al., 2002; de Buron et al., 2013). Such mass die-offs of bivalves are known to influence abiotic factors that negatively affect other organisms in the ecosystem (Cherry et al., 2005; Cooper et al., 2005), however no such phenomena have been observed or studied in the RA area to date.

A12. In which EU Member States has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden.

Response: The Netherlands.

Mulinia lateralis was only first recorded as an introduced species in The Netherlands in 2017 (Craeymeersch et al., 2019; Klunder et al., 2019), although Klunder et al. (2019) suggest that it probably arrived in 2016. With such a recent arrival, it is not yet possible to determine if it will be invasive in the long term. However, Craeymeersch et al. (2019) considered it potentially invasive due to its ability to rapidly colonize defaunated areas, its high fecundity, short generation time, planktonic larvae, and its tolerance for anoxia and temperature extremes. In the Voordelta, dense settlements of up to 6000 ind. m² have been recorded. In its native range it can become dominant when conditions are optimal, achieving average densities as high as 21,000 ind. m² (Santos & Simon, 1980; Chalermwat et al., 1991). This species has now, within just four years of its arrival, spread to many areas of the Netherlands including the Voordelta, the Eems-Dollard estuary, the western part of the Dutch Wadden Sea, and the Westerschelde. Klunder et al. (2019) states that *M. lateralis* seems to have become a successful invader

in the Dutch Wadden Sea, with fast spread to all suitable areas there to be expected, given the current rate of spread since its first detection. In the Westerschelde, in 2019, it was recorded at 18/44 sites sampled (Wallès et al., 2020). See Qu. A11 and Magnitude of Impact section for potential impacts.

A13. Describe any known socio-economic benefits of the organism.

including the following elements:

- Description of known uses for the species, including a list and description of known uses in the risk assessment area and third countries, if relevant.
- Description of social and economic benefits deriving from those uses, including a description of the environmental, social and economic relevance of each of those uses and an indication of associated beneficiaries, quantitatively and/or qualitatively depending on what information is available.

If the information available is not sufficient to provide a description of those benefits for the entire risk assessment area, qualitative data or different case studies from across the risk assessment area or third countries shall be used, if available.

Response: Due to its small size, short generation time, and ease of culture and maintenance, *Mulinia lateralis* is considered potentially important as a model species for bivalve development, genetic and transgenic studies (Calabrese, 1969b; Liu et al., 2020; Yang et al., 2021). It has been used extensively in marine pollution bioassays (His et al., 1999; Cripe, 2006). Within its native range, *M. lateralis* is considered important for recreational hunting and fishing as it provides a food source for birds and fish (Kennedy & Mihursky, 1971; Mendenhall, 2015).

SECTION B – Detailed assessment

Important instructions:

- In the case of lack of information the assessors are requested to use a standardized answer: “No information has been found.” In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable).
- With regard to the scoring of the likelihood of events or the magnitude of impacts see Annexes I and II.
- With regard to the confidence levels, see Annex III.
- Highlight the selected response score and confidence level in **bold** but keep the other scores in normal text (so that the selected score is evident in the final document).

1 PROBABILITY OF INTRODUCTION AND ENTRY

Important instructions:

- **Introduction** is the movement of the species into the risk assessment area (it may be either in captive conditions and/or in the environment, depending on the relevant pathways).
- **Entry** is the release/escape/arrival in the environment, i.e. occurrence in the wild
- Introduction and entry may coincide for species entering through pathways such as “corridor” or “unaided”, but it also may differ. If different, please consider all relevant pathways, both for the introduction into the risk assessment area and the entry in the environment.
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used (see Annex IV). For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document³ and the provided key to pathways⁴.
- For organisms which are already present (recorded or established) in the risk assessment area, the likelihood of introduction and entry should be scored as “very likely” by default.
- Repeated (independent) introductions and entries at separate locations in the risk assessment area should be considered here (see Qu. 1.7).

Qu. 1.1. List relevant pathways through which the organism could be introduced into the risk assessment area and/or enter into the environment. Where possible give details about the specific origins and end points of the pathways as well as a description of any associated commodities.

For each pathway answer questions 1.2 to 1.8 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 1.2a, 1.3a, etc. and then 1.2b, 1.3b etc. for the next pathway.

In this context a pathway is the route or mechanism of introduction and/or entry of the species.

³ <https://circabc.europa.eu/sd/a/7e5f0bd4-34e8-4719-a2f7-c0cd7ec6a86e/2020-CBD-pathways-interpretation.pdf>

⁴ <https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf>

The description of commodities with which the introduction of the species is generally associated shall include a list and description of commodities with an indication of associated risks (e.g. the volume of trade; the likelihood of a commodity being contaminated or acting as vector).

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 1.2-1.9.

Pathway names: No pathway(s) have been proven for *Mulinia lateralis*. This species has not previously been introduced anywhere outside the RA area, and has only recently arrived in NW Europe, possibly as the result of a single introduction (Klunder et al., 2019). *M. lateralis* is free-living and does not attach to substrates; therefore hull fouling, floating debris and shellfish imports are unlikely pathways of introduction, particularly as this species is not part of the fouling community as it lives shallowly buried in soft sediments, so would not adhere to or crawl or burrow into other attached fouling organisms. Unaided natural dispersal is not feasible, as the larvae do not remain in the plankton for sufficient time to drift across the Atlantic. *M. lateralis* is used as a model organism for various experimental studies (see Qu. A13). Thus, escape from confinement (research and ex-situ breeding) is a potential pathway of introduction. However, it was considered an unlikely pathway for entry to the RA area, as the relevant studies are carried out either in the US (where the species is native) or in China, where it was recently (2017) introduced for this purpose (Yang et al., 2021). Similar studies in European laboratories mostly use suitable native species e.g. *Mytilus* spp. or native oyster species (His et al., 1999).

TRANSPORT-STOWAWAY (ship/boat ballast water and sediments)

Kerckhof (2019) suggests that *M. lateralis* was introduced into NW Europe with ballast water, like the bivalve *Ensis leei*, also a species originally from the N. American east coast. In addition, ballast water has been suggested as the pathway for two other recently introduced bivalves, the closely related *Rangia cuneata* and the tellinid *Theora lubrica* (Verween et al., 2006; Faasse et al, 2019).

TRANSPORT-STOWAWAY (other means of transport: marine aggregates & dredging)

The global dredging sector is very important in Europe, particularly in Belgium and The Netherlands. M. Faasse proposed the accidental transfer of dredged materials as a possible pathway (pers. comm., 25th February 2021). This could occur from Canada or Mexico within the native range (but not the US, see Qu. 1.3b.).

Pathway name:

a) TRANSPORT-STOWAWAY (ship/boat ballast water and sediments)

Qu. 1.2a. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: There is no doubt the uptake of larvae or adults in ballast water is accidental. See categorization of pathways in Annex IV.

Qu. 1.3a. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The planktotrophic (free swimming) larvae of *Mulinia lateralis* larvae can be taken in with ballast water. In addition, small juveniles and adults that live only slightly below the surface could also be taken up with any sediments disturbed and accidentally loaded with the water (Gollasch & David, 2019). Entry into the environment will occur during the de-ballasting process.

The propagule pressure will depend on the larval densities in the donor location, the time of the year (i.e. if uptake of ballast water coincides with the peak spawning period), as well as the densities of the adult population. There are large populations of *M. lateralis* in many estuaries along the east coast of N. America, where it can reach average densities of up to 21,000 ind. m² (Santos & Simon, 1980; Walker & Tenore, 1984). The reproductive period of *M. lateralis* varies from just a few months in the Gulf of St. Lawrence to all year round in the Gulf of Mexico (Montagna et al., 1993). Each female produces 3-4 million eggs (for details on reproductive traits please see Qu. 2.7). Calabrese (1970) recorded peak larval densities of 2,500-3,500 ind. m³ from Long Island Sound.

Although no information could be found of *M. lateralis* being detected in ballast water, Gollasch et al. (2002) reported 42 bivalve taxa in ballast water in Europe, including the closely related *Macra corallina*. Ballast water has been specifically implicated in the introduction of several bivalve species e.g. *Ensis leei*, *Rangia cuneata* and *Theora lubrica* (Von Cosel et al., 1982; Verween et al., 2006; Faasse et al., 2019). The lowest estimates of the volumes of ballast water taken up, transferred and discharged into world oceans each year are around 10 billion tonnes (Interwies & Khuchua, 2017), whereas just one cubic metre of ballast water may contain from 21 up to 50,000 zooplankton specimens (Locke et al.,

1991, 1993; Gollasch, 1997) and a heavy bulk carrier can carry more than 130,000 tonnes of ballast water (GloBallast, 2021). In addition, it is estimated that over 100 million tons of ballast tank sediments are being transported annually by ships (Endresen et al., 2003). It is thus evident from the above information that the potential for sufficiently high numbers of *M. lateralis* larvae or adults to travel along this pathway is high.

The transatlantic shipping routes from the east coast of N. America to Northern Europe and the Mediterranean represent a major east-west trade lane, being particularly important for containerized trade, with annual US exports to Europe of 3M TEU (twenty-foot equivalent units). New York, Houston, Savannah, Virginia and Charleston are the main exporting ports (Kaluza et al., 2010; UNCTAD, 2019; JOC, 2020).

Qu. 1.4a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The organism has already entered Europe, putatively via ballast water. Larvae of *Mulinia lateralis* can survive salinities of 7-38 psu and temperatures of 7-33 °C (Calabrese, 1969a). They can remain in the free-swimming planktotrophic phase for up to 22 days (Mann et al., 1991). They are filter-feeders and can feed on a wide variety of phytoplankton and natural seston (particularly bacteria) (Shumway & Newell, 1984; Chalermwat et al., 1991). Thus, there is considerable probability of survival in ballast waters.

Shipping time from the east coast of N. America to the RA area can take 6-30 days (Ports.com, 2021), so the larvae could still be free-swimming when the water is discharged. It is also possible larvae could metamorphose within the ballast water tanks, settle in the ballast sediments, and develop into adults, alongside any adults that had been taken in during the uptake of ballast water.

Adult clams can survive temperatures of -2 °C to 35 °C (Calabrese, 1969a; Kennedy & Mihursky, 1971), anoxic conditions, and salinities of 5-80 psu (Parker, 1975), so should be perfectly able to survive and grow in the ballast tank sediments. *M. lateralis* has a very short generation time (reaching sexual maturity in 1-2 months (Guo & Allen, 1994)) therefore it is likely that, particularly on slower voyages, a significant proportion of any adult clams will be sexually mature.

M. lateralis spawns when water temperatures reach above 16 °C (Calabrese, 1969a). The ballast water temperature is likely to be above this for most of the year for journeys from ports south of Washington DC, to the Mediterranean and Atlantic coasts. Thus, any adults present could release large numbers of larvae into the ballast water.

Qu. 1.5a. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The International Maritime Organization (IMO) Ballast Water Management Convention (BWMC) entered into force in September 2017. It requires ships in international traffic to apply ballast water management measures, in particular:

- ballast water exchange in open seas, away from coastal areas (D-1 standard for an interim period)
- fulfil a certain discharge standard (D-2 standard according to the ship specific application schedule phased in up to 8 September 2024). D-2 standard requires the installation of a certified ballast water treatment device, which enables sterilization to avoid transfers of ballast water mediated species.

Ballast Water Exchange (BWE) is currently practiced and requires ships to exchange a minimum of 95% ballast water volume whenever possible at least 200 nautical miles (nm) from the nearest land and in water depths of at least 200 metres. When this is not possible, the BWE shall be conducted at least 50 nm from the nearest land and in waters at least 200 metres in depth (David et al., 2007; and BWMC Guideline 6). Even though BWE can reduce the concentration of live organisms in ballast by 80–95% (Ruiz & Reid 2007; Darling et al., 2018), its application has severe limitations, primarily dependant on shipping patterns of a port (e.g., shipping routes, length of voyages) and local specifics i.e., distance from nearest shore, water depth (David et al., 2007). This is particularly the case for EU Seas where it is often not possible to meet these conditions. In addition, organisms may still remain in the volume of ballast not exchanged and in ballast sediment, or BWE may not be possible due to weather conditions or other safety restrictions. The survival of zooplanktonic organisms (including *M. lateralis* larvae) is thus not unlikely when only BWE measures are implemented.

As a result, ballast water treatment has been deemed necessary, such that ships shall discharge (in relation to the organism size range of interest for *M. lateralis*): less than 10 viable organisms per cubic metre greater than or equal to 50 micrometres in minimum dimension (IMO D-2 standard). Ballast water treatment options include mechanical (filtration, separation), physical (heat treatment, ozone, UV light) and chemical methods (biocides). Efficiencies of various technologies utilised for ballast water treatment are reviewed in Tsolaki & Diamadopoulos (2009), and can vary with treatment method but the application of many combined methods (e.g., Filtration+UV or Hydroclone+chemical disinfectant) can decrease live zooplankton to undetectable levels, practically diminishing propagule pressure. Under the D-2 standard, all ships shall be required to regularly remove and dispose of sediments from spaces designed to carry ballast water in accordance with the ship’s Ballast Water Management Plan. Sediments will be disposed of at designated sediment reception facilities in ports (GloBallast, 2017).

As such, the survival of *M. lateralis* larvae in ballast water, and adults in sediments, with full implementation of the D-2 standard (i.e. after 2024) is considered unlikely. Until then (i.e. currently), planktonic propagules of the species are likely to survive in ballast water and adults in the sediments.

Qu. 1.6a. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Mulinia lateralis* has already entered Europe undetected, putatively via ballast water (Kerckhof, 2019). The introduction into The Netherlands probably occurred in 2016 or earlier (Klunder et al., 2019) but was not detected until September 2017, during monitoring surveys associated with the Rotterdam harbour extension (Craeymeersch et al., 2019). The probability of observing the initial introduction event, particularly at the larval stage is minimal without targeted monitoring surveys at introduction hotspots (Andersen et al., 2014). The gross morphology of adult *M. lateralis* is similar to some common native species, as described in A.2. The adults are small (10-20 mm in length), while newly metamorphosed juvenile shell lengths range from 200 to 700 µm (Wang & Guo, 2008). Thus, *M. lateralis* could easily be overlooked during casual surveys, or if it settles in inaccessible port areas.

After September 2017, with the BWMC coming into effect and gradually being implemented, detection of larval stages in ballast water during Port State Control inspections may be possible. According to Resolution MEPC.252(67), if initial inspections of ballast water samples indicate non-compliance with the D-2 standard, detailed inspections will be carried out. eDNA methodologies are rapidly becoming one of the fastest and most cost-efficient tools for the detection of NIS⁵ in introduction water samples (Darling et al., 2017; Borrell et al., 2017; Koziol et al., 2019). However, full implementation of the BWMC is not anticipated until 2024. Until then, the risk that *M. lateralis* will enter the RA area undetected in ballast waters and sediments remains likely.

Qu. 1.7a. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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⁵ NIS: non-indigenous species, term used in the Marine Strategy Framework Directive, synonym of “alien species” as used in the framework of Regulation (EU) 1143/2014

Response: *Mulinia lateralis* favours low-energy sheltered coastal waters, estuaries and lagoons. It prefers soft sediments, such as mud and sand, within the low intertidal and shallow subtidal zones (Snelgrove et al., 1993; Klunder et al., 2019). It is quick to colonise disturbed areas, such as sites recently dredged or defaunated for other reasons (Santos & Simon, 1980; Pollack et al., 2018).

In Atlantic Europe, the main ports of entry are located within a relatively confined region, i.e. both coasts of the English Channel, but principally the Le Havre-Hamburg range along the north coast of continental Europe. Ports in northern and western Europe are often situated in or near estuaries that exhibit major fluctuations in salinity that *M. lateralis* can take full advantage of, see Qu. 1.4a for more details.

Conversely in the Mediterranean Sea, potential recipient ports are relatively evenly spaced but slightly more concentrated along the central and western Mediterranean, with a small number in Malta, southern Italy and Spain acting as the main transshipment hubs (Rodrigue, 2020). However most Mediterranean ports are located in coastal areas, not lagoons, thus the main risk areas are more isolated. However, this species can survive on the open coast in Belgium, and in its native range, thus it is feasible that possible points of introduction could be widespread; hence we have proposed a conservatively moderate score of isolated, with low confidence.

Repeated introductions from the native range may continue to occur until full implementation of the D-2 standard has taken place.

Qu. 1.8a. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Regarding existing populations of the organism in the RA area, although not proven, ballast water and sediment transport is considered the most likely pathway of introduction (Kerckhof, 2019). Management measures implemented so far (i.e. BWE) have not proven adequate to prevent the introduction of this and other marine invasive species in EU marine waters. With the recent ratification of the BWMC (September 2017), compliance with the D-2 standard is expected to greatly reduce the likelihood of new introductions of *Mulinia lateralis* into Europe with ballast water and sediments. However, this is not expected before 2024 and there may be difficulties in fully implementing it. For example, there are already some early reports of operational problems with the currently installed Ballast Water Management Systems, presumably due to poor installation and inadequate testing in the field (source: <https://www.seatrade-maritime.com/news/asia/operating-problems-with-60-80-of-ballast-water-treatment-systems-intertanko/>).

A concrete protocol for the verification of ballast water compliance monitoring devices has been proposed by IOC-UNESCO, ICES and ISO to IMO (IOC-UNESCO, ICES, ISO, 2019). This protocol builds on the method presented in documents PPR 6/4 and MEPC 74/4/11 (Denmark) (First et al., 2018)

and suggests a practices for verifying ballast water compliance monitoring devices using laboratory and shipboard tests. The intention is that the protocol can be the basis for the development of a standard for such devices, which may be used during commissioning testing, data gathering during the experience-building phase, compliance testing by Port State Control, or self-monitoring.

Pathway name:

b) TRANSPORT-STOWAWAY (other means of transport: marine aggregates & dredging)

Qu. 1.2b. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional	CONFIDENCE	low
	unintentional		medium
			high

Response: *Mulinia lateralis* has no commercial value; the international dredging activities referred to in this pathway do not include dredging of commercial shellfish beds, and do not intentionally collect this species (but see Qu. 3.3c). See categorization of pathways in Annex IV and guidance notes in the beginning of this section.

Qu. 1.3b. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely	CONFIDENCE	low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response: The global dredging sector is very important in Europe, particularly in Belgium and The Netherlands. European dredging companies have a 90% market share of dredging in worldwide open markets (EuDA, 2013; Van Oord, 2020), and four of the largest dredging companies in the world are Belgian (Jan De Nul, DEME) or Dutch (Boskalis, Van Oord) (Rabobank, 2013). Dutch and Belgian companies have been involved in dredging in Mexico and Canada, at the southern and northern extremes of the native range of *Mulinia lateralis* (Maritime Journal, 2010; Van Oord, 2019; Wikipedia, 2021). However, dredging in the US, which extends over a large part of the native range, is restricted to vessels built, owned and flagged in America (as a result of the ‘Jones Act’) (Frittelli, 2019), and there was no evidence found of US dredging companies operating in Europe. Thus, dredging as a pathway from the native range is possible but somewhat restricted.

Dredging is a major activity in the current invaded range, within the RA area. There is significant dredging and disposal activity all along the Dutch, German and Belgian coasts. For example, the Port of Rotterdam requires intensive maintenance dredging that yields 12-15 million m³ of dredged material annually (Kirichek et al., 2018). Intensive dredging is also taking place in the port of Zeebrugge and the dredgers often move from one operation zone to another, e.g. from Belgium to the Baltic Sea (F. Kerckhof, pers. comm., 17th August 2021). Dredged materials are distributed along the Dutch and Belgian coasts to help prevent coastal erosion and aid land reclamation (ICES, 2018). There are large populations of *M. lateralis* in the sheltered waters such as estuaries, lagoons, harbours and coastal areas in the Gulf of Mexico and, to a lesser extent, in the Gulf of St Lawrence, Canada (see Qu. 1.3a for more detail regarding propagule pressure). Any dredging of these sediments will inevitably collect a high percentage of any *M. lateralis* adults present. The adults are small (10-20 mm in length), while newly metamorphosed juvenile shell lengths range from just 200 to 700 µm (Wang & Guo, 2008), thus can easily be taken up by all forms of dredging. The bulk of the dredged material will be disposed of in the country of origin at designated sites. However, some residual sediment will inevitably be retained in the vessel and on any equipment used. Additionally, larvae of the species may be contained in hopper water. This is water used to fill the hopper (i.e. the storage compartment) of the dredger to increase vessel stability when it is not loaded with sediment. The frequency of events in which European vessels operate in the native range, and then return to Europe without prior extensive cleaning, is unknown, but likely to be very low. Any residual sediment may be washed out in the home port, or may become mixed with waste from the next project (see Qu. 3.6b for more detail). Hopper water with entrained larvae may also be released at the home port or subsequent destinations.

Qu. 1.4b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: During the period of retention on the vessel, the adults will remain in the sediments they were collected with, so should be able to survive, and possibly even reproduce. While in transit, adults can

survive anoxic conditions, salinities of 5-80 psu (Parker, 1975), and temperatures from -2 °C to 35 °C (Calabrese, 1969a; Kennedy & Mihursky, 1971). Larvae in hopper water can also survive in a wide range of temperatures and salinities, even though growth may not be optimal (see Qu 2.1, 2.7). *Mulinia lateralis* can also burrow up to the surface if buried (Maurer et al., 1981), and actively thrives in dredged areas (Flint & Younk, 1983). Shipping time from the east coast of N. America to Europe is estimated to take 6-30 days (Freightos, 2021). If food resources fall below the energy requirements of *M. lateralis*, its inability to catabolize protein reserves can cause mass mortalities (Shumway & Newell, 1984).

Qu. 1.5b. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: No EC regulations regarding the cleaning of vessels and equipment or the exchange of hopper water before returning from overseas could be found. It does not appear to be common practice to clean equipment and exchange hopper water between dredging localities, as contractors considered this to be additional work when new requirements were introduced in Wales, UK (ABPmer, 2019; G. Wynne, B. Wray & S. Vye (Natural Resources Wales), pers. comm. 21st April 2021).

Qu. 1.6b. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: If the vessel and equipment are cleaned on arrival in Europe, *Mulinia lateralis* will be deposited in the port of entry. If the vessel is not cleaned the residual sediments will be mixed with the next dredge load, and would be dumped with a significant amount of sediment, in the designated disposal site. Similarly, larvae in hopper water can also be released at these locations. See Qu. 1.6a for more detail on difficulty of detection.

Qu. 1.7b. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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Response: See Qu. 1.7a. Companies based in The Netherlands and Belgium carry out the majority of overseas dredging, so there is a higher risk of these countries being sites of introduction. However, although it is probable that dredging vessels working overseas would return to their homeport on project completion, it is possible that sometimes they will travel directly to the site of their next contract, which could be anywhere in Europe.

Qu. 1.8b. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Accidental transport with dredge spoils has been proposed as a possible means of introduction, as dredging companies based in the countries within the RA area where *Mulinia lateralis* was first observed are known to carry out major dredging operations in the species' native range. *M. lateralis* larvae and adults/juveniles could potentially survive in hopper water and residual sediments respectively, and be released in home ports or at subsequent dredging destinations. The major unknown factor in this pathway is the frequency with which dredging vessels move between sites in the native range and the RA area.

Qu. 1.9. Estimate the overall likelihood of introduction into the risk assessment area or entry into the environment based on all pathways and specify if different in relevant biogeographical regions in current conditions.

Provide a thorough assessment of the risk of introduction in relevant biogeographical regions in current conditions: providing insight in to the risk of introduction into the risk assessment area.

RESPONSE	very unlikely	CONFIDENCE	low
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	unlikely moderately likely likely very likely		medium high
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Response: *Mulinia lateralis* has already been introduced into the RA area, but possibly only once. Ballast water and sediments is considered the most plausible pathway for this introduction, due to the volume of traffic, and its importance as a vector in the transfer of bivalve molluscs with similar life histories. This theory is supported by its presence in the region of a major shipping hub. However, there is no direct evidence to support ballast water and sediments as the original pathway, and it is possible that this single introduction resulted from a much rarer event such as the accidental transfer of residual dredging waste. The transatlantic shipping routes from the east coast of N. America to Northern Europe and the Mediterranean represent a major east-west trade lane. Despite its current non-native range being limited to the Greater North Sea, further introductions are also likely in the Mediterranean parts of the RA area, where prevailing temperatures favour the survival of planktotrophic larvae likely to be carried by ballast waters (see Risk of Establishment section).

Baltic Sea: moderately likely, low confidence (western part)

Greater North Sea: very likely, high confidence

Celtic Seas: moderately likely, medium confidence

Bay of Biscay and the Iberian coast: moderately likely, medium confidence

Mediterranean Sea: likely, medium confidence

Black Sea: moderately likely, medium confidence

Qu. 1.10. Estimate the overall likelihood of introduction into the risk assessment area or entry into the environment based on all pathways in foreseeable climate change conditions?

Thorough assessment of the risk of introduction in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of introduction (e.g. change in trade or user preferences)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely introduction within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely	CONFIDENCE	low
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	unlikely moderately likely likely very likely		medium high
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Response: Climate change is most likely to affect future introductions as a result of an increase in minimum and maximum Sea Surface Temperatures (SST). The methodology for the developed models is described in Annex IX and considers scenarios RCP 2.6 and RCP 4.5 by 2070.

Ballast water: The rise in maximum SST will increase the likelihood of further introductions into the RA area. In the northern part of the native range there would likely be an extension to the reproductive period, and possibly larger populations on average, providing greater opportunities for larval uptake with ballast water, e.g. in Long Island Sound, an important shipping hub for exports to Europe.

It is anticipated that extreme weather phenomena, such as droughts and storms/flooding, will be more frequent and intense under future climate conditions. Within the native range, this will likely lead to greater fluctuations in salinity, and more frequent defaunation events, facilitating the establishment of opportunistic high-density populations of *Mulinia lateralis*, and leading to a corresponding rise in larval density. More frequent storms leading to greater disturbance of inshore waters, may also increase the likelihood of juvenile and small adults being present in the water-column and thus available for uptake in ballast water. Higher frequency and severity of storms can also increase the amount of time vessels have to spend in port, increasing the likelihood of entrainment (Galil et al., 2019).

The likelihood of introductions into the Greater North Sea and Celtic Seas subregions, will increase as conditions will be favourable over a longer period of the year for survival of larvae discharged with ballast water. It is not anticipated that there will any change in the volume of transatlantic shipping as a result of climate change.

International Dredging: The more intense proliferation of native *M. lateralis* populations, discussed above for ballast water, could also result in a higher risk of *M. lateralis* adults and larvae being present in any residual sediments and water, remaining in European dredging vessels and on equipment, when they return from dredging works in N. America. Dredging activity in the native range may increase in response to rising sea levels, leading to an increase in demand for coastal protection. In addition, an increase in extreme weather events (e.g. flooding with associated terrigenous inputs), may increase the need for dredging to maintain water channels and protect property. As the European dredging sector have a 90% market share of dredging worldwide, it is likely that there will be an increase in the frequency of European dredging vessels operating in the native range.

Baltic Sea: moderately likely, low confidence (western part)

Greater North Sea: very likely, high confidence

Celtic Seas: likely, medium confidence

Bay of Biscay and the Iberian coast: likely, medium confidence

Mediterranean Sea: likely, medium confidence

Black Sea: moderately likely, medium confidence

2 PROBABILITY OF ESTABLISHMENT

Important instructions:

- For organisms which are already established in parts of the risk assessment area or have previously been eradicated, the likelihood of establishment should be scored as “very likely” by default.
- Discuss the risk also for those parts of the risk assessment area, where the species is not yet established.

Qu. 2.1. How likely is it that the organism will be able to establish in the risk assessment area based on similarity of climatic and abiotic conditions in its distribution elsewhere in the world?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species is already established in the RA area, specifically in the Wadden Sea, coastal waters and estuaries in Belgium, the Netherlands and Germany (see Qu. A6).

Mulinia lateralis is a eurythermal and euryhaline species with a latitudinal distribution extending from the Bay of St. Lawrence, Canada to the Gulf of Mexico, Yucatan, Mexico (Turgeon et al., 2009; see also Qu. A3 for details). This distribution corresponds to low winter temperatures of approximately -1 °C to 24 °C (average temperature of the coldest month, retrieved from BIO-ORACLE 2 (Assis et al., 2018)) and high summer temperatures ranging from 17 °C to 30.5 °C (average temperature of the warmest month, retrieved from BIO-ORACLE 2), conditions which are met throughout most of the RA area (see Annex VIII). Under experimental conditions, this temperature range is further extended to -2 °C for adult survival and to 33 °C for larval survival (Calabrese, 1969a). Spawning occurs at different times of the year at different latitudes, e.g. in Canada from mid-July to early September (Sullivan, 1948), in Maryland from May to November with a peak in September (Hanks, 1968), in Texas from January to April, while year-round spawning has also been reported (Montagna et al., 1993). In the Long Island Sound, larvae appear in the water in early July at temperatures ranging between 16 °C and 20 °C but are more abundant at 19-21 °C (Calabrese, 1970). Field observations agree rather well with laboratory results, which indicate that the temperature threshold for larval growth that ensures metamorphosis before the larvae die in the water column lies somewhere between 15 °C and 17.5 °C (Calabrese, 1969a). Thus, temperature does not seem to be a major limiting factor for establishment at a large scale, except perhaps around northern UK waters, where maximum summer temperatures drop below 15 °C. Nevertheless, because *M. lateralis* is primarily an inshore, estuarine species (Walker & Tenore, 1984), coarse grain temperature maps may not accurately reflect local conditions. For example, temperature measurements in marinas and harbours along the UK coast demonstrate water temperatures typically between 16 °C and 22 °C in July – October, all the way up to northern England (Bishop et al., 2015; C. Wood, unpublished data).

The species can also withstand a wide range of salinities, reportedly between 5-80 psu (Parker, 1975 in Montagna et al., 1993). *M. lateralis* can persist equally in low salinity estuarine environments as in hypersaline conditions in lagoons, associated with prolonged drought periods (e.g. in Baffin Bay, Texas, where the species is dominating the infaunal community at salinities up to 60 psu). It is generally observed though that major recruitment events are triggered by big salinity changes caused by freshwater inflow, rather than absolute salinity levels (Montagna & Kalke, 1995; Van Diggelen & Montagna, 2016). On the other hand, its presence along the Dutch and Belgian coasts, as well as the shallow sublittoral in its native range (Cleveland et al., 2002) indicates it can also establish in fully marine areas. Regarding larval development, in laboratory experiments it was shown to be normal between 12.5 to 35 psu, although survival was observed within a much larger range of 7-38 psu (Calabrese 1969a).

Qu. 2.2. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area? Consider if the organism specifically requires another species to complete its life cycle.

RESPONSE	very isolated isolated moderately widespread widespread ubiquitous	CONFIDENCE	low medium high
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Response: *Mulinia lateralis* is an infaunal (i.e. living in soft sediments), opportunistic species that proliferates in shallow, sheltered areas with mixed fine sediments (Walker & Tenore, 1984). It is widespread in coastal bays and often dominates the infaunal assemblages in estuaries and lagoons (Montagna et al., 1993; Reguero & Raz-Guzman, 2018). Even though in its native range it is considered primarily a near-shore, subtidal species, in the RA area it has been repeatedly observed in the intertidal zone and appears capable of establishing there too (Craeymeersch et al., 2019; Klunder et al., 2019). Such habitats are relatively widespread in the RA area; in particular, estuaries and intertidal areas are especially widespread in the North-East Atlantic region while lagoons are more common in the Mediterranean region (Figure 4). Klunder et al. (2019) constructed a habitat suitability map for *M. lateralis* in the Wadden Sea, based on sediment characteristics at a fine scale. This predicted that a considerable part of the Dutch Wadden Sea could provide suitable habitat.

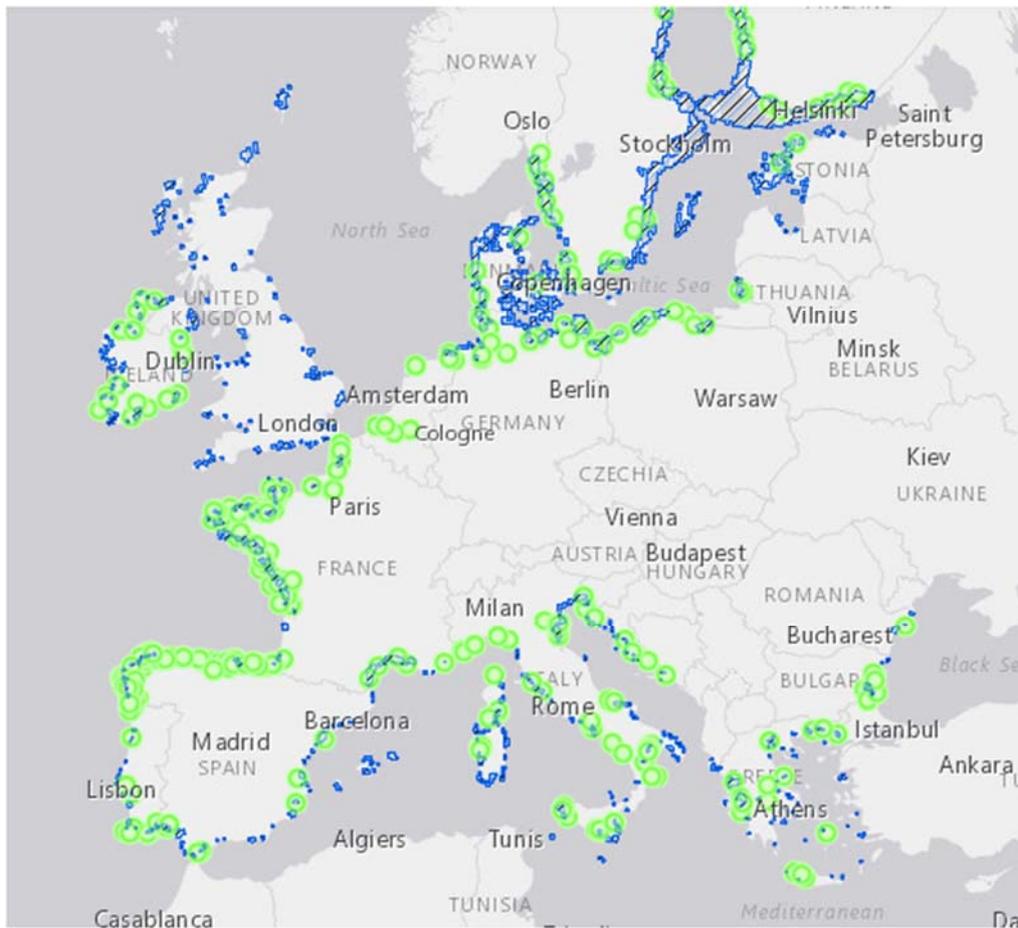


Figure 4: Coastal lagoons (blue polygons) and Estuaries (green circles) in the Natura 2000 network of Europe (source: Natura 2000 Network Viewer).

Qu. 2.3. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Mulinia lateralis* is a typical r-selected species with high fecundity, short generation times and fast maturation (Williams et al., 1986; see also Qu. 2.7). As such, it is a characteristic early colonizer in macrobenthic community succession after periodic or stochastic catastrophic events (e.g., Santos & Simon, 1980; Flint & Younk, 1983) and, according to ecological theory, is not meant to be a good competitor (Rosenberg 1972; 1973; Grassle & Grassle, 1974; McCall, 1977; Rhoads et al., 1978). This is also demonstrated in field studies, where its numerical presence is much reduced in locations with

high bivalve diversity (McKeon et al., 2015) or with the strong presence of bioturbating deposit feeders (Levinton & Bambach, 1970). However, because it can withstand a large range of salinities, it recovers very fast from physical disturbance events and rapidly colonises defaunated habitats, it can persist and dominate in frequently “disturbed” environments, such as estuaries and other transitional systems. Moreover, because it can attain significant densities, has a high filtration rate and is adapted to quickly exploit high concentrations of food resources (Shumway et al., 1983; Craeymeersch et al., 2019), it has the capacity to become an effective competitor for space and food, especially if settlement occurs earlier than for native bivalves with a similar trophic ecology and habitat preference. In the Wadden Sea, with extensive tidal flats and a salinity range of approximately 12-31 psu (Duran-Matute et al., 2014), the species is already established, having been recorded for the first time in 2017 (Klunder et al., 2019). In the Voordelta, in The Netherlands, *M. lateralis* was found together with *Spisula subtruncata* both at high densities at the same site, and likewise in the Westerschelde, it was found with *Cerastoderma edule* both at high densities (Craeymeersch et al., 2019). Thus, competition with functionally similar species (i.e. infaunal suspension feeders) has not prevented establishment.

Qu. 2.4. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: In its native range, *Mulinia lateralis* constitutes an important food source for a variety of organisms both in estuaries and in coastal waters. Predation by various finfish species (drums, croakers, spots) (Blundon & Kennedy, 1982; Pollack et al., 2018), blue crab *Callinectes sapidus* (Williams et al., 1986), rays (Ajemian & Powers, 2012), starfish *Asterias forbesi* (MacKenzie, 1981) and *Luidia clathrata* (McClintock & Lawrence, 1985), diving ducks, e.g., scaup and ruddy ducks (Perry & Uhler, 1982; Stroud et al., 2019), is well documented along the coasts of North America and the Gulf of Mexico. In Chesapeake Bay, it has been suggested that the seasonal fluctuations of the dwarf clams, with very low abundances in the summer, are due to increased predation by crabs and fish but the clams persist in the face of predation by continuous recruitment (Blundon & Kennedy, 1982). Something similar is likely to happen in the RA area, where the species is already established, with predation by native species of crabs, fish, starfish, shore birds and diving ducks.

M. lateralis is reported as the intermediate host for larvae of two parasitic cestode species, *Duplicibothrium minutum* and *Rhodobothrium paucitesticularae*, however no associated pathology to the examined specimens was observed (de Buron et al., 2013). The species is also a host for the protozoan parasite *Perkinsus chesapeaki* (Reece et al., 2008), which infects a wide variety of clam species (Bureson et al., 2005) and is pathogenic to its clam host *Mya arenaria* (Dungan et al., 2002; McLaughlin & Faisal, 1998). No pathogeny has been described though for *M. lateralis* specifically, such that the potential effect of *Perkinsus* infections on the risk of establishment cannot be reliably assessed. The

other two *Perkinsus* species that have been reported in European waters, *P. olseni* and *P. mediterraneus* (Carrasco et al., 2014), can indeed infect clam species but have not been observed in *M. lateralis*.

Brown tide event: In a Texas estuary, a prolonged brown tide event caused the demise of the *M. lateralis* population for two years, although it was not established whether this was due to reproductive failure or direct toxic effects (Montagna et al., 1993). The population rebounded after 2 years in the native range, however recovery may not be possible after such an event in the invaded range with a newly established or isolated population.

Qu. 2.5. How likely is the organism to establish despite existing management practices in the risk assessment area? Explain if existing management practices could facilitate establishment.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: While Ballast Water Exchange (BWE) and Ballast Water Treatment (BWT) can reduce propagule pressure and, consequently, the rate of establishment (see Qu. 1.5a for details), these management practices are not always possible or yet in effect. On the other hand, bivalve transportations for aquaculture purposes (which constitute a pathway of spread) offer suitable habitats to *Mulinia lateralis* in the form of the aquaculture plots themselves and, thus, facilitate establishment. Moreover, seed relaying offers favourable substrates and conditions for settlement and growth, enhancing establishment potential. Dredging activities also enhance the potential for establishment, both at the dredged sites, which are known to be rapidly colonised by the species (Flint & Younk, 1983; Clarke & Miller-Way, 1992) but also at disposal sites with dredge spoils containing *M. lateralis*, or at new dredging locations with contaminated equipment (see Probability of Spread section for details). In a similar way, dredging to remove nuisance species close to shellfish culture plots, sometimes practiced for predatory starfish or the invasive mollusc *Crepidula fornicata*, which heavily infests oyster and mussel beds (Bohn, 2014), may also provide cleared space and promote settlement and establishment of *M. lateralis*.

Qu. 2.6. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: No attempts to eradicate this organism are known. Due to its small size a considerable proportion of the population would be expected to pass through typical commercial towed gear used for removal. Moreover, its burrowing lifestyle, high fecundity, prolonged/continuous recruitment and resistance to disturbance make it a very unlikely candidate for successful eradication as the above characteristics allow the species to maintain low-density “reservoir” populations even under adverse environmental conditions and/or heavy predation (Williams et al., 1986) and recover fast after mass mortality events.

Qu. 2.7. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?

including the following elements:

- a list and description of the reproduction mechanisms of the species in relation to the environmental conditions in the risk assessment area
- an indication of the propagule pressure of the species (e.g. number of gametes, seeds, eggs or propagules, number of reproductive cycles per year) of each of those reproduction mechanisms in relation to the environmental conditions in the risk assessment area.
- If relevant, comment on the likelihood of establishment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in establishment whereas for others high propagule pressure (many thousands of individuals) may not.
- If relevant, comment on the adaptability of the organism to facilitate its establishment and if low genetic diversity in the founder population would have an influence on establishment.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Mulinia lateralis* is a gonochoric (i.e. with separate sexes), broadcast spawner with external fertilisation. It has a short life span, up to 2 years, and reaches sexual maturity at a length of 3 mm and at an age of 60 days (Calabrese, 1969b). The species has free-swimming planktotrophic larvae with pelagic larval duration (PLD) of 7 to 22 days (Mann et al., 1991). It thus has a short generation time of about 3 months (Guo & Allen, 1994) and can spawn more than once a year, depending on ambient temperatures, with a mean fecundity of 3-4 million eggs per female per spawning event (Calabrese, 1969b). Spawning occurs at different times of the year at different latitudes, e.g. in Canada from mid-July to early September (Sullivan, 1948), in Maryland from May to November with a peak in September (Hanks, 1968), in Texas from January to April, while year-round spawning has also been reported (Montagna et al., 1993).

Abiotic parameter thresholds for larval development are outlined in Qu. 2.1. In brief, normal larval development and larval growth in the laboratory are achieved at temperatures not lower than 15 °C and

as high as 32.5 °C, which was the highest temperature tested (Calabrese, 1969a), and at salinities between 12.5 and 37.5 psu. However, satisfactory embryonic development occurred at a more restricted salinity range of 20-32.5 psu (Calabrese, 1969a). Nevertheless, it is generally accepted that it is salinity fluctuations and not absolute salinity values per se that trigger recruitment (Van Diggelen & Montagna, 2016), such that exact salinity thresholds for reproduction are not easy to set. Such conditions are met throughout most of the RA area, at least for the short duration needed for larval development. The only exception is the Baltic Sea, where salinity drops to <10 psu beyond the western Baltic, and northern UK waters, where maximum summer temperatures are close to or below the lower limit for larval development and growth. Abiotic conditions in the Mediterranean Sea are particularly favourable for prolonged or even continuous spawning, with minimum water temperatures >16 °C in the eastern and southern parts of the basin, whereas in Atlantic Europe spawning and recruitment are likely to be more discreet events, limited to the warmer summer months.

Qu. 2.8. If the organism does not establish, then how likely is it that casual populations will continue to occur?

Consider, for example, a species which cannot reproduce in the risk assessment area, because of unsuitable climatic conditions or host plants, but is present because of recurring introduction, entry and release events. This may also apply for long-living organisms.

RESPONSE		CONFIDENCE	
	very unlikely		low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response: Conditions for successful reproduction are encountered throughout most of the RA area. However, long-term establishment most likely requires specific water body types, such as estuaries, lagoons and sheltered inshore waters. Accordingly, the occurrence of casual populations would depend on repeated introductions of either larvae in ballast water or benthic stages in dredged material (and to a lesser extent in ballast sediments), being released at suitable locations. Concerning ballast water, for transatlantic voyages, ballast water exchange is expected to have occurred at sufficient distances away from European coasts such that the risk of introduction is not very high and will be even lower after full implementation of the BWMC. With regards to the transport of aggregates from native donor areas however, the risk of additional introductions remains, as long as operations of European companies continue along areas where *Mulinia lateralis* is native (see relevant pathway in the Probability of Introduction section – Qu. 1.3b, 1.8b). It is worth noting here that an area particularly vulnerable to the occurrence of casual populations is the Baltic Sea, which encompasses a salinity gradient ranging from highly suitable to unsuitable for larval development. Thus, competent larvae entering the Baltic through the high salinity Kattegat can still survive in the western Baltic and eventually settle, even as far as the Baltic proper. Adult clams can survive the salinity in this region but the next generation of larvae would not develop properly. Another area where ephemeral/casual populations may appear is the coast around Ireland, which presents a marginal environment for the species' larval survival in terms of maximum

summer temperature (15-16 °C). Adult clams entering via anthropogenic pathways can survive and may even reproduce locally in a warmer than average year, but long-term establishment doesn't appear likely.

Qu. 2.9. Estimate the overall likelihood of establishment in the risk assessment area under current climatic conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under current climatic conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in current conditions: providing insight in the risk of establishment in (new areas in) the risk assessment area.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species is already established in the southwestern North Sea and further establishment throughout most of the RA area is considered very likely. The population dynamics and persistence of *Mulinia lateralis* populations in its native range are governed by different combinations of reproductive seasonality, abiotic mortality, and predation at different latitudes, as well as local disturbance regimes (Williams et al., 1986). Thus, at lower latitudes, where temperature conditions for spawning and larval development are favourable throughout most of the year, continuous recruitment offsets population losses due to predation and stochastic mass mortality events. On the other hand, at higher latitudes (from Long Island Sound to Canada), where recruitment events are discreet and confined to only a few months per year, populations of the species are described as ephemeral and sporadic, maintained at low density reservoir levels for most of the year.

Similarly, *M. lateralis* is expected to exhibit boom and bust dynamics in Atlantic Europe, where conditions will limit spawning and recruitment to the summer months. Competition and predation by native species have not hindered establishment thus far and are expected to regulate populations of the species in ways similar to what is observed in the native range. In the Mediterranean Sea, should the species be introduced there, it is more likely that populations will become more stable and dominant members of the macrobenthos, especially in the highly variable lagoonal and estuarine systems of the region. The Baltic Sea only offers salinity conditions suitable for establishment in its western part. Establishment is also considered possible in the Black Sea, although areas of freshwater input are fewer in the RA part of this subregion, and low winter temperatures are likely to limit reproduction to the warmer months of the year.

Baltic Sea: moderately likely, low confidence (western part)

Greater North Sea: very likely, high confidence

Celtic Seas: moderately likely, medium confidence

Bay of Biscay and the Iberian coast: likely, medium confidence

Mediterranean Sea: likely, medium confidence

Black Sea: likely, medium confidence

Qu. 2.10. Estimate the overall likelihood of establishment in the risk assessment area under foreseeable climate change conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under foreseeable climate change conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of establishment (e.g. increase in average winter temperature, increase in drought periods)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely establishment within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The response is based on combining physiological tolerances and the results from the distribution modeling (see Qu. 2.1, Qu. 2.9 and Annexes VIII & IX for details). Aspects of climate change most likely to affect future distribution were considered as an increase in minimum and maximum Sea Surface Temperatures (SST). The methodology for the developed models is described in Annex IX and considers scenarios RCP 2.6 and RCP 4.5 by 2070.

Baltic Sea: moderately likely, low confidence (western part)

Greater North Sea: very likely, high confidence

Celtic Seas: likely, medium confidence

Bay of Biscay and the Iberian coast: likely, medium confidence

Mediterranean Sea: likely, medium confidence

Black Sea: moderately likely, medium confidence

The SDM predicted a small reduction in projected suitability for *Mulinia lateralis* for the Mediterranean Sea and a corresponding increase for North-East Atlantic marine subregions under future climate change and, consequently, a small northward shift of the overall suitable area for the species. An increase in sea surface temperature will offer suitable conditions for spawning and larval development for prolonged periods throughout the whole Atlantic Europe and is predicted to extend its potential range further north along the coast of northern England, Scotland and Ireland. Suitable conditions in the Mediterranean are

likely to become even more restricted spatially and temporally, rendering establishment in this marine subregion more localized, especially in the east and south.

Note: Even though the SDM takes into account salinity variability in the form of distance from river mouths, a future scenario for river discharge was not taken into account and would take very elaborate data processing to bring into the model. It is anticipated that extreme weather phenomena, like droughts and storms/flooding, will be more frequent and intense under future climate conditions and this will increase the uncertainty of predictions as well as the population fluctuations of *M. lateralis*.

3 PROBABILITY OF SPREAD

Important instructions:

- Spread is defined as the expansion of the geographical distribution of an alien species within the risk assessment area.
- Repeated releases at separate locations do not represent continuous spread and should be considered in the probability of introduction and entry section (Qu. 1.7).

Qu. 3.1. How important is the expected spread of this organism within the risk assessment area by natural means? (List and comment on each of the mechanisms for natural spread.)

including the following elements:

- a list and description of the natural spread mechanisms of the species in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of spread discussed in relation to the species biology and the environmental conditions in the risk assessment area.

The description of spread patterns here refers to the CBD pathway category “Unaided (Natural Spread)”. It should include elements of the species life history and behavioural traits able to explain its ability to spread, including: reproduction or growth strategy, dispersal capacity, longevity, dietary requirements, environmental and climatic requirements, specialist or generalist characteristics.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Natural larval dispersal: *Mulinia lateralis* can generate a high propagule pressure as it can form dense populations, has a high fecundity, and in some regions of the RA area could reproduce all year round. In its native range in estuaries along the east coast of N. America, it can reach average densities of up to 21,000 ind. m² (Santos & Simon 1980; Walker & Tenore, 1984), and in The Netherlands, up to almost 6000 ind. m² were recorded from the Voordelta (Craeymeersch et al., 2019). Each female produces 3-4 million eggs per spawning event (for more details on reproductive traits please see Qu. 2.7). In Long Island Sound, Calabrese (1970) recorded peak larval densities of 2,500-3,500 ind. m³.

In its native range, the reproductive period of *M. lateralis* varies from just a few months in the Gulf of St. Lawrence to all year round in the Gulf of Mexico (Montagna et al., 1993), depending on the length of time when seawater temperature is above 16 °C (Calabrese et al., 1969a). If this species becomes established in the Mediterranean Sea, the thermal requirements for successful larval development are met throughout most of the year, this would lead to significant potential for natural dispersal. In Atlantic Europe, lower seawater temperatures will limit the period with conditions suitable for reproduction (see Annex VIII), as well as extending the duration of larval development.

M. lateralis larvae remain in the free-swimming planktotrophic phase for 7-22 days (Mann et al., 1991), during which time they could travel hundreds of kilometres. However, Shanks & Brink (2005) reported that in N. Carolina, during a period of upwelling and downwelling, *M. lateralis* larvae actively remained within 5 km of shore despite cross-shelf currents and the exchange of nearshore waters with offshore waters, i.e. they did not behave as passive particles. This phenomenon has been demonstrated for other species with a similar native distribution (e.g. Feng et al., 2017). For *M. lateralis* in particular, a rather limited (spatially) molecular study with populations from Long Island, Virginia and S. Carolina has found that all three populations are rather homogeneous for 19 genetic loci, supporting larval dispersal along the Atlantic coast (Wenne, 1992). However Mann et al. (1991) observed that larvae of this species use their preference for salinity discontinuities or higher salinity water to assist retention in partially mixed estuaries (Mann et al., 1991). Thus, larvae may generally remain entrained in estuaries where they have established. This species is currently limited to the southern part of the North Sea, its invaded range encompassing the full lengths of the Dutch and Belgian coasts, and the German part of the Wadden Sea.

The prevailing currents would tend to carry the larvae from the Wadden Sea eastwards towards Denmark, Norway and the Baltic Sea (De Hauwere, 2016). However, the populations along the Belgian coast appear to be spreading westward. Anthropogenic factors are likely implicated in this spread pattern; this westward spreading of introduced species along the North Sea coasts being rather common e.g. *Petricolaria pholadiformis* and *Ensis leei* (F. Kerckhof, pers. comm., 17th August 2021). However local circulation patterns aided by seasonal wind forcing can vary considerably and can also carry larvae westwards, depending on the timing of spawning (Barney et al., 2003; Lefebvre et al., 2003; Dauvin et al., 2007). If *M. lateralis* continues to spread westward into the English Channel, this may well provide a source of larvae that could be carried to the south coast of the UK, as demonstrated by Lefebvre et al. (2003) for the brittle star *Ophiothrix fragilis*, which has a similar larval duration of 26 days.

M. lateralis is not currently present in the Mediterranean Sea, and it is very unlikely it could disperse there naturally from its current invaded range in the North Sea. However, if it arrived by a human-assisted pathway, it could potentially spread by natural dispersal throughout the Mediterranean. Surface circulation of the Mediterranean consists basically of a separate counter-clockwise movement of the water in each of the two basins. Because of the complexity of the northern coastline and of the numerous islands, many small eddies and other local currents form essential parts of the general circulation (Millot & Taupier-Letage, 2005). Natural dispersal of planktonic larvae can vary at the subregional level as well as seasonally but has been estimated with modelling simulations in the range of 10^2 - 10^3 km for organisms with PLDs similar to *M. lateralis* (Bray et al., 2017; Andrello et al., 2013). Larvae of *M. lateralis* can easily survive the current environmental conditions throughout most of the RA area, see Qu. 2.1 for details. Its preferred salinity range is 18-30 psu (Lippson & Lippson, 1984), and it is especially adapted to mixohaline sites where salinity levels fluctuate. Although, its presence along the Dutch and Belgian coasts indicates it can also establish in fully marine areas. Suitable habitats for *M. lateralis* larvae to settle, consisting of soft sediments on intertidal shores or in the shallow sublittoral, can be found throughout much of the RA area, where there are many estuaries, lagoons and sheltered coastal waters (Craeymeersch et al., 2019; Klunder et al., 2019; Yang et al., 2021).

Natural dispersal of adults: *Mulinia lateralis* juveniles and adults are small, light due to their thin shells, and they live very close to the surface (Chalermwat et al., 1991). Disturbances to the sediment through strong wave action, or bottom trawling, may lead to excavation of large numbers of clams. These can then be transported by bedload transport, tidal currents, or longshore currents, before deposition elsewhere (Cleveland et al., 2002; Prezant et al., 2010). On St Catherine's Island, Georgia, US,

Cleveland et al. (2002) reported a mass exhumation of *M. lateralis*, from a subtidal population, possibly caused by strong wave action or shrimp trawling. The clams were likely transported by the strong tidal currents before deposition in an area covering over 7000 m² of intertidal shore. Rees et al. (1977) also noted storm-induced strandings of several bivalve species along the coast of North Wales. They stated that wave activity could be a factor in the maintenance of soft bottom benthic associations in near-shore waters. This method of transport has also been described for: *Cerastoderma edule* (Richardson et al., 1993); *Mya arenaria* (Emerson & Grant, 1991); *Mercenaria mercenaria*, and several other bivalve species (Prezant et al., 2010). Dispersal is probably over relatively short distances of a few kilometres (Prezant et al., 2010).

A rapid rate of natural spread is predicted, resulting from longer distance larval dispersal along the coast, with some restriction due to larval retention in estuaries, and the short distances involved in adult clam dispersal.

Qu. 3.2a. List and describe relevant pathways of spread other than "unaided". For each pathway answer questions 3.3 to 3.9 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 3.3a, 3.4a, etc. and then 3.3b, 3.4b etc. for the next pathway.

including the following elements:

- a list and description of pathways of spread with an indication of their importance and associated risks (e.g. the likelihood of spread in the risk assessment area, based on these pathways; likelihood of survival, or reproduction, or increase during transport and storage; ability and likelihood of transfer from the pathway to a suitable habitat or host) in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of spread for each pathway discussed in relation to the species biology and the environmental conditions in the risk assessment area.
- All relevant pathways of spread (except "Unaided (Natural Spread)", which is assessed in Qu. 3.1) should be considered. The classification of pathways developed by the Convention of Biological Diversity shall be used (see Annex IV).

Pathway name: Four potential pathways/ vectors were identified and are listed in order of importance:

a) TRANSPORT-STOWAWAY (ship/boat ballast water and sediments)

Kerekhof (2019) suggests that *Mulinia lateralis* was introduced into NW Europe with ballast water, like the bivalve *Ensis leei*, also a species originally from the N. American east coast. In addition, ballast water has been suggested as the pathway for two other recently introduced bivalves, *Rangia cuneata* and *Theora lubrica* (Verween et al., 2006; Faasse et al., 2019).

With respect to the RA area, movement of vessels between ports within its boundaries is less restricted and ballast water regulations do not apply to short journeys within states. This means that the potential of ballast waters and sediments transport to act as a vector of spread of *M. lateralis* within the risk assessment area is significant.

b) TRANSPORT-STOWAWAY (other means of transport: marine aggregates & dredging)

Dredging is carried out throughout the EU for a number of reasons including: keeping waterways and ports navigable; creation of new ports; coastal protection; land reclamation; and the extraction of sediments as sand and gravel for use by the construction industry (EuDA, 2013; Rabobank, 2013). The dredged sediments from ports and channels may be re-used “beneficially“ for coastal protection, land reclamation or offshore construction, or are disposed of in designated disposal sites in the open sea.

The introduction of a closely related bivalve, *Rangia cuneata*, into the Vistula Lagoon in the Baltic Sea, and its spread in the UK and the US have been attributed to dredging activities. (Gallagher & Wells, 1969; Rudinskaya & Gusev, 2012; Willing, 2017).

c) TRANSPORT-CONTAMINANT (contaminant of animals: mariculture)

In shellfish farming, dredging is used extensively in the relaying of shellfish for on-growing or purification (Capelle et al., 2017; DG SANTE, 2018). *M. lateralis* has similar habitat requirements to many cultivated bivalves, so is likely to settle and grow in areas where shellfish are being farmed (McKeon et al., 2015; Craeymeersch et al., 2019). Thus, relaying of shellfish from areas where *M. lateralis* is present is likely to result in transfer of *M. lateralis* larvae or adults, as contaminants of the water or sediment associated with the shellfish.

d) TRANSPORT-STOWAWAY (other means of transport: boat bilge water)

The bilge water of small vessels, such as fishing boats, yachts and motor boats, not subject to the BWMC, can contain bivalve larvae and juveniles (Darbyson et al., 2009; Fletcher et al., 2017). These vessels typically move between marinas and harbours, or sheltered inland waters such as estuaries, that are likely to provide a habitat suited to the establishment of *M. lateralis*. The European Boating Industry (2020) estimates that over 6 million boats are kept in European waters, while 10,000 marinas provide one million berths both inland and in coastal areas.

a) TRANSPORT-STOWAWAY (ship/boat ballast water)

Qu. 3.3a. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional	CONFIDENCE	low
	unintentional		medium high

Response: It can be stated with high certainty that this pathway is unintentional. There is no doubt that uptake of larvae in ballast water is accidental. See categorization of pathways in Annex IV and guidance notes in the beginning of this section.

Qu. 3.4a. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: For reproductive output, ship ballast water and sediment volumes, and potential larval concentrations, see Qu. 1.3a. With respect to spread of the organism within the RA area, transshipment operations constitute the main maritime traffic that will act as the vector for spread. Important transshipment hubs are situated along the southern Mediterranean (serving the rest of the Mediterranean and the Black Sea) and the Le Havre-Hamburg range, serving the UK, the Baltic and Scandinavia (Notteboom & de Langen, 2015). Regional vessel movements are more likely, than those from outside the RA area, to pass over suitable habitat where any larvae or adults released in transit may be able to survive. In particular, small vessels may take routes close to the coast and shelter in estuaries or bays overnight or in bad weather, habitats favoured by *Mulinia lateralis*, see Qu. 1.7a for more details.

Noteworthy, are also larger passenger/ car ferries which operate in most regions, and which have a high traffic volume. Due to the routes taken, measures described in the ballast water convention cannot easily be followed (exchange at depth and distance from shore) so the risk of depositing propagules at suitable locations is higher. These vessels are currently often unchecked, untreated and unregulated, with member states permitted under the BWMC to allow exemptions to apparently low risk inter-regional shipping routes (Olenin et al., 2016). Considering that *M. lateralis* is favoured by water temperatures encountered in many parts of the RA area but has a moderate pelagic larval duration and most likely discreet recruitment periods in Atlantic Europe, it is considered moderately likely that sufficient numbers can be transferred with ballast water along this pathway.

Qu. 3.5a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: As in Qu.1.4.a. Additionally, the shorter duration of sea shipping routes between EU ports, and the ferry routes between many European locations, further increases the likelihood of survival within ballast waters, compared with the transatlantic routes from N. America.

Qu. 3.6a. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: See Q1.5a. BWE for EU short sea-shipping routes is usually restricted to the second criterion of at least 50 nm from the nearest land, and in waters at least 200 metres in depth in the Mediterranean Sea, and even this is often not feasible in northern European Seas (David et al., 2007). Thus, ballast water exchange is not likely to be effective in preventing the spread of *Mulinia lateralis* (and other organisms potentially transferred in ballast water) within European Seas. Regarding the IMO D2 standard, compliance can practically diminish propagule pressure of zooplankton, but full implementation of the BWMC is not expected to happen before 2024. However, even then some of the regulations and requirements under the BWMC may be relaxed for shorter, low-risk shipping routes under regional exemption options.

Qu. 3.7a. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: As in Qu. 1.6a.

Qu. 3.8a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Mulinia lateralis* is typically found in the sheltered, soft sediment habitats common around ports and harbours, which are widely distributed in the RA area. If ballast water exchange occurs in open seas rather than in coastal areas, transfer of planktonic larvae and adults to suitable substrate will be hampered. If, however, untreated ballast water is released in ports, estuaries or other coastal areas, then establishment will be dependent on availability of suitable habitat. Considering: a) the breadth of habitat that characterizes the species; b) the wide distribution of such habitats in the RA area; and c) the initial invasion of the species in The Netherlands, close to Rotterdam (Craeymeersch et al., 2019; Klunder et al., 2019), the third busiest port in the world; there is a high likelihood that *M. lateralis* can transfer to a suitable habitat after release with ballast water.

Qu. 3.9a. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area. (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: Given: a) the amount of maritime traffic between ports and harbours within the RA area (thousands of journeys per year); b) the ballast volume of commercial vessels (10^4 - 10^5 tonnes) (GloBallast, 2021); c) the potentially high densities of both adult *Mulinia lateralis* on the seabed as well as larvae in surface waters; and d) its wide environmental tolerances (see Qu. 1.4a for details); the potential rate of spread of the species via ballast water is high. Ballast water is considered to be one of the main vectors for spread of the closely related mactrid bivalve *Rangia cuneata* that has recently invaded and rapidly spread throughout northern European waters (Faillettaz et al., 2020). In addition, ballast water is the most likely vector for long dispersal jumps to other subregions. However, there is currently no evidence of spread within the RA area from ballast water discharge (i.e. no long dispersal jumps combined with sudden appearance in/close to port areas), and potential recipient sites in the Mediterranean are possibly isolated (see Qu. 1.7a), although regional vessel movements are more likely, than those from outside the RA area, to pass over suitable habitat where any larvae or adults released in

transit may be able to survive. For these reasons we have proposed a conservatively moderate rate of spread, with low confidence, acknowledging the novelty of the invasion and the possibility that the rate of spread may increase in the future. Due to the limited duration of favourable environmental conditions (seawater temperature) for reproduction in the more northerly parts of the NE Atlantic Ocean, the likelihood that dense populations will develop there is lower.

b) TRANSPORT-STOWAWAY (other means of transport: marine aggregates & dredging)

Qu. 3.3b. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional	CONFIDENCE	low
	unintentional		medium
			high

Response: *Mulinia lateralis* has no commercial value. The dredging activities referred to in this pathway do not include dredging of commercial shellfish beds, and do not intentionally collect this species (but see Qu. 3.3c). See categorization of pathways in Annex IV and guidance notes in the beginning of this section.

Qu. 3.4b. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely	CONFIDENCE	low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response: Dredging is carried out for a number of reasons, including: maintenance to keep waterways and ports navigable, creation of new ports, coastal protection, land reclamation, and the extraction of

sediments as sand and gravel, which are used by the construction industry (EuDA, 2013; Rabobank, 2013). The dredged material from ports and channels is usually disposed of in designated areas in the open sea. However, there is a move towards ‘beneficial dredging’ where, whenever possible, dredging waste is re-used for land reclamation, coastal protection, or offshore construction, reducing the need for newly excavated aggregates (ICES, 2016). Thus, dredged materials can be dispersed widely.

Dredging is a major activity in Europe, particularly within The Netherlands, the UK, Denmark and Belgium. For example, over 51 million m³ of marine aggregate was extracted in 2017, approximately 40% as aggregate for construction, 40% for beach replenishment and 19% for land reclamation (ICES, 2018). Maintenance dredging takes place at virtually all harbours, and many marinas throughout the EU. Within the current invaded range of the RA area, there is significant dredging and disposal activity all along the Dutch, German and Belgian coasts. For example, the Port of Rotterdam requires intensive maintenance dredging that yields 12-15 million m³ of dredged material annually (Kirichek et al., 2018). Intensive dredging is also taking place in the port of Zeebrugge and the dredgers often move from one operation zone to another, e.g. from Belgium to the Baltic Sea (F. Kerckhof, pers. comm., 17th August 2021). Large quantities of dredged materials are distributed along the Dutch and Belgian coasts to help prevent coastal erosion and aid land reclamation. *Mulinia lateralis* lives, shallowly buried, in the surface soft sediments of sheltered waters such as estuaries, lagoons, harbours and coastal areas. Dredging activity is high in such areas as they are prone to silt accumulation. Any dredging of these sediments will inevitably collect a high percentage of any *M. lateralis* adults present, but also larvae in ballasting hopper water. The adults are small (10-20 mm in length), while newly metamorphosed juvenile shell lengths range from 200 to 700 µm (Wang & Guo, 2008), thus can easily be taken up by all forms of dredging. For details of the *M. lateralis* propagule pressure generated, see Qu. 1.3a.

M. Faasse proposed the accidental transfer of dredged materials as a possible pathway of spread (pers. comm., 25th February 2021). The introduction of a closely related bivalve, *Rangia cuneata*, into the Vistula Lagoon in the Baltic Sea, and its spread in the UK and the US have been attributed to dredging activities. (Gallagher & Wells, 1969; Rudinskaya & Gusev, 2012; Willing, 2017).

Qu. 3.5b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: During the period of retention on the vessel, the adults will remain in the sediments they were collected with, so should be able to survive, and possibly even reproduce. While in transit, adults can survive anoxic conditions, salinities of 5-80 psu (Parker, 1975), and temperatures from -2 to 35 °C (Calabrese, 1969a; Kennedy & Mihursky, 1971). *Mulinia lateralis* can also burrow up to the surface if

buried (Maurer et al., 1981) and actively thrives in dredged areas (Flint & Younk, 1983). Larvae in hopper water are also likely to survive (Qu 1.4b).

Qu. 3.6b. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The disposal of dredged materials is regulated throughout the EU (summarised by Mink et al., 2006). OSPAR guidelines specify best environmental practice for managing dredged material (OSPAR, 2014). Member states use these guidelines to manage dredging and dumping and to minimise effects on the marine environment. The main management tools are marine licence and control systems. These require assessments of the environmental impact of planned disposal activities in relation to a specific dumpsite, sediment characteristics and contamination load. Dredging activities are also regulated under the Marine Strategy Framework Directive (MSFD) 2008/56/EC, the Water Framework Directive (WFD) 2000/60/EC and the EC Habitats Directive (92/43/EC). However, the licencing procedures in member states tend to focus mainly on the sediment characteristics and the prevention of the disposal of waste containing contaminants such as heavy metals and pathogens, not non-native species. In addition, many maintenance-dredging activities are exempt or have a reduced licencing requirement (Gov.UK, 2021). No EC regulations on the cleaning of dredging vessels and equipment between projects were found. Thus, residual waste intended for disposal at one site could become mixed with a subsequent load and be disposed of accidentally at another location. As the major dredging corporations are based in the North Sea, see Qu. 1.3b, where this species is now established, there is a risk of spread to other areas within the EU where these corporations operate. For example, there are currently major dredging projects throughout the Mediterranean and Black Sea (Dredging Today, 2021, Van Oord, 2020).

Regulations may vary from country to country. For example in Wales, UK, the completion of a Biosecurity Risk Assessment when applying for a marine licence for dredging and/or disposal, is now required for all activities. Marine licencing conditions imposed include the washing of seabed equipment and filter screens at the end of a campaign, the requirement for the circulation and exchange of hopper water away from shore, and the removal of hopper sediment prior to entering a different region of the UK (ABPmer, 2019; G. Wynne, B. Wray & S. Vye (Natural Resources Wales,) pers. comm. 21st April 2021). However, these management practices are not yet statutory, and do not apply to the rest of the UK. In general, restrictions on disposal based on the risk associated with the source areas would be an effective management method, as long as extensive and up-to-date data on the distribution of the high-risk non-native species are available.

Qu. 3.7b. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Any *Mulinia lateralis* accidentally collected would be dumped with a significant amount of sediment, in the designated disposal site. It is unlikely any regular checks will be made at the disposal sites, particularly where the dredge waste is being re-used for land reclamation, offshore construction, or coastal protection. The transportation and release of larvae in hopper water is even less likely to be monitored or detected.

Adult *M. lateralis* are small (10-20 mm in length), and the gross morphology is similar to some common native species, as described in A.2, so may easily be overlooked. Newly metamorphosed juvenile shell lengths range from 200 to 700 µm (Wang & Guo, 2008), so transfers at the larval or juvenile stage, within the associated sediment or water, are very likely to go undetected.

Qu. 3.8b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Mulinia lateralis* will be deposited along with the sediment or hopper water it was collected with, so the immediate habitat will be very suitable. If the waste is being used for coastal protection or land reclamation, the deposition will probably be made close to shore in a sheltered area. Licensed dredged waste disposal sites may be further offshore, but again the seabed is likely to consist of soft sediments from previous disposals. *M. lateralis* can also burrow up to the surface if buried during the deposition (Maurer et al., 1981).

Qu. 3.9b. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area. (please provide quantitative data where possible).

RESPONSE	very slowly	CONFIDENCE	low
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	slowly moderately rapidly very rapidly		medium high
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Response: Given the high frequency and volumes involved in maintenance and capital dredging activities, the likely high survival rate during transit, and deposition commonly being into suitable habitat, there is a high risk of spread. This is especially the case in the Greater North Sea area where the waste is normally re-used ‘beneficially’ for coastal protection or land reclamation, e.g. in The Netherlands, Denmark and Belgium (ICES, 2016). Under these circumstances, disposal sites may be greater distances away from the initial collection site, than would be the case for designated waste disposal sites. Although the disposal site is likely to be within the same member state, or a close neighbouring member state. Long dispersal events with dredge spoils are probably likely to be more sporadic compared with spread via ballast water/sediment due to the higher volume and frequency of commercial ship journeys.

c) TRANSPORT-CONTAMINANT (contaminant of animals: mariculture)

Qu. 3.3c. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: *Mulinia lateralis* is not grown commercially for food, thus its introduction into commercial shellfish beds is unintentional. In addition, as it will compete with the cultivated shellfish for space and food, so would be considered a nuisance species.

Qu. 3.4c. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Cultivated shellfish are frequently moved between sites locally, regionally and internationally, for the purposes of: restocking; to enhance production; relaying for fattening purposes; relaying for cleansing; or to keep fresh and alive before consumption (McKindsey et al., 2007; Brenner et al., 2014; Muehlbauer et al., 2014). It is known that many non-native species can hitchhike with these movements, including the Manila clam, *Ruditapes philippinarum* (McKindsey et al., 2007, and references therein).

Production of bivalve molluscs in the EU averages 500,000 tonnes annually (350,000 tonnes of mussels, 100,000 tonnes of oysters and 50,000 tonnes of clams), France and Spain are the most important countries in terms of production volume and value (DG SANTE, 2018). Spain, Ireland, France, Italy and the UK are the most important regarding trade of live bivalve molluscs for farming and relaying. France is particularly important in terms of the number of movements of live bivalve molluscs for relaying before putting on the market, and the natural production of seed spat of Pacific oysters and mussels that is subject to movements either within France or to other member states. There are also a large number of movements from Spain, particularly from Galicia to Italy, for relaying and purification (Robert et al., 2013; DG SANTE, 2018). Mussel bottom culture is typically practiced on shallow mudflats in areas where there are extensive naturally occurring mussel seed beds. In the Netherlands, Germany, UK, and Ireland, seed fished from natural beds is the main source for bottom culture (Kamermans & Smaal, 2002).

Mulinia lateralis is likely to establish and thrive in habitats typically used for benthic shellfish cultivation, e.g. in Chesapeake Bay in its native range, it is found in association with several commercial species (Reece et al., 2008), and in the Wadden Sea, it was found to be abundant amongst the edible clam, *Spisula subtruncata* (Craeymeersch et al., 2019). *Mytilus edulis*, the Blue mussel, is produced in the Western part of the Dutch Wadden Sea and in the Oosterschelde estuary, both areas where *Mulinia* has already invaded (Robert et al., 2013; Craeymeersch et al., 2019; Faasse et al., 2019). For details of the *M. lateralis* propagule pressure generated, see Qu. 1.3a.

The relaying of spat for on-growing, or adult bivalves for fattening or cleansing, is carried out by dredging the donor site (using a variety of methods), then relaying the bivalves at the new location (Diaz et al., 2012; Gaspar et al., 2013; Robert et al., 2013; Capelle et al., 2014). Although *M. lateralis* does not attach to other shellfish, juveniles and adults present as contaminants in the sediment would be collecting as by-catch during the dredging process, also larvae may be captured in water taken up during the dredging.

Qu. 3.5c. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: In order to successfully transfer the target commercial bivalve species, the dredging, on-board conditions, and transit time will all be optimized to ensure the target species survives and remains healthy. This process will also enable the survival of *Mulinia lateralis*, a species with wide environmental tolerances, see Qu. 1.4a for more details. Reproduction during transit is unlikely as the period between dredging and relaying of the target species is short, particularly for spat, as the shellfish need to be kept alive and healthy; although some larvae may metamorphose.

Qu. 3.6c. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: At the EU level, COUNCIL REGULATION (EC) No 708/2007 concerning use of alien and locally absent species in aquaculture defines the procedures to be followed that minimize the risk of introducing non-target alien species accompanying commercial shellfish spat and stocks. It requires a permit procedure, involving risk assessment for the non-target species and a quarantine period for the translocated stock. Importantly, in relation to spread within the RA area, the regulation does not apply to movements of locally absent species within the member states (i.e. in this case cultivated native species of bivalves) “except for cases where, on the basis of scientific advice, there are grounds for foreseeing environmental threats due to the translocation, Art. 2 para. 2.” Additionally, movements of the bivalves *Magallana gigas* and *Ruditapes philippinarum*, listed in Annex IV of the Council Regulation, which could be contaminated with *M. lateralis*, are permitted without any risk assessment or quarantine. However, local/national legislation exists that can limit the translocation possibilities of species like *M. gigas*, e.g. see WG-AS & Gittenberger (2018) for the trilateral Wadden Sea area. With regard to mussel relaying in Wales, UK a walkover survey of the source site is required checking for before a licence is granted (G. Wynne, B. Wray & S. Vye (Natural Resources Wales,) pers. comm. 21st April 2021). Moreover, if the import region is a Natura 2000 area, regulations can be much stricter as they aim to protect the conservation objectives of the protected area first.

Where the commercial shellfish are cleaned before transfer, removing any sediment, this will significantly reduce the level of contamination by *Mulinia lateralis* larvae and juveniles, as these would not be attached to the shellfish. Sorting by size may also limit contamination of adult commercial stocks as *M. lateralis* only grows to 10-20 mm.

In general, restrictions on transfers based on the risk associated with the source areas is an effective management method, as long as extensive and up-to-date data on the distribution of the high-risk non-native species are available. However, delays in identifying new non-native species, and their distribution in the RA area, can mean that spread, particularly within member states, is not prevented. For example, in The Netherlands, mussel seed is fished from seed beds which generally occur in the Wadden Sea, it is then transported to culture plots in the Wadden Sea and the Oosterschelde (Robert et al., 2013), although movement is not permitted in the other direction. This offers a possible explanation of the finding of *M. lateralis* in the Oosterschelde. Visual inspections of the dredged shellfish are unlikely to detect *M. lateralis*, unless it is present in large numbers, due to its small size, and larvae would not be visible. Moves within the commercial shellfish sector towards hatchery culture of spat, and rope culture for mussels, will reduce the need for dredging (Robert et al., 2013; Smaal et al., 2019).

Qu. 3.7c. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Perfunctory visual inspections during bivalve aquaculture operations are likely to miss adult *Mulinia lateralis* as they are small (10-20 mm in length), and the gross morphology is similar to some common native species, as described in A.2. Newly metamorphosed juvenile shell lengths range from 200 to 700 µm (Wang & Guo, 2008), so transfers at the larval or juvenile stage, within the associated sediment or water, are very likely to go undetected.

Qu. 3.8c. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: If bivalve seed/stock contaminated with *Mulinia lateralis* is relayed on cultivation plots without any prior management measures, the likelihood of transfer to other suitable habitats is very high (the cultivation plots themselves are suitable habitats, see Qu. 3.4c). These plots are often situated in coastal areas in close proximity to additional suitable natural habitat to which individuals may spread.

Qu. 3.9c. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area. (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: Shellfish movements are a likely mechanism of spread in the RA area, particularly at the local level, where spat is dredged and then relayed at nearby sites for on-growing. However, considering the degree of regulation of the industry and the fact that in many cases transfers are predominantly conducted within member states, spread to distant locations through this pathway may be less important than spread through other pathways such as ballast water and sediments.

d) TRANSPORT-STOWAWAY (other means of transport: boat bilge water)

Qu. 3.3d. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: Bilge water is not taken up intentionally; it results from rough seas, leaks in the hull, rain, washing of equipment on deck, or other interior spillage. Thus, there is no doubt the presence of any *Mulinia lateralis* in bilge water is accidental. See categorization of pathways in Annex IV.

Qu. 3.4d. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway

- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: It depends on:

- The number of yachts and vessels arriving in a hub;
- Volume of bilge water;
- Theoretical distance, time to first discharge (assuming constant and linear travel);
- Propagule pressure; and
- Survival of the species.

An indication of the number of recreational vessels in the RA area is given by the European Boating Industry (2020), which estimates that over 6 million boats are kept in European waters while 10,000 marinas provide one million berths, both inland and in coastal areas. Fletcher et al. (2017) recorded bilge water volumes of between 0.9 and 29 l from 15 motorboats sampled, and 0.2-200 l from 15 yachts, including a large catamaran, in New Zealand. Fletcher et al. (2020) noted that yachts in particular, have a high likelihood of bilge water being on board when leaving port (i.e. high-risk source regions for *Mulinia lateralis* larvae). Extrapolating from CPS Authority et al. (2017), approximately 30% of these vessels travel distances >100 km from their home port. Assuming travel speeds of 5 knots (Fletcher et al., 2017) considerable distances can be travelled within the RA area within a matter of days, which significantly increases the likelihood that sufficient viable propagules of *M. lateralis* can spread along this pathway from already established populations in the RA area. For propagule pressure, see Qu. 1.3a.

Qu. 3.5d. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Analysis of samples collected from yachts and motorboats in Canada and New Zealand, found bivalve larvae and juveniles in the bilge water (Darbyson et al., 2009; Fletcher et al., 2017). Considering: the pelagic larval duration of *Mulinia lateralis* (7 to 22 days); the relatively short travel times of small vessels within the RA area; the tolerance of the organism to salinities down to 5 psu; and its tolerance of oil pollution (a common contaminant of bilge water (Grassle & Grassle, 1974); the

likelihood of survival along this pathway is assessed as high. As adults are unlikely to be taken in with the small water incursions that accumulate as bilge water, it is unlikely that *M. lateralis* will reproduce during transport.

Qu. 3.6d. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Legislation regarding discharge of bilge water only relates to large commercial vessels and is included under the BWMC, see Qu. 3.6a. For smaller vessels, there is currently no EC legislation in place specifically covering bilge water. However, since 2008 some member states are requiring the installation of holding tanks in recreational vessels for waste water, although this is mainly to control ‘black water’ or ‘grey water’ from the toilets or washing facilities, and does not generally cover bilge water (Noonsite, 2019). Most recreational vessels now have bilge water pumps that operate automatically so the boat owner cannot control the location of discharge (Fletcher et al., 2017).

Qu. 3.7d. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: See response Qu. 1.6a. In order to reach Good Environmental Status (GES) targets with reference to Descriptor D2, most EU states are already designing or implementing national/regional NIS-targeted monitoring programmes. Monitoring should focus on introduction hotspots (e.g. ports, marinas, aquaculture plots) and this will increase the detectability of *Mulinia lateralis* entering the RA area through bilge water, particularly if molecular methods are employed (Hayes et al., 2004).

Qu. 3.8d. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Recreational vessels spend substantial periods of time stationary; berthed in marinas and ports, or on moorings in estuaries (Ashton et al., 2006). At other times, they generally remain in sheltered inland waters. Thus, if viable propagules are discharged with untreated bilge water they are likely to transfer to a suitable habitat consisting of soft sediments on intertidal shores or in the shallow sublittoral, see Qu. 1.7a for more detail on habitat.

Qu. 3.9d. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area. (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: Small vessels travelling within the RA area can easily spread this organism while in transit, through automatic bilge water discharge, or at the next destination. However, compared to the transport of ballast water by larger vessels, the volumes of water transported are much smaller, the distances covered are generally much shorter, and the voyages less frequent. So the contribution of this pathway to the overall potential rate of spread is assessed as relatively lower than ballast water (see Qu. 3.9a).

Qu. 3.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?

RESPONSE	very easy easy with some difficulty difficult very difficult	CONFIDENCE	low medium high
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Response: Naturally dispersing organisms are very difficult to contain, especially species such as *Mulinia lateralis* with high fecundity, relatively long pelagic larval duration, and the capability to

develop extremely dense populations in a very short time (See Qu. 2.7 for the biological characteristics of the species). Currently this species is restricted to the Greater North Sea region, and there is no evidence of repeated introductions into the RA area. Thus, it may be feasible to delay the rapid spread of the species, especially long dispersal jumps, if restrictions based on the risk associated with the source areas are rapidly adopted by the industry. The current legal instruments and levels of implementation of voluntary measures are not sufficient to ensure containment of the organism, when transferred by ballast water (but this can change with full implementation of the D-2 Standard), dredging, bivalve movements, or bilge water.

Qu. 3.11. Estimate the overall potential rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (indicate any key issues and provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in current conditions, providing insight in the risk of spread into (new areas in) the risk assessment area.

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: Unaided dispersal and multiple pathways of human-aided spread create a considerable potential for spread. This is illustrated by the known spread of the species along approximately 450 km of coastline during the 4 years since its arrival.

Ballast water and sediment transport is the most likely vector to facilitate rapid spread via long distance transport of the species within European waters, until the BWMC is fully implemented. However, there is a lack of evidence of *Mulinia lateralis* being transported in ballast water and of the likelihood of it being transported to suitable habitats in other subregions, see Qu 1.7a and Qu 3.9a.

The movements of dredging waste may already have contributed to the current spread of *Mulinia lateralis* in the Greater North Sea region. Bivalve transfers are another likely mechanism of spread in the RA area, especially within member states but potentially also between marine regions/subregions. Particular attention is needed when transferring oyster consignments between Atlantic Europe and the Mediterranean. Finally, bilge waters, a vector that has been overlooked until recently, appears to be able to transport viable propagules of the species in the relatively short duration of intra-European journeys.

Due to the difficulty of predicting the potential for long dispersal jumps via ballast water or the other pathways, we have proposed a conservative rate of spread of moderately, with low confidence.

Qu. 3.12. Estimate the overall potential rate of spread in relevant biogeographical regions in foreseeable climate change conditions (provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if rates of spread are likely slowed down or accelerated.

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: The rate of spread is not expected to change significantly under foreseeable climate change conditions. An overall rise in sea surface temperatures will increase the potential for adult populations and larval production in the Greater North Sea and Celtic Seas subregions, with a corresponding increase in the likelihood of spread via all pathways. Whereas in the Mediterranean Sea, an increase in SST may result in fewer source populations (see Qu. 2.10 for more detail), and thus reduce the likelihood of spread. Higher frequency and severity of storms can increase the amount of time vessels have to spend in port, increasing the likelihood of entrainment in ballast water (Galil et al., 2019).

Dredging activity may increase in response to rising sea levels, leading to an increase in demand for coastal protection. In addition, an increase in extreme weather events (e.g. flooding with associated terrigenous inputs), may increase the need for dredging to maintain water channels and protect property. Heat waves can cause mass mortality of aquaculture bivalves, leading to increased shellfish transfers to replenish the stocks (Rodrigues et al., 2015). More shellfish movements may be associated with a higher risk of introduction if the stocks/seed originate from areas with a high risk of contamination with *Mulinia lateralis* and the necessary precautions are not taken.

4 MAGNITUDE OF IMPACT

Important instructions:

- Questions 4.1-4.5 relate to biodiversity and ecosystem impacts, 4.6-4.8 to impacts on ecosystem services, 4.9-4.13 to economic impact, 4.14-4.15 to social and human health impact, and 4.16-4.18 to other impacts. These impacts can be interlinked, for example, a disease may cause impacts on biodiversity and/or ecosystem functioning that leads to impacts on ecosystem services and finally economic impacts. In such cases the assessor should try to note the different impacts where most appropriate, cross-referencing between questions when needed.
- Each set of questions starts with the impact elsewhere in the world, then considers impacts in the risk assessment area (=EU excluding outermost regions) separating known impacts to date (i.e. past and current impacts) from potential future impacts (including foreseeable climate change).
- Only negative impacts are considered in this section (socio-economic benefits are considered in Qu. A.7)
- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable).

Biodiversity and ecosystem impacts

Qu. 4.1. How important is the impact of the organism on biodiversity at all levels of organisation caused by the organism in its non-native range excluding the risk assessment area?

including the following elements:

- Biodiversity means the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems
- impacted chemical, physical or structural characteristics and functioning of ecosystems

RESPONSE	NA	CONFIDENCE	
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Response: There is no evidence to suggest that this species is non-native anywhere else in the world outside the RA area.

Qu. 4.2. How important is the current known impact of the organism on biodiversity at all levels of organisation (e.g. decline in native species, changes in native species communities, hybridisation) in the risk assessment area (include any past impact in your response)?

Discuss impacts that are currently occurring or are likely occurring or have occurred in the past in the risk assessment area. Where there is no direct evidence of impact in the risk assessment area (for

example no studies have been conducted), evidence from outside of the risk assessment area can be used to infer impacts within the risk assessment area.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: *Mulinia lateralis* has only been present within the RA area since 2016, and its current distribution is limited to the southern North Sea (see Qu. A6). No studies of impact have been reported from the RA area, so it is not yet possible to reliably assess its current impact on biodiversity. However, this species' rapid population growth within the RA, its population dynamics within the native range, and the known impacts on biodiversity from other invasive bivalve species, indicate that *M. lateralis* is likely already having some impact.

Competition: In 2017, dense populations of up to almost 6000 ind. m² were recorded from the Voordelta (southwestern Dutch coastal waters), and again in 2018 at densities of 1000 ind. m² (Craeymeersch et al., 2019). In 2018, *M. lateralis* was recorded at high density (820 ind. m²) in the Westerschelde (Craeymeersch et al., 2019). Subsequently, in 2019, it was recorded there at 18/44 sites sampled, at an average of five ind. m² (Wallis et al., 2020). In its native range in estuaries along the east coast of N. America, it can reach average densities of up to 21,000 ind. m² (Santos and Simon 1980; Walker & Tenore, 1984). It is possible that such dense settlements are competing with native bivalves for space and food, but see Qu. 4.3.

Impacts of similar species (i.e. shallowly buried, infaunal suspension feeders with an r-strategy) in the Greater North Sea region:

Ensis leei: In Belgium, declines in abundance of other bivalves such as *Macra stultorum*, *Cerastoderma edule*, *Spisula subtruncata*, *Ensis magnus* and tellinids have been observed since the introduction of *E. leei* (Gollasch et al., 2015).

Rangia cuneata: sympatric with *M. lateralis* in their native range (temperate west Atlantic, Gulf of Mexico), this mactrid bivalve has recently invaded and rapidly spread throughout northern European waters (Faillettaz et al., 2020). It has managed to establish numerical dominance in many of the invaded locations (estuaries, tidal reaches, brackish waters) with its numbers exceeding those of native species such as *Macoma balthica* and *Cerastoderma glaucum* in the Gulf of Gdansk (Janas et al., 2014).

Qu. 4.3. How important is the potential future impact of the organism on biodiversity at all levels of organisation likely to be in the risk assessment area?

See comment above. The potential future impact shall be assessed only for the risk assessment area. A potential increase in the distribution range due to climate change does not *per se* justify a higher impact score.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Competition: Most bivalve species largely utilize the same food source and are therefore competitors for food. Whether food competition leads to an introduced species out-competing indigenous species is dependent on many factors such as filtration capacity, selection and absorption efficiency, and energy demand (Shumway & Newell 1984). *Mulinia lateralis* is adapted to quickly exploit high concentrations of phytoplankton and natural seston (particularly bacteria) but has a relatively high-energy demand and an apparent inability to catabolize protein during prolonged periods of starvation, which may be the cause of observed mass mortalities in its native range (Shumway and Newell, 1984; Chalermwat et al., 1991).

Within its native range, *M. lateralis* is considered an opportunistic species that tends to rapidly colonise disturbed areas at high population densities, but can just as quickly disappear (see Qu. A.11 and Qu. 2.3). According to McKeon et al. (2015), from studies in its native range, *M. lateralis* reaches its greatest densities when released from competition with other bivalves. However, in the Voordelta, in The Netherlands, *M. lateralis* was found together with *Spisula subtruncata* (a native species considered to be threatened (Cardoso et al., 2007)), both at high densities at the same site, and likewise in the Westerschelde, where *M. lateralis* was found with *Cerastoderma edule* both at high densities (Craeymeersch et al., 2019). Both native species are summer spawners, with the main spatfall occurring from June to September (Smaal et al., 1997; Cardoso et al., 2007). Thus, there is considerable overlap likely with *M. lateralis* during spat settlement (presuming its reproductive period in the North Sea is similar to that in the northern part of its native range (Calabrese, 1970)). Therefore, *M. lateralis* may well compete successfully both for space and food with native bivalves. Whether this will lead to native species' declines cannot be predicted with any certainty due to the novelty of the invasion, however increased settlement seems likely as the species establishes dense populations and expands its range. In addition, its colonisation of recently defaunated areas may prevent native opportunistic species from establishing at those sites.

Shellfish disease: In its native range, *M. lateralis* is a host for the protozoan parasite *Perkinsus chesapeaki*, recently recorded from the Mediterranean and Atlantic coasts of France and Spain, which can infect clams and cockles. According to Carrasco et al. (2014), no clear evidence of relevant host reaction was observed in *Cerastoderma edule* tissues, although *P. chesapeaki* seemed to cause damage to the structure of gill lamellae, see Qu. 2.4. If *P. chesapeaki* is present in the *M. lateralis* populations in the RA area, this disease could infect and lead to a decline in native clam or cockle populations.

Hybridisation: *M. lateralis* can hybridise with other clams e.g. *Spisula solidissima* (Lindell et al., 2006), whether it will hybridise naturally with clams native to the RA area, such as other *Spisula* species, is not known.

Trophic interactions: Many native species could potentially utilise *M. lateralis* as a food resource. *M. lateralis* lives very close to the sediment surface and has a thin shell, so is easily available to a wide variety of predators (see Qu. 2.4). Craeymeersch et al. (2019) anticipate many native species in the North

Sea will be able to feed on *Mulinia*. Whether *M. lateralis* would provide equivalent energy and nutrient levels as current native prey species would require detailed investigation. In its native range, *M. lateralis* is an important food source for wildfowl during the winter (Calabrese, 1970) and it was found to be an adequate replacement nutritionally for *Ischadium recurvum*, the Hooked Mussel (Wells-Berlin et al., 2015). Baffin Bay, a hypersaline estuary in the Gulf of Mexico, supports an important recreational and commercial fishery for black drum *Pogonias cromis*, this benthic predator is highly reliant on *M. lateralis* as a food source, the biomass of which varies with the rapid changes in salinity that occur there (Pollack et al., 2018; Rubio et al., 2018). The sudden crashes in *M. lateralis* populations could lead to starvation of predators such as crabs and fish that have come to rely on it (Walker & Tenore, 1984). For example, wintering scaup (*Aythya affinis*) populations in Lake Pontchartrain, Louisiana, declined precipitously a year after a hurricane caused mass mortalities of their preferred bivalve prey, i.e. the clams *Rangia cuneata* and *M. lateralis* (Stroud et al., 2019).

Mass mortality events: Mass die-offs of bivalves can affect the ecosystem in 3 ways:

a) Food web structure, nutrient cycling and transfer of energy to higher trophic levels - The interaction between infaunal filter feeders, such as *M. lateralis*, and resuspended microphytobenthos and detritus can be particularly important in estuaries (e.g. Jones et al., 2016). The sudden loss of large amounts of bivalve biomass can disrupt the transfer of carbon and energy in the system.

b) Water quality - Mass die-offs of bivalves are known to influence abiotic factors that negatively affect other organisms in the ecosystem through organic matter decomposition with associated reduced dissolved oxygen and elevated ammonia concentrations, sometimes to toxic levels (e.g. Cherry et al., 2005; Cooper et al., 2005), although such impacts are less likely to be severe or long-lived in well-flushed environments.

c) Habitat alteration - In its native range, very dense settlements of *M. lateralis* that then die off can leave large amounts of dead shells in areas of predominantly soft sediments; these can also be transported to local shores (Levinton, 1970; Powell et al., 1986). Changes in habitat complexity and seabed structural properties can in turn greatly affect the communities living within soft sedimentary environments (Bodis et al., 2014). Although this may be considered a positive impact as shell deposits can increase species' richness through provision of shelter and substrate for other species (Gutiérrez et al., 2003). However, Nicastro et al. (2009) found that even large depositions of shells of the non-native gastropod, *Maoricolpus roseus*, into a soft-sediment habitat within a dynamic coastal lagoon, did not affect the community structure.

Regional differences: In the Mediterranean Sea, should the species be introduced there, the impacts will probably be greater as it is likely that *M. lateralis* populations will become more dominant members of the macrobenthos throughout the year, especially in the highly variable lagoonal and estuarine systems (with low diversity) of the region, see Qu. 2.9. Interaction with the dominant bivalve *Abra ovata* is possible. As a result, strong trophic interactions are more likely to develop but also population crashes of dense settlements are more likely to produce negative effects.

Qu. 4.4. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism currently in the risk assessment area?

including the following elements:

- native species impacted, including red list species, endemic species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000

- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

The Netherlands: The Wadden Sea is the largest unbroken system of intertidal sand and mud flats in the world; it stretches from The Netherlands to Denmark, and is recognised as a UNESCO World Heritage Site. It is known for its habitat variability and unique (benthic) biodiversity. The invasion of non-native species can alter the synergetic community currently present in this ecosystem, which provides vital support to migratory birds as a staging, moulting and wintering area. The availability of food and a low level of disturbance are essential factors that contribute to the key role of the property in supporting the survival of migratory species (UNESCO, 2021). The Voordelta, Oosterschelde, Westerschelde and North Sea coastal zone are all designated as Natura 2000 sites under the Habitat Directive and the European Birds Directive. *Mulinia lateralis* is present in all of these areas (see Qu. A8).

Belgium: Most of the Belgian coast is designated either under the European Birds Directive and/or the Habitat Directive, the largest area is the Vlaamse Banken between Ostend and the French border. *M. lateralis* has been found scattered along the whole Belgian coast.

Germany: The Wadden Sea – see The Netherlands above.

The habitat types where *M. lateralis* has established in these member states are Estuaries (X01), Marine littoral sediment (A2), and Marine sublittoral sediment (A5). The broadscale habitat features suitable for establishment of *M. lateralis* are littoral sand and muddy sand, mud, and mixed sediments; and sublittoral, sand, mud, and mixed sediments; corresponding to the EUNIS level 3 codes: A2.2, A2.3, A2.4, A5.2, A5.3, A5.4.

Changes through competition to infaunal bivalve populations, such as *Cerastoderma edule*, *Macra stultorum* or *Spisula subtruncata*, have the potential to affect the overwintering success of protected diving seabirds such as the common eider (*Somateria mollissima*) and common scoter (*Melanitta nigra*), which depend upon these prey resources for their survival (Bräger et al., 1995). With respect to the environmental status of the sites it has invaded, its impact could be related to the MSFD descriptors D1 (biodiversity), D2 (NIS), D3 (fishing), and D4 (food webs). Yet, the degree impact on GES, based on the MSFD descriptors by *M. lateralis* has not been quantitatively assessed. The only evident measured impact is presently the indicator D2 (criterion D2C1: number of new NIS per 6 years).

Qu. 4.5. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.3. and 4.4.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: The conservation status of many estuarine and lagoonal Natura 2000 sites, MPAs and SSSIs could be degraded. Any decline in conservation value is likely to be greater in the Mediterranean Sea, should the species be introduced there, as it is more likely that, due to the potential for prolonged recruitment, year-round reproductive populations will develop, especially in the highly variable lagoonal and estuarine systems of the region, see Qu. 2.9. In addition to the habitats noted in Qu. 4.4, the biodiversity of saline (X02) and brackish lagoon habitats (X03) within the Mediterranean, along the Atlantic coast, and in the Black Sea could be impacted. Considering that lagoonal habitats are important as nursery areas for many fish populations (Pérez-Ruzafa et al., 2011; Newton et al., 2018), as well as feeding grounds for waterfowl, changes in trophic interactions and the overall food web could have short to medium term effects on their conservation value, which is already degraded/unfavourable in large parts of the RA area (EEA – Habitats Directive reporting under Article 17).



Figure 5: Conservation status of coastal lagoons (Habitat 1150) for the period 2013-2018 for EU28. (Source: European Environment Agency, available at <https://nature-art17.eionet.europa.eu/article17/>)

With respect to the environmental status of any conservation sites if invaded, the impact of *M. lateralis* could be related to the MSFD descriptors D1 (biodiversity), D2 (NIS), D3 (fishing), and D4 (food webs).

Ecosystem Services impacts

Qu. 4.6. How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?

- For a list of services use the CICES classification V5.1 provided in Annex V.
- Impacts on ecosystem services build on the observed impacts on biodiversity (habitat, species, genetic, functional) but focus exclusively on reflecting these changes in relation to their links with socio-economic well-being.
- Quantitative data should be provided whenever available and references duly reported.

RESPONSE	NA	CONFIDENCE	
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Response: There is no evidence to suggest that the species is non-native anywhere else in the world outside the RA area.

Qu. 4.7. How important is the impact of the organism on provisioning, regulating, and cultural services currently in the different biogeographic regions or marine subregions where the species has established in the risk assessment area (include any past impact in your response)?

- See guidance to Qu. 4.6.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: *Mulinia lateralis* has only been present within the RA area since 2016, and its current distribution is limited to the southern North Sea (see Qu. A6). No studies of impact have been reported from the RA area, so it is not yet possible to reliably assess its current impact on ecosystem services. However, this species' rapid population growth within the RA, its population dynamics within the native range, and the known impacts on ecosystem services from other invasive bivalve species, indicate that *M. lateralis* is likely already having some impact.

Potential causes of as yet unidentified impacts:

Provisioning: Biomass - Although competition for food and space with cultivated bivalves is a potential source of loss, it is unclear if *M. lateralis* is a strong competitor to commercial species (see Qu. 2.3). The Wadden Sea and Oosterschelde are important areas for shellfish culture.

Regulation and Maintenance: In the North Sea, where this species is established, it is vulnerable to mass die-offs resulting from storms, low salinity events, or seawater temperatures being too low to sustain annual reproduction. Such events could affect water quality (see Qu. A11, Qu. 2.9 and Qu. 4.3.)

Qu. 4.8. How important is the impact of the organism on provisioning, regulating, and cultural services likely to be in the different biogeographic regions or marine subregions where the species can establish in the risk assessment area in the future?

- See guidance to Qu. 4.6.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Potential causes of impacts:

Provisioning: Biomass - Although competition for food and space with cultivated bivalves is a potential source of loss, it is unclear if *Mulinia lateralis* is a strong competitor to commercial species (see Qu. 2.3). These effects are likely to be greater in subregions where abiotic conditions are most suitable for year-round reproductive populations to establish, i.e. the Mediterranean, and the Bay of Biscay and the Iberian coast subregions (see Qu. 2.9).

Regulation and maintenance:

Water - In the North Sea, where this species is established, it is vulnerable to mass die-offs resulting from storms, low salinity events, or seawater temperatures being too low to sustain annual reproduction. Such events could affect water quality (see Qu. A11, Qu. 2.9 and Qu. 4.3). These impacts are likely to be greater in subregions where abiotic conditions are not optimal, i.e. the Greater North Sea, Celtic Seas, Baltic Sea and Black Sea (see Qu. 2.9).

Lifecycle maintenance - Maintaining nursery populations and habitats. By altering trophic interactions in lagoons, it has the potential to affect the nursery function of these habitats, especially if it undergoes population fluctuations tied to seasonal and/or stochastic events.

Pest and disease control - *M. lateralis* may facilitate the spread of the pathogenic parasite *P. chesapeaki* to commercial clam species (see Qu. 4.11), or cestode parasites to elasmobranchs (see Qu. 4.16). This will have most effect in the Mediterranean and the Bay of Biscay and Iberian coast subregions where commercial clam growing is greatest (see Qu. 4.11).

Cultural:

Physical and experiential - It may affect the amenity and recreational value of infested areas by reducing recreational harvests of other shellfish such as clams and cockles, if *M. lateralis* replaces these species on intertidal shores.

Economic impacts

Qu. 4.9. How great is the overall economic cost caused by the organism within its current area of distribution (excluding the risk assessment area), including both costs of / loss due to damage and the cost of current management.

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the world these should be reported here. The assessment of the potential costs of / loss due to damage shall describe those costs quantitatively and/or qualitatively depending on what information is available. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage. As far as possible, it would be useful to separate costs of / loss due to the organism from costs of current management.

RESPONSE		CONFIDENCE	
	minimal		low
	minor		medium
	moderate		high
	major		
	massive		

Outside of its native range, *Mulinia lateralis* is not known to be present, other than within the RA area.

Impacts as a result of trophic interactions in the native range: The Baffin Bay complex (Texas) supports a large commercial Black Drum fishery (Grubbs et al., 2013; Olsen, 2016) which was compromised in a 2012 emaciation event due to reduced *M. lateralis* availability (its preferred prey), linked to extreme salinity conditions in the bay in 2012 (Pollack et al., 2018). (See also Qu. 4.3). Baffin Bay saw roughly a 33% drop in production by weight in 2012. Considering that the coastwide fishery of Black Drum was estimated as worth approximately \$1.5 million per year at the time and the Baffin Bay fishery contributes roughly 50% to the overall landings (Grubbs et al., 2013), the economic impact is estimated as moderate.

Other potential causes of unidentified losses in the native range: Although competition for food and space with cultivated bivalves is a potential source of loss, it is unclear if *M. lateralis* is a strong competitor to commercial species, see Qu. 2.3. *M. lateralis* is a host to the parasitic pathogen *Perkinsus chesapeakei*, which also infects the commercial clam *Mya arenaria* and is likely to affect its growth, reproduction and possibly mortality (Dungan et al., 2002).

Qu. 4.10. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism currently in the risk assessment area (include any past costs in your response)?

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the EU these should be reported here. Assessment of the potential costs of damage on human health, safety, and the economy, including the cost of non-action. A full economic assessment at EU scale might not be possible, but qualitative data or different case studies from across the EU (or third countries if relevant) may provide useful information to inform decision making. In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. In this case, no score and

confidence should be given and the standardized “score” is N/A (not applicable). Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	N/A	CONFIDENCE	
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Response: No information has been found on the issue. *Mulinia lateralis* has only been present within the RA area since 2016, and its current distribution is limited to the southern North Sea (see Qu. A6). No studies of impact have yet been reported from the RA area, and no economic costs have yet been identified or estimated, so it is not possible to reliably assess its current economic impact.

Qu. 4.11. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.10.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Competition: This species is not itself of direct commercial importance (Calabrese, 1970). It may compete with commercially grown bivalves for food and space in natural habitats, potentially reducing the crop harvest, but it is unclear if it is a strong competitor (see Qu. 2.3).

Shellfish disease: Perkinsosis is a disease affecting numerous mollusc species worldwide, causing significant mortalities and economic losses. The main causative species of disease in oysters is *Perkinsus marinus*, and in clams is *P. olseni*, currently these are the only species classed as notifiable in Europe. However, *Mulinia lateralis* is a host for *P. chesapeaki*, recently recorded from the Mediterranean and Atlantic coasts of France and Spain, that can infect commercial clams and cockles including *Ruditapes decussatus*, *R. philippinarum* and *Cerastoderma edule*, and possibly affect production (Dungan et al., 2002; Reece et al., 2008; Arzul et al., 2012; Carrasco et al., 2014; Ruano et al., 2015).

According to Carrasco et al. (2014), no clear evidence of relevant host reaction was observed in *C. edule* tissues, although *P. chesapeaki* seemed to cause damage to the structure of gill lamellae. Carrasco et al. (2014) conclude that the impact of *P. chesapeaki* in *C. edule*, as well as in other cohabiting bivalves such as clams, with high commercial value in the region, needs to be urgently addressed. Park et al. (2010) found that the fecal discharge (faeces and pseudofaeces) and decomposition of infected clam tissue could be the two major routes of transmission for *Perkinsus* parasites. Thus, dense populations of *M. lateralis*, either alive or after a mass die-off, could significantly mediate the spread of these parasites.

Despite a relatively low production in Europe, clams are a high value seafood product, economically important in many European countries, particularly in Spain, Italy and Portugal. Annual production of clams averages 50,000 tonnes (DG SANTE, 2018), with the main producer of clams being Italy. Clams in aquaculture alone (36,000 tonnes in 2019) are worth 240 million Euros (FAO, Fisheries Statistics, 2021). Any potential impacts of *M. lateralis* would be indirect, to the extent that transport of adult, parasite-infested individuals would facilitate the spread of *Perkinsus* species to commercial clams. Considering the high value of the clam industry in Europe, moderate economic losses are a plausible scenario, although this estimate comes with high uncertainty.

Qu. 4.12. How great are the economic costs / losses associated with managing this organism currently in the risk assessment area (include any past costs in your response)?

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable).

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No specific management plans are in place for this particular organism in Europe. For marine invasive species introduced by ballast water there are considerable management measures at various stages of implementation (see also Management Annex). These costs are not specific for *Mulinia lateralis* and therefore not included in the score. Within the Wadden Sea, management measures are in place regarding shellfish transportation (WG-AS & Gittenberger, 2019) again these are not specific to this species.

Qu. 4.13. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.12.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: *Mulinia lateralis* is already established in the Greater North Sea, and eradication would likely not be an economically viable option due to the open nature of the marine environment and life history traits of the species. However, measures to prevent its spread to other regions of the RA area, particularly the Mediterranean may be considered, given the likelihood of greater impact on biodiversity and clam fisheries in that region. Costs related to shipping (ballasts/fouling) will be borne by the shipping companies. Significant costs can be associated with the ratification of relevant legislation, e.g. the Ballast Water Management Convention, in ensuring its compliance, related to planning, monitoring, enforcement and capacity building. However, these costs are not specific to *M. lateralis* and will prevent/reduce the introduction of a wide range of species carried by ballast water.

If management is taken forward then likely costs would include:

Dredging: Introduction of regulations regarding the cleaning of dredging vessels and equipment, such as water exchange and sediment removal from hoppers, between projects; and regulations regarding the ‘beneficial’ use of dredging spoil. There would be significant costs associated with the ratification of any such legislation, and in ensuring compliance, related to planning, monitoring and enforcement. Operational costs would be borne by the dredging companies. However, these costs are not specific to *M. lateralis* and will prevent/reduce the introduction and spread of a wide range of species. To prevent spread to new sites within the RA area, dredging sites would need to be tested for *M. lateralis* (and other NIS). Currently, under Marine Licensing regulations these sites are initially checked for chemical contaminants and pathogens, but not NIS.

Shellfish movements and disease: Introduction of further controls over shellfish relaying and movements of other commercial shellfish between natural habitats to include testing for a range of NIS. Current regulations relate to pathogens and diseases. These costs would not be specific to *M. lateralis* and will prevent/reduce the spread of a wide range of species. A ban on imports or restrictions in the movement of shellfish seed/stock from areas where *M. lateralis* is present could have potentially significant economic implications for shellfish producers. Monitoring of clam and cockle populations for *P. chesapeaki* infections - This is not currently a notifiable species, although other Perkinsus species are. Detection of infested *M. lateralis* specimens among commercial clam beds may warrant the early harvesting of clams, with associated costs and economic losses (Andrews & Ray, 1988).

Conservation status: As this species would be very difficult to eradicate or contain once it is present in a designated conservation area, and direct impact on biodiversity is considered to be ‘minor’ (see Qu. 4.3) any monitoring carried out would not result in management costs directly attributable to this species. However, *M. lateralis* should be included in MSFD D2 monitoring programmes within the RA area, with particular awareness of its trophic interactions with species of conservation importance.

With regards to the cost of monitoring, an indicative estimate comes from Denmark, where the cost of a proposed hotspot monitoring programme for all marine NIS (covering 13 ports and three areas with discharges of cooling water) was estimated at approximately €125,000 for the period 2015-2017 (Andersen et al., 2014). In the UK, a broad initial estimate of monitoring costs for MSFD D2 alone (considering that existing or new surveys for other descriptors will also contribute to the monitoring of NIS) suggests that they would be less than €974,000 over 10 years (DEFRA, 2012) with an additional €103,000 euros for drafting legislation and guidance.

Social and human health impacts

Qu. 4.14. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism for the risk assessment area and for third countries, if relevant (e.g. with similar eco-climatic conditions).

The description of the known impact and the assessment of potential future impact on human health, safety and the economy, shall, if relevant, include information on

- illnesses, allergies or other affections to humans that may derive directly or indirectly from a species;
- damages provoked directly or indirectly by a species with consequences for the safety of people, property or infrastructure;
- direct or indirect disruption of, or other consequences for, an economic or social activity due to the presence of a species.

Social and human health impacts can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	N/A	CONFIDENCE	
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Response: No information has been found on the issue.

Qu. 4.15. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism in the future for the risk assessment area.

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable).

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: There may be an impact on the recreational harvesting of bivalves such as clams and cockles, if *Mulinia lateralis* replaces these species on intertidal shores. Other recreational activities, such as fishing and bird-watching may be affected if *M. lateralis* becomes a dominant prey resource and predator populations follow its fluctuations.

Other impacts

Qu. 4.16. How important is the organism in facilitating other damaging organisms (e.g. diseases) as food source, a host, a symbiont or a vector etc.?

RESPONSE		CONFIDENCE	
	minimal		low
	minor		medium
	moderate		high
	major		
	massive		

Response: *Mulinia lateralis* is a host for the parasitic pathogen *Perkinsus chesapeaki* that can infect other clam and cockle species See Qu. 4.11. *M. lateralis* is also reported as the intermediate host for larvae of two elasmobranch parasitic cestode species, *Duplicibothrium minutum* and *Rhodobothrium paucitesticularae*, see Qu. 2.4. In the native range of *M. lateralis*, the Cownose ray, is the host for both species. No information could be found as to whether either species could potentially infect any elasmobranchs within the RA area.

Qu. 4.17. How important might other impacts not already covered by previous questions be resulting from introduction of the organism?

RESPONSE	NA	CONFIDENCE	

Response: No additional impacts could be identified.

Qu. 4.18. How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area?

RESPONSE		CONFIDENCE	
	minimal		low
	minor		medium
	moderate		high
	major		
	massive		

Response:

Predation: Within its native range, a wide variety of species prey on *Mulinia lateralis*. Something similar is likely to happen in the RA area, with predation by native species of crabs, fish, starfish, shore birds

and diving ducks. In the port of Zeebrugge many Oystercatchers have been observed preying on the abundant *M. lateralis* living on an intertidal mudflat (F. Kerckhof, pers. comm., 17th August 2021). In the colder parts of its native range predation by crabs and fish is probably the major factor controlling adult population size, at least during warmer months, and numbers increase drastically in the absence of predators (Virnstein, 1977). Wildfowl feeding may become an important regulator (Wells-Berlin et al., 2015), see Qu. 2.4 for more detail. Such trophic interactions however are likely to mediate impacts, rather than temper them, if specific predator-prey relationships develop (as in Qu. 4.3).

Parasites/pathogens: The parasitic pathogen *Perkinsus chesapeaki* or tapeworm infections may effect some control over abundance, although no pathology has been reported from the native range, see Qu 2.4 for more detail.

Qu. 4.19. Estimate the overall impact in the risk assessment area under current climate conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, in current conditions.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: As *Mulinia lateralis* has not previously invaded anywhere else in the world outside the RA area, and as it has only been recorded in the RA area since 2017, there is very little evidence available regarding its likely overall impact. The bulk of the information regarding the likely behavior and potential impacts of *M. lateralis* in the RA area, presented in this assessment, have been inferred from its life-history traits and population dynamics throughout its native range along the east coast of N. America. What little early indicators of impact there are regarding its recent invasion of the RA area, come from the Greater North Sea subregion, an area corresponding climatically to the northern limit of this species' native range, and thus may not be indicative of impacts in other subregions.

No impacts have yet been reported from the RA area. However, *M. lateralis* appears to have the potential to cause minor to moderate environmental impacts within the RA area, with the greatest impacts being predicted for the Mediterranean, if invaded, as populations are more likely to become dominant members of the macrobenthos throughout the year. The habitats primarily affected are estuaries and lagoons, which are both of high conservation value.

Biodiversity: Initial indications are that population densities could be sufficient to impact on native bivalve biodiversity, through competition for space and food; and affect trophic interactions by potentially altering predator/prey relationships e.g. with crabs, fish and shorebirds (Craeymeersch et al., 2019; Klunder et al., 2019). It is likely that mass die-offs, typical of this species, will occur which could have multiple effects on: food web structure; nutrient cycling; the transfer of energy to higher trophic levels; water quality; and habitat structure through the deposition of dead shells. These impacts are likely

to be greater in subregions where abiotic conditions are not optimal, i.e. the Greater North Sea, Celtic Seas, Baltic Sea and Black Sea. Other potential impacts on biodiversity include facilitating the spread of the protozoan parasite *Perkinsus chesapeaki* to clams and cockles or cestode parasites to elasmobranchs.

Ecosystem services: Competition for food and space with, and the possible spread of *P. chesapeaki* to, cultivated bivalves are potential sources of economic loss. By altering trophic interactions in lagoons, *M. lateralis* has the potential to affect the nursery function of these habitats, especially if it undergoes population fluctuations tied to seasonal and/or stochastic events.

Economy: Competition for food and space with, and the possible spread of *P. chesapeaki* to cultivated bivalves are potential sources of economic loss.

Social and human health: Recreational harvests of other shellfish such as clams and cockles could also be reduced, if *M. lateralis* replaces these species on intertidal shores.

Baltic Sea: minor, low confidence (western part)

Greater North Sea: minor, low confidence

Celtic Seas: minor, low confidence

Bay of Biscay and the Iberian coast: moderate, low confidence

Mediterranean Sea: moderate, low confidence

Black Sea: minor, low confidence

Qu. 4.20. Estimate the overall impact in the risk assessment area in foreseeable climate change conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, under future conditions.

- See also guidance to Qu. 4.3.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: See Qu. 4.19.

An increase in sea surface temperature will offer suitable conditions for spawning and larval development for longer periods throughout the Greater North Sea and Celtic Seas; this could reduce the likelihood of mass die-offs in those subregions, but increase the effects of competition on native bivalve biodiversity. In addition, it is anticipated that extreme weather phenomena, like droughts and storms/flooding, will be more frequent and intense under future climate conditions, this may lead to higher salinity variations and mechanical substrate disturbance, factors that facilitate colonisation by *Mulinia lateralis*.

Baltic Sea: minor, low confidence (western part)

Greater North Sea: moderate, low confidence

Celtic Seas: moderate, low confidence

Bay of Biscay and the Iberian coast: moderate, low confidence

Mediterranean Sea: moderate, low confidence

Black Sea: minor, low confidence

RISK SUMMARIES			
	RESPONSE	CONFIDENCE	COMMENT
Summarise Introduction and Entry*	very unlikely unlikely moderately likely likely very likely	low medium high	<i>Mulinia lateralis</i> has already been introduced into the RA area, but possibly only once. Ballast water and sediments is considered the most plausible pathway due to the volume of traffic, its importance as a vector in the transfer of bivalve molluscs with similar life histories, and its presence in the region of a major shipping hub. However, it is possible that this single introduction resulted from a much rarer event such as the accidental transfer of residual dredging waste or hopper water.
Summarise Establishment*	very unlikely unlikely moderately likely likely very likely	low medium high	The species is already established in the southeastern North Sea and further establishment throughout most of the RA area is considered very likely. <i>M. lateralis</i> is expected to exhibit boom and bust dynamics in Atlantic Europe, where conditions will limit spawning and recruitment to the summer months. Establishment is considered likely up as far as northern England and Ireland. In the Mediterranean Sea, should the species be introduced there, it is more likely that due to the potential for prolonged recruitment, year-round, dense populations will develop, especially in the highly variable lagoonal and estuarine systems of the region. The Baltic Sea only offers salinity conditions suitable for establishment in its western part. Localised establishment is also considered possible in the Black Sea, although conditions there are sub-optimal. Under future climate change a small northward shift and slight increase of the overall suitable area for the species is expected.
Summarise Spread*	very slowly slowly moderately rapidly	low medium high	There is considerable potential for spread through unaided larval dispersal on ocean currents, and multiple pathways of human-aided

	very rapidly		<p>spread (ballast water and sediments, dredging activities, shellfish transfers, and boat bilge water).</p> <p>Ballast water and sediment transport may facilitate long distance transport of larvae and adults within European waters, until the BWMC is fully implemented.</p> <p>Dredging activities, bivalve transfers, and movements of small boats containing bilge waters, usually involve shorter distances, generally remaining within member states.</p> <p>However, international dredging contracts and bivalve transfers do also take place between marine regions/subregions, e.g. with the transfer of oyster consignments between Atlantic Europe and the Mediterranean.</p> <p>Under future climate conditions, an overall rise in SST will increase the potential for adult populations and larval production in the Greater North Sea and Celtic Seas, with a corresponding increase in the likelihood of spread via all pathways. Whereas in the Mediterranean Sea, an increase in SST may result in fewer source populations in the south and east of the region, and thus reduce the likelihood of spread. There may also be an increased demand for dredging and shellfish movements.</p>
Summarise Impact*	minimal minor moderate major massive	low medium high	<p><i>Mulinia lateralis</i> appears to have the potential to cause minor to moderate environmental impacts within the RA area, with the greatest impacts being predicted for the Mediterranean. The habitats primarily affected are estuaries and lagoons, which are both of high conservation value.</p> <p><i>M. lateralis</i> may impact native bivalve biodiversity, through competition for space and food, and affect trophic interactions by potentially altering predator/prey relationships. Mass die-offs, typical of this species, could affect</p>

			<p>food web structure, nutrient cycling, the transfer of energy to higher trophic levels, water quality, and habitat structure. It may also facilitate spread of the protozoan parasite <i>Perkinsus chesapeaki</i> to clams and cockles or cestode parasites to elasmobranch species. <i>M. lateralis</i> also has the potential to affect the nursery function of lagoonal habitats.</p> <p>There may be economic losses in the cultivation of bivalves through competition for space and food, and the possible spread of <i>P. chesapeaki</i>. Recreational harvests of other shellfish such as clams and cockles could also be reduced.</p>
Conclusion of the risk assessment (overall risk)	low moderate high	low medium high	<p><i>Mulinia lateralis</i> is an opportunistic, infaunal bivalve already introduced in the North Sea, either by ballast water/sediments or by dredger vessels operating in its native range. Further spread via natural dispersal is expected to proceed relatively quickly, and the human-mediated pathways are likely to cause secondary introductions into the other RA subregions.</p> <p>While dense populations may be ephemeral in Atlantic Europe, the species is expected to persist more in lagoons and estuaries of the northern and western Mediterranean.</p> <p>Minor to moderate impacts may be anticipated, through competition and altered trophic interactions with native species, primarily in estuaries and lagoons. The ecosystem services and conservation value of these habitats are likely to be moderately affected, especially after mass die-offs of <i>M. lateralis</i>, while the bivalve aquaculture sector may be at risk by increased spread of <i>Perkinsus</i> parasites.</p>

*in current climate conditions and in foreseeable future climate conditions

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Distribution Summary

Please answer as follows:

- Yes if recorded, established or invasive
- if not recorded, established or invasive
- ? Unknown; data deficient

The columns refer to the answers to Questions A5 to A12 under Section A.

For data on marine species at the Member State level, delete Member States that have no marine borders.

In all other cases, provide answers for all columns.

EU-Member States, and United Kingdom

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Belgium	Yes	Yes	Yes	Yes	
Bulgaria	-	-	Yes	Yes	
Croatia	-	-	Yes	Yes	
Cyprus	-	-	-	-	
Denmark	-	-	Yes	Yes	
Estonia	-	-	-	-	
Finland	-	-	-	-	
France	-	-	Yes	Yes	
Germany	Yes	Yes	Yes	Yes	
Greece	-	-	Yes	Yes	
Ireland	-	-	No (casual)	No (casual)	
Italy	-	-	Yes	Yes	
Latvia	-	-	-	-	
Lithuania	-	-	-	-	
Malta	-	-	-	-	
Netherlands	Yes	Yes	Yes	Yes	
Poland	-	-	-	-	
Portugal	-	-	Yes	Yes	
Romania	-	-	-	Yes	
Slovenia	-	-	Yes	Yes	
Spain	-	-	Yes	Yes	
Sweden	-	-	-	Yes	
United Kingdom	-	-	Yes	Yes	

Marine regions and subregions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Baltic Sea			Yes (limited)	Yes (limited)	
Black Sea			Yes	Yes	
North-east Atlantic Ocean	Yes	Yes	Yes	Yes	
Bay of Biscay and the Iberian Coast			Yes	Yes	
Celtic Sea			Yes	Yes	
Greater North Sea	Yes	Yes	Yes	Yes	
Mediterranean Sea			Yes	Yes	
Adriatic Sea			Yes	Yes	
Aegean-Levantine Sea			Yes	Yes	
Ionian Sea and the Central Mediterranean Sea			Yes (limited)	Yes (limited)	
Western Mediterranean Sea			Yes	Yes	

ANNEX I Scoring of Likelihoods of Events

(taken from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Description	Frequency
Very unlikely	This sort of event is theoretically possible, but is never known to have occurred and is not expected to occur	1 in 10,000 years
Unlikely	This sort of event has occurred somewhere at least once in the last millennium	1 in 1,000 years
Moderately likely	This sort of event has occurred somewhere at least once in the last century	1 in 100 years
Likely	This sort of event has happened on several occasions elsewhere, or on at least once in the last decade	1 in 10 years
Very likely	This sort of event happens continually and would be expected to occur	Once a year

ANNEX II Scoring of Magnitude of Impacts

(modified from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Biodiversity and ecosystem impact	Ecosystem Services impact	Economic impact (Monetary loss and response costs per year)	Social and human health impact, and other impacts
	<i>Question 5.1-5</i>	<i>Question 5.6-8</i>	<i>Question 5.9-13</i>	<i>Question 5.14-18</i>
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected ⁶	Up to 10,000 Euro	No social disruption. Local, mild, short-term reversible effects to individuals.
Minor	Some ecosystem impact, reversible changes, localised	Local and temporary, reversible effects to one or few services	10,000-100,000 Euro	Significant concern expressed at local level. Mild short-term reversible effects to identifiable groups, localised.
Moderate	Measurable long-term damage to populations and ecosystem, but reversible; little spread, no extinction	Measurable, temporary, local and reversible effects on one or several services	100,000-1,000,000 Euro	Temporary changes to normal activities at local level. Minor irreversible effects and/or larger numbers covered by reversible effects, localised.
Major	Long-term irreversible ecosystem change, spreading beyond local area	Local and irreversible or widespread and reversible effects on one / several services	1,000,000-10,000,000 Euro	Some permanent change of activity locally, concern expressed over wider area. Significant irreversible effects locally or reversible effects over large area.
Massive	Widespread, long-term population loss or extinction, affecting several species with serious ecosystem effects	Widespread and irreversible effects on one / several services	Above 10,000,000 Euro	Long-term social change, significant loss of employment, migration from affected area. Widespread, severe, long-term, irreversible health effects.

⁶ Not to be confused with “no impact”.

ANNEX III Scoring of Confidence Levels

(modified from Bacher et al., 2017)

Each answer provided in the risk assessment must include an assessment of the level of confidence attached to that answer, reflecting the possibility that information needed for the answer is not available or is insufficient or available but conflicting.

The responses in the risk assessment should clearly support the choice of the confidence level.

Confidence level	Description
Low	There is no direct observational evidence to support the assessment, e.g. only inferred data have been used as supporting evidence <i>and/or</i> Impacts are recorded at a spatial scale which is unlikely to be relevant to the assessment area <i>and/or</i> Evidence is poor and difficult to interpret, e.g. because it is strongly ambiguous <i>and/or</i> The information sources are considered to be of low quality or contain information that is unreliable.
Medium	There is some direct observational evidence to support the assessment, but some information is inferred <i>and/or</i> Impacts are recorded at a small spatial scale, but rescaling of the data to relevant scales of the assessment area is considered reliable, or to embrace little uncertainty <i>and/or</i> The interpretation of the data is to some extent ambiguous or contradictory.
High	There is direct relevant observational evidence to support the assessment (including causality) <i>and</i> Impacts are recorded at a comparable scale <i>and/or</i> There are reliable/good quality data sources on impacts of the taxa <i>and</i> The interpretation of data/information is straightforward <i>and/or</i> Data/information are not controversial or contradictory.

ANNEX IV CBD pathway categorisation scheme

Overview of CBD pathway categorisation scheme showing how the 44 pathways relate to the six main pathway categories. All of the pathways can be broadly classified into 1) those that involve intentional transport (blue), 2) those in which the taxa are unintentionally transported (green) and 3) those where taxa moved between regions without direct transportation by humans and/or via artificial corridors (orange and yellow). **Note that the pathways in the category “Escape from confinement” can be considered intentional for the introduction into the risk assessment area and unintentional for the entry into the environment.**



ANNEX V Ecosystem services classification (CICES V5.1, simplified) and examples

For the purposes of this risk assessment, please feel free to use what seems as the most appropriate category / level / combination of impact (Section – Division – Group), reflecting information available.

Section	Division	Group	Examples (i.e. relevant CICES “classes”)
Provisioning	Biomass	Cultivated <i>terrestrial</i> plants	<p>Cultivated terrestrial plants (including fungi, algae) grown for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from cultivated plants, fungi, algae and bacteria for direct use or processing (excluding genetic materials); Cultivated plants (including fungi, algae) grown as a <u>source of energy</u></p> <p><i>Example: negative impacts of non-native organisms to crops, orchards, timber etc.</i></p>
		Cultivated <i>aquatic</i> plants	<p>Plants cultivated by in- situ aquaculture grown for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from in-situ aquaculture for direct use or processing (excluding genetic materials); Plants cultivated by in- situ aquaculture grown as an <u>energy source</u>.</p> <p><i>Example: negative impacts of non-native organisms to aquatic plants cultivated for nutrition, gardening etc. purposes.</i></p>
		Reared animals	<p>Animals reared for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from reared animals for direct use or processing (excluding genetic materials); Animals reared to provide <u>energy</u> (including mechanical)</p> <p><i>Example: negative impacts of non-native organisms to livestock</i></p>
		Reared <i>aquatic</i> animals	<p>Animals reared by in-situ aquaculture for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from animals grown by in-situ aquaculture for direct use or processing (excluding genetic materials); Animals reared by in-situ aquaculture as an <u>energy source</u></p> <p><i>Example: negative impacts of non-native organisms to fish farming</i></p>
		Wild plants (terrestrial and aquatic)	<p>Wild plants (terrestrial and aquatic, including fungi, algae) used for <u>nutrition</u>; <u>Fibres and other materials</u> from wild plants for direct use or processing (excluding genetic materials); Wild plants (terrestrial and aquatic, including fungi, algae) used as a <u>source of energy</u></p> <p><i>Example: reduction in the availability of wild plants (e.g. wild berries, ornamentals) due to non-native organisms (competition, spread of disease etc.)</i></p>
		Wild animals (terrestrial and aquatic)	<p>Wild animals (terrestrial and aquatic) used for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from wild animals for direct use or processing (excluding genetic materials); Wild animals (terrestrial and aquatic) used as a <u>source of energy</u></p>

			<i>Example: reduction in the availability of wild animals (e.g. fish stocks, game) due to non-native organisms (competition, predations, spread of disease etc.)</i>
	Genetic material from all biota	Genetic material from plants, algae or fungi	<u>Seeds, spores and other plant materials</u> collected for maintaining or establishing a population; Higher and lower plants (whole organisms) used to <u>breed new strains or varieties</u> ; Individual genes extracted from higher and lower plants for the <u>design and construction of new biological entities</u> <i>Example: negative impacts of non-native organisms due to interbreeding</i>
		Genetic material from animals	Animal material collected for the purposes of maintaining or establishing a population; Wild animals (whole organisms) used to breed new strains or varieties; Individual genes extracted from organisms for the design and construction of new biological entities <i>Example: negative impacts of non-native organisms due to interbreeding</i>
	Water ⁷	Surface water used for nutrition, materials or energy	Surface water for <u>drinking</u> ; Surface water used as a material (<u>non-drinking purposes</u>); Freshwater surface water, coastal and marine water used as an <u>energy source</u> <i>Example: loss of access to surface water due to spread of non-native organisms</i>
		Ground water for used for nutrition, materials or energy	Ground (and subsurface) water for <u>drinking</u> ; Ground water (and subsurface) used as a material (<u>non-drinking purposes</u>); Ground water (and subsurface) used as an <u>energy source</u> <i>Example: reduced availability of ground water due to spread of non-native organisms and associated increase of ground water consumption by vegetation.</i>
Regulation & Maintenance	Transformation of biochemical or physical inputs to ecosystems	Mediation of wastes or toxic substances of anthropogenic origin by living processes	<u>Bio-remediation</u> by micro-organisms, algae, plants, and animals; <u>Filtration/sequestration/storage/accumulation</u> by micro-organisms, algae, plants, and animals <i>Example: changes caused by non-native organisms to ecosystem functioning and ability to filtrate etc. waste or toxics</i>
		Mediation of nuisances of anthropogenic origin	<u>Smell reduction</u> ; <u>noise attenuation</u> ; <u>visual screening</u> (e.g. by means of green infrastructure) <i>Example: changes caused by non-native organisms to ecosystem structure, leading to reduced ability to mediate nuisances.</i>
	Regulation of physical, chemical, biological conditions	Baseline flows and extreme event regulation	Control of <u>erosion</u> rates; Buffering and attenuation of <u>mass movement</u> ; <u>Hydrological cycle and water flow regulation</u> (Including flood control, and coastal protection); <u>Wind</u> protection; <u>Fire</u> protection

⁷ Note: in the CICES classification provisioning of water is considered as an abiotic service whereas the rest of ecosystem services listed here are considered biotic.

			<p><i>Example: changes caused by non-native organisms to ecosystem functioning or structure leading to, for example, destabilisation of soil, increased risk or intensity of wild fires etc.</i></p>
		<p>Lifecycle maintenance, habitat and gene pool protection</p>	<p>Pollination (or 'gamete' dispersal in a marine context); <u>Seed dispersal</u>; Maintaining <u>nursery populations and habitats</u> (Including gene pool protection)</p> <p><i>Example: changes caused by non-native organisms to the abundance and/or distribution of wild pollinators; changes to the availability / quality of nursery habitats for fisheries</i></p>
		<p>Pest and disease control</p>	<p>Pest control; Disease control</p> <p><i>Example: changes caused by non-native organisms to the abundance and/or distribution of pests</i></p>
		<p>Soil quality regulation</p>	<p><u>Weathering processes</u> and their effect on soil quality; <u>Decomposition and fixing processes</u> and their effect on soil quality</p> <p><i>Example: changes caused by non-native organisms to vegetation structure and/or soil fauna leading to reduced soil quality</i></p>
		<p>Water conditions</p>	<p>Regulation of the <u>chemical condition</u> of freshwaters by living processes; Regulation of the chemical condition of salt waters by living processes</p> <p><i>Example: changes caused by non-native organisms to buffer strips along water courses that remove nutrients in runoff and/or fish communities that regulate the resilience and resistance of water bodies to eutrophication</i></p>
		<p>Atmospheric composition and conditions</p>	<p>Regulation of <u>chemical composition</u> of atmosphere and oceans; Regulation of <u>temperature and humidity</u>, including ventilation and transpiration</p> <p><i>Example: changes caused by non-native organisms to ecosystems' ability to sequester carbon and/or evaporative cooling (e.g. by urban trees)</i></p>
Cultural	Direct, in-situ and outdoor interactions with living systems that depend on presence in the environmental setting	<p>Physical and experiential interactions with natural environment</p>	<p>Characteristics of living systems that that enable activities promoting health, recuperation or enjoyment through <u>active or immersive interactions</u>;</p> <p>Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through <u>passive or observational interactions</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that make it attractive for recreation, wild life watching etc.</i></p>
		<p>Intellectual and representative interactions with natural environment</p>	<p>Characteristics of living systems that enable <u>scientific investigation</u> or the creation of traditional ecological knowledge;</p> <p>Characteristics of living systems that enable <u>education and training</u>;</p> <p>Characteristics of living systems that are resonant in terms of <u>culture or heritage</u>;</p> <p>Characteristics of living systems that enable <u>aesthetic experiences</u></p>

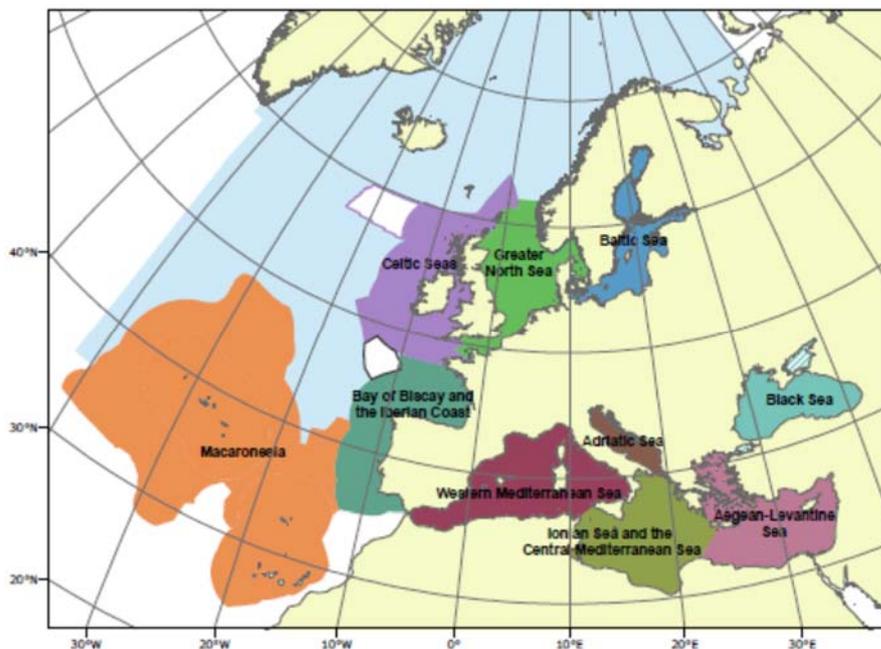
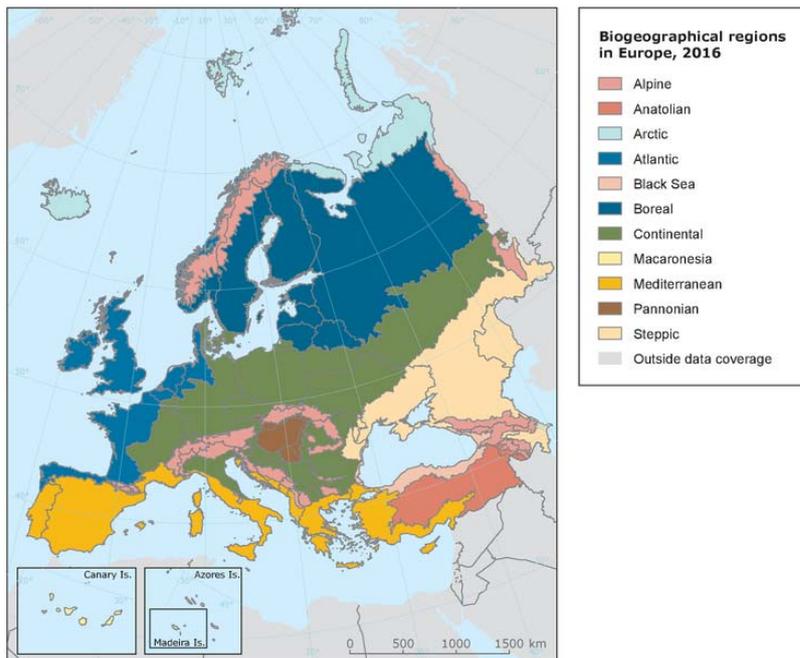
			<i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have cultural importance</i>
	Indirect, remote, often indoor interactions with living systems that do not require presence in the environmental setting	Spiritual, symbolic and other interactions with natural environment	Elements of living systems that have <u>symbolic meaning</u> ; Elements of living systems that have <u>sacred or religious meaning</u> ; Elements of living systems used for <u>entertainment or representation</u> <i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have sacred or religious meaning</i>
		Other biotic characteristics that have a non-use value	Characteristics or features of living systems that have an <u>existence value</u> ; Characteristics or features of living systems that have an <u>option or bequest value</u> <i>Example: changes caused by non-native organisms to ecosystems designated as wilderness areas, habitats of endangered species etc.</i>

ANNEX VI EU Biogeographic Regions and MSFD Subregions

See <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2> ,
http://ec.europa.eu/environment/nature/natura2000/biogeog_regions/

and

<https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions-1/technical-document/pdf>



ANNEX VII Delegated Regulation (EU) 2018/968 of 30 April 2018

see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>

ANNEX VIII *Mulinia lateralis* physiological requirements and thresholds

(text and analysis specifically developed for the purposes of this risk assessment)

Mulinia lateralis is a eurythermal and euryhaline species with a latitudinal distribution extending from the Bay of St. Lawrence, Canada, to the Gulf of Mexico, Yucatan, Mexico (Turgeon et al., 2009; see also Qu. A3 for details). This distribution corresponds to low winter temperatures of approximately -1 °C to 24 °C (mean temperature of the coldest month, retrieved from BIO-ORACLE 2 (Assis et al., 2018)) and high summer temperatures ranging from 17 °C to 30.5 °C (mean temperature of the warmest month, retrieved from BIO-ORACLE 2), conditions which are met throughout most of the RA area. Spawning occurs at different times of the year at different latitudes, e.g. in Canada from mid-July to early September (Sullivan, 1948), in Maryland from May to November with a peak in September (Hanks, 1968), in Texas from January to April, while year-round spawning has also been reported (Montagna et al., 1993). In the Long Island Sound, larvae appear in the water in early July at temperatures ranging between 16 °C and 20 °C but are more abundant at 19-21 °C (Calabrese, 1970). Field observations agree rather well with laboratory results, which indicate that the temperature threshold for larval growth that ensures metamorphosis before the larvae die in the water column lies somewhere between 15 °C and 17.5 °C (Calabrese, 1969a). Thus, temperature doesn't seem to be a major limiting factor for establishment at a large scale, except perhaps around northern UK waters, where maximum summer temperatures drop below 15°C. Nevertheless, because *M. lateralis* is primarily an inshore, estuarine species (Walker & Tenore, 1984), coarse grain temperature maps may not accurately reflect local conditions. For example, temperature measurements in marinas and harbours along the UK coast demonstrate water temperatures from 16-22°C in July – October, every year, all the way up to northern England (Bishop et al., 2015; C. Wood, unpublished data).

Regarding upper temperature limits for larval development, in the laboratory normal development and growth were achieved at temperatures as high as 32.5 °C (Calabrese, 1969b). Considering potential discrepancies between large-scale modelled data and local measurements, especially in shallow waters, this generally agrees well with the condition the species is faced with in the wild.

For modeling purposes, conditions unsuitable for establishment were defined as:

Mean temperature of the warmest month (LtMax) <15 °C

Mean temperature of the warmest month >32.5 °C

The same temperature limits were used for the calculation of growing degree days (GDD) in the Modeling Annex.

The species can also withstand a wide range of salinities, reportedly between 5-80 psu (Parker, 1975 in Montagna et al., 1993). This extremely wide range does not necessarily mean that *M. lateralis* from all geographical areas are able to withstand such extreme salinities, nor does it mean that all stages of reproduction are accomplished at the extremes of the salinity range.

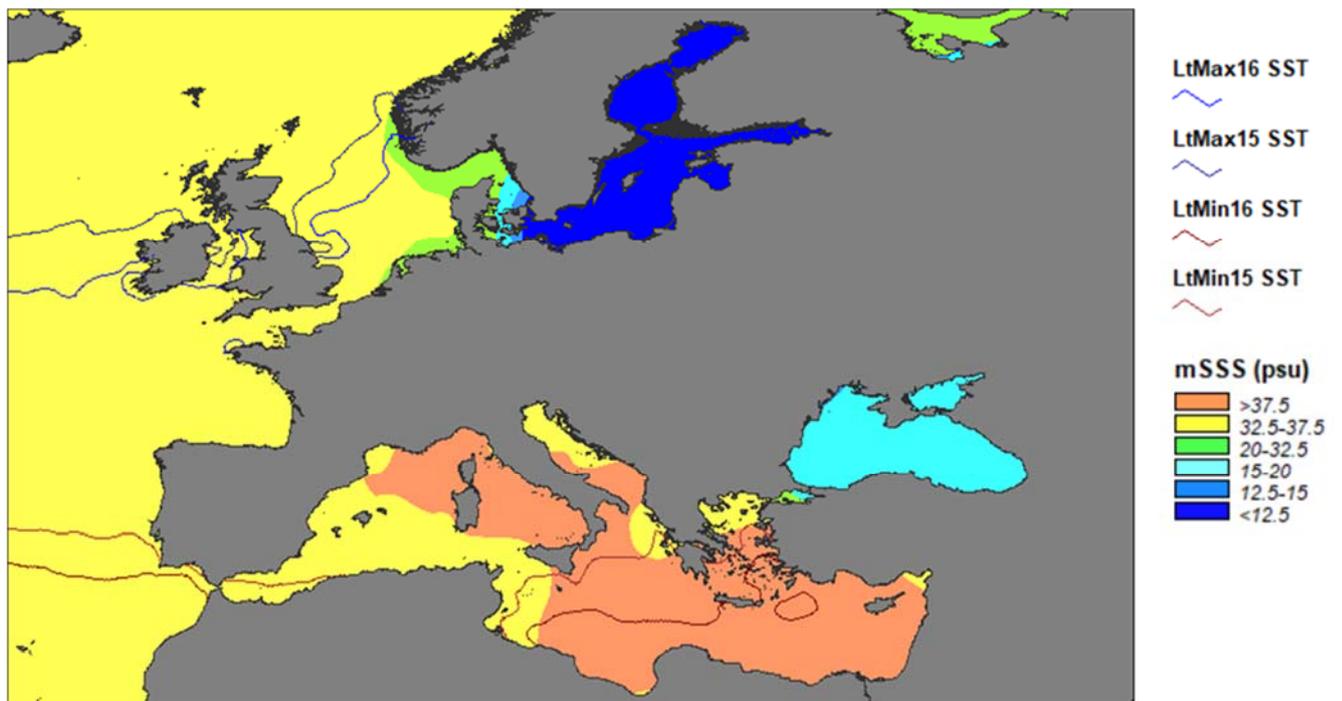
M. lateralis can persist equally in low salinity estuarine environments as in hypersaline conditions in lagoons, associated with prolonged drought periods (e.g. in Baffin Bay, Texas, where the species is dominating the infaunal community at salinities between 40-60 psu). It is generally observed though that major recruitment events are triggered by big salinity changes caused by freshwater inflow, rather than absolute salinity levels (Montagna & Kalke, 1995; Van Diggelen & Montagna, 2016). On the other

hand, its presence along the Dutch and Belgian coasts, as well as in the shallow sublittoral in its native range (Cleveland et al., 2002) indicates it can also establish in fully marine areas. Regarding larval development, in laboratory experiments it was shown to be normal between 12.5 and 37.5 psu. Larval survival was observed within a larger range of 7-38 psu (Calabrese, 1969), however satisfactory embryonic development occurred at a more restricted salinity range of 20-32.5 psu (Calabrese, 1969a). Thus, as long as spawning and embryonic development take place at appropriate salinities, larvae can disperse and will survive and grow in different water bodies.

It is apparent from the above that, when it comes to salinity, it is difficult to set a hard limit for larval development, especially considering that large scale salinity maps are modeling products themselves and do not capture small scale salinity variations near the coast. **For the purpose of defining the unsuitable background for modeling, a limit of 12.5 psu was tentatively set** for mean surface salinity. However, in an effort to capture the influence of salinity variability we employ an additional parameter, namely distance to the nearest river mouth (see Annex IX, the Species Distribution Model).

The above-mentioned potential limitations to establishment are displayed in Figure 1. This map intends to serve as a first indication of rough geographical limits set by low summer temperatures (blue contour lines of 15°C and 16°C temperature of the warmest month LtMax), and broad salinity ranges (mSSS), as well as indicating areas where continuous recruitment is likely (red contour line of 15°C and 16°C temperature of the coldest month LtMin).

Figure 1.



ANNEX IX Species Distribution Model

Projection of environmental suitability for *Mulinia lateralis* establishment in Europe

Björn Beckmann, Marika Galanidi, Christine Wood and Dan Chapman

22 June 2021

Aim

To project the suitability for potential establishment of *Mulinia lateralis* in Europe, under current and predicted future climatic conditions.

Data for modelling

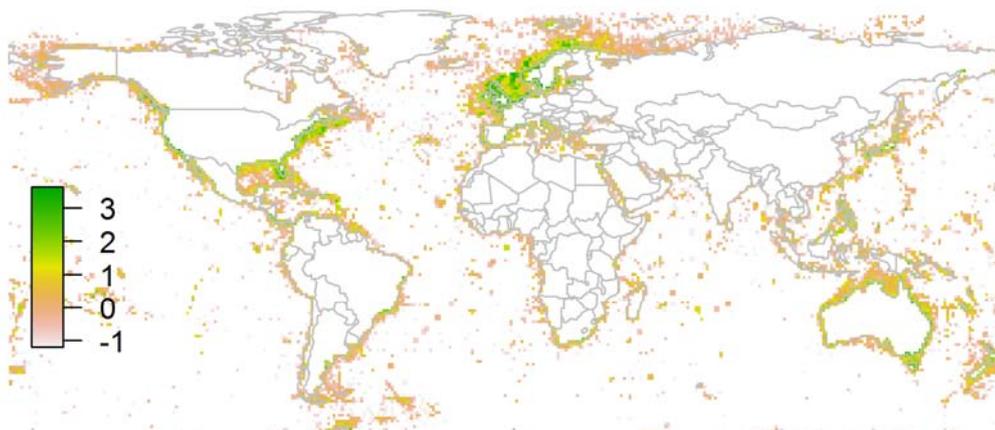
Species occurrence data were obtained from the Global Biodiversity Information Facility (GBIF) (195 records), iNaturalist (161 records), the Biodiversity Information Serving Our Nation database (BISON) (121 records), and additional records from the risk assessment team. We scrutinised occurrence records and removed any dubious ones and records where the georeferencing was too imprecise or outside of the coverage of the predictor layers. The records were gridded at a 0.25 x 0.25 degree resolution for modelling, yielding 77 grid cells with occurrences (Figure 1a). As a proxy for recording effort, the density of *Bivalvia* records held by GBIF was also compiled on the same grid (Figure 1b).

Figure 1. (a) Occurrence records obtained for *Mulinia lateralis* and used in the modelling, showing native and invaded distributions. (b) The recording density of *Bivalvia* on GBIF, which was used as a proxy for recording effort.

(a) Species distribution used in modelling



(b) Estimated recording effort (log10-scaled)



Predictors describing the marine environment were selected from the ‘Bio-ORACLE2’ set of GIS rasters providing geophysical, biotic and environmental data for surface and benthic marine realms (Tyberghein et al., 2012; Assis et al., 2018), supplemented by variables calculated from MARSPEC monthly sea surface temperature data (Sbrocco & Barber, 2013). Both were originally at 5 arcminute resolution (0.083 x 0.083 degrees of longitude/latitude) and aggregated to a 0.25 x 0.25 degree grid for use in the model.

Based on the biology of *Mulinia lateralis*, the following variables were used in the modelling:

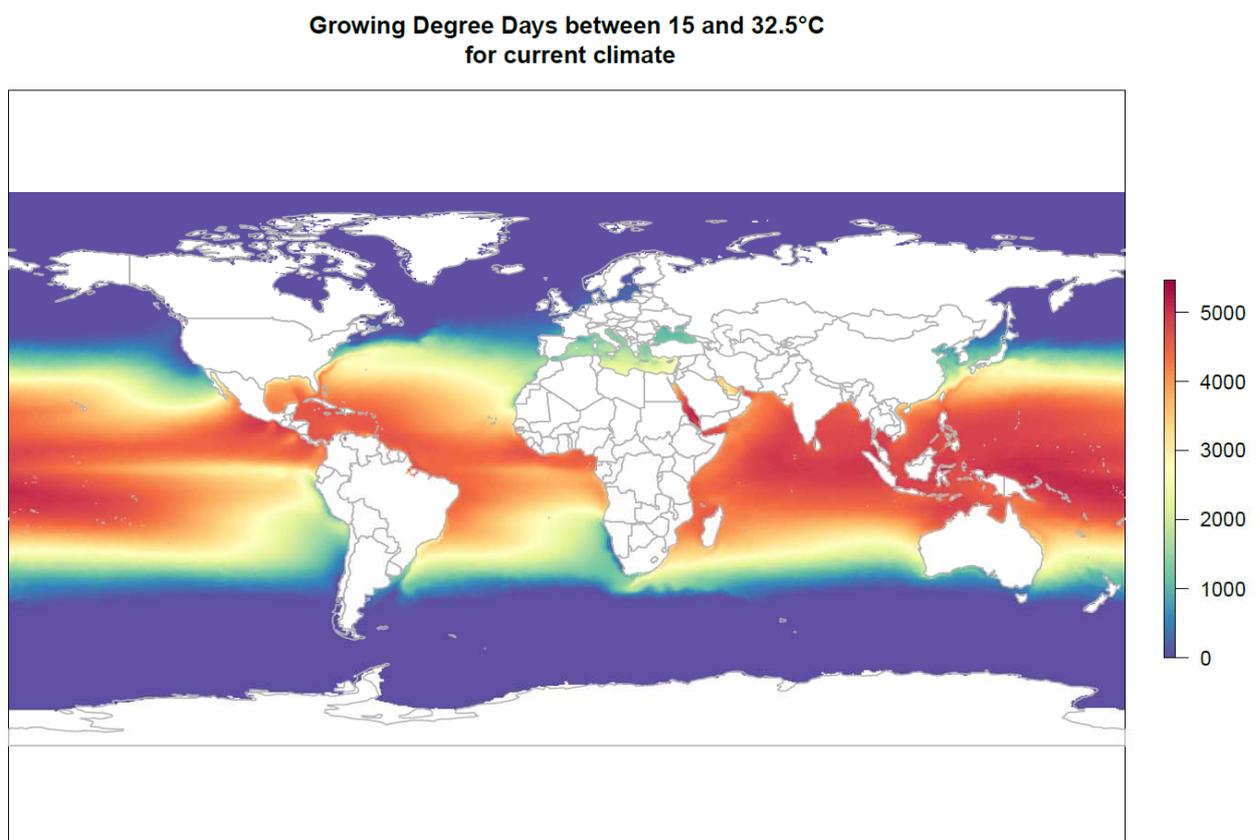
- Maximum long-term temperature (templtmax_ss)
- Minimum long-term temperature (templtmin_ss)
- Mean salinity (salinitymean_ss)
- Mean bathymetry (bathymean)

- Growing Degree Days between 15 and 32.5°C (gdd_15_32.5)
- Sea distance to nearest river mouth with average discharge >10 m³/s (sea_dist_10cms), log+1 transformed to increase normality.

All parameters (except depth) are measured at the sea surface.

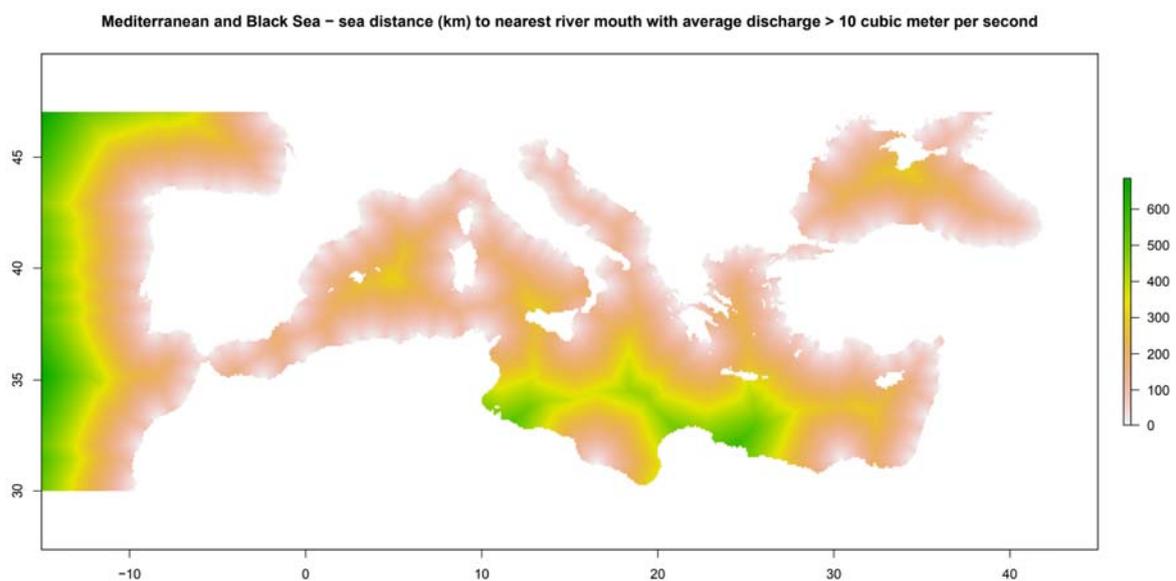
Growing Degree Days (GDD) have been widely used as a measure of the “biologically useful” sum of warmth between threshold temperatures where processes of interest occur (McMaster & Wilhelm, 1997). For *Mulinia lateralis*, larval development occurs between approximately 15 and 32.5°C (see Risk Assessment for details). GDD are usually calculated from daily temperature data, but since these are not available for the marine environment at a global scale, GDD were calculated from MARSPEC monthly mean temperature data. First, all grid cells with temperatures less than 15°C or more than 32.5°C were excluded for each month. Then, any positive difference from the lower temperature threshold was multiplied by the number of days in the month. For example, a grid cell with a temperature of 17°C in January had a value of 17°C - 15°C = 2°C x 31 days = 62 GDD for January. Finally, GDD values for all months were summed to produce an annual total for each grid cell (Figure 2).

Figure 2. Annual Growing Degree Days (GDD) between 15°C and 32.5°C for the current climate, calculated from MARSPEC monthly mean sea surface temperatures.



The distance to the nearest large river mouth (with average discharge $>10 \text{ m}^3/\text{s}$) was used as a proxy for presence of brackish water (in addition to the Bio-ORACLE2 salinity layer mentioned above). The location of rivers and their average discharge was taken from the “HydroRIVERS” dataset, version 1.0 (Lehner et al., 2008). Point locations of river mouths were “rasterized” to the model grid, summing discharge per 0.25×0.25 degree grid cell in case there were several river mouths per cell. Endorheic rivers (not flowing into the oceans) were excluded. For each model grid cell, distance-by-sea (in meters) to the nearest cell with average discharge $> 10 \text{ m}^3/\text{sec}$ was then calculated using “gridDistance” in the R package “raster”, version 3.4.5 (Hijmans, 2020). Figure 3 shows the Mediterranean and Black Sea areas of the final layer (with distances in km).

Figure 3. Proximity to the nearest river mouth with average discharge $> 10 \text{ m}^3/\text{sec}$. Layer created as a proxy for salinity variation.



To estimate the effect of climate change on the potential distribution of *Mulinia lateralis*, equivalent modelled future conditions for the 2070s under the Representative Concentration Pathways (RCP) 2.6 and 4.5 were also obtained. These represent low and medium emissions scenarios, respectively. Projections for the 2070s were calculated as averages of projections for the 2040s and 2090s (which are the time periods available on Bio-ORACLE).

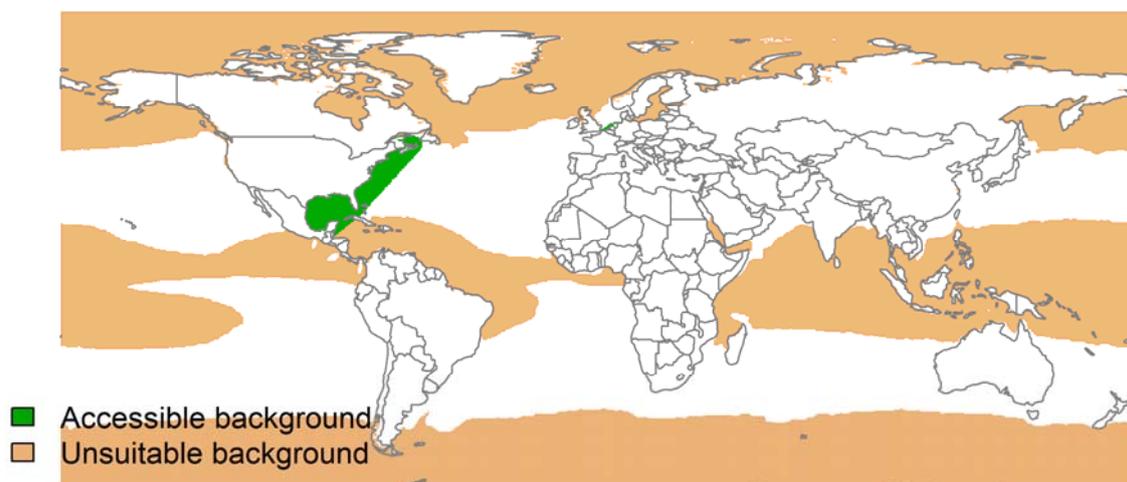
Species distribution model

A presence-background (presence-only) ensemble modelling strategy was employed using the BIOMOD2 R package version 3.4.6 (Thuiller et al., 2009; Thuiller et al., 2020). These models contrast the environment at the species' occurrence locations against a random sample of the global background environmental conditions (often termed 'pseudo-absences') in order to characterise and project suitability for occurrence. This approach has been developed for distributions that are in equilibrium with the environment. Because invasive species' distributions are not at equilibrium and subject to dispersal constraints at a global scale, we took care to minimise the inclusion of locations suitable for the species but where it has not been able to disperse to (Chapman et al., 2019). Therefore the background sampling region included:

- The area accessible by native *Mulinia lateralis* populations, in which the species is likely to have had sufficient time to disperse to all locations. Based on presumed maximum dispersal distances, the accessible region was defined as a 400 km buffer around the native range occurrences (the Pacific Ocean was excluded from this area because *Mulinia lateralis* occurs only on the Atlantic coasts of North America and historically could not readily disperse to the Pacific side); AND
- A 100 km buffer around the non-native occurrences, encompassing regions likely to have had high propagule pressure for introduction by humans and/or dispersal of the species; AND
- Regions where we have an *a priori* expectation of high unsuitability for the species so that absence is assumed irrespective of dispersal constraints (see Figure 4). The following rules were applied to define a region expected to be highly unsuitable for *Mulinia lateralis* at the spatial scale of the model:
 - Minimum long-term temperature (templtmin_ss) > 25 °C
 - Maximum long-term temperature (templtmax_ss) < 15 °C
 - Mean salinity (salinitymean_ss) < 12.5 psu

Altogether, 2.6% of occurrence grid cells were located in the unsuitable background region.

Within the unsuitable background region, 10 samples of 5000 randomly sampled grid cells were obtained. In the accessible background (comprising the accessible areas around native and non-native occurrences as detailed above), the same number of pseudo-absence samples were drawn as there were presence records (77), weighting the sampling by a proxy for recording effort (Figure 1(b)). **Figure 4.** The background from which pseudo-absence samples were taken in the modelling of *Mulinia lateralis*. Samples were taken from a 400 km buffer around the native range and a 100 km buffer around non-native occurrences (together forming the accessible background), and from areas expected to be highly unsuitable for the species (the unsuitable background region). Samples from the accessible background were weighted by a proxy for recording effort (Figure 1(b)).



Each dataset (i.e. combination of the presences and the individual background samples) was randomly split into 80% for model training and 20% for model evaluation. With each training dataset, five

statistical algorithms were fitted with the default BIOMOD2 settings and rescaled using logistic regression, except where specified below:

- Generalised linear model (GLM)
- Generalised boosting model (GBM)
- Generalised additive model (GAM) with a maximum of four degrees of freedom per smoothing spline
- Artificial neural network (ANN)
- Multivariate adaptive regression splines (MARS)
- Random forest (RF)
- Maxent

Since the total background sample was larger than the number of occurrences, prevalence fitting weights were applied to give equal overall importance to the occurrences and the background. Normalised variable importance was assessed and variable response functions were produced using BIOMOD2's default procedure.

Model predictive performance was assessed by the following three measures:

- AUC, the area under the receiver operating characteristic curve (Fielding & Bell, 1997). Predictions of presence-absence models can be compared with a subset of records set aside for model evaluation (here 20%) by constructing a confusion matrix with the number of true positive, false positive, false negative and true negative cases. For models generating non-dichotomous scores (as here) a threshold can be applied to transform the scores into a dichotomous set of presence-absence predictions. Two measures that can be derived from the confusion matrix are sensitivity (the proportion of observed presences that are predicted as such, quantifying omission errors), and specificity (the proportion of observed absences that are predicted as such, quantifying commission errors). A receiver operating characteristic (ROC) curve can be constructed by using all possible thresholds to classify the scores into confusion matrices, obtaining sensitivity and specificity for each matrix, and plotting sensitivity against the corresponding proportion of false positives (equal to $1 - \text{specificity}$). The use of all possible thresholds avoids the need for a selection of a single threshold, which is often arbitrary, and allows appreciation of the trade-off between sensitivity and specificity. The area under the ROC curve (AUC) is often used as a single threshold-independent measure for model performance (Manel et al., 2001). AUC is the probability that a randomly selected presence has a higher model-predicted suitability than a randomly selected absence (Allouche et al., 2006).
- Cohen's Kappa (Cohen, 1960). This measure corrects the overall accuracy of model predictions (ratio of the sum of true presences plus true absences to the total number of records) by the accuracy expected to occur by chance. The Kappa statistic ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random. Advantages of Kappa are its simplicity, the fact that both commission and omission errors are accounted for in one parameter, and its relative tolerance to zero values in the confusion matrix (Manel et al., 2001). However, Kappa has been criticised for being sensitive to prevalence (the proportion of sites in which the species was recorded as present) and may therefore be inappropriate for comparisons of model accuracy between species or regions (McPherson et al., 2004; Allouche et al., 2006).

- TSS, the true skill statistic (Allouche et al., 2006). TSS is defined as sensitivity + specificity - 1, and corrects for Kappa's dependency on prevalence. TSS compares the number of correct forecasts, minus those attributable to random guessing, to that of a hypothetical set of perfect forecasts. Like Kappa, TSS takes into account both omission and commission errors, and success as a result of random guessing, and ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random (Allouche et al., 2006).

An ensemble model was created by first rejecting poorly performing algorithms with relatively extreme low AUC values and then averaging the predictions of the remaining algorithms, weighted by their AUC. To identify poorly performing algorithms, AUC values were converted into modified z-scores based on their difference to the median and the median absolute deviation across all algorithms (Iglewicz & Hoaglin, 1993). Algorithms with $z < -2$ were rejected. In this way, ensemble projections were made for each dataset and then averaged to give an overall suitability, as well as its standard deviation.

Projections were classified into suitable and unsuitable regions using a "lowest presence threshold" (Pearson et al., 2007), setting the cut-off as the lowest value at which 98% of all presence records are classified correctly under the current climate (here 0.77). In order to express the sensitivity of classifications to the choice of this threshold, thresholds at which 95% and 99% of records are classified correctly (here 0.89 and 0.72 respectively) were used in the calculation of error bars in Figures 11 and 12 below in addition to taking account of uncertainty in the projections themselves.

We also produced limiting factor maps for Europe following Elith et al. (2010). For this, projections were made separately with each individual variable fixed at a near-optimal value. These were chosen as the median values at the occurrence grid cells. Then, the most strongly limiting factors were identified as the one resulting in the highest increase in suitability in each grid cell.

Results

The ensemble model suggested that suitability for *Mulinia lateralis* was most strongly determined by Mean bathymetry (bathymean), accounting for 23.9% of variation explained, followed by Maximum long-term temperature (temptlmax_ss) (23.7%), Minimum long-term temperature (temptlmin_ss) (22.4%), Growing Degree Days between 15 and 32.5°C (gdd_15_32.5) (17.1%), Sea distance to nearest river mouth with average discharge >10 m³/s (sea_dist_10cms) (10.8%) and Mean salinity (salinitymean_ss) (2%) (Table 1, Figure 5). **Table 1.** Summary of the cross-validation predictive performance (ROC, Kappa, TSS) and variable importance of the fitted model algorithms and the ensemble (AUC-weighted average of the best performing algorithms). Results are the average from models fitted to 10 different background samples of the data.

Algorithm	AUC	Kappa	TSS	Used in the ensemble	Variable importance (%)					
					Mean bathymetry (bathymean)	Maximum long-term temperature (temptlmax_ss)	Minimum long-term temperature (temptlmin_ss)	Growing Degree Days between 15 and 32.5°C (gdd_15_32.5)	Sea distance to nearest river mouth with average discharge >10 m ³ /s (sea_dist_10cms)	Mean salinity (salinitymean_ss)
GLM	0.986	0.749	0.960	yes	23	14	17	27	18	2
GAM	0.996	0.748	0.987	yes	12	20	24	26	15	3
GBM	0.993	0.759	0.968	yes	45	30	22	1	2	0
ANN	0.976	0.674	0.936	yes	27	26	15	18	13	3
MARS	0.966	0.734	0.930	no	18	11	27	22	19	3
RF	0.996	0.772	0.975	yes	14	29	34	14	7	2
Maxent	0.894	0.719	0.784	no	7	10	32	25	18	10
Ensemble	0.996	0.774	0.986		24	24	22	17	11	2

Figure 5. Partial response plots from the fitted models. Thin coloured lines show responses from the algorithms in the ensemble, while the thick black line is their ensemble. In each plot, other model variables are held at their median value in the training data. Some of the divergence among algorithms is because of their different treatment of interactions among variables.

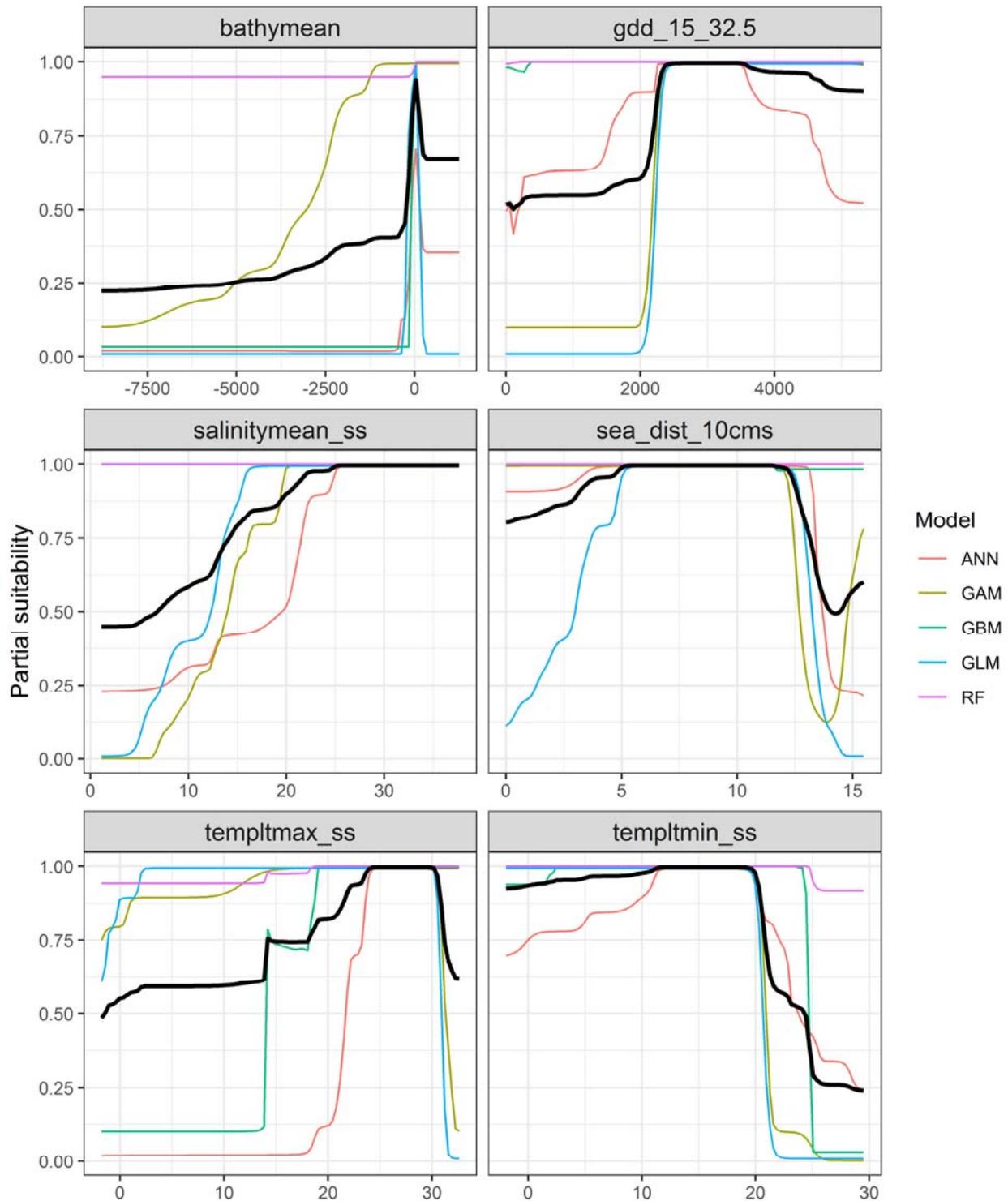
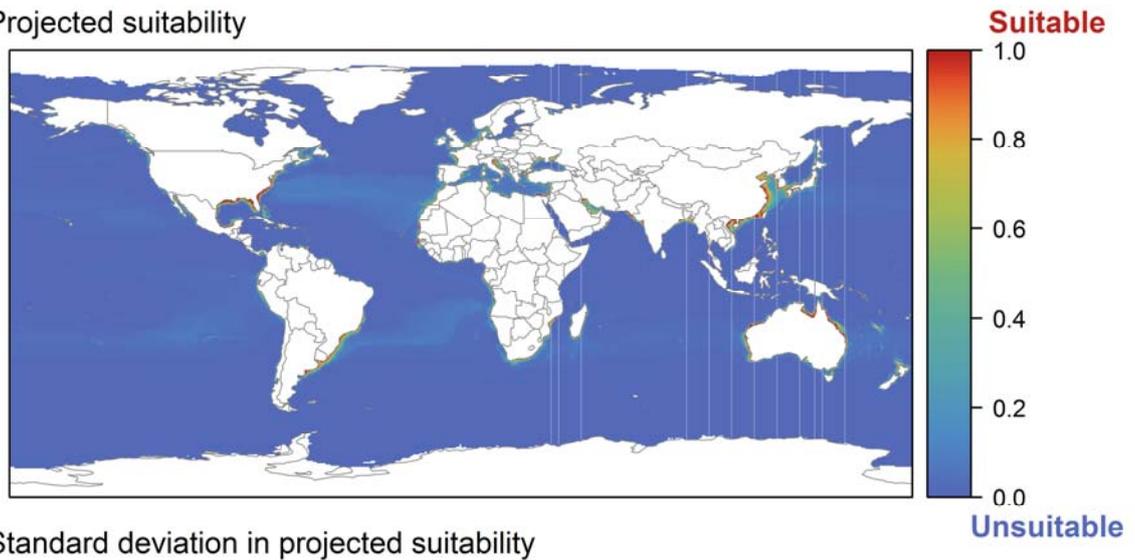


Figure 6. (a) Projected global suitability for *Mulinia lateralis* establishment in the current climate. For visualisation, the projection has been aggregated to a 0.5 x 0.5 degree resolution, by taking the maximum suitability of constituent higher resolution grid cells. Values > 0.77 are suitable for the species, with 98% of global presence records above this threshold. Values below 0.77 indicate lower relative suitability. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

(a) Projected suitability



(b) Standard deviation in projected suitability

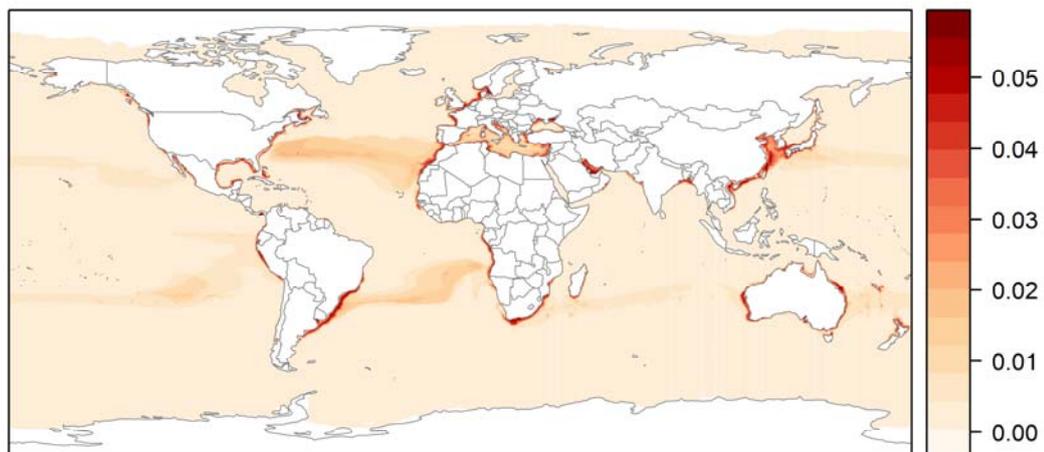


Figure 7. (a) Projected current suitability for *Mulinia lateralis* establishment in Europe and the Mediterranean region. Values > 0.77 are suitable for the species, with 98% of global presence records above this threshold. Values below 0.77 indicate lower relative suitability. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

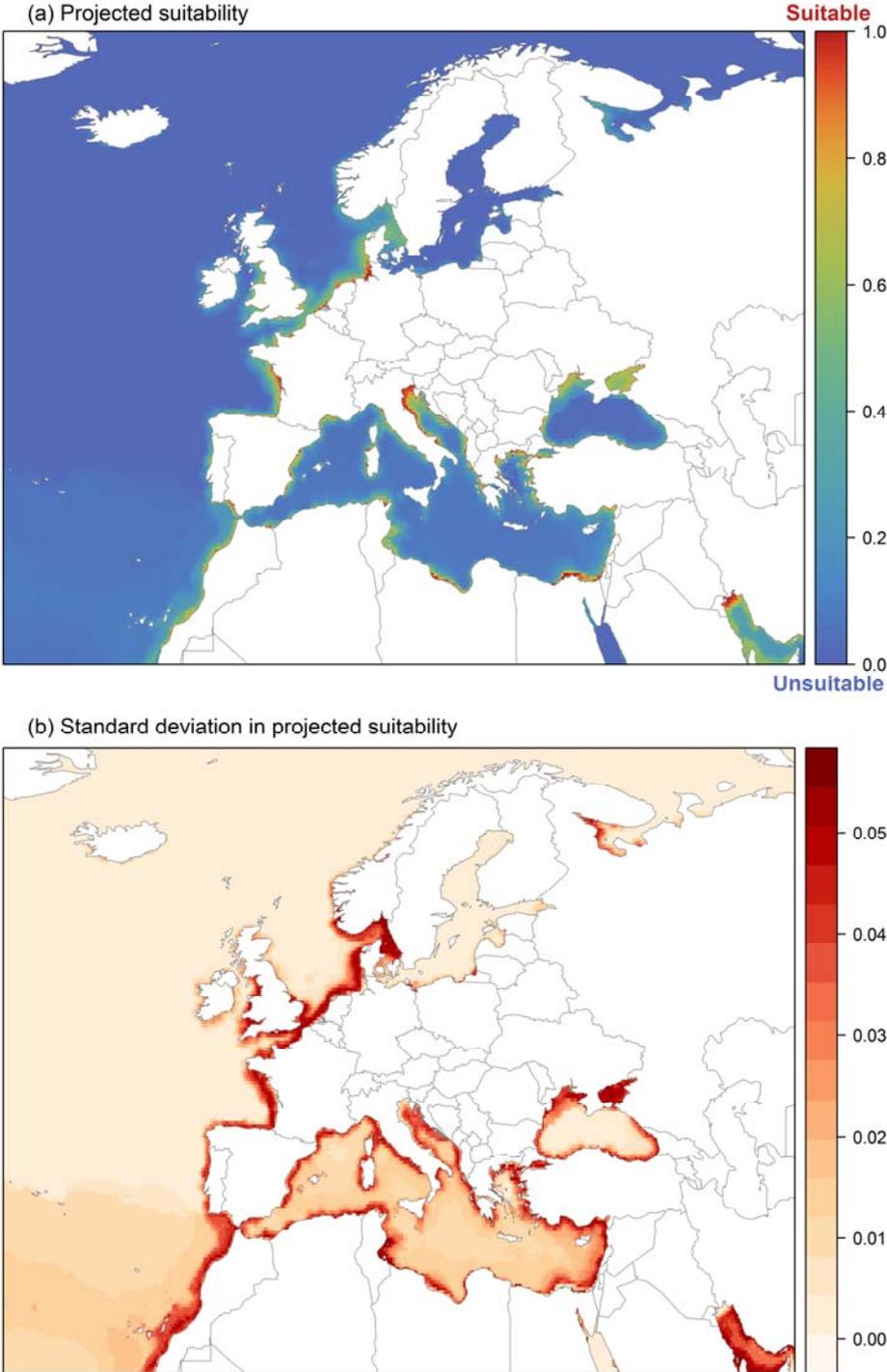


Figure 8. The most strongly limiting factors for *Mulinia lateralis* establishment estimated by the model in Europe and the Mediterranean region in current climatic conditions.

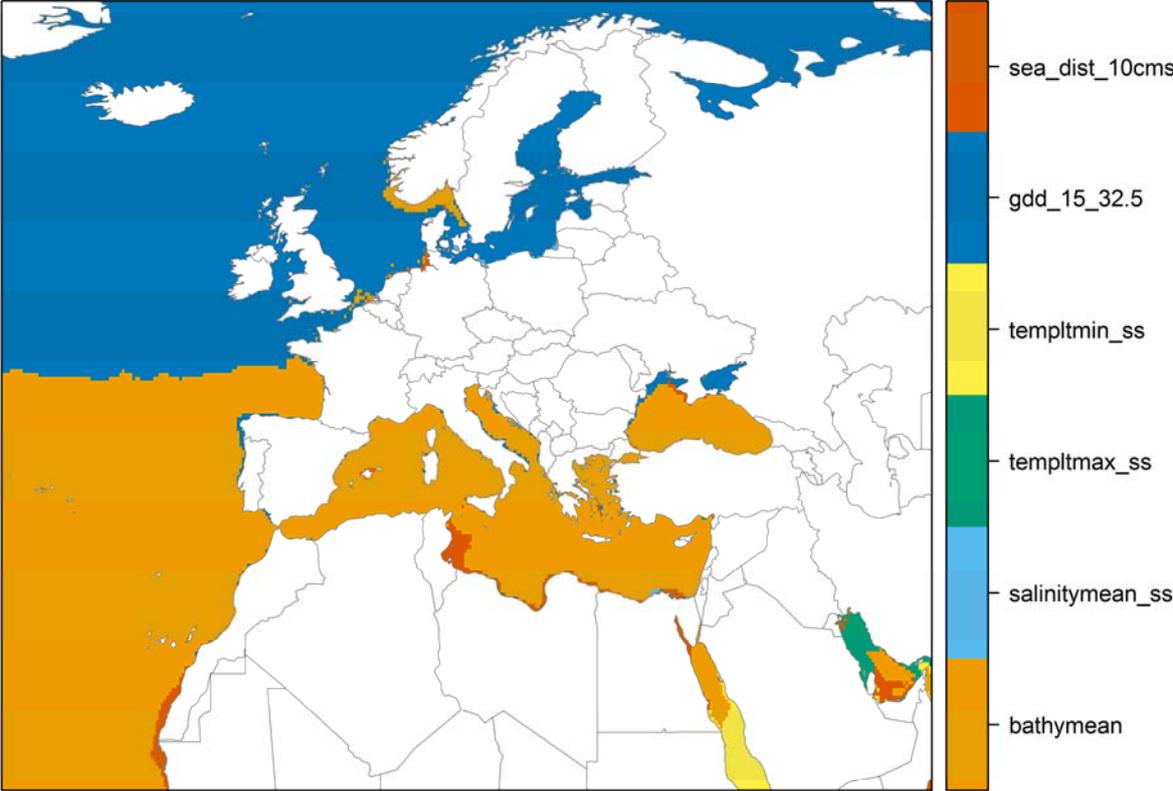


Figure 9. (a) Projected suitability for *Mulinia lateralis* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP 2.6. Values > 0.77 are suitable for the species, with 98% of global presence records above this threshold under current climate. Values below 0.77 indicate lower relative suitability. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

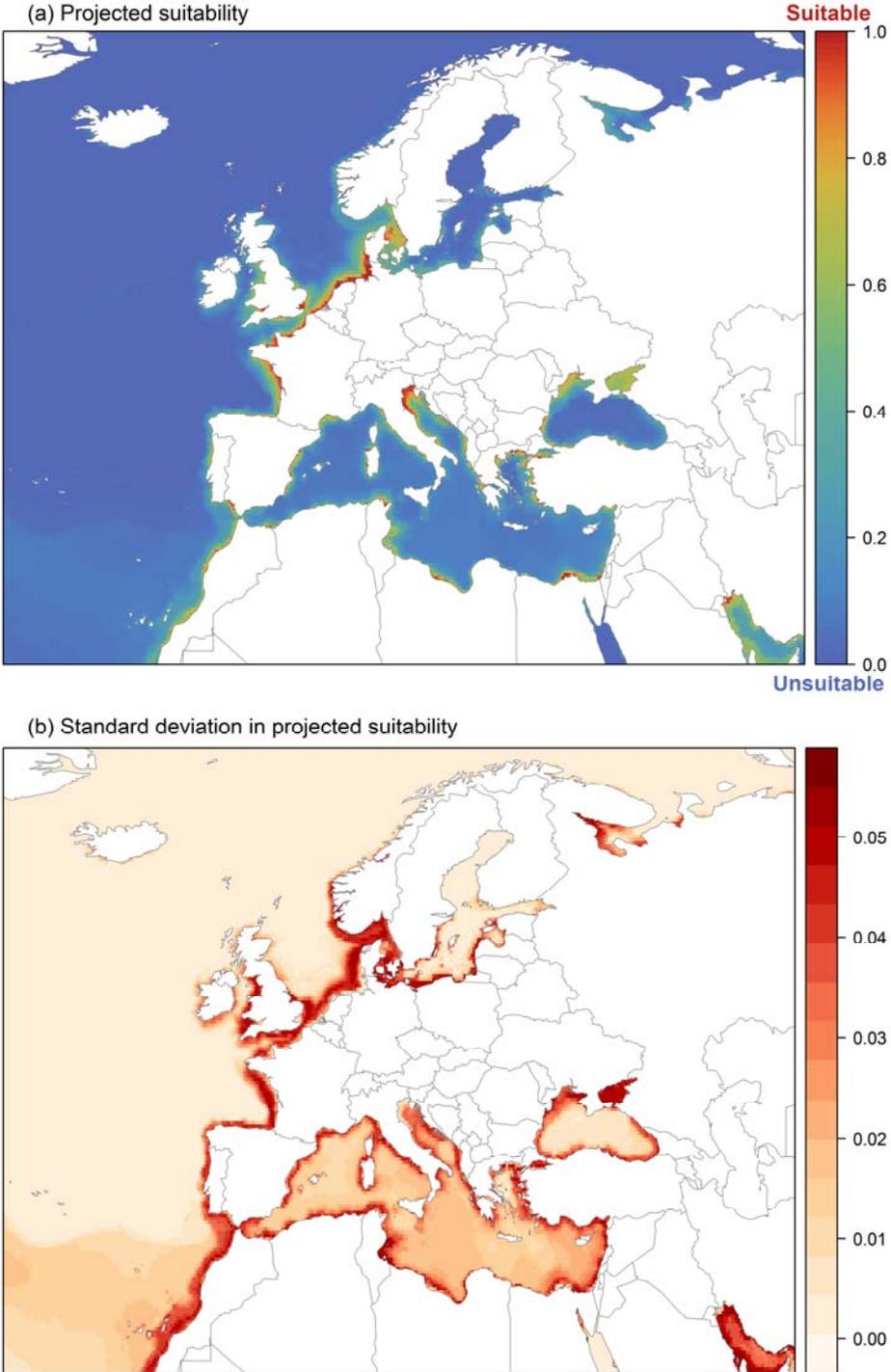


Figure 10. (a) Projected suitability for *Mulinia lateralis* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP 4.5. Values > 0.77 are suitable for the species, with 98% of global presence records above this threshold under current climate. Values below 0.77 indicate lower relative suitability. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

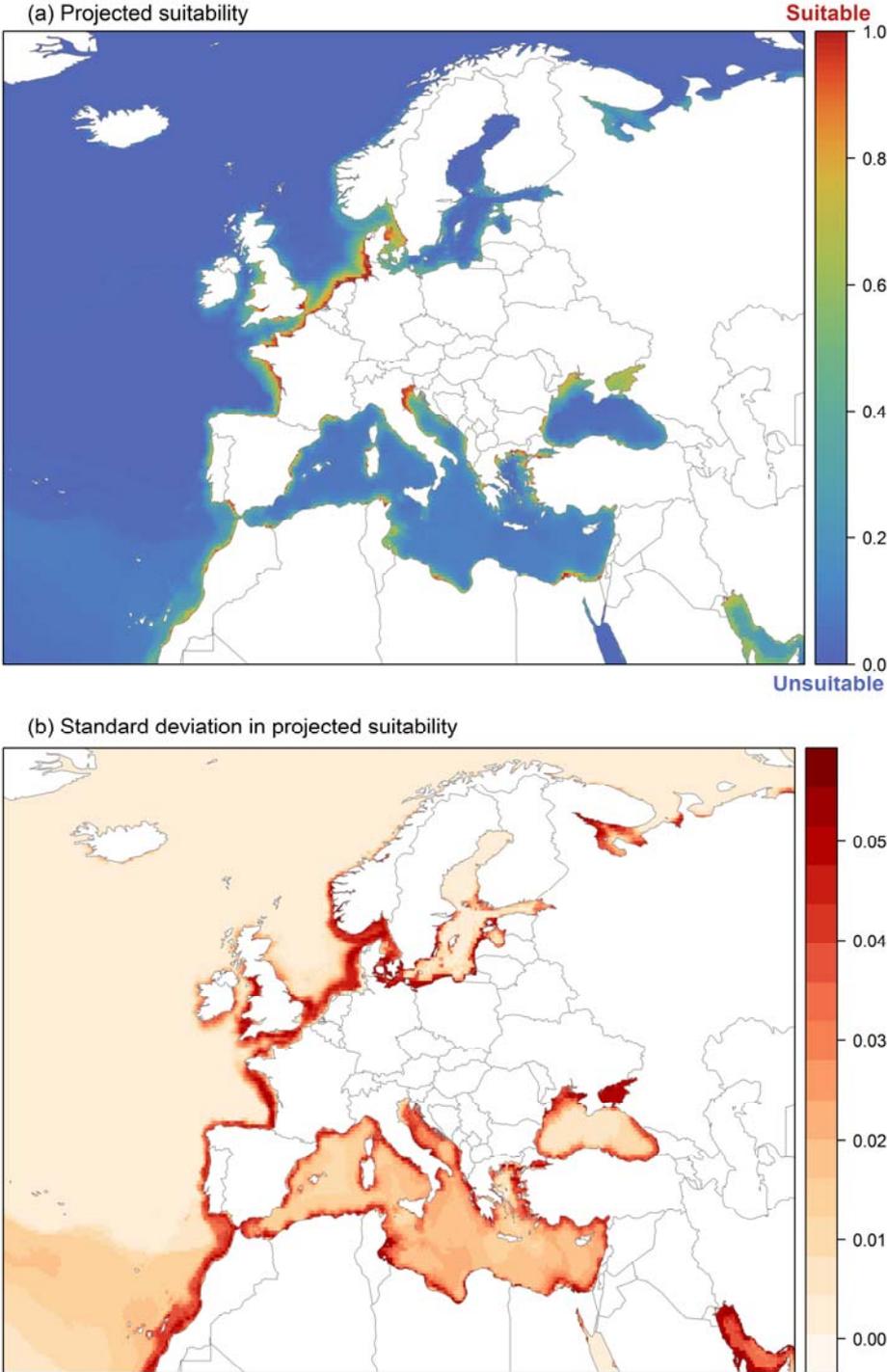


Figure 11. Variation in projected suitability for *Mulinia lateralis* establishment among marine subregions of Europe. The bar plots show the proportion of grid cells in each region classified as suitable (with values > 0.77) in the current climate and projected climate for the 2070s under two RCP emissions scenarios. Error bars indicate uncertainty due to both the choice of classification threshold and uncertainty in the projections themselves (cf. part (b) of Figures 7, 9 and 10). The location of each region is also shown. Macaronesia is excluded as it is not part of the study area.

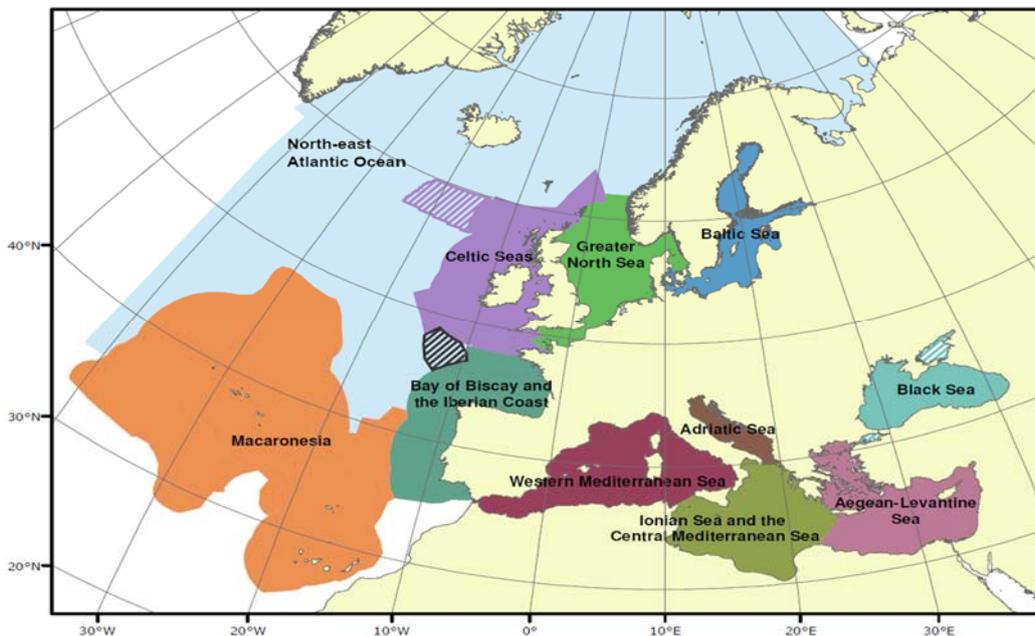
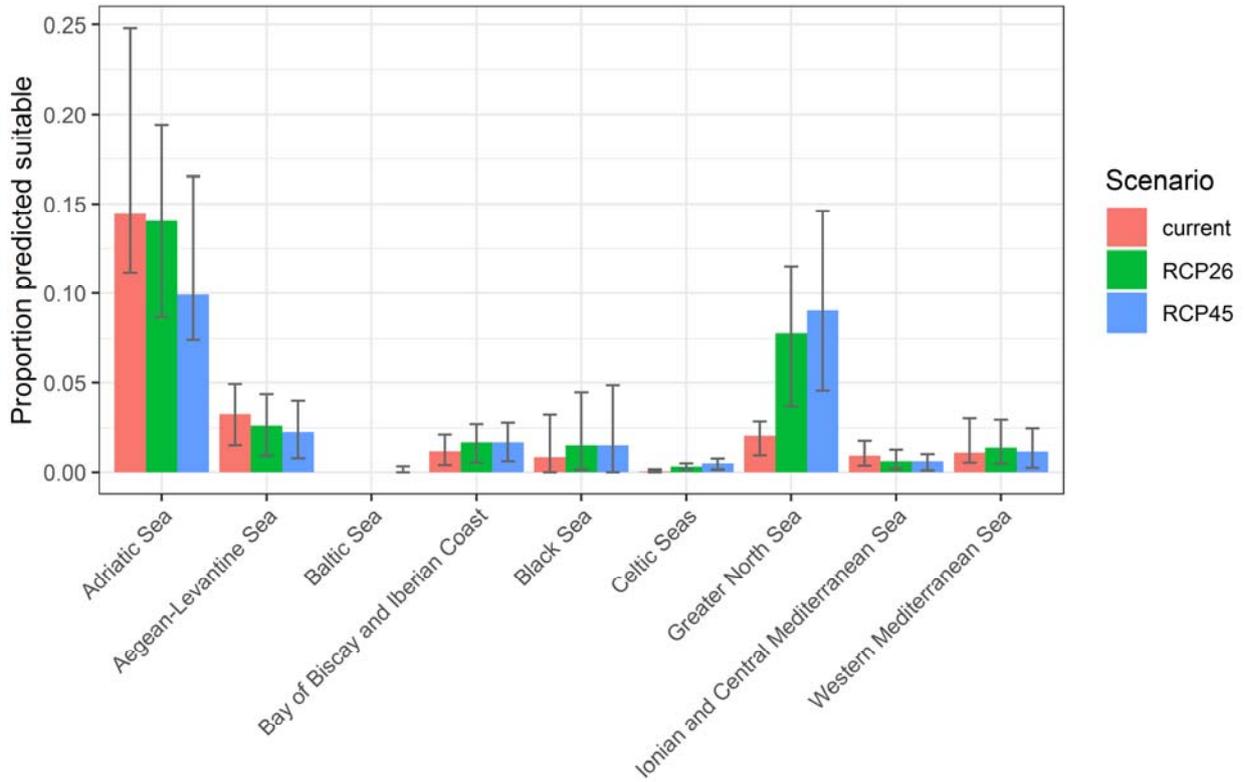


Table 2. Variation in projected suitability for *Mulinia lateralis* establishment among marine subregions of Europe (numerical values of Figure 11 above). The numbers are the proportion of grid cells in each region classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios.

	current climate			2070s RCP2.6			2070s RCP4.5		
	lower	central estimate	upper	lower	central estimate	upper	lower	central estimate	upper
Adriatic Sea	0.11	0.14	0.25	0.09	0.14	0.19	0.07	0.10	0.17
Aegean- Levantine Sea	0.01	0.03	0.05	0.01	0.03	0.04	0.01	0.02	0.04
Baltic Sea	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Bay of Biscay and Iberian Coast	0.00	0.01	0.02	0.00	0.02	0.03	0.01	0.02	0.03
Black Sea	0.00	0.01	0.03	0.00	0.01	0.04	0.00	0.01	0.05
Celtic Seas	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
Greater North Sea	0.01	0.02	0.03	0.04	0.08	0.12	0.05	0.09	0.15
Ionian and Central Mediterranean Sea	0.00	0.01	0.02	0.00	0.01	0.01	0.00	0.01	0.01
Western Mediterranean Sea	0.00	0.01	0.03	0.00	0.01	0.03	0.00	0.01	0.02

Figure 12. Variation in projected suitability for *Mulinia lateralis* establishment among the territorial coastal waters of European Union countries and the UK (from osm-boundaries.com). The bar plots show the proportion of grid cells in each country classified as suitable (with values > 0.77) in the current climate and projected climate for the 2070s under two RCP emissions scenarios. Error bars indicate uncertainty due to both the choice of classification threshold and uncertainty in the projections themselves (cf. part (b) of Figures 7, 9 and 10). The location of each region is also shown.

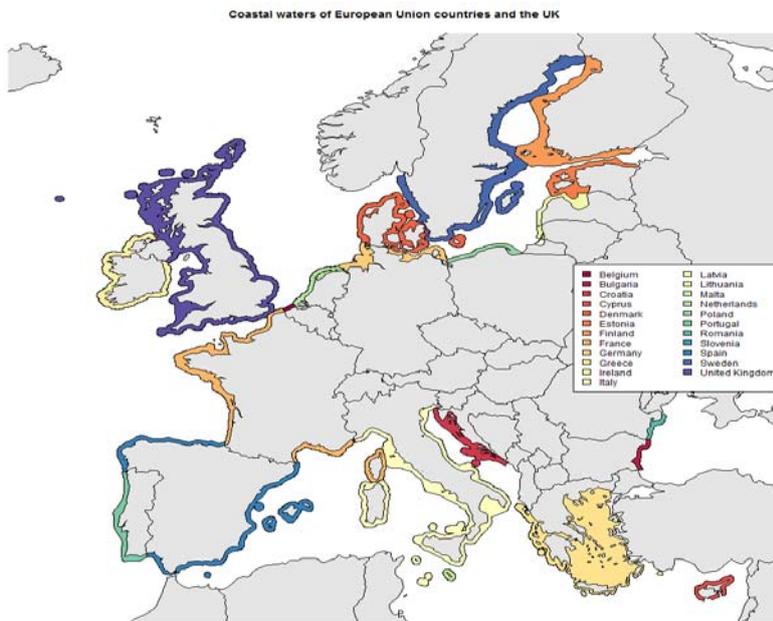
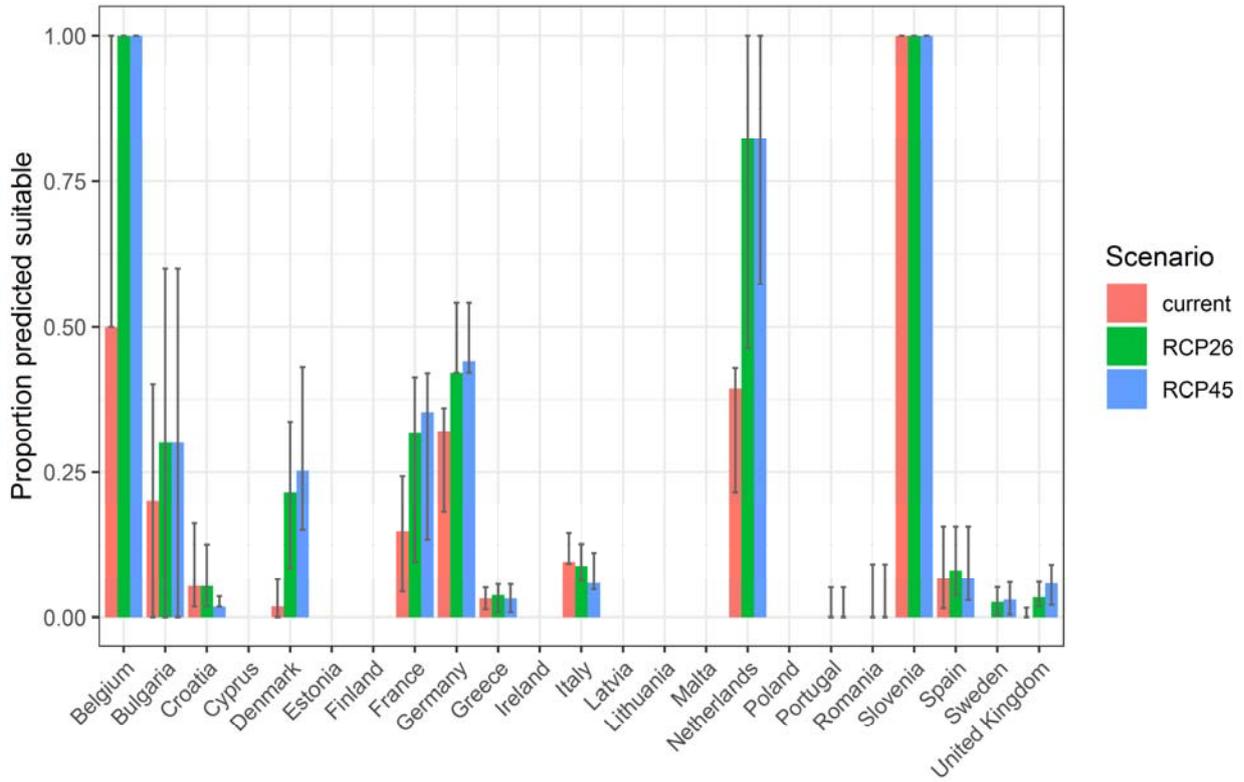


Table 3. Variation in projected suitability for *Mulinia lateralis* establishment among territorial waters of European Union countries and the UK (numerical values of Figure 12 above). The numbers are the proportion of grid cells in each country classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios.

	current climate			2070s RCP 2.6			2070s RCP 4.5		
	lower	central estimate	upper	lower	central estimate	upper	lower	central estimate	upper
Belgium	0.50	0.50	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Bulgaria	0.00	0.20	0.40	0.00	0.30	0.60	0.00	0.30	0.60
Croatia	0.02	0.05	0.16	0.02	0.05	0.12	0.02	0.02	0.04
Cyprus	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Denmark	0.00	0.02	0.07	0.08	0.21	0.34	0.15	0.25	0.43
Estonia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Finland	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
France	0.04	0.15	0.24	0.10	0.32	0.41	0.13	0.35	0.42
Germany	0.18	0.32	0.36	0.42	0.42	0.54	0.42	0.44	0.54
Greece	0.01	0.03	0.05	0.01	0.04	0.06	0.01	0.03	0.06
Ireland	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Italy	0.09	0.10	0.14	0.06	0.09	0.13	0.05	0.06	0.11
Latvia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Lithuania	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Malta	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Netherlands	0.21	0.39	0.43	0.46	0.82	1.00	0.57	0.82	1.00
Poland	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Portugal	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.05
Romania	0.00	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.09
Slovenia	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Spain	0.01	0.07	0.15	0.04	0.08	0.15	0.03	0.07	0.15
Sweden	0.00	0.00	0.00	0.00	0.03	0.05	0.00	0.03	0.06
UK	0.00	0.00	0.02	0.02	0.03	0.06	0.02	0.06	0.09

Caveats to the modelling

To remove spatial recording biases, the selection of the background sample from the accessible background was weighted by the density of *Bivalvia* records on the Global Biodiversity Information Facility (GBIF). While this is preferable to not accounting for recording bias at all, it may not provide the perfect measure of recording bias.

There was substantial variation among modelling algorithms in the partial response plots (Figure 5). In part this will reflect their different treatment of interactions among variables. Since partial plots are made with other variables held at their median, there may be values of a particular variable at which this does not provide a realistic combination of variables to predict from.

Other variables potentially affecting the distribution of the species, such as sediment composition, were not included in the model, due to the lack of suitable global layers.

M. lateralis is primarily an inshore, estuarine species and coarse grain temperature maps may not accurately reflect local conditions, e.g. water temperature in shallow, sheltered areas or in harbours and marinas. Finally, even though the model takes into account salinity variability in the form of distance from river mouths, a future scenario for river discharge was not taken into account and would take very elaborate data processing to bring into the model. We used the same predictor layer for both current and future climate conditions.

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Annex with evidence on measures and their implementation cost and cost-effectiveness

Species (scientific name)	<i>Mulinia lateralis</i> (Say, 1822)
Species (common name)	Dwarf surf clam, Coot clam, Mactre naine, Amerikaanse strandschelp
Author(s)	Marika Galanidi, Christine A. Wood
Reviewer	Francis Kerckhof, Argyro Zenetos
Date completed	15/10/2021

Summary¹

Highlight of measures that provide the most cost-effective options to prevent the introduction, achieve early detection, rapidly eradicate and manage the species, including significant gaps in information or knowledge to identify cost-effective measures.

Mulinia lateralis is a small infaunal bivalve, native to the west Atlantic, which recently entered the RA area and has a limited distribution along the southern North Sea region, from Germany to Belgium. It is an opportunistic species that can attain significant densities in lagoons and estuaries and is prone to mass mortality events. Prevention of new introductions and limiting subsequent spread are the best opportunities for management. Once established in the wild there are no effective methods to eradicate or control this species.

Prevention: The main pathways of introduction and spread of *M. lateralis* are ship-related (i.e. ballast water, dredging/aggregate extraction operations, and bilge water of recreational vessels), and bivalve transfers. The risk of further introductions and spread through ballast waters will be greatly reduced once the Ballast Water Management Convention is fully implemented (presumably by 2024), as compliance with the IMO D2 standard can decrease larval concentrations to undetectable levels. Current EU-wide regulations in place concerning the dredging and aggregate extraction sector are not adequate to prevent new introductions and the spread of *M. lateralis* and marine alien species in general. Local regulations recently adopted in Wales, UK, regarding marine licencing conditions require thorough cleaning of vessels and dredging equipment in between projects or when moving to different regions, and are expected to prove much more effective. Shellfish transfers between and within EU countries is mainly relevant as a pathway for spread within the RA area, unless national/regional regulations provide a stricter regulatory framework than the EC regulations, especially with respect to clam and mussel transfers, which may be a main pathway for the target species. Restrictions on transfers based on the risk associated with the source areas are generally practiced by the industry and provide an effective management method, as long as extensive and up-to-date data on the distribution of the high-risk non-indigenous species (NIS) are available and risks are communicated to shellfish growers. Bilge waters of recreational and other small vessels are not managed for invasive alien species (IAS) but this pathway of spread could be addressed with awareness raising campaigns to boat owners and the promotion/adoption of relatively simple measures, such as discharge of bilge water in the source region or retention

and subsequent treatment. Current levels of compliance with other, voluntary biosecurity measures by leisure craft owners however, indicate that voluntary guidelines are not sufficiently adopted to eliminate the risk of IAS spread.

Early detection: Surveillance for early detection will rely on formal monitoring campaigns, targeting marine alien species in general, or *M. lateralis* specifically, in introduction hotspots (i.e., ports, marinas, aquaculture areas, dredging locations), and on the vectors themselves, within the context of preventative measures. Existing monitoring schemes for the implementation of the Marine Strategy Framework Directive (MSFD) are likely to be a useful source of sightings, detection however may be delayed by the long processing times required to analyse large amounts of sediment samples. Environmental DNA (eDNA) methodologies offer the fastest, as well as most cost-efficient option for the early detection of both pelagic (larvae) and benthic life stages, both in water and in sediment samples. Citizen scientist observations have already contributed to our knowledge of the current spread of the species but refer to washed up specimens of already established populations.

Rapid eradication: Due to the infaunal existence of the species in open water habitats and unaided larval dispersal, eradication is not considered feasible. If population removals were to be attempted, towed fishing gear like trawls and dredges would be employed. Due to the small adult size, and the ability of the species to rapidly colonise defaunated habitats, eradication is very unlikely to be achieved. Moreover, dredging outside commercial operations and cultured bivalve beds is likely to have serious negative impacts on local species and habitats. If *M. lateralis* is detected during a shellfish transfer (e.g. to an on-growing site), the transfer must stop and bivalves should not be relayed. They may be placed in quarantine to ensure removal of the invader or be destroyed.

Management: As above, population control efforts in the wild are regarded as very unlikely to be effective. The best way to manage the species, once established, is to prevent long dispersal jumps by managing the pathways of secondary introduction and spread. Where *M. lateralis* infected with the parasite *Perkinsus* is detected among cultivated or commercially harvested bivalve beds, the recommended action would be for close monitoring of all clam species for infection and early harvesting of clams, alongside the invader.

Detailed assessment			
	Description of measures ²	Assessment of implementation cost and cost-effectiveness (per measure) ³	Level of confidence ⁴
Methods to achieve prevention of intentional and unintentional introduction ⁵	<p>Managing the pathway “TRANSPORT-STOWAWAY (ship/boat ballast water and sediment)”</p> <p>Regulation (EU) No 1143/2014 (EU, 2014) on the prevention and management of the introduction and spread of invasive alien species recognises the IMO Ballast Water Management Convention (BWMC) (IMO, 2004) as one of the possible management measures for invasive species of concern. The BWMC entered into force in September 2017 and requires ships in international traffic to apply ballast water management measures, such as ballast water exchange (D-1 standard for an interim period) and fulfil certain discharge standards (D-2 standard according to the ship specific application schedule). D-2 states that ships shall discharge less than 10 viable organisms per cubic metre greater than or equal to 50 micrometres in minimum dimension. This can be achieved through mechanical (filtration,</p>	<p>Ballast Water Exchange (BWE) can reduce the concentration of live organisms in ballast by 80–95% (Ruiz & Reid, 2007). Its application, however, has severe limitations, primarily dependant on geography (David et al., 2007), weather conditions, and other safety restrictions (ship instability). In addition, BWE effectiveness is influenced by tank structure and the skill and experience of the ship crew, while it cannot be used to manage ballast water of vessels on shorter coastal (non-transoceanic) voyages (Bailey, 2015). Costs related to Ballast Water Management (BWM) and the implementation of the IMO’s D2 Standard are not specific to <i>M. lateralis</i> but refer to all marine NIS. The cost of installing Ballast Water Management Systems will be borne by shipping companies. Installation costs can vary from €140K-€1.675m per ship (figure reported in DEFRA (2012), after consultation with the UK Marine Coastguard Agency). Efficiencies of various technologies utilised for ballast water treatment are reviewed in Tsolaki & Diamadopoulos (2009) and can vary with treatment method, but the application of many combined methods (e.g., Filtration+UV or Hydroclone+chemical disinfectant) can decrease live zooplankton to undetectable levels, practically diminishing propagule pressure.</p>	High

	<p>separation), physical (heat treatment, ozone, UV light) and chemical methods (biocides).</p> <p>Full implementation of the BWMC is expected to take some years (as late as 2024 – see http://maritime-executive.com/article/imo-pushes-back-ballast-water-compliance-dates).</p> <p>Eventually, most ships will need to install an on-board ballast water treatment system, and all ships will be required to carry a ballast water record book and an international ballast water management certificate.</p> <p>Under the D-2 standard, all ships will also be required to regularly remove and dispose of sediments from spaces designed to carry ballast water at designated sediment reception facilities in ports.</p>	<p>Significant costs can be associated with the ratification of the BWMC in ensuring its compliance, related to, for example, planning, monitoring, enforcement and capacity-building. As an example, in Croatia, approximately €1.26 million will be incurred by the state for institutional capacity building and in fulfilment of its flag State and port State obligations (Interwies & Khuchua, 2017). This is broken down into €470k from flag state obligations (with 150 registered ships in 2015) and €482k from port state obligations (with 6 major ports). The remaining €308k are preparatory phase costs. Compliance costs will need to be incurred anyway to enable trade with other countries. Additionally, Regulations A4 and A3 of the BMWC enable the granting of exemptions from the requirement for BWM (provisions made in the BWMC for regular routes and other cases – Olenin et al., 2016). Such requests must fulfil certain criteria and require a risk assessment according to BWMC guidelines (Stuer-Lauridsen et al., 2018).</p>	
	<p>Managing the pathway “TRANSPORT-STOWAWAY (other means of transport: marine aggregates & dredging)</p> <p>The disposal of dredged materials is regulated throughout the EU (summarised by Mink et al., 2006). OSPAR guidelines specify best environmental practice for managing dredged material (OSPAR, 2014). The main management tools are marine</p>	<p>Licensing procedures in member states tend to focus mainly on the sediment characteristics and the prevention of the disposal of waste containing contaminants such as heavy metals and pathogens, not non-native species. Thus, they would be ineffective in preventing the spread of <i>M. lateralis</i> in the RA area. Moreover, no EC regulations on the cleaning of dredging vessels and equipment <u>between</u> projects were found. Additional local or national regulations may apply that introduce further biosecurity measures for the effective</p>	<p>Medium</p>

	<p>licence and control systems. These require assessments of the environmental impact of planned disposal activities in relation to a specific dumpsite, sediment characteristics and contamination load. Dredging activities are also regulated under the Marine Strategy Framework Directive (MSFD) 2008/56/EC, the Water Framework Directive (WFD) 2000/60/EC and the EC Habitats Directive (92/43/EC). The ballast water and sediments carried in ballast tanks of dredger vessels is regulated under the Ballast Water Management Convention (see above).</p>	<p>prevention of NIS spread via dredging operations. For example, in Wales, UK, marine licencing conditions recently imposed include the washing of seabed equipment and filter screens at the end of a campaign, the requirement for the circulation and exchange of hopper water (i.e. water used to fill the storage compartment) away from shore, and the removal of hopper sediment prior to entering a different region of the UK (ABPmer, 2019; G. Wynne, B. Wray & S. Vye (Natural Resources Wales) pers. comm. 21 April 2021). Such measures can prove effective, particularly to reduce spread between the existing North Sea populations and other RA subregions (e.g. Baltic Sea, Celtic Seas) but are expected to increase the cost of operations.</p> <p>No quantitative information on relevant costs was found but any management costs would not be specific to <i>M. lateralis</i> and would cover other organisms potentially transferred through this pathway.</p>	
<p>Reducing spread</p>	<p>Managing the pathway “CONTAMINANT ON ANIMALS - Mariculture”</p> <p>1. COUNCIL REGULATION (EC) No 708/2007 concerning use of alien and locally absent species in aquaculture defines the procedures to be followed. According to the regulation, all aquaculture operators who intend to introduce an alien species or translocate a locally absent species must first apply</p>	<p>The measures are cost-effective as the regulations and industry practices are already in effect. However, the bivalves <i>M. gigas</i> and <i>R. philippinarum</i>, listed in Annex IV of Council Regulation 708/2007, constitute an exception and can be moved without any risk assessment or quarantine; also, the regulation does not apply to movements of locally absent species within the Member States “except for cases where, on the basis of scientific advice, there are grounds for foreseeing environmental threats due to the translocation, Art. 2 para. 2.”</p>	<p>High</p> <p>These are well established and widely approved practices, some of them already translated into legislation with demonstrated effectiveness. Some</p>

	<p>for a permit from the competent authority of the Member State where the transfer will take place. The Regulation specifies the information to be provided by the applicant and the type of assessment that the competent authority must perform before granting the permit.</p> <p>2. The ICES Code of Practice on the Introductions and Transfers of Marine Organisms (ICES, 2005) recommends the procedure for introduced or transferred species which are part of current commercial practice. The procedure states clearly that:</p> <p>a) All products should originate from sources in areas that meet current codes, such as the OIE International Aquatic Animal Health Code or equivalent EU directives.</p> <p>b) If required, there should be inspection, disinfection, quarantine or destruction of the introduced organisms and transfer material (e.g., transport water, packing material, and containers) based on OIE or EU directives.</p> <p>3. Use locally harvested or hatchery produced seed for non-native cultivated bivalves such as <i>Magallana gigas</i> and</p>	<p>Additional local, national or European regulations may apply that limit the translocation possibilities of species like <i>M. gigas</i> and <i>R. philippinarum</i> in certain areas (e.g., for the Wadden Sea see Gittenberger et al., 2017; WG-AS & Gittenberger, 2018). More stringent controls for these species could take the form of the prohibition of imports from areas where <i>M. lateralis</i> is known to be present. Alternatively, such imports could be subjected to quarantine measures. In many cases voluntary or mandatory measures to protect against non-native species are already implemented (see below), such that stakeholder acceptance and compliance are expected to be high. This would be the case in particular, where a pathogen posing a risk to the commercial operation might be suspected or identified (e.g. <i>Perkinsus</i> parasites).</p> <p>Regarding the relaying of mussels and clams within Member States for on-growing or purification, restrictions based on risks associated with source areas are in place in certain areas (e.g. in Wales and The Netherlands) and are an effective management method, as long as extensive and up-to-date data on the distribution of the high-risk non-native species are available.</p> <p>As a matter of standard industry practices, there are sampling and monitoring protocols for the inspection of shellfish areas between which translocations take place (e.g. Wilson & Smith., 2008; Gittenberger, 2014; MEC, 2015). For example, if high risk non-native species (based on a pre-established species list) are detected</p>	<p>uncertainty remains however in relation to the detectability of <i>M. lateralis</i> individuals during routine inspections by industry standards and the degree of compliance with legislation and codes of conduct at the EU scale.</p>
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	<p><i>Ruditapes philippinarum</i>. Both species are naturalised in parts of the RA area.</p>	<p>during transportation, the import stops and bivalves are not relayed. They may be returned to the harvesting area or placed in quarantine. If IAS are detected on a relaying site, the plots must be cleaned and the mussels placed in quarantine.</p> <p>Any cleaning or removal procedure during transportation on board a dredger vessel (e.g. washing over a certain sieve size to remove larvae and juveniles, hand-picking of adults) would not guarantee the complete removal/eradication of risk organisms.</p>	
<p>Reducing spread</p>	<p>Managing the pathway “Bilge water” Bilge water is generally defined as any seawater that accumulates within the hull of a vessel, including uncontained water on the deck area. Bilge water volumes in recreational vessels can range between <1l for small motorboats to >200l for a large catamaran (Fletcher et al., 2017) and are typically pumped out of the vessel untreated. Discharge of bilge water for large commercial vessels is regulated under Marpol Annex I (MEPC.1/Circ.642), which requires an integrated bilge water treatment system (IMO, 2008).</p> <p>There are currently no legal acts or official guidance for the prevention of NIS transportation with bilge water by small vessels.</p>	<p>Requiring discharge within the source region seems the most practical option available, with no associated costs and minimal logistical constraints. Desirable vessel operator behaviours can be promoted through awareness campaigns to educate boaters about possible risks from bilge water, e.g. the “Check Clean Dry” campaign already implemented in the UK in relation to aquatic hitchhikers.</p> <p>http://www.nonnativespecies.org//checkcleandry/index.cfm?</p>	<p>Low</p> <p>These methods are not currently practiced in the marine environment and are based on expert opinion.</p>

	<p>Potential mitigation measures (Fletcher et al., 2017):</p> <ul style="list-style-type: none"> • Restrictions on the location of discharge. • Retention of bilge water for subsequent disposal. • Treatment of bilge water prior to discharge. • Awareness campaigns to boaters. • Industry codes of practice. 		
<p>Methods to achieve early detection⁶</p>	<p>Monitoring and early detection In order to reach Good Environmental Status targets with reference to Descriptor D2 for alien species (i.e. “Non-indigenous species introduced by human activities are at levels that do not adversely alter the ecosystems”), most EU states are already designing or implementing national/regional NIS-targeted monitoring programs (see ICES, (2017) for national reports in France, Germany, Sweden, Denmark, Norway; see Minchin (2014) for Ireland). Monitoring should focus on introduction hotspots (e.g., ports, marinas, aquaculture plots, aggregate extraction sites) and specific natural hotspots on a yearly basis (ICES, 2017). Molecular tools and DNA barcoding of zooplankton samples may represent a further early</p>	<p>One of the potential caveats of monitoring based on morphological identification, especially if it’s not specifically targeting IAS, is the long processing times needed to analyse large amounts of samples (Klunder et al., 2019). On the other hand, Rapid Assessment Surveys carried out by experienced specialists with the appropriate methods and targeted species lists, can prove effective at early NIS detection even in subtidal soft sediments (Buschbaum et al., 2012). Nevertheless, the efficacy of early detection methods in detecting <i>M. lateralis</i> (including early life stages) at monitoring hotspots for new introduction events, will be the highest if eDNA methodologies are implemented for ballast and sea water samples and sediments, as well as potentially contaminated aggregates. This would be particularly effective in detecting new introductions via dredger vessels with EU home ports returning from the native area, as the potential donor regions where such operations take place are very specific (i.e. eastern coast of Mexico and Bay of St. Lawrence, Canada).</p>	<p>Medium</p> <p>Monitoring of dredging activities and equipment specifically for the purposed of NIS detection has not been implemented. The logistics, effectiveness and uptake of proposed methods are unknown.</p>

	<p>detection approach that could be extended to ballast water, hopper water and bilge water monitoring (Zaiko et al., 2015; Fletcher et al., 2017). Furthermore, eDNA methodologies for the detection of macro-organisms in sediments are continuously being developed and refined (Xie et al., 2018; Sakata et al., 2020) and could be applied to ballast tank sediments, and port sediments, as well as dredged material.</p>	<p>Additionally, the complete mitochondrial genome of <i>M. lateralis</i> has been sequenced and is available for the development of specific assays (Liu et al., 2020). With regards to the cost of monitoring, an indicative estimate comes from Denmark, where the cost of a proposed hotspot monitoring program for all marine NIS (covering 13 ports and three areas with discharges of cooling water) was estimated at approximately €125k for the period 2015-2017 (Andersen et al., 2014). In the UK, a broad initial estimate of monitoring costs for MSFD D2 alone (considering that existing or new surveys for other descriptors will also contribute to the monitoring of NIS) suggests that they would be less than €961k over 10 years (DEFRA, 2012), with an additional €100k for drafting legislation and guidance. In New Zealand, surveillance of 11 ports for high risk NIS every six months cost approximately NZ\$ 2 million annually (Arthur et al., 2015). The costs of eDNA methodologies can vary considerably depending on the approach employed (e.g. Borrell et al., 2017) but generally nucleic acids-based tools are more cost-efficient compared to traditional sampling and morphological identification approaches (Darling & Frederick, 2018), provided that the necessary infrastructure is already available and the method is established and sensitive. These are general monitoring costs and not specific to <i>M. lateralis</i>.</p>	
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	<p>Early warning systems / awareness raising</p> <p>Citizen scientist observation networks are a suitable platform for this species, which can be found intertidally, is frequently washed up on the shore, and attracts the attention of shell collectors. Early warning systems to inform shellfish growers should be designed due to the potential danger <i>M. lateralis</i> poses for the spread of <i>Perkinsus</i> parasites.</p>	<p>Its small size and similarity with common native species mean that <i>M. lateralis</i> can get easily misidentified by non-experts at the juvenile stage.</p> <p>On the other hand, many of the recent Belgian records refer to adult individuals from citizen scientist observations through a well organised reporting and validation platform (Waarnemingen, 2021). However, if individuals of the species have been washed up on the shore and are grown enough to be identifiable by the public, this likely means that the population is already established.</p>	<p>Medium</p>
<p>Methods to achieve eradication⁷</p>	<p>Mechanical removal</p> <p>This could be attempted with different types of towed bottom gears, such as trawls and dredges. Different types of trawls have been used to sample <i>M. lateralis</i> in scientific surveys (e.g. Reguero & Raz-Guzman, 2018) and dredges used for commercial clam species could also be employed.</p>	<p><i>Mulinia lateralis</i> is not commercially harvested, hence information on the effectiveness of different fishing gears in removing the species was not found. <i>Mulinia</i> however is generally small (up to 21 mm in the invaded range - Craeymeersch et al., 2019) and a considerable proportion of the population would be expected to pass through typical commercial gear. Given the very small size at which it can reproduce (3 mm – Calabrese, 1969) and the ability of the species to rapidly colonise defaunated habitats (Flint & Younk, 1983), eradication is very unlikely to be achieved. On the contrary, by removing competition, dredging may allow the remaining <i>M. lateralis</i> individuals to opportunistically and rapidly re-colonise the dredged area, possibly reaching even higher densities.</p> <p>Moreover, dredging outside commercial operations and cultured bivalve beds is likely to have serious negative impacts on local species and habitats. Finally, the use of this measure will likely require special permits or</p>	<p>Medium</p>

		changes in fisheries regulations (local spatial/temporal restrictions) and is unlikely to be allowed in protected areas due to potential damage to protected features.	
	<p>Physical/chemical methods Other methods that are commonly considered for the eradication and/or population control of marine organisms involve chemical treatment with a variety of substances (from salt, to chlorine, to specialised biocides) and physical methods such as the application of heated water, sometimes in combination with physical containment (i.e. plastic sheets) (see McEnnulty et al., 2001).</p>	None of these methods are applicable to <i>Mulinia lateralis</i> because of its infaunal existence in open water habitats (as opposed to the majority of bivalve species for which such control methods have been applied which are either freshwater and/or biofouling species). The broadcast application of chemicals has detrimental effects on native species while containment with physical barriers is only realistic for visible organisms with limited/clustered distribution.	Low
Methods to achieve management⁸	<p>Population removal Dredging would be the best option if population removals were attempted. In the case where <i>M. lateralis</i> infected with the parasite <i>Perkinsus</i> were detected among cultivated or harvested bivalve beds the recommended action would be close monitoring of all clam species for infection and the early harvesting of clams, alongside the invader (Andrews & Ray, 1988).</p>	As in the previous section, population control efforts in the wild are regarded as ineffective.	Low

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Guidelines for Completing the Annex

1 Provide a brief summary description of the most cost-effective methods drawing on the reviews in the detailed assessments

2 Provide a description of the potential method. This should be based on the available key scientific evidence which should be gathered from sources including articles and reviews in technical and scientific journals, internet searches, online databases, grey literature and relevant books and personal communications from scientists, stakeholders, conservation practitioners and governmental bodies. This information should include a full bibliographic list detailing the literature and sources considered.

3 Provide an assessment of the likely cost and effectiveness of the method. Where information is available, consider the following range of questions, accepting that not all questions will be appropriate in all circumstances.

- How effective has this approach proven to be in the past or in an analogous situation?
- How publically acceptable is the approach likely to be?
- Over what period of time would this approach need to be applied to be effective?
- What is the direct cost of implementing this approach?
- How likely are the methods used in the approach to be available?
- How likely is it that relevant licences or other approvals to undertake the approach would be difficult to obtain?
- How likely is it that health and safety issues would prevent the use of this approach?
- How significant is the environmental harm caused by this approach?
- How significant is the economic harm caused by this approach?
- How significant is the social harm caused by this approach?
- How likely is it that the approach will be criticised on welfare grounds?
- How likely is it that the approach will be acceptable to other stakeholders?

Where available, factual information on the costs of specialist equipment, or case studies of management costs from across the Union or third countries should be provided. When describing case studies, if the information is available then provide both total cost and the area over which control was undertaken so that a cost per unit area might be derived. Where such quantitative information is not available, then any qualitative information from the literature is acceptable to help guide decision making. It is accepted that in the majority of cases the information required to assess the potential total cost of management at a member state level is unlikely to be available. This would normally require information on the extent and abundance of the species which is beyond the scope of this assessment. Assessors are not expected to extrapolate the potential total costs of management at a member state level, only to report on the information provided within the literature.

4 Provide an overall assessment of the confidence that can be applied to the information provided for this method. This confidence should relate to the quality of the available information using the guidance below. It should NOT relate to the confidence in the effectiveness of the method

The confidence scores are:

- **High:** Information comes from published material, or current practices based on expert experience applied in one of the EU countries or third country with similar environmental, economic and social conditions.
- **Medium:** Information comes from published data or expert opinion, but it is not commonly applied, or it is applied in regions that may be too different from Europe (e.g. tropical regions) to guarantee that the results will be transposable.
- **Low:** data are not published in reliable information sources and methods are not commonly practiced or are based solely on opinion; This is for example the case of a novel situation where there is little evidence on which to base an assessment.

If there are further factors beyond these that have determined the chosen level of confidence, then provide a brief written description to support the choice of the level of confidence.

5 Measures for preventing the species being introduced intentionally or unintentionally into the territory of a Member State (cf. Articles 7(1)(a), 7(2), 13, 15), i.e. preventing a species entering by blocking its pathways, including pre-border prevention and prevention of escape or release into the environment and secondary spread whenever relevant. Measures for preventing the species reproducing while in containment should be included, if appropriate. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

6 Measures to run an effective surveillance system for achieving an early detection of a new occurrence (cf. Article 16). This section assumes that the species is not currently present in a Member State, or part of a Member State's territory.

7 Measures to achieve eradication. Preferably, but not exclusively, used at an early stage of invasion, after an early detection of a new occurrence (cf. Article 17). Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

8 Measures to achieve management of the species once it has become widely spread within a Member State territory (cf. Article 19). These measures can be aimed at eradication, population control or containment of a population of the species. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

The development and completion of this template forms part of the EU project **No 07.0202/2020/834529/ETU/ENV.D.2** Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention.

**Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention"
Contract No 07.0202/2020/834529/ETU/ENV.D.2¹**

Name of organism: *Obama nungara* Carbayo, Álvarez-Presas, Jones & Riutort, 2016

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Risk Assessment Area: The risk assessment area is the territory of the European Union 27 and the United Kingdom, excluding the EU-outermost regions.

Peer review 1: Dr Richard Shaw, CABI, Egham, UK

Peer review 2: Dr Brian Boag, James Hutton Institute, Invergowrie Dundee, UK

Date of completion: 15/10/2021

¹ This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA). A number of amendments have been introduced to ensure compliance with Regulation (EU) 1143/2014 on IAS and relevant legislation, including the Delegated Regulation (EU) 2018/968 of 30 April 2018, supplementing Regulation (EU) No 1143/2014 of the European Parliament and of the Council with regard to risk assessments in relation to invasive alien species (see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>).

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SECTION A – Organism Information and Screening

A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?

including the following elements:

- the taxonomic family, order and class to which the species belongs;
- the scientific name and author of the species, as well as a list of the most common synonym names;
- names used in commerce (if any)
- a list of the most common subspecies, lower taxa, varieties, breeds or hybrids

As a general rule, one risk assessment should be developed for a single species. However, there may be cases where it may be justified to develop one risk assessment covering more than one species (e.g. species belonging to the same genus with comparable or identical features and impact). It shall be clearly stated if the risk assessment covers more than one species, or if it excludes or only includes certain subspecies, lower taxa, hybrids, varieties or breeds (and if so, which subspecies, lower taxa, hybrids, varieties or breeds). Any such choice must be properly justified.

Response: *Obama nungara* Carbayo, Álvarez-Presas, Jones & Riutort, 2016

Phylum Platyhelminthes, Class Rhabditophora (previously Turbellaria), Order Tricladida, Family Geoplanidae, Subfamily Geoplaninae

Synonyms: *Obama marmorata pro parte*

This species was originally mistaken as *Obama marmorata* when first found in Europe (Lago-Barcia *et al.*, 2015) but subsequently identified as a new species *Obama nungara* (Carbayo *et al.*, 2016).

The organism is a single taxonomic entity. There are no known varieties, breeds or hybrids. Through molecular analyses of the COI gene, Justine *et al.* (2020) found *O. nungara* to be composed of three clades. They termed these: ‘Brazil’ - currently confined to Brazil, ‘Argentina 2’ - found in Argentina and Spain; ‘Argentina 1’ - found in Argentina and in Spain, Portugal, France, UK, Italy, Belgium, and Switzerland. As to date there is no information on morphological or physiological differences between the clades, this risk assessment will apply to all

Common names: The ‘Obama flatworm’. Note there is no connection to the former US President Barack Obama. The genus name *Obama* comes from the Tupi language and means leaf-animal. In French, ‘Marron plate’.

A2. Provide information on the existence of other species that look very similar [that may be detected in the risk assessment area, either in the environment, in confinement or associated with a pathway of introduction]

Include both native and non-native species that could be confused with the species being assessed, including the following elements:

- other alien species with similar invasive characteristics, to be avoided as substitute species (in this case preparing a risk assessment for more than one species together may be considered);

- other alien species without similar invasive characteristics, potential substitute species;
- native species, potential misidentification and mis-targeting

Response: *Obama nungara* was formally described in 2016 as a new species (Carbayo *et al.*, 2016). The flatworm measures typically 50 – 80 mm in length and 5 mm in width, with brown dorsal colouration, with multiple longitudinal black striae giving a ‘netted’, reticulated appearance, and a beige sole (Justine *et al.*, 2020; Soors *et al.*, 2019). The dorsal colour is variable ranging from orange to almost black. Multiple eyes extend in a lateral band on each side of the body, one third of the flatworm’s body width (Carbayo *et al.*, 2016). Justine *et al.* (2020) gives detailed descriptions suitable for the non-specialist along with photographs of the different colour forms. Egg cocoons are shiny spheroidal capsules which are red when newly laid, turning dark brown to black within a few days (Justine *et al.*, 2020). They measure c. 5 mm in diameter. Juveniles are light cream in colour with fine dark brown stipling (Justine *et al.*, 2020).

Obama nungara may be confused with other terrestrial flatworms. Superficially, this may include small native species and invasive alien species such as *Arthurdendyus triangulatus* and *Bipalium kewense*. Native flatworm species are mostly small (< 50 mm length), innocuous animals whereas *O. nungara* can be distinguished from alien flatworms by dorsal colouration (e.g., the pale marginal edge of *A. triangulatus*) and body/head shape (e.g., the shovelhead shape of *Bipalium* spp.).

Justine *et al.* (2020), on the experience of citizen science records, says that *O. nungara* may be confused with: 1) *Parakontikia ventrolineata*, an alien species native to Australia, but this species is smaller and has longitudinal lines on its back and belly; 2) *Platydemus manokwari* but the body shape is different and *P. manokwari* has a clear, pale single longitudinal line on its back and two conspicuous eyes.

Obama nungara may be confused with other *Obama* spp., in particular *Obama marmorata* but this species has not been found within the risk assessment area. *Obama marmorata* is up to 100 mm long, 10 mm wide, with the dorsum covered with green brown longitudinal striae on an orange to ivory background (Carbayo *et al.*, 2016).

A3. Does a relevant earlier risk assessment exist? Give details of any previous risk assessment, including the final scores and its validity in relation to the risk assessment area.

Response: There is no quantitative or qualitative risk assessment for *O. nungara*.

A4. Where is the organism native?

including the following elements:

- an indication of the continent or part of a continent, climatic zone and habitat where the species is naturally occurring
- if applicable, indicate whether the species could naturally spread into the risk assessment area

Response: *Obama nungara* is native to South America, and particularly to Argentina, south-eastern Brazil and Uruguay (Carbayo *et al.*, 2016; Lago-Barcia *et al.*, 2015; Negrete *et al.*, 2020). The specimens found in Europe show most affinity to the Argentinian clade (Justine *et al.*, 2020).

As with other invasive flatworms, *O. nungara* is synanthropic, found in gardens and other modified habitats in its native range (Lago-Barcia *et al.*, 2019; Negrete *et al.*, 2020; Carbayo *et al.*, 2016). In a study on the coexistence of several terrestrial flatworm species in South American Atlantic Forest, *O. nungara* was found in human-disturbed areas (Boll and Leal-Zanchet, 2016). Similarly *O. nungara* has also been collected from human-disturbed native forest habitats (Lago-Barcia *et al.*, 2019). However, Negrete *et al.* (2020) collected many specimens in undisturbed native forests from Tucumán Province, north-western Argentina. It is possible that this is close to the natural habitat of *O. nungara*. However, L. Negrete (2021, pers. comm.) commented that this area is very close to routes with intense trade between provinces, which could suggest *O. nungara* may have been introduced there and has found ideal conditions for establishment. In that same area (Tucumán) large number *Bipalium kewense* (a definite introduction) were found, which would support this conjecture. It is not therefore clear exactly what and where is the native habitat of *O. nungara*.

Obama nungara is a predatory, soil-dwelling organism that naturally disperses by creeping on the substrate surface. In common with other terrestrial flatworms, especially these of a synanthropic nature, it is usually found under wood, rocks and debris on the soil surface. It could not naturally spread into the risk assessment area.

A5. What is the global non-native distribution of the organism outside the risk assessment area?

Response: *Obama nungara* is native to South America and has been recorded from Argentina, Brazil, Uruguay and Chile, with the latter likely to be an introduction. Outside of South America, *O. nungara* has only been recorded in Europe and two records from the USA in 2020, in North Carolina (www.inaturalist.org/observations/68472771) and South Carolina (L. Winsor 2021, unpubl.). Most records in Europe are from the risk assessment area but *O. nungara* has established in the wild also in Guernsey (Álvarez-Presas *et al.*, 2014; Carbayo *et al.*, 2016; Webster, 2020), Madeira Island and São Miguel in the Azores (Lago-Barcia *et al.*, 2020; Justine *et al.*, 2020) and Switzerland (Justine *et al.*, 2020).

A6. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species been recorded and where is it established? The information needs be given separately for recorded (including casual or transient occurrences) and established occurrences. “Established” means the process of an alien species successfully producing viable offspring with the likelihood of continued survival².

² Convention on Biological Diversity, Decision VI/23

A6a. Recorded: List regions

A6b. Established: List regions

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

- Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea.

Comment on the sources of information on which the response is based and discuss any uncertainty in the response.

For delimitation of EU biogeographical regions please refer to <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2> (see also Annex VI).

For delimitation of EU marine regions and subregions consider the Marine Strategy Framework Directive areas; please refer to <https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions/technical-document/pdf> (see also Annex VI).

Response (6a): *Obama nungara* has been recorded in the Atlantic, Continental and Mediterranean biogeographic regions (see A.8 for references).

Response (6b): *Obama nungara* has also established in the Atlantic, Continental and Mediterranean biogeographic regions (see A.8 for references).

A7. In which biogeographic region(s) or marine subregion(s) in the risk assessment area could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A7a. Current climate: List regions

A7b. Future climate: List regions

With regard to EU biogeographic and marine (sub)regions, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (7a): *Obama nungara* is predicted to have a potentially wide distribution in Europe. Under the current climate it could establish in the Atlantic, Mediterranean and Black Sea biogeographical regions and to a lesser extent in the Continental region, with small incursions into Pannonian and Alpine regions (see Annex VIII ‘Projection of environmental suitability for *Obama nungara* establishment in Europe’).

Response (7b): Under climate change scenarios RCP 2.6 (likely range of 0.4-1.6°C global warming increase) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase), *O. nungara* could establish in greater areas of the Atlantic and Continental regions as its range increases northwards and eastwards. The Pannonian region is also markedly more vulnerable to flatworm establishment. However, the Black Sea region only shows a slight increase in the area suitable for *O. nungara* under these climate change scenarios, whilst the flatworm’s range in the Mediterranean declines in the southern localities (see Annex VIII ‘Projection of environmental suitability for *Obama nungara* establishment in Europe’)

A8. In which EU Member States has the species been recorded and in which EU Member States has it established? List them with an indication of the timeline of observations. The information needs be given separately for recorded and established occurrences.

A8a. Recorded: List Member States

A8b. Established: List Member States

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden

The description of the invasion history of the species shall include information on countries invaded and an indication of the timeline of the first observations, establishment and spread.

Response (8a): *Obama nungara* has been recorded in the wild or as interceptions in the following Member States:

Austria (J-L Justine 2020, unpubl.), Belgium (Soors *et al.*, 2019), France (Justine *et al.*, 2020), Germany (J-L Justine 2020, unpubl.; Kutschera and Ehnes, 2021), Ireland (Justine *et al.*, 2020), Italy (Carbayo *et al.*, 2016), Netherlands (Justine *et al.*, 2020; Aldred, 2016), Portugal (Justine *et al.*, 2020), Slovakia (L. Winsor 2021, unpubl.) and Spain (Carbayo *et al.*, 2016).

Response (8b): *Obama nungara* has established in France, Belgium, Spain, Portugal and Italy (Justine and Winsor, 2020), and almost certainly in other countries adjoining these but this has not been confirmed to date.

Before being described as a separate species, *O. nungara* was first recorded as an unknown species in Guernsey, the Channel Islands, in 2008 and England, in 2009 (Carbayo *et al.*, 2016). It was subsequently found in Spain in 2010 (Álvarez-Presas *et al.*, 2014), where it was initially misidentified as *Obama marmorata* (Lago-Barcia *et al.*, 2015). Carbayo *et al.* (2016) described the flatworm specimens as a new species, *O. nungara*, listing many additional records from Spain, principally from two regions, Asturias and Cantabria on Spain's Atlantic coast, and from the Catalonia region on Spain's north-east Mediterranean coast, as well as detailing a record from Italy (2012). Lago-Barcia *et al.* (2019) extended the known distribution on the Iberian Peninsula, collecting specimens from the Porto region, Portugal. In 2016, there was a record of *O. nungara* being intercepted in the UK on plants that came from the Netherlands (Aldred, 2016).

The most comprehensive study of *O. nungara* distribution is from France (Justine *et al.*, 2020), where 530 records were collected through citizen science from 2013-2018, and the flatworm has established in 72 of the 96 administrative Departments in Metropolitan France, as well as Corsica. From these 530 citizen science records, *O. nungara* was the most recorded flatworm species in France. *Obama nungara* was found established in Belgium in 2017 (Soors *et al.*, 2019).

A9. In which EU Member States could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A9a. Current climate: List Member States

A9b. Future climate: List Member States

With regard to EU Member States, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (9a): There have been no experimental assessments on the environmental requirements of *O. nungara*. The most comprehensive study of *O. nungara* distribution in Europe is from Metropolitan France (Justine *et al.*, 2020), where the species has achieved a widespread distribution especially along the Atlantic and Mediterranean coasts. There were fewer records in the central plains and mountainous regions of France and none from above 500 m altitude, which the authors attribute to the likelihood of freezing. However, *O. nungara* was found above 500 m at a location on São Miguel in the Azores (Lago-Barcia *et al.*, 2020). Given the wide distribution in France, Soors *et al.* (2019) commented that *O. nungara* has a broad temperature tolerance within a temperate climate.

Using species occurrence data, Negrete *et al.* (2020) modelled and mapped the potential distribution of *O. nungara* using MaxEnt software, under both current and climate change scenarios. They found that the most important factors determining the distribution of *O. nungara* (accounting for 60% of the variance) were annual mean temperature (moderate probability of establishment 9 - 17°C), temperature range, temperature seasonality and precipitation of the coldest quarter (moderate probability of establishment 140 - 330 mm). In Europe, *O. nungara* was most suited to the climate type Cfb (temperate, oceanic) of the Köppen-Geiger climate classification; whereas in South America Cfa (humid, subtropical) fitted best with the flatworm's distribution. The model predicted that under the current climate, *O. nungara* has the potential to expand its range in Europe, to establish in Luxembourg, the Netherlands, Germany, and Denmark, as well as regions of Croatia and Greece on the east coast of the Adriatic.

The model developed as part of this risk assessment is presented in detail in Annex VIII. The model is an ensemble of six algorithms. The factors that most strongly predicted *O. nungara* distribution were minimum temperature of the coldest month (38% of variation), human influence index (24%), mean temperature of the warmest quarter (18%), annual precipitation (11%), precipitation seasonality (5%)

and climatic moisture index (4%). The flatworm is predicted capable of establishing in large areas (>50% of the region) in Belgium, France, Italy, Luxembourg, the Netherlands and Portugal. Significant areas (>20%) of Croatia, Germany, Greece and Spain, as well as the United Kingdom, are also at risk of this species establishing (Annex VIII).

Response (9b): Negrete *et al.* (2020) modelled the distribution of *O. nungara* under RCP 2.6 and RCP 8.5 climate change scenarios. Under both RCP 2.6 and 8.5, *O. nungara* was predicted to expand its current potential range in Europe by 35%, which would include the following Member States: Luxembourg, the Netherlands, Denmark, Ireland, Croatia, and Greece.

The species distribution model predictions (Annex VIII) under RCP 2.6 and RCP 4.5 show a similar expansion of range to the north and east as with the Negrete *et al.* (2020) under both climate change scenarios, with Austria, the Czech Republic, Denmark, Hungary and Ireland at significant risk with >20% of area suitable for *O. nungara* establishment. Conversely, some Mediterranean countries (Greece, Italy, Portugal and Spain) may see the areas suitable for *O. nungara* establishment decline under both climate change scenarios. Predictions under RCP 2.6 and 8.5, gave the same general trends with the RCP 8.5 predictions more pronounced.

A10. Is the organism known to be invasive (i.e. to threaten or adversely impact upon biodiversity and related ecosystem services) anywhere outside the risk assessment area?

Response: No evidence of impact is available for *O. nungara* so far, despite other species of alien flatworms being known for their invasive potential due to their predatory impact on soil fauna, but this may be due to the relatively small initial populations.

Outside of the risk assessment area, *O. nungara* has established in the wild in Guernsey (Álvarez-Presas *et al.*, 2014; Carbayo *et al.*, 2016; Webster, 2020), Madeira Island and in São Miguel in the Azores (Lago-Barcia *et al.*, 2020; Justine *et al.*, 2020), Switzerland (Justine *et al.*, 2020), Chile and the US.

A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea

Response: No evidence of impact is available for the species so far in any EU biogeographical region. *Obama nungara* is a predator of soil invertebrates including earthworms and molluscs (Boll and Leal-Zanchet, 2016). To date, there have been no studies assessing the impact of *O. nungara*. Consequently, it is not yet known whether *O. nungara* will have a negative effect on European soil fauna (Negrete *et al.*, 2020). The greatest densities found so far are in populations occurring within the Atlantic biogeographical region (Justine *et al.*, 2020).

A12. In which EU Member States has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden

Response: No evidence of impact is available for the species so far in any EU Member State (please see Qu. A11). High numbers of *O. nungara* have been collected by gardeners in France. In one garden, 944 specimens were collected from May to August from an area of c. 300 m²; whereas in another 1,442 were collected from November to May from a garden of c. 175 m² (Justine *et al.*, 2020). However, the impact of *O. nungara* on soil fauna has not been assessed.

A13. Describe any known socio-economic benefits of the organism.

including the following elements:

- Description of known uses for the species, including a list and description of known uses in the risk assessment area and third countries, if relevant.
- Description of social and economic benefits deriving from those uses, including a description of the environmental, social and economic relevance of each of those uses and an indication of associated beneficiaries, quantitatively and/or qualitatively depending on what information is available.

If the information available is not sufficient to provide a description of those benefits for the entire risk assessment area, qualitative data or different case studies from across the risk assessment area or third countries shall be used, if available.

Response: There are no known socio-economic benefits. *Obama nungara* could possibly benefit gardeners and farmers by feeding on pest snails and slugs. However, experience with other snail-feeding flatworms such as *P. manokwari*, suggests that the long-term deleterious effects on native mollusc biodiversity outweighs any benefits (Gerlach *et al.*, 2020). Similarly, *O. nungara* will feed on other flatworm species (e.g. *Dolichoplana carvalhoi*) (Boll and Leal-Zanchet, 2016) and therefore there is the tentative possibility of biocontrol of other invasive flatworm species but this remains to be tested and, as above, there is potential for significant non-target effects on indigenous flatworm species.

SECTION B – Detailed assessment

Important instructions:

- In the case of lack of information the assessors are requested to use a standardized answer: “No information has been found.” In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable).
- With regard to the scoring of the likelihood of events or the magnitude of impacts see Annexes I and II.
- With regard to the confidence levels, see Annex III.
- Highlight the selected response score and confidence level in **bold** but keep the other scores in normal text (so that the selected score is evident in the final document).

1 PROBABILITY OF INTRODUCTION AND ENTRY

Important instructions:

- **Introduction** is the movement of the species into the risk assessment area (it may be either in captive conditions and/or in the environment, depending on the relevant pathways).
- **Entry** is the release/escape/arrival in the environment, i.e. occurrence in the wild
- Introduction and entry may coincide for species entering through pathways such as “corridor” or “unaided”, but it also may differ. If different, please consider all relevant pathways, both for the introduction into the risk assessment area and the entry in the environment.
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used (see Annex IV). For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document³ and the provided key to pathways⁴.
- For organisms which are already present (recorded or established) in the risk assessment area, the likelihood of introduction and entry should be scored as “very likely” by default.
- Repeated (independent) introductions and entries at separate locations in the risk assessment area should be considered here (see Qu. 1.7).

Qu. 1.1. List relevant pathways through which the organism could be introduced into the risk assessment area and/or enter into the environment. Where possible give details about the specific origins and end points of the pathways as well as a description of any associated commodities.

For each pathway answer questions 1.2 to 1.8 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 1.2a, 1.3a, etc. and then 1.2b, 1.3b etc. for the next pathway.

In this context a pathway is the route or mechanism of introduction and/or entry of the species.

³ <https://circabc.europa.eu/sd/a/7e5f0bd4-34e8-4719-a2f7-c0cd7ec6a86e/2020-CBD-pathways-interpretation.pdf>

⁴ <https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf>

The description of commodities with which the introduction of the species is generally associated shall include a list and description of commodities with an indication of associated risks (e.g. the volume of trade; the likelihood of a commodity being contaminated or acting as vector).

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 1.2-1.9.

A main pathway described in this risk assessment for *O. nungara* is “contaminant nursery material” (unintentional) because of the species being considered as associate to the plant trade, the flatworm has been found in nurseries and the recognition of nursery material as a pathway for other invasive flatworms. There is no evidence in the literature of any other pathway being significant for introduction to the risk assessment area. Some thought was given as to whether botanical gardens (unintentional) should also be considered as a pathway as they have been implicated in the introduction of other flatworm species, e.g. *Bipalium kewense* (Winsor, 1983), *Platydemus manokwari* (Justine *et al.*, 2014) and *Arthurdendyus triangulatus* (Boag and Neilson, 2014). However, the pathways are very similar and represent unintentional introduction via trade and movement of living plants, which may be ultimately to a garden centre or botanic garden (in some cases, the same site may function as both). This is different from a plant species being intentionally brought into a botanical garden and escaping into the wild, as under the CBD ‘escape from confinement’ pathways. There is a suggestion of importation to the island of São Miguel on soil on machinery (Lago-Barcia *et al.*, 2020), but this pathway is considered here not active for introduction or entry into the risk assessment area and will be considered under spread within the risk assessment area. EU restrictions on the movement of soil (e.g. under EU Commission Implementing Regulation (EU) 2019/2072) and phytosanitary procedures for the cleansing of imported machinery make this pathway more pertinent within the risk assessment area, where such restrictions are not in place.

Pathway name: **Contaminant nursery material**

It is not known how *O. nungara* was introduced to the risk assessment area but terrestrial flatworms are most commonly introduced via accidental importation in plants for planting (nursery stock) (Sluys, 2016) and this would seem the most likely pathway (Lago-Barcia *et al.*, 2019). The proximity of early records to garden centres, nurseries and gardens (Carbayo *et al.*, 2016; Justine *et al.*, 2020; Álvarez-Presas *et al.*, 2014) strongly support this contention.

Qu. 1.2a. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional	CONFIDENCE	low
	unintentional		medium
			high

Response: This pathway is unintentional. Terrestrial flatworms have been intercepted in the UK on plant material, particularly tree ferns (*Dicksonia* spp.), from Australia and New Zealand (Matthews, 2005; Cannon and Baker, 2007). Although within the risk assessment area, there is a record of *O. nungara*

being intercepted in the UK on plants that came from the Netherlands (Aldred, 2016), which serves as another example of this potential pathway for introduction.

Qu. 1.3a. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Terrestrial flatworms are cryptic, nocturnal animals that shelter under debris on the soil surface during the day. Some species, such as *O. nungara*, are synanthropic, being found in man-disturbed habitats, which includes gardens and nurseries. It is postulated that *O. nungara* likely sheltered in plant containers or plant material that was exported from Argentina to Europe (Justine *et al.*, 2020; Lago-Barcia *et al.*, 2019; Justine and Winsor, 2020). Experience with other invasive flatworms would suggest that the number of individuals would be low and sporadic, with one or two individual flatworms in a contaminated shipment. Flatworm egg cocoons may also be present and they could give rise to several individuals; in theory, a single egg cocoon could give rise to a population. As *O. nungara* is not associated with a particular host plant but is rather an opportune contaminant, it is difficult to determine which nursery plant species are likely to be contaminated with flatworms and thus form the precise pathway of introduction. Europe imported US \$32 million worth of plants for planting from South America in 2002 (van Uffelen and de Groot, 2005). It is also difficult to determine if *O. nungara* was repeatedly imported with nursery material from South America or arrived only once and was then disseminated throughout Europe. There are two genetically distinct clades of *O. nungara* found in Europe. One found only in Spain and the other much more widespread being found in Spain, Portugal, France, UK, Italy, Belgium, and Switzerland (Justine *et al.*, 2020). This would suggest at least two separate introductions. It should also be considered that phytosanitary procedures in the EU have changed with the adoption in 2019 of EU Commission Implementing Regulation (EU) 2019/2072. The current procedures should minimise the likelihood of contamination with invasive alien flatworms but much depends on compliance, inspection and policing of these phytosanitary requirements.

Qu. 1.4a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Within a potted plant or enclosed in a plant’s root ball, *O. nungara* would be in a ‘protected’ environment and able to survive for extended periods. The conditions necessary to keep the plant healthy would presumably match those of the source location and therefore be amenable to the flatworm. Other invasive flatworm species such as *A. triangulatus* and *P. manokwari* can survive long periods (months) without food as they metabolise their own tissues and degrow but it is not confirmed that *O. nungara* can do likewise. Egg cocoons may be more tolerant of physical disruption and desiccation than adult flatworms during transportation, although this has not been tested for *O. nungara* or other flatworm species.

Qu. 1.5a. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Plant health inspection and phytosanitary measures are the most practical means of preventing invasion by cryptic flatworm species. Legislation in the EU requires that growing media must be free from soil or subjected to suitable treatment to ensure freedom from pests (EU Commission Implementing Regulation (EU) 2019/2072). However, legislation is dependent on plant health certification by exporting producers and only a small proportion of the plant trade can be directly inspected by the importing country.

Aside from plant health inspection, there are no existing management practices implemented against invasive flatworms. Hot water treatment has been suggested for flatworm management for imported plants for planting (Justine *et al.*, 2014; Murchie and Moore, 1998; Sugiura, 2008a), but has not been used for this specific purpose.

If *O. nungara* makes it into a plant consignment shipment undetected, it is likely that it will survive the onward journey.

Qu. 1.6a. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Obama nungara* has already been introduced into the risk assessment area undetected. Terrestrial flatworms are cryptic animals that shelter in crevices in plant material or within root balls and growing media. *Obama nungara* is brown to black and would be difficult to detect in transit. Egg cocoons are similarly dark-coloured and at c. 5 mm diameter (Justine *et al.*, 2020), smaller than the adult flatworms and difficult to see. In most cases, terrestrial flatworms have been detected by gardeners post-introduction (Justine *et al.*, 2020), underneath plant pots, plastic and other items on the soil surface in their own gardens. However, laypeople or nursery workers may not be able to detect the species if they are not familiar with this group.

Qu. 1.7a. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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Response: Plants transported for nursery stock are mostly imported to wholesalers and then disseminated to multiple retail points. Most large towns would have several garden centres or nurseries. Assuming that infested plants are bought and then planted in the consumers’ gardens, potentially, there would be many thousands of possible points of introduction into the environment within the risk assessment area.

Qu. 1.8a. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely	CONFIDENCE	low
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	unlikely moderately likely likely very likely		medium high
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Response: *Obama nungara* has already been introduced at multiple localities within the risk assessment area including on both continental areas and islands, e.g. in Corsica (and outside the risk assessment area, the islands of Guernsey, Madeira and São Miguel). Such a pattern of introduction is indicative of importation via the plant trade. Whilst no one knows for certain how *O. nungara* was introduced to the risk assessment area, there is agreement amongst researchers that plant trade was involved (Justine and Winsor, 2020; Negrete *et al.*, 2020; Justine *et al.*, 2020; Soors *et al.*, 2019). Therefore, the likelihood of introduction through this pathway is considered very likely and confidence is scored as medium due to the lack of data on the specific pathway of introduction.

Qu. 1.9. Estimate the overall likelihood of introduction into the risk assessment area or entry into the environment based on all pathways and specify if different in relevant biogeographical regions in current conditions.

Provide a thorough assessment of the risk of introduction in relevant biogeographical regions in current conditions: providing insight in to the risk of introduction into the risk assessment area.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Obama nungara* has already been introduced to several countries in the risk assessment area (in particular France, Spain and Belgium) therefore it is scored as very likely with high confidence. This flatworm has been found at nurseries and garden centres, and in domestic gardens (Carbayo *et al.*, 2016; Justine *et al.*, 2020; Álvarez-Presas *et al.*, 2014), which supports the view that traded live plants were the initial pathway of introduction into the risk assessment area and of entry into the environment.

The first records of *O. nungara* in Europe were from 2008 (Guernsey) and from 2010 in the risk assessment area (Spain) (Carbayo *et al.*, 2016). Eschen *et al.* (2015) commented that previously the EU permitted sufficient soil to maintain plants-for-planting in transit, but did not specify quantities, which in effect allowed a whole ecosystem to be transplanted, leading to a high risk of non-native organisms to be accidentally introduced. Plant health legislation has been updated recently (EU Commission Implementing Regulation (EU) 2019/2072) and there are requirements for plants-for-planting to be bare-rooted or treated to ensure freedom from pests and diseases. If this legislation is comprehensively implemented, the likelihood of inadvertently importing *O. nungara* should be minimal. However, the volume and diversity of trade in plants, and the limited capacities of biosecurity authorities in the EU,

makes it almost impossible to monitor, police and completely ensure that consignments are flatworm-free.

Qu. 1.10. Estimate the overall likelihood of introduction into the risk assessment area or entry into the environment based on all pathways in foreseeable climate change conditions?

Thorough assessment of the risk of introduction in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of introduction (e.g. change in trade or user preferences)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely introduction within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Obama nungara* has already been introduced to the risk assessment area and the likelihood is that climate change will increase the risk of this species being introduced to more regions in the future. There are two reasons for this. First, climate change will enable the flatworm to establish in more regions and countries around the world (e.g. North America), some of which will export plants to the risk assessment area. Models predicted an 83% increase of the suitable area at a global level for *O. nungara* under the RCP 2.6 compared to the current predicted distribution (Negrete *et al.*, 2020). Second, as the climate within Europe changes, the planting preferences of gardeners will likely adjust, potentially increasing the market for sub-tropical plant species native to South America within Europe. Relating the likelihood of this happening with the RCP 2.6 and the RCP 4.5 is difficult because of the many interacting and unknown factors involved, hence the confidence level is ‘low’.

2 PROBABILITY OF ESTABLISHMENT

Important instructions:

- For organisms which are already established in parts of the risk assessment area or have previously been eradicated, the likelihood of establishment should be scored as “very likely” by default.
- Discuss the risk also for those parts of the risk assessment area, where the species is not yet established.

Qu. 2.1. How likely is it that the organism will be able to establish in the risk assessment area based on similarity of climatic and abiotic conditions in its distribution elsewhere in the world?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: As *Obama nungara* has already established in the risk assessment area, in France, Belgium, Spain, Portugal and Italy (Justine and Winsor, 2020) the response is that establishment in the risk assessment area is very likely and confidence is high. It is likely that it has established in other countries in the risk assessment area where it has been introduced, but there is no published evidence available for those countries. The Species Distribution Model (Annex VIII) demonstrates that significant regions of the risk assessment are climatically suitable for establishment of *O. nungara*.

Qu. 2.2. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area? Consider if the organism specifically requires another species to complete its life cycle.

RESPONSE	very isolated isolated moderately widespread widespread ubiquitous	CONFIDENCE	low medium high
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Response: *Obama nungara* has been found most often in gardens and disturbed habitats (Justine *et al.*, 2020; Lago-Barcia *et al.*, 2019; Negrete *et al.*, 2020). So, the habitats necessary for the survival, development and multiplication of the organism are widespread in the risk assessment area. The habitat preferences and natural locations of *O. nungara* are unknown, so it is difficult to specify details beyond

this. In the UK and Ireland, *A. triangulatus* was initially found mostly in gardens but migrated out from these to surrounding farmland, in particular pasture (Murchie *et al.*, 2003). Flatworms may be unlikely to establish widely in arable cropping habitats due the likelihood of physical damage when the soil is cultivated.

The flatworm is a generalist predator, recorded as feeding on snails, slugs, earthworms and other flatworms, in Brazil (Boll and Leal-Zanchet, 2016). *Obama nungara*'s prey range within Europe has not been fully assessed but Justine *et al.* (2020) reported *O. nungara* feeding on a snail (*Theba pisana*) and an unidentified earthworm species in France. *Obama nungara* fed on an unidentified earthworm, the snail *Cochlicella acuta* and part of a defrosted cockroach in the laboratory (Carbayo *et al.*, 2016). Given the presumably wide prey range, it is assumed that prey availability will not be a limiting factor in the flatworm's establishment.

Qu. 2.3. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Obama nungara* is actually established in the risk assessment area despite potential competitors so the response is very likely with a high confidence. Indigenous *Microplana* spp. flatworms in the risk assessment area are smaller than *O. nungara* and there is no evidence that they can outcompete alien flatworm species. Where invasive flatworms have established, they have often become the dominant flatworm species. This would seem to be the case with *O. nungara*, e.g. in a citizen science survey of flatworms in France, *O. nungara* was the most recorded species (Justine *et al.*, 2020). The potentially broad prey range of *O. nungara* (see Qu. 2.2) also makes it less vulnerable to competition with existing, more specialized predators.

There is a theory that invasive flatworms in Europe may be exploiting an underdeveloped predatory niche, given the comparatively rich native flatworm fauna in Asia, South America and Australasia (Boag and Yeates, 2001; Boag *et al.*, 2010).

Qu. 2.4. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely	CONFIDENCE	low medium high
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	likely very likely		
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Response: Terrestrial flatworms are generally considered unpalatable to vertebrate predators. Although birds and shrews have been found feeding on other species of terrestrial flatworm in the risk assessment area (Cannon *et al.*, 1999; Justine *et al.*, 2014), they are not specialist predators and are unlikely to prevent establishment. Predatory beetles and other generalist invertebrate predators may also attack terrestrial flatworms (Gibson *et al.*, 1997) but their impact at the population level remains unknown. There have been no specific studies on which species predate *O. nungara* so a medium confidence is given.

Qu. 2.5. How likely is the organism to establish despite existing management practices in the risk assessment area? Explain if existing management practices could facilitate establishment.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Obama nungara* is already established in the risk assessment area. As *O. nungara* is synanthropic, disturbance of the habitat and the provision of refuges on the soil surface through gardening or building activities will likely benefit this species. However, direct cultivation of the soil is likely to be harmful to flatworms and limits their establishment. In terms of management of flatworm species, there are no specific management methods widely practiced in the risk assessment area.

Qu. 2.6. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Obama nungara* has already established in many regions in the risk assessment area. *Obama nungara* is a cryptic soil-dwelling animal and difficult to target for eradication as they are hidden in the soil or under refuges on the soil surface and hard to detect. Flatworms are hermaphrodites and produce egg cocoons containing multiple offspring, so a single surviving egg cocoon could give rise to a

population. There have been no formal large-scale eradication programmes for terrestrial flatworm species anywhere in the world. Local eradication of *A. triangulatus* by lay-persons has been attempted but not been successful (A.K. Murchie 2021, unpubl.).

Qu. 2.7. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?

including the following elements:

- a list and description of the reproduction mechanisms of the species in relation to the environmental conditions in the risk assessment area
- an indication of the propagule pressure of the species (e.g. number of gametes, seeds, eggs or propagules, number of reproductive cycles per year) of each of those reproduction mechanisms in relation to the environmental conditions in the risk assessment area.
- If relevant, comment on the likelihood of establishment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in establishment whereas for others high propagule pressure (many thousands of individuals) may not.
- If relevant, comment on the adaptability of the organism to facilitate its establishment and if low genetic diversity in the founder population would have an influence on establishment.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Obama nungara* is hermaphrodite and reproduces sexually via egg cocoons, which typically measure c. 3-5 mm diameter and each contain 3-6 offspring (Negrete *et al.*, 2020; Justine *et al.*, 2020). A single cocoon could give rise to a population. The impact of limited genetic diversity on the ability of *O. nungara* to establish is unknown. Inbreeding depression has been observed in freshwater flatworms (Benazzi and Forli, 2009). However, in an exclusively self-fertilising flatworm, no effect was found on fitness (Benazzi, 1991). Justine and Winsor (2020) commented that *O. nungara* reproduces very quickly and numbers can build up rapidly, e.g. 1,442 specimens were collected from a garden area of c. 175 m² within 7 months (Justine *et al.*, 2020). However, there has been no specific research on *O. nungara*'s fecundity.

Other invasive flatworm species such as *A. triangulatus* and *P. manokwari* can reabsorb tissues when food is scarce, to enable them to survive long periods (months) of starvation. Flatworms can also retreat deep into the soil or within rotten logs during dry periods, where they survive in mucus lined chambers. It seems very likely that *O. nungara* will have similar survival characteristics but this is not been studied.

Qu. 2.8. If the organism does not establish, then how likely is it that casual populations will continue to occur?

Consider, for example, a species which cannot reproduce in the risk assessment area, because of unsuitable climatic conditions or host plants, but is present because of recurring introduction, entry and release events. This may also apply for long-living organisms.

RESPONSE	N/A very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Obama nungara* has already established in many regions in the risk assessment area.

Qu. 2.9. Estimate the overall likelihood of establishment in the risk assessment area under current climatic conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under current climatic conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in current conditions: providing insight in the risk of establishment in (new areas in) the risk assessment area.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Obama nungara* has already established in the risk assessment area, in many regions of France, Spain and parts of Portugal, Belgium and Italy so the response is very likely with high confidence. The flatworm has established predominantly in the Atlantic and Mediterranean biogeographical regions, and to a lesser extent in the Continental biogeographical region. The Species Distribution Model (SDM) also predicted that *O. nungara* could establish in the Black Sea biogeographical region (Annex VIII). The SDM predicted that 48% of the Atlantic biogeographical region, 38% of the Mediterranean, 9% of the Continental and 29% of the Black Sea region would be suitable for establishment. This is bearing in mind that the Continental biogeographical region is considerably larger than, for example, the Atlantic biogeographical region.

Qu. 2.10. Estimate the overall likelihood of establishment in the risk assessment area under foreseeable climate change conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under foreseeable climate change conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of establishment (e.g. increase in average winter temperature, increase in drought periods)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely establishment within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Under climate change scenarios, *O. nungara* has the potential to increase its range northwards and eastwards into areas of Austria, the Czech Republic, Denmark, Germany, Hungary and Ireland (Annex VIII ‘Projection of environmental suitability for *Obama nungara* establishment in Europe’). Under RCP 4.5, *O. nungara* could potentially establish in southern Sweden. Some Mediterranean countries (Greece, Italy, Portugal and Spain) may become less suitable for *O. nungara* establishment under climate change. The climatic suitability for *O. nungara* was most strongly determined by temperature, human influence and precipitation. Increased annual temperatures will benefit this species and allow northward range expansion, provided soil moisture is maintained. The reduction in the southern range around the Mediterranean is likely due to changes in patterns of precipitation, with correspondingly drier and more arid conditions, which would prohibit *O. nungara* survival.

3 PROBABILITY OF SPREAD

Important instructions:

- Spread is defined as the expansion of the geographical distribution of an alien species within the risk assessment area.
- Repeated releases at separate locations do not represent continuous spread and should be considered in the probability of introduction and entry section (Qu. 1.7).

Qu. 3.1. How important is the expected spread of this organism within the risk assessment area by natural means? (List and comment on each of the mechanisms for natural spread.)

including the following elements:

- a list and description of the natural spread mechanisms of the species in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of spread discussed in relation to the species biology and the environmental conditions in the risk assessment area.

The description of spread patterns here refers to the CBD pathway category “Unaided (Natural Spread)”. It should include elements of the species life history and behavioural traits able to explain its ability to spread, including: reproduction or growth strategy, dispersal capacity, longevity, dietary requirements, environmental and climatic requirements, specialist or generalist characteristics.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Terrestrial flatworms move by creeping on the soil surface. *Obama nungara*'s life stages (egg cocoon, juvenile and adult) are all soil-bound and there is no specific dispersal phase. The general pattern of dispersal of invasive terrestrial flatworms is that of man-made spread via movement of infested potted plants by nurseries, garden centers and gardeners, followed by local colonization of the surrounding area by natural movement. This appears to be what is happening in France (Justine and Winsor, 2020). The exact rate of natural movement by *O. nungara* is not known (has not been studied) and aside from locomotory dispersal, there is no evidence of natural movement by other means (e.g. birds or water). However, for a comparable flatworm such as *A. triangulatus* movement was assessed by tracking the spread of established populations in transects of traps. The maximum rate of movement in the wild was 15 m in 7 days, whereas Gibson and Cosens (1998) found the flatworm to disperse 17 m in 30 days. Such movement is random and represents the locomotory capability of the species. The moving front of invasion into a new area for *P. manokwari* was found to be c. 20-30 m per year in a garden setting (Winsor, 1990) and this might be a more appropriate measure than the ability of individuals to disperse. Either way the importance of natural spread is considered minor with high confidence.

Qu. 3.2a. List and describe relevant pathways of spread other than "unaided". For each pathway answer questions 3.3 to 3.9 (copy and paste additional rows at the end of this section

as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 3.3a, 3.4a, etc. and then 3.3b, 3.4b etc. for the next pathway.

including the following elements:

- a list and description of pathways of spread with an indication of their importance and associated risks (e.g. the likelihood of spread in the risk assessment area, based on these pathways; likelihood of survival, or reproduction, or increase during transport and storage; ability and likelihood of transfer from the pathway to a suitable habitat or host) in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of spread for each pathway discussed in relation to the species biology and the environmental conditions in the risk assessment area.
- All relevant pathways of spread (except “Unaided (Natural Spread)”, which is assessed in Qu. 3.1) should be considered. The classification of pathways developed by the Convention of Biological Diversity shall be used (see Annex IV).

The principal pathway for spread of *O. nungara* is likely to be movement of ‘contaminated nursery material’ (unintentional) from nurseries, garden centres, DIY stores, supermarkets and between gardeners. *Obama nungara* was first found at garden centres and nurseries in Guernsey (Carbayo *et al.*, 2016) and a specimen of *O. nungara* was discovered at a garden centre in Oxfordshire (UK), crawling out of a *Heuchera* plant pot imported from the Netherlands (Aldred, 2016). An example pathway is as follows. A flatworm infested consignment of potted plants from South America is disseminated to wholesalers in the risk assessment area and then to retail outlets. The flatworms may establish within premises along the way where they can shelter in new material, or be transported onwards in the original material. Contaminated stock is bought by gardeners, who may then move plants amongst premises. There was some discussion as to whether this pathway overlaps with the ‘contaminant on plants’ pathway. For example, a gardener may buy infested nursery stock from a garden centre, which allows *O. nungara* to establish and proliferate in their garden. They then move plants from their garden to another site, inadvertently carrying flatworms in the plant’s root balls. However, the flatworms are not attached to the plants in the manner of a phytophagous insect pest but are sheltering within the root ball, soil attached to the plant, or within plant pots.

Flatworms may also be moved in contaminated soil, either on its own (‘transportation of habitat material’ - unintentional) or stuck to machinery as stowaway on “machinery/equipment” (unintentional).

Pathway name: Contaminant nursery material

Qu. 3.3a. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: This pathway is unintentional. The association of *O. nungara* with nurseries and gardens indicates inadvertent transfer along this pathway within the risk assessment area. Gardeners do not want to buy nursery material that contains flatworms. This is non-intentional transfer and if known it can adversely affect the reputation of the business or botanic garden concerned (Boag and Neilson, 2014).

Qu. 3.4a. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Trade in plants within countries offers numerous opportunities for spread of *O. nungara*. A single fertilised flatworm or an egg cocoon could give rise to a population. As dispersal by this pathway is unintentional, flatworms are usually unnoticed until they have established at a premises or in gardens. Therefore, it is difficult to assess the numbers being spread at any one time. This is particularly so if gardeners move plants between different locations or informally pass them on to neighbours and fellow gardeners. The apparently rapid spread of *O. nungara* in France (Justine *et al.*, 2020) and the discovery of *O. nungara* at nurseries in other countries suggests this is the major route of dissemination. As the timeframe is just one year then the highest likelihood category is not justified and confidence is only medium.

Qu. 3.5a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: If *O. nungara* is transferred with nursery plants containing growing media within the risk assessment area, and providing these plants are not exposed to temperature extremes (e.g. direct sunlight, frost), it is likely that flatworms will survive. Reproduction is probably unlikely during transit or storage as mating may be disturbed but there are no barriers to fertilised flatworms laying egg cocoons and these being transported. During storage in the garden centres or nurseries the species is also well able to survive and reproduce.

Qu. 3.6a. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: National phytosanitary measures within Member States may be implemented at nurseries / garden centres to control flatworms, if a consignment is suspected to be contaminated, but are rarely executed in practice. In such a case, use of hot-water treatment shows promise for flatworm management (Sugiura, 2008a; Murchie and Moore, 1998; Justine *et al.*, 2014). However, we know of no instances where any routine management is practiced against invasive flatworms. As existing management practices are not widely applied in the risk assessment area, it is likely that the flatworm’s survival would not be affected.

Qu. 3.7a. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Obama nungara* is a cryptic, nocturnal soil-dwelling species. It can shelter within plant pots, root balls and within plant material making it very difficult to detect unless the plants are uprooted and the growing media and root balls examined directly by qualified personnel. The spread in France was largely undetected until flatworms had established in gardens. It is very likely that its spread would be undetected and confidence is high.

Qu. 3.8a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Obama nungara* is a synanthropic species, often found in man-made and disturbed habitats (Lago-Barcia *et al.*, 2019; Negrete *et al.*, 2020; Carbayo *et al.*, 2016) and a polyphagous predator (Boll and Leal-Zanchet, 2016). Gardens would seem to provide an ideal habitat (Justine *et al.*, 2020). It is very likely that *O. nungara* is unintentionally spread with infested nursery stock to domestic gardens, botanic gardens, parks and landscaped areas. This is essentially direct transfer to the environment and has been demonstrated widely in France.

Qu. 3.9a. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area. (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: *Obama nungara* could spread rapidly through the plant trade network but there are no explicit quantitative data (studies have not been conducted). The flatworms are opportunistic contaminants that do not require a specific host plant. This means that if they infested a nursery or garden centre, then there is great opportunity for secondary spread. Most large towns in Europe have plant nurseries, garden centres or DIY stores that sell containerised plants. The rate of spread is difficult to predict as although most garden centres will operate locally, some may trade on the internet, whilst customers may purchase plants on holiday or travelling. A record of *O. nungara* in Scotland was due to a gardener buying a contaminated plant in southern England (H. Jones 2021, pers. comm.). Therefore, although such spread is likely to be mostly localised, there will be some with random long-distance transfers.

As transfer of plants is human-mediated and likely to be via cars and vans, environmental conditions are unlikely to have an impact on this means of transfer *per se* and the environmental conditions in the risk assessment area where the potted plants are likely to end up are very likely to pose no restrictions on this rapid spread.

Pathway name: Transportation of habitat material (soil, vegetation,...)

Qu. 3.3b. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional	CONFIDENCE	Low
	unintentional		medium high

Response: Terrestrial flatworms may be spread with contaminated soil or material left on the soil surface or plant material that they have sheltered beneath. Such transport is unintentional.

Qu. 3.4b. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely	CONFIDENCE	Low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response: Movement of topsoil and compost have spread other terrestrial flatworms, such as *A. triangulatus* (Cannon *et al.*, 1999; Christensen and Mather, 1998; Moore *et al.*, 1998). Although there is no specific data for *O. nungara*, as it shelters under soil refuges in the same way as *A. triangulatus*, including items such as bagged soil / compost, it is reasonable to assume that this flatworm species could be spread in a similar manner. Compost heaps in gardens may have a rich invertebrate fauna including earthworms and molluscs that *O. nungara* may be attracted to and prey on. Mature compost is a valuable growing media and soil conditioner in domestic gardens. As such, it is likely to be distributed in the garden and may be moved to other sites or given to neighbours.

Qu. 3.5b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	Low medium high
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Response: Soil can provide a microhabitat that retains moisture and buffers temperature fluctuations. *Obama nungara* would likely survive transport and storage in soil especially if the volume is large. Reproduction is less likely due to the disturbance, so flatworms would probably not mate in transit but fertilised adults could lay egg cocoons. *Obama nungara* is likely to survive storage of soil if it is left outdoors and retains moisture. If the soil is kept indoors it may dry out over time, which would be detrimental for the flatworms.

Qu. 3.6b. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	Low medium high
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Response: If soil is sterilised by steaming *O. nungara* would be killed. This is sometimes used for large quantities of commercial topsoil but would not be a commonplace approach for domestic soil/compost or for smaller quantities moved within a region. It has also been suggested that soil or compost could be heated by placing in a glasshouse to kill *A. triangulatus* (A.K. Murchie 2021, unpubl.). However, this has not been tested. Subsequent sieving or cultivation of the soil would be detrimental to *O. nungara* as the flatworm could be physically damaged. These options for management along this pathway can only be applied if a consignment is known to be contaminated, rather than as a routine preventive measure.

Qu. 3.7b. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Obama nungara* has mostly been detected in the wild after it has established, or intercepted emerging from containerised plants (Justine *et al.*, 2020; Aldred, 2016). Detecting *O. nungara* in a contaminated consignment of soil / compost would be very difficult and laypersons may not recognise the flatworms, even if seen. It is likely that the flatworm will be buried in cracks and crevices within the soil / compost, and being a nocturnal, brown and cryptic species, they are not easily noticed. Their egg cocoons are black and c. 5 mm in diameter, so even more difficult to detect. Environmental DNA technologies could be used to detect invasive flatworms in soil but this has not been tested as yet.

Qu. 3.8b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The movement of contaminated topsoil or compost between gardens and in landscaping would directly transfer *O. nungara* into a suitable habitat. This is providing that the habitat contained suitable refuges on the soil surface (stones, wood, plant pots, plastic sheeting etc.) and prey (e.g. earthworms, slugs, snails). Transfer is therefore, very likely with high confidence.

Qu. 3.9b. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: This pathway can be quite diverse and vary from movement of contaminated compost between neighbouring gardeners to large quantities of topsoil moved for landscaping purposes onto new construction developments. Larger quantities of topsoil and compost can be split into multiple sub-lots and disseminated extensively to different recipients. Due to the diverse nature of this pathway, it is difficult to quantify the potential for spread of *O. nungara*. So far, we have no direct reports of movement along this pathway for *O. nungara*, but evidence from other invasive alien flatworms suggests it is a significant risk (Moore *et al.*, 1998; Christensen and Mather, 1998). It is however, likely that the environmental conditions in the receiving area along with the large volume of soil likely to be transported will mean spread will not be limited by environmental conditions.

Pathway name: machinery/equipment (transport-stowaway)

Qu. 3.3c. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: Transfer along this pathway is unintentional. Terrestrial flatworms and flatworm egg cocoons can be carried in soil remnants left on machinery or equipment. In addition, flatworms are covered in mucus and may adhere directly to machinery and equipment.

Qu. 3.4c. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Obama nungara* was found on São Miguel in the Azores, in an area free from imported plants, which led Lago-Barcia *et al.* (2020) to suggest that it was transported with machinery used in building work or with tourists visiting the nearby beauty spot. Other invasive flatworms are reported to have been translocated in soil on machinery, boots or stuck to farming materials and equipment (Sugiura *et al.*, 2006; Okochi *et al.*, 2004; Moore *et al.*, 1998; Boag *et al.*, 1999). This is random and sporadic spread. There are no data available to estimate the propagule pressure, although in theory a single egg cocoon could spawn a population. The rate of spread along this pathway will be determined by the volume of movement of machines or equipment.

Qu. 3.5c. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The longer the distance travelled the less likely flatworms will survive as they will be subject to mechanical damage and desiccation. Reproduction is unlikely. Egg cocoons embedded in soil on machinery are more likely to survive than adults, being smaller and immobile. Adult flatworms only may survive short-range transport along this pathway. These limitations mean that survival and reproduction are unlikely in this pathway but confidence remains low as there has been no research to date.

Qu. 3.6c. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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For general biosecurity purposes, machinery should be washed clear of soil when moving between locations. However, this is only likely to occur where there is significant distance involved or movement over state boundaries. Power-washing should dislodge flatworms and may physically damage them. Therefore cleaning equipment and machinery should be straightforward but much depends on how often it is implemented and how thorough the washing. These management practices, if implemented, would make survival on this pathway unlikely.

Qu. 3.7c. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Obama nungara* is a cryptic nocturnal species, so will hide away during the day. It is brown and therefore may be difficult to detect on machinery or equipment carrying soil. Laypersons may not recognise the species as problematic. Egg capsules are 5 mm diameter and black, so are also difficult to detect unless examined close-up. Other flatworms have been detected on farming machinery. For example, *A. triangulatus* has been seen on hay and silage bales and the equipment used to move these (Boag *et al.*, 1999; Moore *et al.*, 1998).

Qu. 3.8c. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Machinery and equipment used for horticultural or agricultural purposes are likely to be moving between similar habitats and may transport *O. nungara* directly to new habitats. For example, an egg cocoon embedded in soil in the tread of a tractor could be dropped off as soon as the tractor starts working in a new area. *Obama nungara* has mostly been found in gardens within the risk assessment area (Justine *et al.*, 2020) but there seems no reason why they could not establish in horticultural sites or on the margins of agricultural land and being subsequently further spread with machinery. However, terrestrial flatworms are susceptible to physical damage so would not survive intensive cultivation practices or longer transport or storage periods.

Qu. 3.9c. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: Agricultural and horticultural machinery and equipment are predominantly used locally, although sometimes they can be moved over long distances between sites. Aside from the suggestion that contaminated building machinery may have been the source of *O. nungara* on São Miguel in the Azores (Lago-Barcia *et al.*, 2020), there are no quantitative data on the spread of *O. nungara* along this pathway. As with the other pathways above, it is likely to be random and sporadic but more limited in nature.

Qu. 3.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?

RESPONSE	very easy easy with some difficulty difficult very difficult	CONFIDENCE	low medium high
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Response: *Obama nungara* shelters during the day under debris / materials on the soil surface, including plant pots and containers (terrestrial flatworms seem to have a particular affinity for plastic as it retains moisture and provide a smooth resting place). The flatworm is not therefore associated with a particular host plant or commodity but is inadvertently moved around as a contaminant of potted and containerised plants, soil or compost and machinery / material where it has sheltered. The possibilities for spread within the risk assessment area are therefore large and include the plant trade, domestic movement of plants, movement of soil / compost, and potentially any machinery or material that has been left on the soil surface. The potential individual routes and commodities within pathways are numerous and are both commercial and domestic. The flatworms are difficult to detect and there are no universal control measures that could be applied to bulk quantities of either plants, soil or compost.

Qu. 3.11. Estimate the overall potential rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (indicate any key issues and provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in current conditions, providing insight in the risk of spread into (new areas in) the risk assessment area.

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: *Obama nungara* was first detected in France in 2013 but is now established in 72 of the 96 administrative Departments in Metropolitan France in the Atlantic, Continental and Mediterranean Regions (Justine *et al.*, 2020). It may be that the flatworm was present for some time before its first detection. For example, the highly noticeable non-native flatworms *Bipalium* spp. and *Diversibipalium* spp. escaped attention for 20 years in France (Justine *et al.*, 2018). However, the pattern of spread in France and the notification of this species from surrounding countries do indicate that spread has been

rapid. This is principally driven by human movement of contaminated materials but local natural movement from gardens to surrounding farmland and wild areas is probable.

Qu. 3.12. Estimate the overall potential rate of spread in relevant biogeographical regions in foreseeable climate change conditions (provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if rates of spread are likely slowed down or accelerated.

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: As spread is mostly man-made, there is little reason to suggest that the potential rate of spread will slow due to climate change. Indeed, it seems likely in the areas affected that plant trade in exotic ornamentals may increase as the region’s climate becomes hotter and wetter. It is feasible that gardeners may seek out new plant species and varieties to grow as the climate changes. In addition, milder, wetter conditions may facilitate the natural spread of *O. nungara* from gardens to the surrounding habitat. The Atlantic biogeographical region would be most affected by this scenario under current and future climatic conditions.

4 MAGNITUDE OF IMPACT

Important instructions:

- Questions 4.1-4.5 relate to biodiversity and ecosystem impacts, 4.6-4.8 to impacts on ecosystem services, 4.9-4.13 to economic impact, 4.14-4.15 to social and human health impact, and 4.16-4.18 to other impacts. These impacts can be interlinked, for example, a disease may cause impacts on biodiversity and/or ecosystem functioning that leads to impacts on ecosystem services and finally economic impacts. In such cases the assessor should try to note the different impacts where most appropriate, cross-referencing between questions when needed.
- Each set of questions starts with the impact elsewhere in the world, then considers impacts in the risk assessment area (=EU excluding outermost regions) separating known impacts to date (i.e. past and current impacts) from potential future impacts (including foreseeable climate change).
- Only negative impacts are considered in this section (socio-economic benefits are considered in Qu. A.7)
- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable).

Biodiversity and ecosystem impacts

Qu. 4.1. How important is the impact of the organism on biodiversity at all levels of organisation caused by the organism in its non-native range excluding the risk assessment area?

including the following elements:

- Biodiversity means the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems
- impacted chemical, physical or structural characteristics and functioning of ecosystems

RESPONSE	N/A	CONFIDENCE	low medium high
	minimal minor moderate major massive		

Response: No information has been found on the issue. Outside of its native range and the risk assessment area, *O. nungara* has established in the wild in Guernsey (Álvarez-Présas *et al.*, 2014; Carbayo *et al.*, 2016; Webster, 2020), Madeira Island and in São Miguel in the Azores, Switzerland (Lago-Barcia *et al.*, 2020; Justine *et al.*, 2020), Chile and the US. Whilst there is concern expressed in the literature that *O. nungara* will impact on native species, there have been no studies to assess this to date (Justine and Winsor, 2020; Justine *et al.*, 2020; Negrete *et al.*, 2020). These concerns relate to how

similar species of invasive flatworms such as *A. triangulatus* and *P. manokwari* have negatively impacted on earthworm and snail biodiversity, respectively, through predation (Murchie and Gordon, 2013; Gerlach *et al.*, 2020). Given *O. nungara*'s broad prey range and potential to establish in large areas of the risk assessment, these concerns are justified.

Qu. 4.2. How important is the current known impact of the organism on biodiversity at all levels of organisation (e.g. decline in native species, changes in native species communities, hybridisation) in the risk assessment area (include any past impact in your response)?

Discuss impacts that are currently occurring or are likely occurring or have occurred in the past in the risk assessment area. Where there is no direct evidence of impact in the risk assessment area (for example no studies have been conducted), evidence from outside of the risk assessment area can be used to infer impacts within the risk assessment area.

RESPONSE	N/A minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: *Obama nungara* has established in France, Belgium, Spain, Portugal and Italy (Justine and Winsor, 2020). As a potentially numerous predator in the soil ecosystem, there is much concern that it will impact upon native species. The prey range of *O. nungara* has been assessed in Brazil, where it fed on snails, slugs, earthworms and other flatworms, when they were presented to flatworms in the laboratory (Boll and Leal-Zanchet, 2016). However, there have been no studies assessing the impact of *O. nungara* predation in the field in the risk assessment area. Other earthworm-feeding and snail-feeding flatworms such as *A. triangulatus* and *P. manokwari* have had demonstrable negative effects on soil fauna. For example, *A. triangulatus* reduced anecic earthworm biomass by 74% in a field study in Northern Ireland (Murchie and Gordon, 2013), whilst *P. manokwari* has been implicated in the extinction of indigenous snail fauna on Pacific islands (Sugiura, 2008b). Due to the lack of data on *O. nungara*, this question has been scored 'N/A', as it refers to 'current known impact' rather than potential impact (please see Qu. 4.3 below).

Qu. 4.3. How important is the potential future impact of the organism on biodiversity at all levels of organisation likely to be in the risk assessment area?

See comment above. The potential future impact shall be assessed only for the risk assessment area. A potential increase in the distribution range due to climate change does not *per se* justify a higher impact score.

RESPONSE	minimal	CONFIDENCE	low
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	minor moderate major massive		medium high
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Response: There are no specific data on the future impact of *O. nungara* on soil biodiversity in the risk assessment area. However, there are relevant examples from other invasive alien flatworms which have had negative effects on soil biodiversity where they have established, and that are reported here as example of the potential future impact of *O. nungara*. *Arthurdendyus triangulatus* is a predator of earthworms. In field experiments where flatworm density was manipulated, *A. triangulatus* at 0.8 individuals per m² reduced earthworm biomass by 20%. *Arthurdendyus triangulatus* predation had a disproportionate impact on *Lumbricus terrestris* whose biomass was reduced by 74% (Murchie and Gordon, 2013). Similarly, the snail predator *P. manokwari* has had devastating impacts on endemic snail species on Pacific islands where it has been introduced and potentially could impact snail biodiversity in the risk assessment area (Murchie and Beckmann, 2020). Both snails and earthworms are important food resources for other species, including many birds and mammals, so predation on these invertebrates by *O. nungara* would have consequences further up the food chain.

It has to be expected that *O. nungara*, as a generalist predator feeding on earthworms and molluscs, would have a similar impact such as *A. triangulatus* and *P. manokwari*. High numbers of *O. nungara* are likely to outcompete other soil predators, including indigenous flatworms but no data are available on such an impact. Justine *et al.* (2020) commented that the ecological impact of *O. nungara* was likely to be high, based on the impact of other flatworms, *O. nungara*'s broad prey range and the locally high population densities in France, but this has yet to be studied. The score of moderate is based on expert opinion in the absence of hard data so the confidence is set as low.

Qu. 4.4. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism currently in the risk assessment area?

including the following elements:

- native species impacted, including red list species, endemic species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: There are no data on the decline in conservation value caused by presence of *O. nungara*. At the present time, *O. nungara* is mostly restricted to gardens. However, there is one record of *O. nungara* from a high value natural ecosystem in Spain (Lago-Barcia *et al.*, 2020) and as a generalist predator of earthworms and snails, *O. nungara* has the potential to impact on endemic and conservation value species.

Qu. 4.5. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.3. and 4.4.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: *Obama nungara* is a predator of earthworms, terrestrial gastropods and other soil-dwelling invertebrates. In the European region, 22% of the 2,469 native species of gastropods (snails and slugs) are considered threatened (critically endangered, endangered or vulnerable), with 97 species critically endangered (European Commission, 2020). *Obama nungara* has already established in the Mediterranean region, which includes areas of high snail endemicity (Cuttelod *et al.*, 2011) and therefore the flatworm could have a future impact on indigenous snail species. For earthworms, there is no comparable Europe-wide red list and the conservation status of most species is unknown. The only assessment is by the Federal Agency for Nature Conservation (Bundesamt für Naturschutz) who produced an earthworm red list for Germany, with 14 of the 47 earthworm species assessed as ‘extremely rare’ (Phillips *et al.*, 2017). As with snails, there is therefore a potential for *O. nungara* predation to have an impact on rare earthworm species but data are lacking. The score of moderate is based on expert opinion in the absence of hard data so the confidence is set as low.

Ecosystem Services impacts

Qu. 4.6. How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?

- For a list of services use the CICES classification V5.1 provided in Annex V.
- Impacts on ecosystem services build on the observed impacts on biodiversity (habitat, species, genetic, functional) but focus exclusively on reflecting these changes in relation to their links with socio-economic well-being.
- Quantitative data should be provided whenever available and references duly reported.

RESPONSE	N/A minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No information has been found on the issue.

Outside of its native range and the risk assessment area, *O. nungara* has established in the wild in Guernsey (Álvarez-Presas *et al.*, 2014; Carbayo *et al.*, 2016; Webster, 2020), Madeira Island and in São Miguel in the Azores, Switzerland (Lago-Barcia *et al.*, 2020; Justine *et al.*, 2020), Chile and the US. As these records are comparatively recent, no impacts on ecosystem services have been reported so far but likewise, there have been no specific studies on this issue. Please see Qu. 4.8 below for potential future impacts.

Qu. 4.7. How important is the impact of the organism on provisioning, regulating, and cultural services currently in the different biogeographic regions or marine sub-regions where the species has established in the risk assessment area (include any past impact in your response)?

- See guidance to Qu. 4.6.

RESPONSE	N/A minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No information has been found on the issue.

Most records of *O. nungara* in the risk assessment area are from France and the impact on ecosystem services has not been evaluated (Justine *et al.*, 2020). Please see Qu. 4.8 below for potential future impacts.

Qu. 4.8. How important is the impact of the organism on provisioning, regulating, and cultural services likely to be in the different biogeographic regions or marine sub-regions where the species can establish in the risk assessment area in the future?

- See guidance to Qu. 4.6.

RESPONSE	minimal	CONFIDENCE	low
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	minor moderate major massive		medium high
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Response: There are no data on the impact of *O. nungara* on ecosystem services. However, where established, the flatworm can build up large population levels (Justine *et al.*, 2020) and as a generalist, apex predator in the soil ecosystem, there is future potential that *O. nungara* could have a significant impact on other soil fauna, with knock-on effects on ecosystem services.

If *O. nungara* predation reduces earthworm densities in the future this will have an effect on ‘soil quality regulation’. Earthworms perform a valuable role in aerating and draining the soil, as well as recycling nutrients for plant growth (Edwards and Bohlen, 1996). ‘Water conditions’ could also be potentially affected if reduced earthworm densities change the hydrological aspects of the soil and increase run-off to neighbouring waterways. Snail predation could have an impact on populations of wild snails used as food (‘wild animals used for nutritional purposes’). The presence of invasive alien flatworms can also cause distress to gardeners and others working on the soil, and impact upon their ‘physical and experiential interactions with natural environment’. Especially if present in large numbers, the flatworms are viewed as undesirable, harmful and uncontrollable species. There are numerous examples on social media of people exclaiming upset at having found an invasive alien flatworm in their locality.

Economic impacts

Qu. 4.9. How great is the overall economic cost caused by the organism within its current area of distribution (excluding the risk assessment area), including both costs of / loss due to damage and the cost of current management.

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the world these should be reported here. The assessment of the potential costs of / loss due to damage shall describe those costs quantitatively and/or qualitatively depending on what information is available. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage. As far as possible, it would be useful to separate costs of / loss due to the organism from costs of current management.

RESPONSE	N/A minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No information has been found on the issue.

Obama nungara is not considered a pest within its native South American distribution range. Outside of that and the risk assessment area, *O. nungara* has established in Guernsey (Álvarez-Presas *et al.*, 2014; Carbayo *et al.*, 2016; Webster, 2020), Madeira Island and in São Miguel in the Azores, Switzerland (Lago-Barcia *et al.*, 2020; Justine *et al.*, 2020), Chile and the US. At the present time, there are no reports of any economic damage or costs associated with management.

Qu. 4.10. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism currently in the risk assessment area (include any past costs in your response)?

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the EU these should be reported here. Assessment of the potential costs of damage on human health, safety, and the economy, including the cost of non-action. A full economic assessment at EU scale might not be possible, but qualitative data or different case studies from across the EU (or third countries if relevant) may provide useful information to inform decision making. In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable). Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: *Obama nungara* has established in France, Belgium, Spain, Portugal and Italy (Justine and Winsor, 2020). At the present time, there are no reports of any economic damage. However, one online retailer denied the presence of *O. nungara* in a shipment despite expert assessment, presumably to safeguard their reputation (H. Jones 2021, unpubl.). Gardeners and consumers are increasingly aware of invasive alien flatworms through magazine articles and online fora, and contamination of products with flatworms could have a potentially serious economic impact on gardening businesses but there are no studies on this or quantitative data available. These possible reputational costs coupled with the extra time spent dealing with investigations of possible contamination are unlikely at present to exceed 100,000 euros per year so a score of minor with low confidence is given.

Qu. 4.11. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.10.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: *Obama nungara* is a predator of molluscs and earthworms. Predation on snails may cause biodiversity losses but could also impact on heliciculture (snail farming). Within some parts of Europe, snails (*Cornu aspersum* and *Helix pomatia*, and others) are farmed and collected from the wild as food (Conte, 2015; Waldhorn, 2020). It is feasible that if *O. nungara* spread and established in snail farming / collecting regions, it could impact on snail production for food. Predation on earthworms could have knock-on effects on crop yield. *Arthurdendyus triangulatus* is a flatworm species that predaes upon earthworms, and has established in the UK, Ireland and the Faroe Islands. Using figures of a 20% decline in earthworm biomass caused by flatworm predation (Murchie and Gordon, 2013) times the contribution of earthworms to ryegrass above ground biomass of 34% (van Groenigen *et al.*, 2014), the predicted decline in grass yield was 7% in Northern Ireland (Murchie, 2018). The economic impact in Northern Ireland would be in the region of €17 per ha per annum as pasture underpins large dairy and beef production. The concern is that *O. nungara* could be an equivalent predator of earthworms to *A. triangulatus*, and cause similar losses but, unlike *A. triangulatus*, has already established extensively in gardens in France and continental Europe. Permanent grassland covers c. 50 million ha in the EU27 (Huyghe *et al.*, 2014). If *O. nungara* had a similar effect as *A. triangulatus* across pasture production in the EU27, the economic impact could potentially run into several 100 million euros. Nonetheless a moderate score is given for the immediate future as recorded costs are likely to be much lower and no data exist on the impact of *O. nungara* on earthworms.

Qu. 4.12. How great are the economic costs / losses associated with managing this organism currently in the risk assessment area (include any past costs in your response)?

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable).

RESPONSE	N/A minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No information has been found on the issue.

As far as we are aware there have been no management practices implemented aside from actions taken by local gardeners to physically collect and destroy *O. nungara* (Justine *et al.*, 2020).

Qu. 4.13. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.12.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: If *O. nungara* were to cause significant problems that necessitated widespread management the costs would be substantial. The flatworm is widely established in gardens and as a soil-dwelling species difficult to target with treatments. In addition, there are no known pesticide treatments or biological control agents. Management therefore would rely on greater plant health inspections to prevent further spread, and physical barriers and heat treatment at nurseries and garden centres, as has been recommended for *A. triangulatus* (EPPO, 2001). Research would also be required into novel approaches for management. This would likely be more than 1m euros across the risk assessment area each year.

Social and human health impacts

Qu. 4.14. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism for the risk assessment area and for third countries, if relevant (e.g. with similar eco-climatic conditions).

The description of the known impact and the assessment of potential future impact on human health, safety and the economy, shall, if relevant, include information on

- illnesses, allergies or other affections to humans that may derive directly or indirectly from a species;
- damages provoked directly or indirectly by a species with consequences for the safety of people, property or infrastructure;
- direct or indirect disruption of, or other consequences for, an economic or social activity due to the presence of a species.

Social and human health impacts can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: The presence of *O. nungara* in gardens would be distressing to gardeners in a local affected area, as they see this species as a harmful and insidious alien species. This would be a mild short-term upset in most cases. Presence of *O. nungara* in nursery produce could result in reputational damage for nurseries or garden centres.

Qu. 4.15. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism in the future for the risk assessment area.

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable).

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Another snail-eating flatworm, *P. manokwari*, is a paratenic host of parasitic nematodes, *Angiostrongylus* spp. (rat lungworms) (Asato *et al.*, 2004) which have snails as their intermediate host, so there is potential that *O. nungara* could also host and transmit parasites (Negrete *et al.*, 2020). Flatworms, as snail predators, may bioaccumulate nematodes in their tissues and if consumed by humans lead to the potentially fatal condition of angiostrongyliasis. However, human angiostrongyliasis is recorded mainly in tropical and subtropical regions (Barratt *et al.*, 2016) and there is no evidence to date (no studies) that *O. nungara* has hosted lungworm parasites.

Terrestrial flatworms feed by secreting neurotoxins to subdue prey (Stokes *et al.*, 2014) and digestive enzymes to break down prey tissue. Exposure to these through handling of flatworms may cause skin irritation (Blackshaw and Stewart, 1992). However, this is felt mostly as mild dermabrasion.

Due to the lack of data on this topic and that angiostrongyliasis is mainly a tropical disease, which is unlikely to arise in the risk assessment area, the score is ‘minor’ with ‘low’ confidence.

Other impacts

Qu. 4.16. How important is the organism in facilitating other damaging organisms (e.g. diseases) as food source, a host, a symbiont or a vector etc.?

RESPONSE	N/A minimal minor	CONFIDENCE	low medium high
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	moderate major massive		
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Response: No information has been found on the issue.

As mentioned in Qu. 4.15, *O. nungara* could potentially carry nematodes and other parasitic organisms (Negrete *et al.*, 2020). It is possible that flatworms could increase the likelihood of heart lungworm in dogs (*Angiostrongylus vasorum*) but there is no evidence or information for this.

Qu. 4.17. How important might other impacts not already covered by previous questions be resulting from introduction of the organism?

RESPONSE	N/A minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No information has been found on the issue.

Qu. 4.18. How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area?

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: *Obama nungara* may be predated upon by native birds, shrews and insects, but there are no specific predators likely to provide population control (Justine and Winsor, 2020). Similarly, there are no known parasites or pathogens capable of limiting *O. nungara* populations, that we are aware of, either in the flatworm's natural range or in the invaded regions.

There is a lack of data on the impact of *O. nungara* and the score of 'major' is based on expert opinion and hence confidence is 'low'.

Qu. 4.19. Estimate the overall impact in the risk assessment area under current climate conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, in current conditions.

RESPONSE	Minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: *Obama nungara* was first discovered in Europe in 2008 but the species was only described in 2016 (Carbayo *et al.*, 2016). The most comprehensive assessment of *O. nungara*'s distribution in Europe was published in 2020 (Justine *et al.*, 2020). There are therefore relatively few scientific papers on *O. nungara* compared to other flatworm species such as *A. triangulatus*, *P. manokwari* and *B. kewense*.

Aside from a laboratory assessment of *O. nungara*'s predatory capabilities from Brazil (Boll and Leal-Zanchet, 2016), there are no European or field assessments of the impact on soil fauna by *O. nungara*. Assessing the impact of *O. nungara* on biodiversity and ecosystem services, the economy and human health, therefore relies heavily on information from other similar species, principally *A. triangulatus* and *P. manokwari*.

Some factors are however clear. *Obama nungara* is well established in the risk assessment area and is spreading. *Obama nungara* is synanthropic and ecologically suited to man-disturbed habitats. The flatworm can achieve high local densities. One gardener in south-western France collected 924 specimens from an area of c. 300 m² over the course of the summer; whereas another gardener in north-western France collected 1,442 specimens from 175 m² (Justine *et al.*, 2020). *Obama nungara* is a predator of molluscs and earthworms (Boll and Leal-Zanchet, 2016) and this flexibility of prey utilization may allow greater niche expansion than if it was limited to just one food source. This broad prey range could either alleviate the predatory impact of *O. nungara* on any particular species or worsen it. If *O. nungara* feeds on many prey species then the impact on any one particular species may be lessened. Alternatively, *A. triangulatus* preferentially predate anecic earthworms but was able to sustain itself on other earthworm species, thereby circumventing the normal predator / prey cycles, and having a greater impact on the anecic earthworm species (Murchie and Gordon, 2013).

Whilst data are very limited, there is potential that *O. nungara* could have the same impact in continental Europe as *A. triangulatus* has had in regions of the UK, Ireland and Faroe Islands. This is very likely to lead to a decline in earthworm species, with knock-on effects on pasture yield and earthworm feeding wildlife as well as other soil fauna that fall within its prey range. This is likely to be most damaging in the Atlantic biogeographic region, as this region is most suited to flatworm establishment coupled with a relatively high proportion of permanent grassland. *Obama nungara* also has potential to impact on endemic snails, particularly in the Mediterranean biogeographic region, with a potential extinction risk

Scoring the impact of *O. nungara* in the risk assessment area is problematic due to the relatively recent introduction of the flatworm and the lack of scientific studies on it in the field in Europe. Given the densities that can be achieved by *O. nungara* in France, its broad prey range and the known impacts of other similar flatworm species with particular reference to rare and highly-localised endemic species (snails, slugs and earthworms species) that could be driven to extinction, expert opinion assessed the impact as ‘major’ but with ‘low’ confidence.

Qu. 4.20. Estimate the overall impact in the risk assessment area in foreseeable climate change conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, under future conditions.

- See also guidance to Qu. 4.3.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Climate change predictions suggest that *O. nungara* could establish in substantially more regions of the risk assessment area and extend its range northwards and eastwards (Annex VIII). In the Atlantic biogeographic region this may enable more pasture to be affected (including in Germany, the Netherlands and Denmark). There is some contraction of the predicted range in southern regions but it is comparatively small compared to the expansion. The impacts on biodiversity, ecosystem services and human health are likely to increase under climate change and mostly occur in the same biogeographic regions.

RISK SUMMARIES			
	RESPONSE	CONFIDENCE	COMMENT
Summarise Introduction and Entry*	very unlikely unlikely moderately likely likely likely very likely	low medium high	<i>Obama nungara</i> has already been introduced to the risk assessment area. The most likely pathway was the plant trade, in particular plants for planting.
Summarise Establishment*	very unlikely unlikely moderately likely likely likely very likely	low medium high	<i>Obama nungara</i> has definitely established in France, Belgium, Spain, Portugal and Italy, and probably other countries in the risk assessment area. Climate change will substantially increase the regions in the risk assessment area suitable for flatworm establishment.
Summarise Spread*	very slowly slowly moderately rapidly very rapidly	low medium high	<i>Obama nungara</i> is spread by human-assisted transport followed by natural dispersal. Flatworms can be inadvertently spread with plants for planting, soil or compost, and equipment that has been in contact with the soil surface. First detected in France in 2013, <i>O. nungara</i> has spread to 72 of the 96 administrative Departments in Metropolitan France within less than a decade.
Summarise Impact*	minimal minor moderate major massive	low medium high	Little is known about the impact of <i>O. nungara</i> as a predator of soil fauna, given its comparatively recent establishment in Europe. <i>Obama nungara</i> is a predator of earthworms and molluscs, and can occur in large numbers. The species therefore could potentially reduce both mollusc and earthworm biodiversity and densities, including endemic European species, with a risk of extinction of rare isolated species. Reductions of earthworm densities can have negative effect on ecosystem services and knock-on effects on pasture productivity. Climate change will probably increase the impact of <i>O. nungara</i> in the risk assessment area.
Conclusion of the risk assessment (overall risk)	low moderate high	low medium high	<i>Obama nungara</i> has established in the risk assessment area and is spreading. In some domestic gardens it has achieved high densities. It is a predator of molluscs and earthworms with no known natural enemies or means of control other than phytosanitary measures. There is a high risk that <i>O. nungara</i> could impact

			<p>biodiversity in a similar way to the invasive flatworm <i>Arthurdendyus triangulatus</i> and deplete native/endemic snail and earthworm populations within the risk assessment area with possible extinctions of rare species. However, there have been no studies on this to date.</p>
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*in current climate conditions and in foreseeable future climate conditions

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Distribution Summary

Please answer as follows:

- Yes if recorded, established or invasive
- if not recorded, established or invasive
- ? Unknown; data deficient

The columns refer to the answers to Questions A5 to A12 under Section A.

For data on marine species at the Member State level, delete Member States that have no marine borders.

In all other cases, provide answers for all columns.

Member States and the United Kingdom

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Austria	Yes	?	No	Yes	
Belgium	Yes	Yes	Yes	Yes	?
Bulgaria			?	?	
Croatia			Yes	Yes	
Cyprus			No	No	
Czech Republic			No	Yes	
Denmark			No	Yes	
Estonia			No	No	
Finland			No	No	
France	Yes	Yes	Yes	Yes	Yes
Germany	Yes	?	Yes	Yes	?
Greece			Yes	Yes	
Hungary			No	Yes	
Ireland	Yes	?	No	Yes	
Italy	Yes	Yes	Yes	Yes	?
Latvia			No	?	
Lithuania			No	?	
Luxembourg			Yes	Yes	
Malta					
Netherlands	Yes	?	Yes	Yes	?
Poland			No	Yes	
Portugal	Yes	Yes	Yes	Yes	?
Romania			No	?	
Slovakia	Yes		?	Yes	
Slovenia			Yes	Yes	
Spain	Yes	Yes	Yes	Yes	?
Sweden			No	Yes	
United Kingdom	Yes	?	Yes	Yes	?

Biogeographical regions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Alpine			?	Yes	
Atlantic	Yes	Yes	Yes	Yes	Yes
Black Sea			Yes	Yes	
Boreal			No	?	
Continental	Yes	Yes	Yes	Yes	
Mediterranean	Yes	Yes	Yes	Yes	Yes
Pannonian			?	Yes	
Steppic			No	?	

ANNEX I Scoring of Likelihoods of Events

(taken from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Description	Frequency
Very unlikely	This sort of event is theoretically possible, but is never known to have occurred and is not expected to occur	1 in 10,000 years
Unlikely	This sort of event has occurred somewhere at least once in the last millennium	1 in 1,000 years
Moderately likely	This sort of event has occurred somewhere at least once in the last century	1 in 100 years
Likely	This sort of event has happened on several occasions elsewhere, or on at least once in the last decade	1 in 10 years
Very likely	This sort of event happens continually and would be expected to occur	Once a year

ANNEX II Scoring of Magnitude of Impacts

(modified from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Biodiversity and ecosystem impact	Ecosystem Services impact	Economic impact (Monetary loss and response costs per year)	Social and human health impact, and other impacts
	<i>Question 5.1-5</i>	<i>Question 5.6-8</i>	<i>Question 5.9-13</i>	<i>Question 5.14-18</i>
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected ⁵	Up to 10,000 Euro	No social disruption. Local, mild, short-term reversible effects to individuals.
Minor	Some ecosystem impact, reversible changes, localised	Local and temporary, reversible effects to one or few services	10,000-100,000 Euro	Significant concern expressed at local level. Mild short-term reversible effects to identifiable groups, localised.
Moderate	Measureable long-term damage to populations and ecosystem, but reversible; little spread, no extinction	Measureable, temporary, local and reversible effects on one or several services	100,000-1,000,000 Euro	Temporary changes to normal activities at local level. Minor irreversible effects and/or larger numbers covered by reversible effects, localised.
Major	Long-term irreversible ecosystem change, spreading beyond local area	Local and irreversible or widespread and reversible effects on one / several services	1,000,000-10,000,000 Euro	Some permanent change of activity locally, concern expressed over wider area. Significant irreversible effects locally or reversible effects over large area.
Massive	Widespread, long-term population loss or extinction, affecting several species with serious ecosystem effects	Widespread and irreversible effects on one / several services	Above 10,000,000 Euro	Long-term social change, significant loss of employment, migration from affected area. Widespread, severe, long-term, irreversible health effects.

⁵ Not to be confused with “no impact”.

ANNEX III Scoring of Confidence Levels

(modified from Bacher et al. 2017)

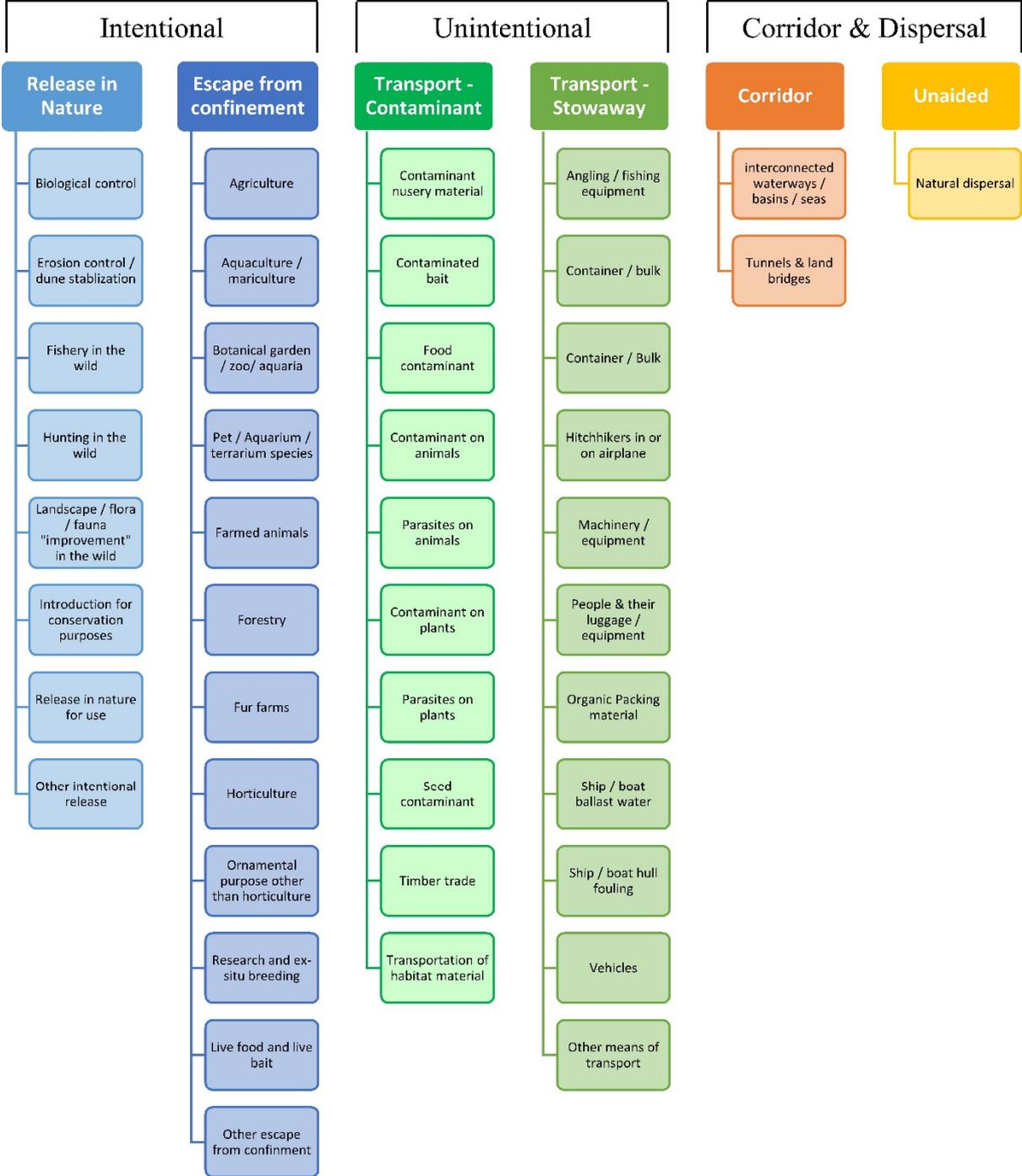
Each answer provided in the risk assessment must include an assessment of the level of confidence attached to that answer, reflecting the possibility that information needed for the answer is not available or is insufficient or available but conflicting.

The responses in the risk assessment should clearly support the choice of the confidence level.

Confidence level	Description
Low	There is no direct observational evidence to support the assessment, e.g. only inferred data have been used as supporting evidence <i>and/or</i> Impacts are recorded at a spatial scale which is unlikely to be relevant to the assessment area <i>and/or</i> Evidence is poor and difficult to interpret, e.g. because it is strongly ambiguous <i>and/or</i> The information sources are considered to be of low quality or contain information that is unreliable.
Medium	There is some direct observational evidence to support the assessment, but some information is inferred <i>and/or</i> Impacts are recorded at a small spatial scale, but rescaling of the data to relevant scales of the assessment area is considered reliable, or to embrace little uncertainty <i>and/or</i> The interpretation of the data is to some extent ambiguous or contradictory.
High	There is direct relevant observational evidence to support the assessment (including causality) <i>and</i> Impacts are recorded at a comparable scale <i>and/or</i> There are reliable/good quality data sources on impacts of the taxa <i>and</i> The interpretation of data/information is straightforward <i>and/or</i> Data/information are not controversial or contradictory.

ANNEX IV CBD pathway categorisation scheme

Overview of CBD pathway categorisation scheme showing how the 44 pathways relate to the six main pathway categories. All of the pathways can be broadly classified into 1) those that involve intentional transport (blue), 2) those in which the taxa are unintentionally transported (green) and 3) those where taxa moved between regions without direct transportation by humans and/or via artificial corridors (orange and yellow). **Note that the pathways in the category “Escape from confinement” can be considered intentional for the introduction into the risk assessment area and unintentional for the entry into the environment.**



ANNEX V Ecosystem services classification (CICES V5.1, simplified) and examples

For the purposes of this risk assessment, please feel free to use what seems as the most appropriate category / level / combination of impact (Section – Division – Group), reflecting information available.

Section	Division	Group	Examples (i.e. relevant CICES “classes”)
Provisioning	Biomass	Cultivated <i>terrestrial</i> plants	<p>Cultivated terrestrial plants (including fungi, algae) grown for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from cultivated plants, fungi, algae and bacteria for direct use or processing (excluding genetic materials); Cultivated plants (including fungi, algae) grown as a <u>source of energy</u></p> <p><i>Example: negative impacts of non-native organisms to crops, orchards, timber etc.</i></p>
		Cultivated <i>aquatic</i> plants	<p>Plants cultivated by in- situ aquaculture grown for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from in-situ aquaculture for direct use or processing (excluding genetic materials); Plants cultivated by in- situ aquaculture grown as an <u>energy source</u>.</p> <p><i>Example: negative impacts of non-native organisms to aquatic plants cultivated for nutrition, gardening etc. purposes.</i></p>
		Reared animals	<p>Animals reared for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from reared animals for direct use or processing (excluding genetic materials); Animals reared to provide <u>energy</u> (including mechanical)</p> <p><i>Example: negative impacts of non-native organisms to livestock</i></p>
		Reared <i>aquatic</i> animals	<p>Animals reared by in-situ aquaculture for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from animals grown by in-situ aquaculture for direct use or processing (excluding genetic materials); Animals reared by in-situ aquaculture as an <u>energy source</u></p> <p><i>Example: negative impacts of non-native organisms to fish farming</i></p>
		Wild plants (terrestrial and aquatic)	<p>Wild plants (terrestrial and aquatic, including fungi, algae) used for <u>nutrition</u>; <u>Fibres and other materials</u> from wild plants for direct use or processing (excluding genetic materials); Wild plants (terrestrial and aquatic, including fungi, algae) used as a <u>source of energy</u></p> <p><i>Example: reduction in the availability of wild plants (e.g. wild berries, ornamentals) due to non-native organisms (competition, spread of disease etc.)</i></p>
		Wild animals (terrestrial and aquatic)	<p>Wild animals (terrestrial and aquatic) used for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from wild animals for direct use or processing (excluding genetic materials); Wild animals (terrestrial and aquatic) used as a <u>source of energy</u></p>

			<i>Example: reduction in the availability of wild animals (e.g. fish stocks, game) due to non-native organisms (competition, predations, spread of disease etc.)</i>
	Genetic material from all biota	Genetic material from plants, algae or fungi	<u>Seeds, spores and other plant materials</u> collected for maintaining or establishing a population; Higher and lower plants (whole organisms) used to <u>breed new strains or varieties</u> ; Individual genes extracted from higher and lower plants for the <u>design and construction of new biological entities</u> <i>Example: negative impacts of non-native organisms due to interbreeding</i>
		Genetic material from animals	Animal material collected for the purposes of maintaining or establishing a population; Wild animals (whole organisms) used to breed new strains or varieties; Individual genes extracted from organisms for the design and construction of new biological entities <i>Example: negative impacts of non-native organisms due to interbreeding</i>
	Water ⁶	Surface water used for nutrition, materials or energy	Surface water for <u>drinking</u> ; Surface water used as a material (<u>non-drinking purposes</u>); Freshwater surface water, coastal and marine water used as an <u>energy source</u> <i>Example: loss of access to surface water due to spread of non-native organisms</i>
		Ground water for used for nutrition, materials or energy	Ground (and subsurface) water for <u>drinking</u> ; Ground water (and subsurface) used as a material (<u>non-drinking purposes</u>); Ground water (and subsurface) used as an <u>energy source</u> <i>Example: reduced availability of ground water due to spread of non-native organisms and associated increase of ground water consumption by vegetation.</i>
Regulation & Maintenance	Transformation of biochemical or physical inputs to ecosystems	Mediation of wastes or toxic substances of anthropogenic origin by living processes	<u>Bio-remediation</u> by micro-organisms, algae, plants, and animals; <u>Filtration/sequestration/storage/accumulation</u> by micro-organisms, algae, plants, and animals <i>Example: changes caused by non-native organisms to ecosystem functioning and ability to filtrate etc. waste or toxics</i>
		Mediation of nuisances of anthropogenic origin	<u>Smell reduction</u> ; <u>noise attenuation</u> ; <u>visual screening</u> (e.g. by means of green infrastructure) <i>Example: changes caused by non-native organisms to ecosystem structure, leading to reduced ability to mediate nuisances.</i>
	Regulation of physical, chemical, biological conditions	Baseline flows and extreme event regulation	Control of <u>erosion</u> rates; Buffering and attenuation of <u>mass movement</u> ; <u>Hydrological cycle and water flow regulation</u> (Including flood control, and coastal protection); <u>Wind</u> protection; <u>Fire</u> protection

⁶ Note: in the CICES classification provisioning of water is considered as an abiotic service whereas the rest of ecosystem services listed here are considered biotic.

			<p><i>Example: changes caused by non-native organisms to ecosystem functioning or structure leading to, for example, destabilisation of soil, increased risk or intensity of wild fires etc.</i></p>
		<p>Lifecycle maintenance, habitat and gene pool protection</p>	<p>Pollination (or 'gamete' dispersal in a marine context); <u>Seed dispersal</u>; Maintaining <u>nursery populations and habitats</u> (Including gene pool protection)</p> <p><i>Example: changes caused by non-native organisms to the abundance and/or distribution of wild pollinators; changes to the availability / quality of nursery habitats for fisheries</i></p>
		<p>Pest and disease control</p>	<p>Pest control; Disease control</p> <p><i>Example: changes caused by non-native organisms to the abundance and/or distribution of pests</i></p>
		<p>Soil quality regulation</p>	<p><u>Weathering processes</u> and their effect on soil quality; <u>Decomposition and fixing processes</u> and their effect on soil quality</p> <p><i>Example: changes caused by non-native organisms to vegetation structure and/or soil fauna leading to reduced soil quality</i></p>
		<p>Water conditions</p>	<p>Regulation of the <u>chemical condition</u> of freshwaters by living processes; Regulation of the chemical condition of salt waters by living processes</p> <p><i>Example: changes caused by non-native organisms to buffer strips along water courses that remove nutrients in runoff and/or fish communities that regulate the resilience and resistance of water bodies to eutrophication</i></p>
		<p>Atmospheric composition and conditions</p>	<p>Regulation of <u>chemical composition</u> of atmosphere and oceans; Regulation of <u>temperature and humidity</u>, including ventilation and transpiration</p> <p><i>Example: changes caused by non-native organisms to ecosystems' ability to sequester carbon and/or evaporative cooling (e.g. by urban trees)</i></p>
Cultural	Direct, in-situ and outdoor interactions with living systems that depend on presence in the environmental setting	<p>Physical and experiential interactions with natural environment</p>	<p>Characteristics of living systems that that enable activities promoting health, recuperation or enjoyment through <u>active or immersive interactions</u>;</p> <p>Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through <u>passive or observational interactions</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that make it attractive for recreation, wild life watching etc.</i></p>
		<p>Intellectual and representative interactions with natural environment</p>	<p>Characteristics of living systems that enable <u>scientific investigation</u> or the creation of traditional ecological knowledge;</p> <p>Characteristics of living systems that enable <u>education and training</u>;</p> <p>Characteristics of living systems that are resonant in terms of <u>culture or heritage</u>;</p> <p>Characteristics of living systems that enable <u>aesthetic experiences</u></p>

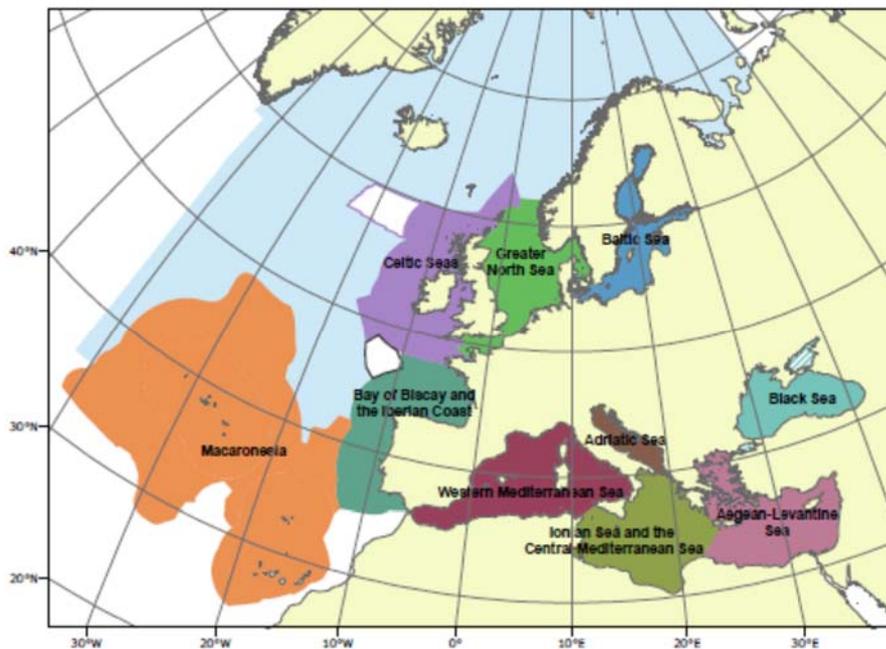
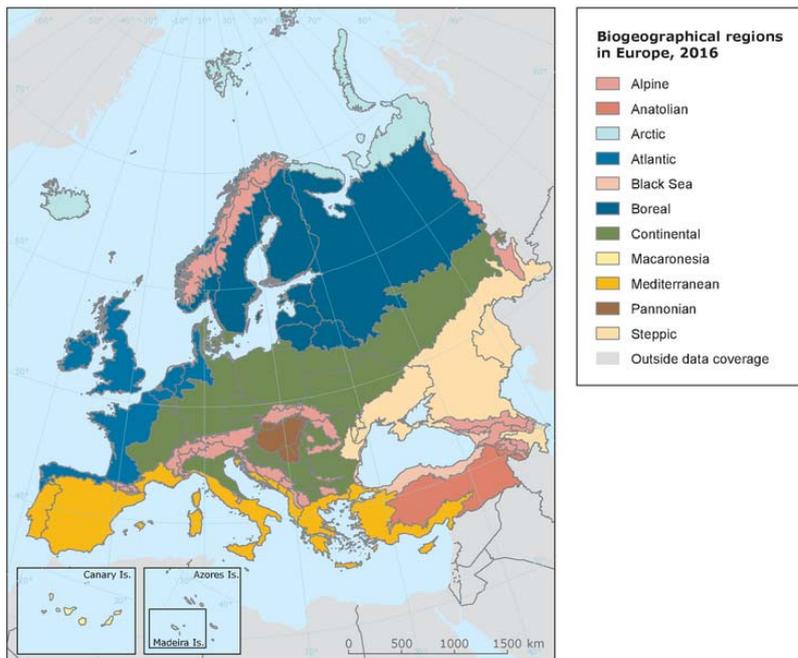
			<i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have cultural importance</i>
	Indirect, remote, often indoor interactions with living systems that do not require presence in the environmental setting	Spiritual, symbolic and other interactions with natural environment	Elements of living systems that have <u>symbolic meaning</u> ; Elements of living systems that have <u>sacred or religious meaning</u> ; Elements of living systems used for <u>entertainment or representation</u> <i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have sacred or religious meaning</i>
		Other biotic characteristics that have a non-use value	Characteristics or features of living systems that have an <u>existence value</u> ; Characteristics or features of living systems that have an <u>option or bequest value</u> <i>Example: changes caused by non-native organisms to ecosystems designated as wilderness areas, habitats of endangered species etc.</i>

ANNEX VI EU Biogeographic Regions and MSFD Subregions

See <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2> ,
http://ec.europa.eu/environment/nature/natura2000/biogeog_regions/

and

<https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions-1/technical-document/pdf>



ANNEX VII Delegated Regulation (EU) 2018/968 of 30 April 2018

see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>

ANNEX VIII Projection of environmental suitability for *Obama nungara* establishment in Europe

Björn Beckmann, Archie Murchie, Leigh Winsor, Jean-Lou Justine and Dan Chapman

21 September 2021

Aim

To project the suitability for potential establishment of *Obama nungara* in Europe, under current and predicted future climatic conditions.

Data for modelling

Species occurrence data were obtained from the Global Biodiversity Information Facility (GBIF) (543 records), iNaturalist (79 records), and additional records from the risk assessment team. We scrutinised occurrence records from regions where the species is not known to be established and removed any dubious records or where the georeferencing was too imprecise (e.g. records referenced to a country or island centroid) or outside of the coverage of the predictor layers (e.g. small island or coastal occurrences). The remaining records were gridded at a 0.25 x 0.25 degree resolution for modelling, yielding 233 grid cells with occurrences (Figure 1a). As a proxy for recording effort, the density of Platyhelminthes records held by GBIF was also compiled on the same grid (Figure 1b).

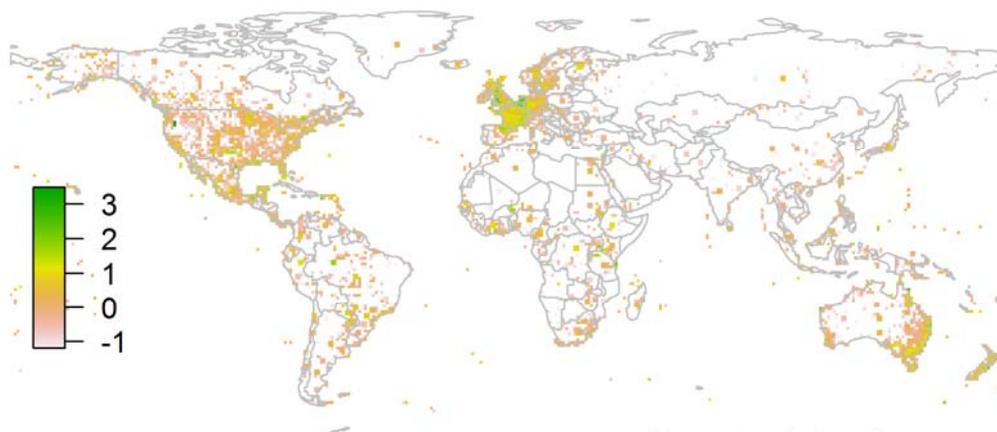
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Figure 1. (a) Occurrence records obtained for *Obama nungara* and used in the modelling, showing native and invaded distributions. (b) The recording density of Platyhelminthes on GBIF, which was used as a proxy for recording effort.

(a) Species distribution used in modelling



(b) Estimated recording effort (log10-scaled)



Climate data were selected from the 'Bioclim' variables contained within the WorldClim database (Hijmans et al., 2005), originally at 5 arcminute resolution (0.083 x 0.083 degrees of longitude/latitude) and aggregated to a 0.25 x 0.25 degree grid for use in the model.

Based on the biology of *Obama nungara*, the following climate variables were used in the modelling:

- Minimum temperature of the coldest month (Bio6)
- Mean temperature of the warmest quarter (Bio10)
- Annual precipitation (Bio12)
- Precipitation seasonality (Bio15)
- Climatic moisture index (CMI): ratio of mean annual precipitation to potential evapotranspiration, log+1 transformed. For its calculation, monthly potential evapotranspirations were estimated from the WorldClim monthly temperature data and solar

radiation using the simple method of Zomer et al. (2008) which is based on the Hargreaves evapotranspiration equation (Hargreaves, 1994).

To estimate the effect of climate change on the potential distribution, equivalent modelled future climate conditions for the 2070s under the Representative Concentration Pathways (RCP) 2.6 and 4.5 were also obtained. These represent low and medium emissions scenarios, respectively. The above variables were obtained as averages of outputs of eight Global Climate Models (BCC-CSM1-1, CCSM4, GISS-E2-R, HadGEM2-AO, IPSL-CM5A-LR, MIROC-ESM, MRI-CGCM3, NorESM1-M), downscaled and calibrated against the WorldClim baseline (see http://www.worldclim.org/cmip5_5m).

The following habitat layers were also used:

- Human influence index (HII): As many non-native invasive species associate with anthropogenically disturbed habitats. We used the Global Human Influence Index Dataset of the Last of the Wild Project (Wildlife Conservation Society - WCS & Center for International Earth Science Information Network - CIESIN - Columbia University, 2005), which is developed from nine global data layers covering human population pressure (population density), human land use and infrastructure (built-up areas, nighttime lights, land use/land cover) and human access (coastlines, roads, railroads, navigable rivers). The index ranges between 0 and 1 and was $\ln+1$ transformed for the modelling to improve normality.

Species distribution model

A presence-background (presence-only) ensemble modelling strategy was employed using the BIOMOD2 R package version 3.4.6 (Thuiller et al., 2020, Thuiller et al., 2009). These models contrast the environment at the species' occurrence locations against a random sample of the global background environmental conditions (often termed 'pseudo-absences') in order to characterise and project suitability for occurrence. This approach has been developed for distributions that are in equilibrium with the environment. Because invasive species' distributions are not at equilibrium and subject to dispersal constraints at a global scale, we took care to minimise the inclusion of locations suitable for the species but where it has not been able to disperse to (Chapman et al. 2019).

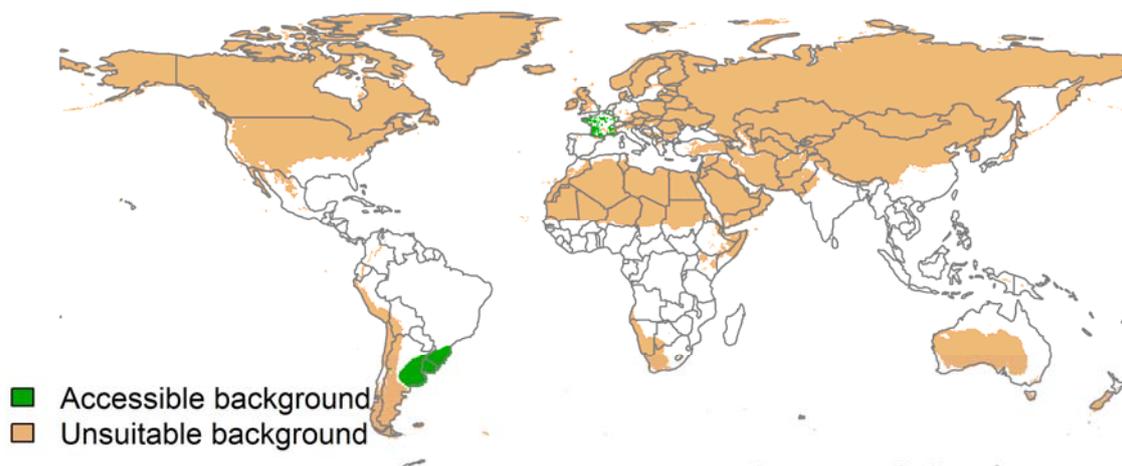
Therefore the background sampling region included:

- The area accessible by native *Obama nungara* populations, in which the species is likely to have had sufficient time to disperse to all locations. Based on presumed maximum dispersal distances, the accessible region was defined as a 300km buffer around the native range occurrences; AND
- A 30km buffer around the non-native occurrences, encompassing regions likely to have had high propagule pressure for introduction by humans and/or dispersal of the species; AND
- Regions where we have an *a priori* expectation of high unsuitability for the species so that absence is assumed irrespective of dispersal constraints (see Figure 2). The following rules were applied to define a region expected to be highly unsuitable for *Obama nungara* at the spatial scale of the model:
 - Minimum temperature of the coldest month (Bio6) < -4°C
 - Mean temperature of the warmest quarter (Bio10) < 15.5°C
 - Annual precipitation (Bio12) < 350mm

Altogether, 0.9% of occurrence grid cells were located in the unsuitable background region.

Within the unsuitable background region, 10 samples of 5000 randomly sampled grid cells were obtained. In the accessible background (comprising the accessible areas around native and non-native occurrences as detailed above), the same number of pseudo-absence samples were drawn as there were presence records (233), weighting the sampling by a proxy for recording effort (Figure 1(b)).

Figure 2. The background from which pseudo-absence samples were taken in the modelling of *Obama nungara*. Samples were taken from a 300km buffer around the native range and a 30km buffer around non-native occurrences (together forming the accessible background), and from areas expected to be highly unsuitable for the species (the unsuitable background region). Samples from the accessible background were weighted by a proxy for recording effort (Figure 1(b)).



Each dataset (i.e. combination of the presences and the individual background samples) was randomly split into 80% for model training and 20% for model evaluation. With each training dataset, seven statistical algorithms were fitted with the default BIOMOD2 settings and rescaled using logistic regression, except where specified below:

- Generalised linear model (GLM)
- Generalised boosting model (GBM)
- Generalised additive model (GAM) with a maximum of four degrees of freedom per smoothing spline
- Artificial neural network (ANN)
- Multivariate adaptive regression splines (MARS)
- Random forest (RF)
- Maxent

Since the total background sample was larger than the number of occurrences, prevalence fitting weights were applied to give equal overall importance to the occurrences and the background. Normalised variable importance was assessed and variable response functions were produced using BIOMOD2's default procedure.

Model predictive performance was assessed by the following three measures:

- AUC, the area under the receiver operating characteristic curve (Fielding & Bell 1997). Predictions of presence-absence models can be compared with a subset of records set aside for model evaluation (here 20%) by constructing a confusion matrix with the number of true positive, false positive, false negative and true negative cases. For models generating non-dichotomous scores (as here) a threshold can be applied to transform the scores into a dichotomous set of presence-absence predictions. Two measures that can be derived from the confusion matrix are sensitivity (the proportion of observed presences that are predicted as such, quantifying omission errors), and specificity (the proportion of observed absences that are predicted as such, quantifying commission errors). A receiver operating characteristic (ROC) curve can be constructed by using all possible thresholds to classify the scores into confusion matrices, obtaining sensitivity and specificity for each matrix, and plotting sensitivity against the corresponding proportion of false positives (equal to $1 - \text{specificity}$). The use of all possible thresholds avoids the need for a selection of a single threshold, which is often arbitrary, and allows appreciation of the trade-off between sensitivity and specificity. The area under the ROC curve (AUC) is often used as a single threshold-independent measure for model performance (Manel et al. 2001). AUC is the probability that a randomly selected presence has a higher model-predicted suitability than a randomly selected absence (Allouche et al. 2006).
- Cohen's Kappa (Cohen 1960). This measure corrects the overall accuracy of model predictions (ratio of the sum of true presences plus true absences to the total number of records) by the accuracy expected to occur by chance. The Kappa statistic ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random. Advantages of Kappa are its simplicity, the fact that both commission and omission errors are accounted for in one parameter, and its relative tolerance to zero values in the confusion matrix (Manel et al. 2001). However, Kappa has been criticised for being sensitive to prevalence (the proportion of sites in which the species was recorded as present) and may therefore be inappropriate for comparisons of model accuracy between species or regions (McPherson et al. 2004, Allouche et al. 2006).
- TSS, the true skill statistic (Allouche et al. 2006). TSS is defined as sensitivity + specificity - 1, and corrects for Kappa's dependency on prevalence. TSS compares the number of correct forecasts, minus those attributable to random guessing, to that of a hypothetical set of perfect forecasts. Like Kappa, TSS takes into account both omission and commission errors, and success as a result of random guessing, and ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random (Allouche et al. 2006).

An ensemble model was created by first rejecting poorly performing algorithms with relatively extreme low AUC values and then averaging the predictions of the remaining algorithms, weighted by their AUC. To identify poorly performing algorithms, AUC values were converted into modified z-scores based on their difference to the median and the median absolute deviation across all algorithms (Iglewicz & Hoaglin 1993). Algorithms with $z < -2$ were rejected. In this way, ensemble projections were made for each dataset and then averaged to give an overall suitability, as well as its standard deviation.

Projections were classified into suitable and unsuitable regions using a “lowest presence threshold” (Pearson et al. 2007), setting the cut-off as the lowest value at which 98% of all presence records are classified correctly under the current climate (here 0.71). In order to express the sensitivity of classifications to the choice of this threshold, thresholds at which 95% and 99% of records are classified correctly (here 0.79 and 0.62 respectively) were used in the calculation of error bars in Figures 9 and 10 below in addition to taking account of uncertainty in the projections themselves.

We also produced a limiting factor map for Europe following Elith et al. (2010). For this, projections were made separately with each individual variable fixed at a near-optimal value. These were chosen as the median values at the occurrence grid cells. Then, the most strongly limiting factors were identified as the ones resulting in the highest increase in suitability in each grid cell.

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Results

The ensemble model suggested that suitability for *Obama nungara* was most strongly determined by Minimum temperature of the coldest month (Bio6), accounting for 42.3% of variation explained, followed by Human influence index (HII) (21.8%), Mean temperature of the warmest quarter (Bio10) (15.9%), Annual precipitation (Bio12) (11.8%), Climatic moisture index (CMI) (5.1%) and Precipitation seasonality (Bio15) (3.2%) (Table 1, Figure 3).

Table 1. Summary of the cross-validation predictive performance (AUC, Kappa, TSS) and variable importance of the fitted model algorithms and the ensemble (AUC-weighted average of the best performing algorithms). Results are the average from models fitted to 10 different background samples of the data.

Algorithm	AUC	Kappa	TSS	Used in the ensemble	Variable importance (%)					
					Minimum temperature of the coldest month (Bio6)	Human influence index (HII)	Mean temperature of the warmest quarter (Bio10)	Annual precipitation (Bio12)	Climatic moisture index (CMI)	Precipitation seasonality (Bio15)
GLM	0.979	0.655	0.939	yes	37	26	16	14	3	5
GAM	0.975	0.657	0.937	yes	29	23	15	23	5	6
GBM	0.972	0.658	0.934	yes	40	35	17	6	1	1
ANN	0.979	0.660	0.942	yes	43	22	26	5	2	2
MARS	0.980	0.649	0.936	yes	55	10	18	3	15	0
RF	0.971	0.619	0.926	yes	53	16	7	18	4	4
Maxent	0.972	0.647	0.915	yes	41	21	12	14	7	5
Ensemble	0.977	0.654	0.940		42	22	16	12	5	3

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Figure 3. Partial response plots from the fitted models. Thin coloured lines show responses from the algorithms in the ensemble, while the thick black line is their ensemble. In each plot, other model variables are held at their median value in the training data. Some of the divergence among algorithms is because of their different treatment of interactions among variables.

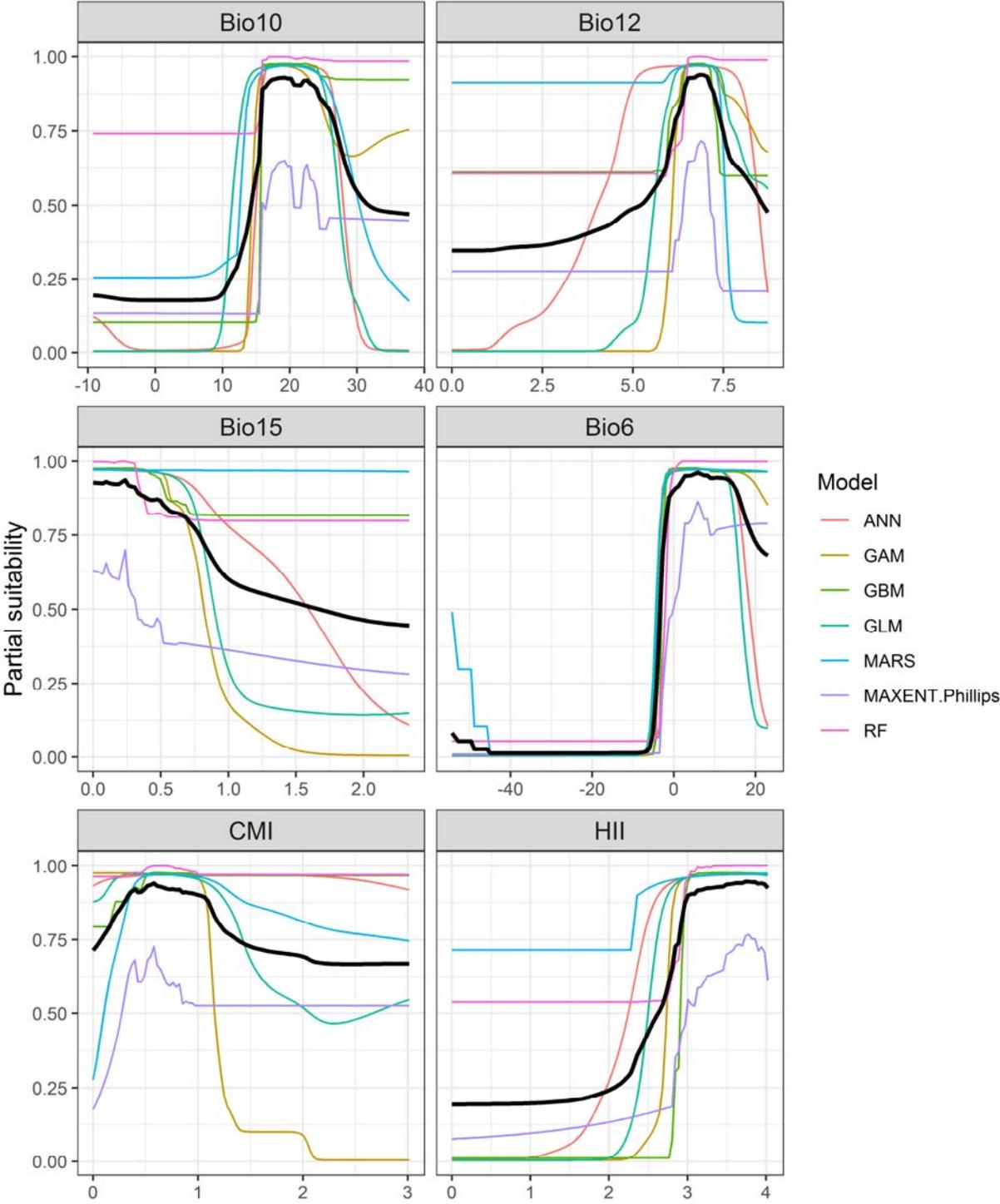
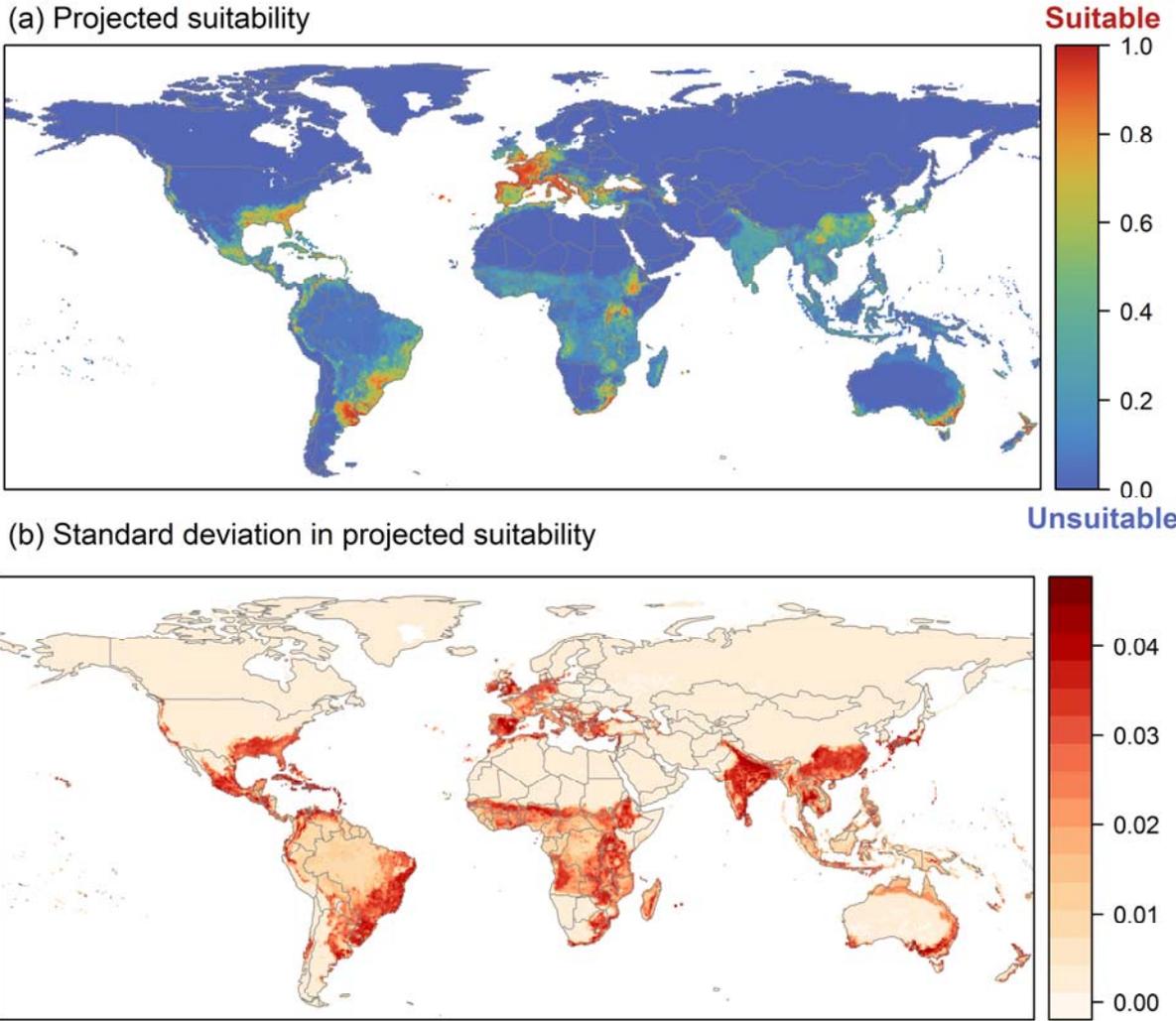


Figure 4. (a) Projected global suitability for *Obama nungara* establishment in the current climate. For visualisation, the projection has been aggregated to a 0.5 x 0.5 degree resolution, by taking the maximum suitability of constituent higher resolution grid cells. Values > 0.71 are suitable for the species, with 98% of global presence records above this threshold. Values below 0.71 indicate lower relative suitability. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.



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Figure 5. (a) Projected current suitability for *Obama nungara* establishment in Europe and the Mediterranean region. Values > 0.71 are suitable for the species, with 98% of global presence records above this threshold. Values below 0.71 indicate lower relative suitability. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

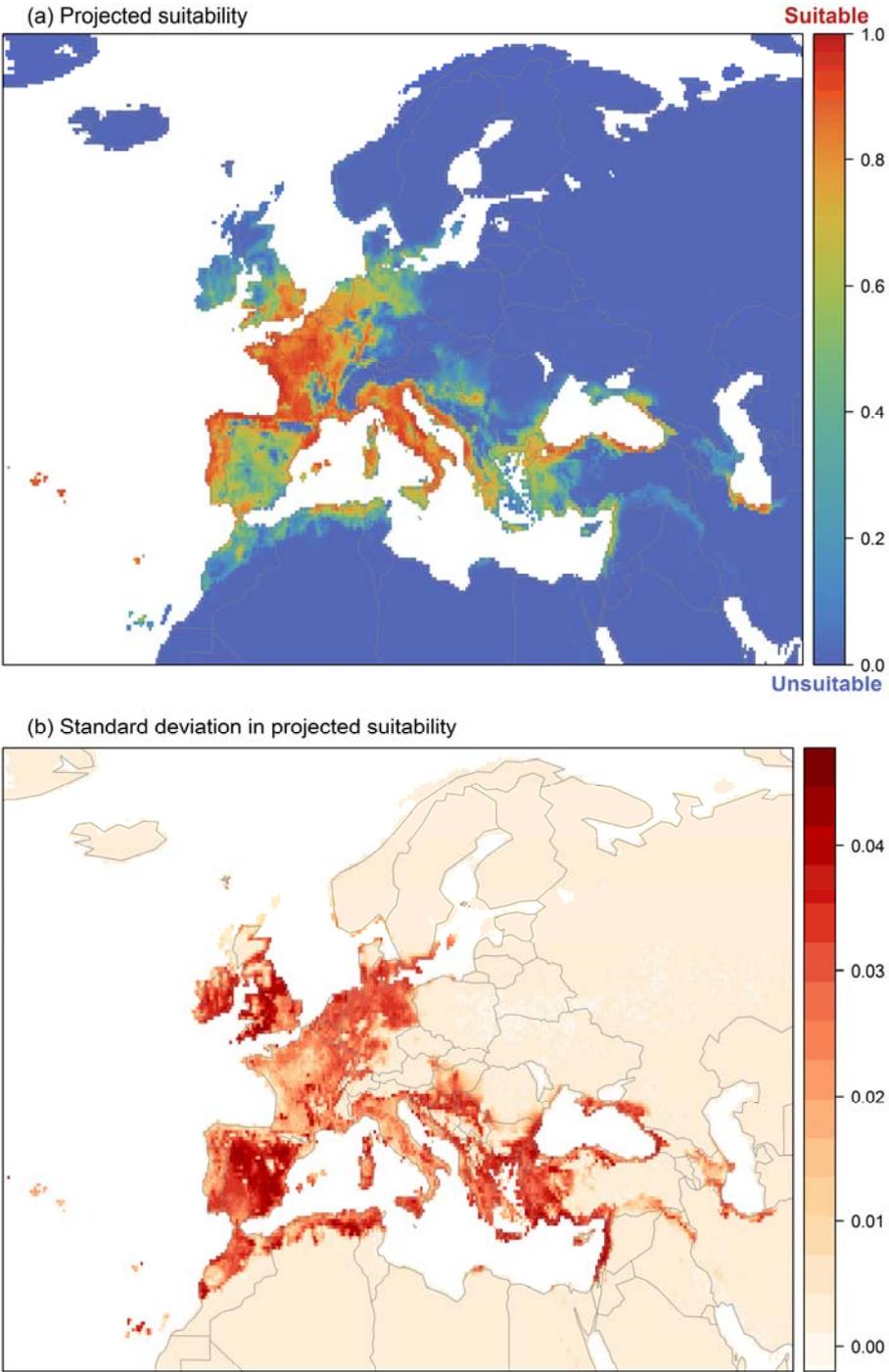
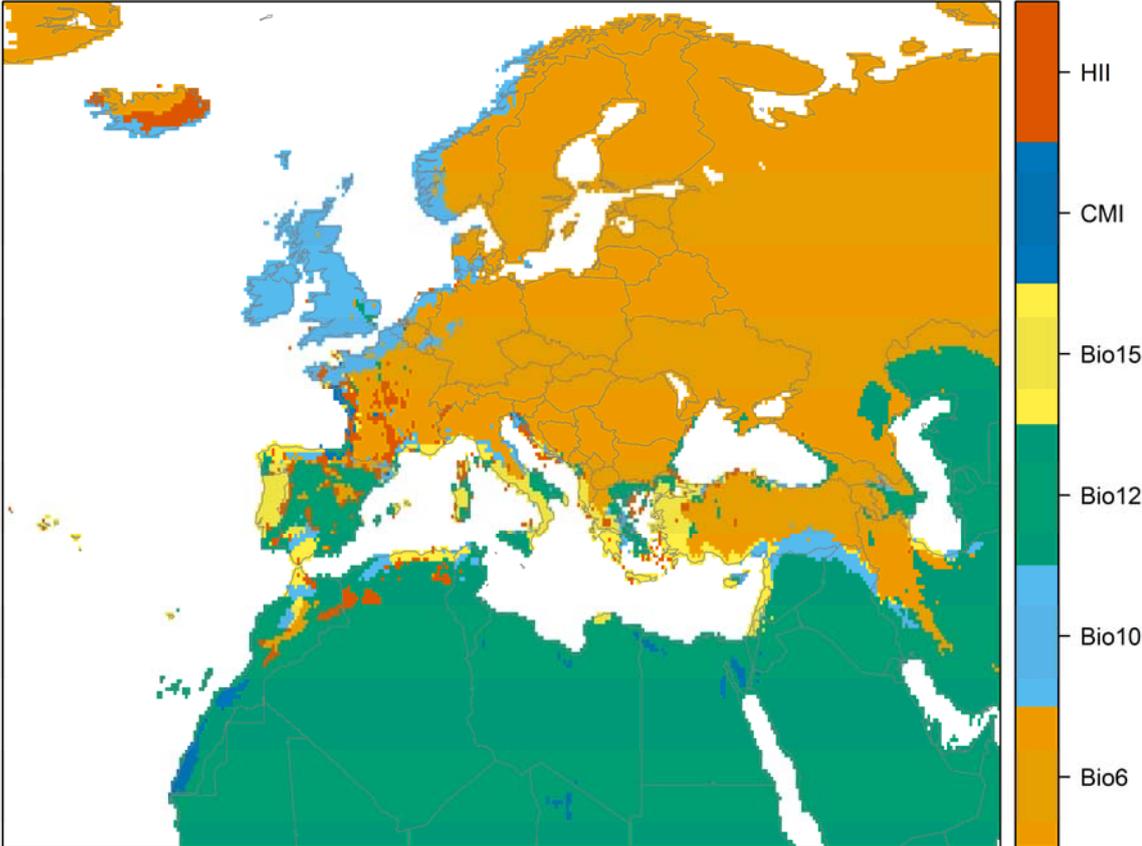


Figure 6. The most strongly limiting factors for *Obama nungara* establishment estimated by the model in Europe and the Mediterranean region in current climatic conditions.



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Figure 7. (a) Projected suitability for *Obama nungara* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP2.6. Values > 0.71 are suitable for the species, with 98% of global presence records above this threshold under current climate. Values below 0.71 indicate lower relative suitability. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

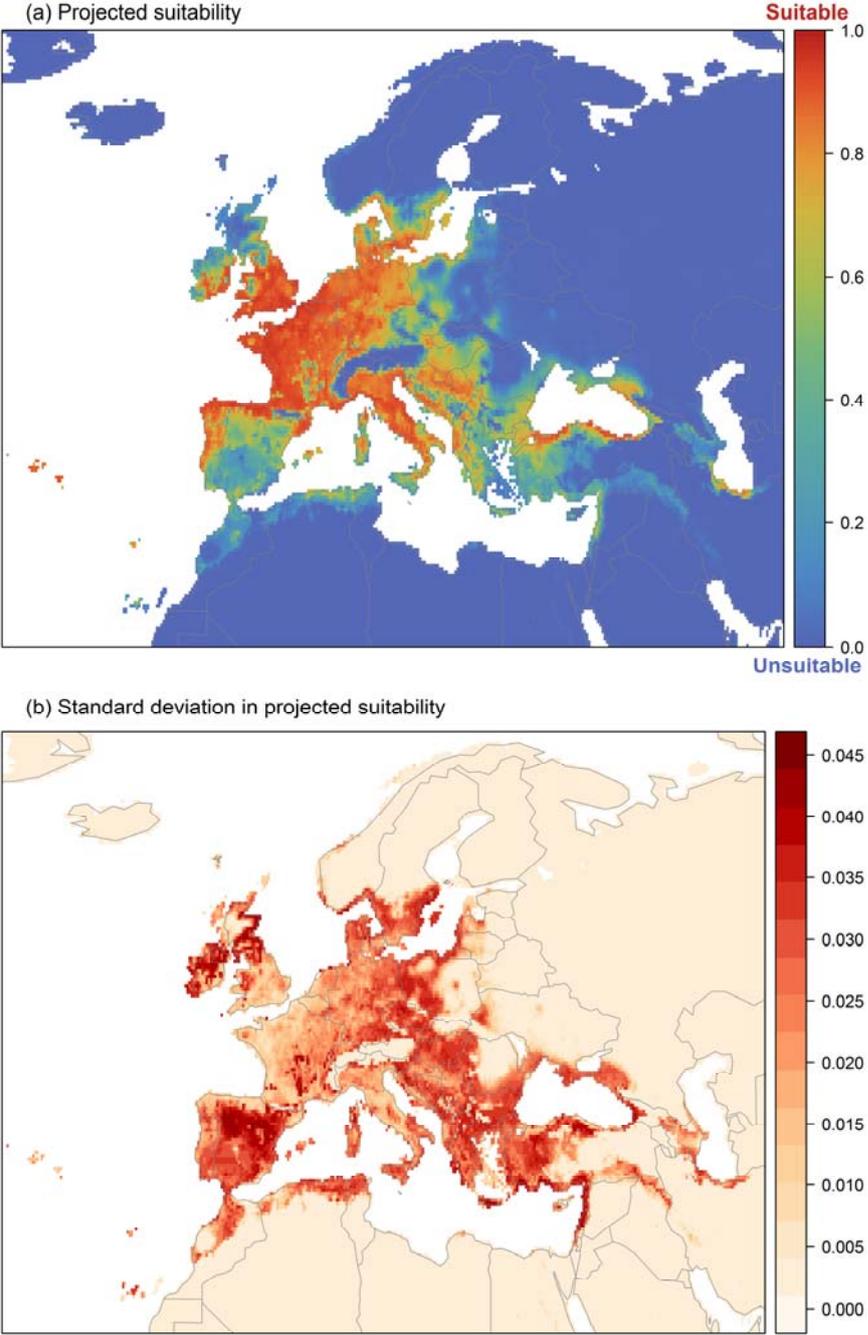


Figure 8. (a) Projected suitability for *Obama nungara* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP4.5. Values > 0.71 are suitable for the species, with 98% of global presence records above this threshold under current climate. Values below 0.71 indicate lower relative suitability. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

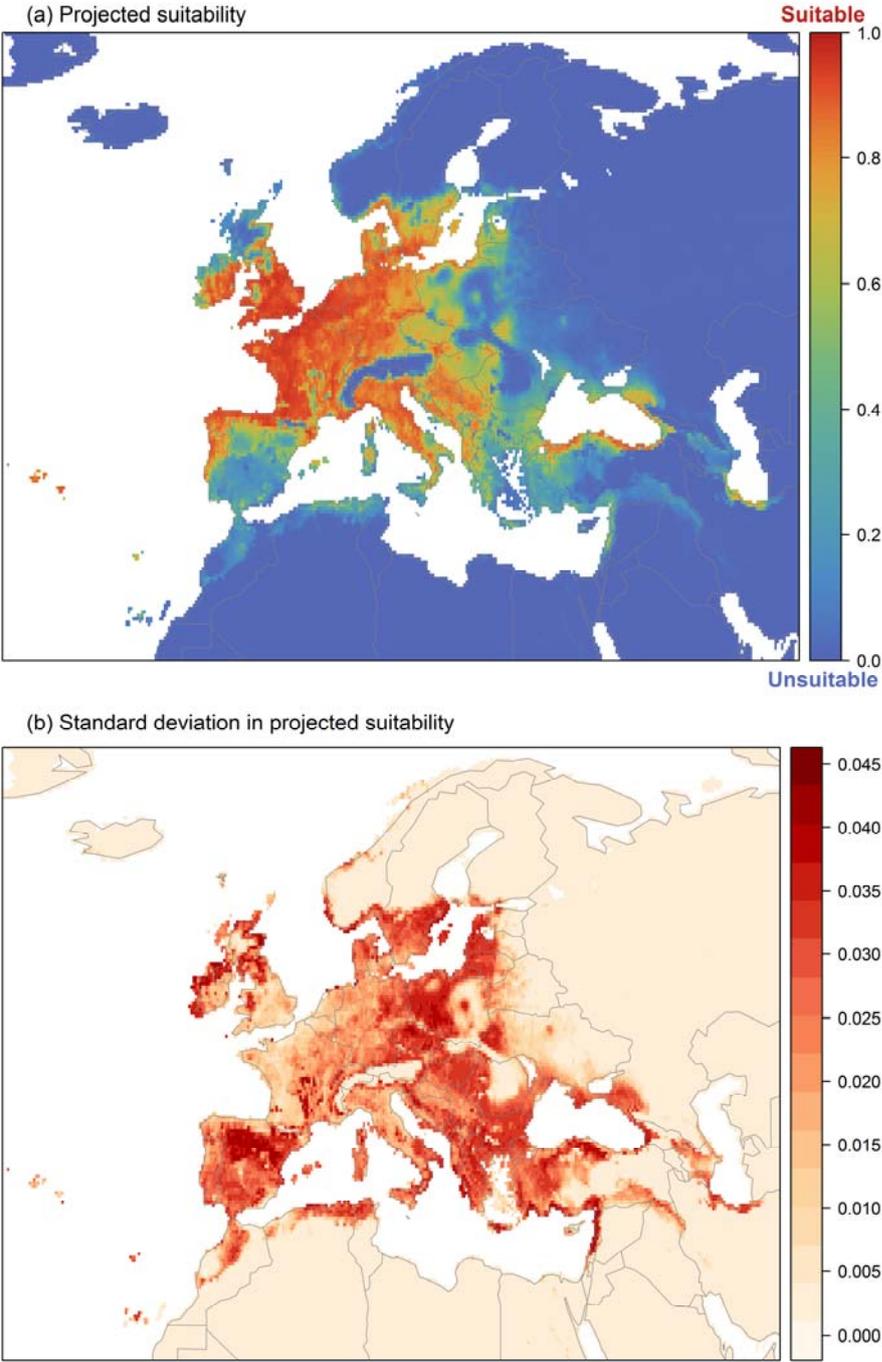
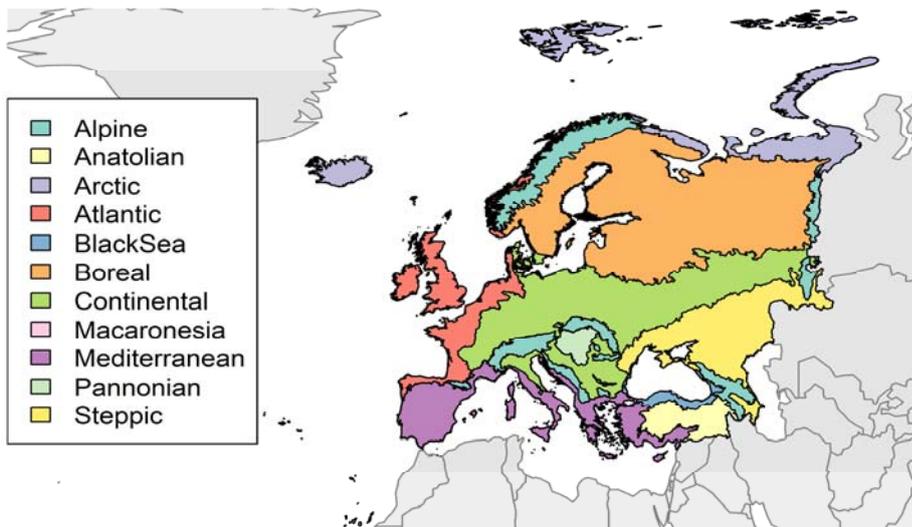
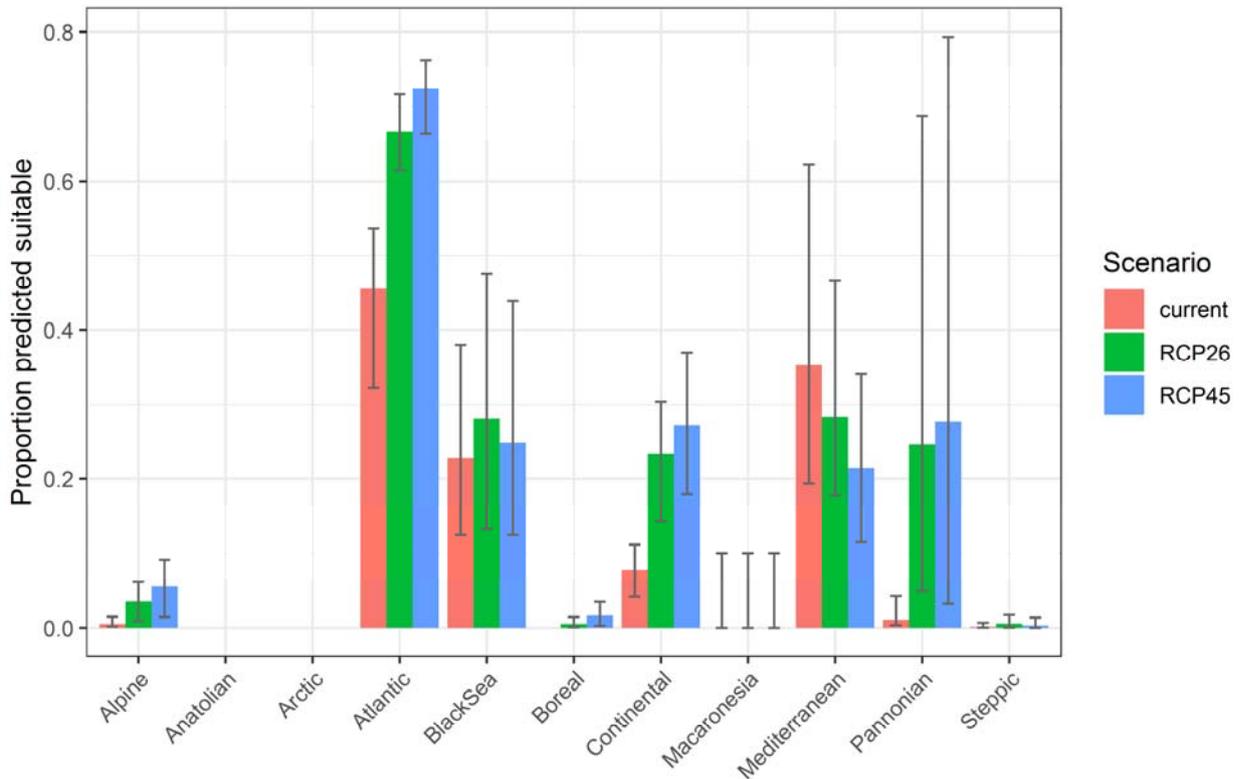


Figure 9. Variation in projected suitability for *Obama nungara* establishment among Biogeographical Regions of Europe (<https://www.eea.europa.eu/data-and-maps/data/biogeographical-regions-europe-3>). The bar plots show the proportion of grid cells in each region classified as suitable (with values > 0.71) in the current climate and projected climate for the 2070s under two RCP emissions scenarios. Error bars indicate uncertainty due to both the choice of classification threshold (cf. p.6) and uncertainty in the projections themselves (cf. part (b) of Figures 5, 7 and 8). The location of each region is also shown. The Arctic and Macaronesian regions are not part of the study area, but are included for completeness.



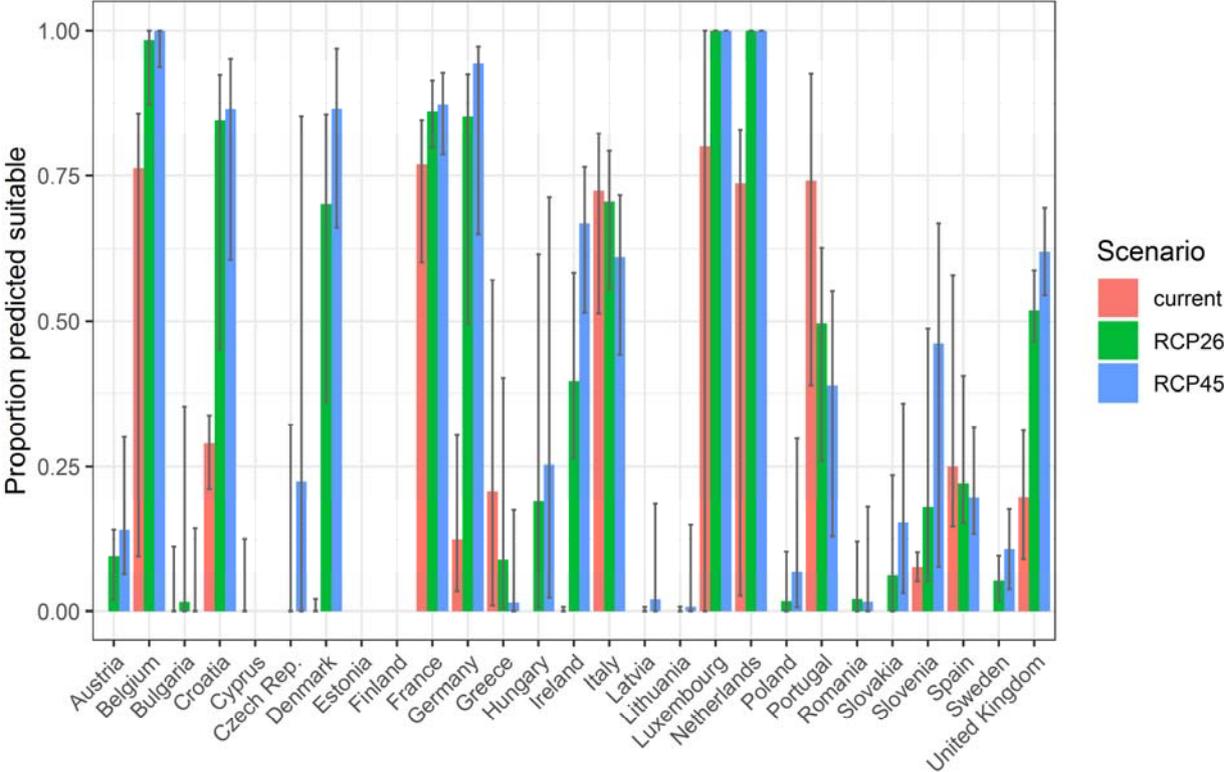
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Table 2. Variation in projected suitability for *Obama nungara* establishment among Biogeographical regions of Europe (numerical values of Figure 9 above). The numbers are the proportion of grid cells in each region classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. The Arctic and Macaronesian biogeographical regions are not part of the study area, but are included for completeness.

	current climate			2070s RCP2.6			2070s RCP4.5		
	lower	central estimate	upper	lower	central estimate	upper	lower	central estimate	upper
Alpine	0.00	0.00	0.01	0.01	0.03	0.06	0.01	0.06	0.09
Anatolian	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Arctic	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Atlantic	0.32	0.45	0.54	0.61	0.67	0.72	0.66	0.72	0.76
Black Sea	0.12	0.23	0.38	0.13	0.28	0.48	0.12	0.25	0.44
Boreal	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.02	0.03
Continental	0.04	0.08	0.11	0.14	0.23	0.30	0.18	0.27	0.37
Macaronesia	0.00	0.00	0.10	0.00	0.00	0.10	0.00	0.00	0.10
Mediterranean	0.19	0.35	0.62	0.18	0.28	0.47	0.11	0.21	0.34
Pannonian	0.00	0.01	0.04	0.05	0.25	0.69	0.03	0.28	0.79
Steppic	0.00	0.00	0.01	0.00	0.01	0.02	0.00	0.00	0.01

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Figure 10. Variation in projected suitability for *Obama nungara* establishment among European Union countries and the UK. The bar plots show the proportion of grid cells in each country classified as suitable (with values > 0.71) in the current climate and projected climate for the 2070s under two RCP emissions scenarios. Error bars indicate uncertainty due to both the choice of classification threshold (cf. p.6) and uncertainty in the projections themselves (cf. part (b) of Figures 5, 7 and 8). Malta has been excluded because the Human Influence Index dataset lacks coverage for Malta.



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Table 3. Variation in projected suitability for *Obama nungara* establishment among European Union countries and the UK (numerical values of Figure 10 above). The numbers are the proportion of grid cells in each country classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios.

	current climate			2070s RCP2.6			2070s RCP4.5		
	lower	central estimate	upper	lower	central estimate	upper	lower	central estimate	upper
Austria	0.00	0.00	0.00	0.02	0.10	0.14	0.06	0.14	0.30
Belgium	0.10	0.76	0.86	0.87	0.98	1.00	0.94	1.00	1.00
Bulgaria	0.00	0.00	0.11	0.00	0.02	0.35	0.00	0.00	0.14
Croatia	0.21	0.29	0.34	0.45	0.85	0.92	0.61	0.87	0.95
Cyprus	0.00	0.00	0.12	0.00	0.00	0.00	0.00	0.00	0.00
Czech Rep.	0.00	0.00	0.00	0.00	0.00	0.32	0.00	0.22	0.85
Denmark	0.00	0.00	0.02	0.36	0.70	0.86	0.66	0.87	0.97
Estonia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Finland	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
France	0.60	0.77	0.85	0.80	0.86	0.91	0.79	0.87	0.93
Germany	0.03	0.12	0.30	0.50	0.85	0.92	0.65	0.94	0.97
Greece	0.01	0.21	0.57	0.00	0.09	0.40	0.00	0.01	0.17
Hungary	0.00	0.00	0.00	0.01	0.19	0.61	0.02	0.25	0.71
Ireland	0.00	0.00	0.01	0.26	0.40	0.58	0.51	0.67	0.76
Italy	0.51	0.72	0.82	0.55	0.71	0.79	0.44	0.61	0.72
Latvia	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.02	0.19
Lithuania	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.15
Luxembourg	0.00	0.80	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Netherlands	0.03	0.74	0.83	1.00	1.00	1.00	1.00	1.00	1.00
Poland	0.00	0.00	0.00	0.00	0.02	0.10	0.01	0.07	0.30
Portugal	0.39	0.74	0.93	0.26	0.50	0.63	0.13	0.39	0.55
Romania	0.00	0.00	0.00	0.00	0.02	0.12	0.00	0.02	0.18
Slovakia	0.00	0.00	0.00	0.00	0.06	0.23	0.03	0.15	0.36
Slovenia	0.05	0.08	0.10	0.05	0.18	0.49	0.08	0.46	0.67
Spain	0.15	0.25	0.58	0.15	0.22	0.40	0.13	0.20	0.32
Sweden	0.00	0.00	0.00	0.02	0.05	0.10	0.04	0.11	0.18
UK	0.09	0.20	0.31	0.47	0.52	0.59	0.54	0.62	0.69

Caveats to the modelling

To remove spatial recording biases, the selection of the background sample from the accessible background was weighted by the density of Platyhelminthes records on the Global Biodiversity Information Facility (GBIF). While this is preferable to not accounting for recording bias at all, it may not provide the perfect measure of recording bias.

There was substantial variation among modelling algorithms in the partial response plots (Figure 3). In part this will reflect their different treatment of interactions among variables. Since partial plots are made with other variables held at their median, there may be values of a particular variable at which this does not provide a realistic combination of variables to predict from.

Other variables potentially affecting the distribution of the species, such as types of land cover were not included in the model.

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Annex with evidence on measures and their implementation cost and cost-effectiveness

Species (scientific name)	<i>Obama nungara</i> Carbayo, Álvarez-Presas, Jones & Riutort, 2016
Species (common name)	Obama flatworm
Author(s)	Archie K. Murchie, Leigh Winsor, Jean-Lou Justine
Reviewer	Brian Boag, Richard Shaw
Date completed	15/10/2021

Summary¹

Highlight of measures that provide the most cost-effective options to prevent the introduction, achieve early detection, rapidly eradicate and manage the species, including significant gaps in information or knowledge to identify cost-effective measures.

Obama nungara is an alien terrestrial flatworm, originally from South America, that has established in France, Belgium, Spain, Portugal and Italy (Justine and Winsor, 2020). It has been recorded most often from domestic gardens. *Obama nungara* is a potentially problematic species because it is a predator of earthworms, snails and other invertebrates found in the soil. If established in a region, eradication and management options are very limited. Prevention of introduction, prevention of establishment and prevention of spread are by far the best options.

Unintentional introduction: Legislative control and phytosanitary practices at exporting plant producers would be the first step to prevent introduction of *O. nungara*. If *O. nungara* is found, detention notices should be enforced to prevent further stock movement. Plants from the same consignment should be traced and thoroughly examined for flatworms. Good biosecurity practices should be in place. Machinery and equipment should be hosed down with water to remove soil before moving between sites. Gardeners and others should not move soil, compost or plants from infested sites.

Detection: An education and awareness programme should be implemented to raise the profile of *O. nungara* and the potential risk it poses to the environment within the industry. Importing nurseries, botanic gardens or garden centres should inspect their stock regularly for non-indigenous flatworms. Pictorial guides should be produced to enable identification by laypersons.

Eradication: Eradication is only likely to be feasible in very small, controlled areas before the species has entered the wild. If on concrete, biocides could be used, but on a soil-base, removal and scorching would be necessary. If *O. nungara* spreads to the wild, eradication will be virtually impossible.

Long-term Management: Management options are limited. There are no chemical or classical biological control methods currently available for invasive alien flatworms. Spread may be limited by good biosecurity measures, cleaning machinery and equipment before moving between sites. Soil, compost and rooted plants should not be moved from infested gardens or other sites.

Detailed assessment			
	Description of measures ²	Assessment of implementation cost and cost-effectiveness (per measure) ³	Level of confidence ⁴
Methods to achieve prevention of intentional and unintentional introduction⁵	<p>Plant health inspection Terrestrial flatworms are mostly introduced via inadvertent transport by the plant trade, particularly plants-for-planting. Regular plant health inspections and surveillance are necessary to prevent establishment. Current EU Commission Implementing Regulation (EU) 2019/2072 stipulates the special requirements for growing medium attached to plants to sustain them and this largely prohibits soil but includes plants imported bare root or maintained on an artificial media. If implemented correctly, this should reduce the likelihood of <i>O. nungara</i> being carried in plant containers. Legislation to guarantee that exported plants are free from infestation by invasive flatworms is required.</p> <p>EPPO (2001) guidelines for <i>Arthurdendyus triangulatus</i> specify that:</p>	<p>Plant health inspection Plant health inspections and surveillance are important universal controls for crop pests and pathogens, as well as preventing incursion of invasive species. They are widely accepted and expected across the horticulture sector therefore any inspections for <i>O. nungara</i> will probably be accepted by stakeholders with minimal objections. It is likely that surveillance for <i>O. nungara</i> would be incorporated into routine surveillance. However, examination for soil-borne or cryptic pests such as <i>O. nungara</i> is likely to be very time-consuming, expensive and inaccurate. Plant inspections at source would be more effective than portal inspections. Environmental DNA technologies could aid in detection but have not been developed as yet.</p> <p>There would be an incremental cost from increasing the level of plant health inspection at ports of entry as well as the cost of training inspectors in new monitoring and control</p>	<p>Medium</p> <p>(some information from published sources on terrestrial flatworms but not applied to <i>O. nungara</i>)</p>

	<p>1) Plants are grown on slatted benches 2) The consignment must come from a place of production found free from flatworms 3) The consignment must have been subjected to a disinfestation treatment to eliminate flatworms.</p> <p>Hot-water treatment Hot water treatment may be used to phytosanitise plants for terrestrial flatworms, either by immersion (Sugiura, 2008; Murchie and Moore, 1998) or as a drench (Justine <i>et al.</i>, 2014). The temperature will depend on the flatworm species. For <i>A. triangulatus</i>, immersion in water at 34°C, resulted in death after 5 minutes (Murchie and Moore, 1998). No data are available for <i>O. nungara</i>. Treatment at source would be preferable, as this would meet the requirement that the consignment has been subject to disinfestation treatment (as above). The guidelines above can be stipulated by importers and National Plant Protection Organisations.</p>	<p>measures which could be passed on to the industry.</p> <p>Hot-water treatment Hot water treatment is used for nematode control and could be effective for <i>O. nungara</i>. The method is likely acceptable to the public for <i>O. nungara</i> management as these measures have been agreed for other invasive flatworms and hot water is the recommended method of euthanising them when found in domestic settings. However, on a commercial scale it requires specialist equipment and risks damaging the plants. There may be some health and safety implications of hot water treatment such as scalding but these are likely to be routine. Justine <i>et al.</i> (2014) considered that drenching may be more practical in a commercial setting. There is little information on whether hot-water treatment has been used for flatworm control at any nurseries or producers. Placing compost in glasshouses to raise the temperature has been occasionally used against <i>A. triangulatus</i> but its effectiveness against <i>O. nungara</i>, a sub-tropical species, would be smaller. There are few environmental implications for heat-treatment control as measures will take place in a managed environment at a local scale. However, any washings must be filtered or reheated before disposal to prevent any contamination from plant</p>	
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		<p>pathogens to the surrounding environment. Eradication at site would need to be implemented as rapidly as possible.</p>	
<p>Methods to achieve early detection⁶</p>	<p>Awareness and education (detection by industry and public)</p> <p>UK and Irish guidelines (MAFF, 1996; Kelly, 2012) for nurseries and garden centres suggest the following methods to detect terrestrial flatworms:</p> <ol style="list-style-type: none"> 1) Check regularly under matting or pots standing directly on the ground for flatworms or their egg cocoons. 2) Lift plants from their pots frequently to check for the presence of flatworms or their egg cocoons. Standing potted plants in water overnight has been practised by some gardeners as a means of detecting <i>A. triangulatus</i> (B. Boag 2005, pers. comm.). 3) Set traps (e.g. gravel or sand filled polythene bags) along the edges of the holding especially where it adjoins private gardens. Regularly check these for the presence of flatworms or their egg cocoons. 4) Inspect all outgoing consignments of plants carefully whether for export or not. <p>For <i>A. triangulatus</i>, pictorial identification guides have been produced and it is</p>	<p>Awareness and education (detection by industry and public)</p> <p>As <i>O. nungara</i> is likely to be spread from nurseries, garden centres and botanic gardens, detection at these sites would be most cost-effective. Raising awareness of invasive flatworms and their potential impact to industry and consumers, would be one of the most effective ways to detect this species. The methods proposed are low-cost and easily implemented as part of normal nursery practice. Awareness and vigilance are key. If a nursery or garden centre were found to disseminate <i>O. nungara</i> in their plants, this would cause significant reputational damage with consequent loss in consumer confidence. There is therefore a clear commercial incentive for producers to be involved.</p> <p>Citizen science approaches have been used to map the spread of invasive flatworms, including <i>O. nungara</i> (Moore <i>et al.</i>, 1998; Justine <i>et al.</i>, 2020; Justine <i>et al.</i>, 2015). This is a valuable approach that has become increasingly effective with the development of mobile phone cameras and online recording applications.</p>	<p>High</p> <p>(professionally produced guidelines for effective invasive flatworm detection published and available on several websites)</p>

	<p>recommended that similar was done for <i>O. nungara</i>. Environmental DNA (eDNA) technologies could be used for detection by, for example, analysing water samples passed through growing media held in plant pots.</p>	<p>Large adult flatworms are distinctive from other soil organisms such as earthworms and insect larvae. However, for species identification specialist training would be required. A citizen science approach would cost tens of thousands of euros, but this would comprise many hours of individual unpaid activity, rather than direct costs to government or industry. There are environmental benefits of engaging the public in this process, as it aids in the overall awareness of invasive species and other issues. The health and safety and welfare issues are minimal. However, members of the public should handle flatworms wearing gloves or using forceps in case of skin irritation.</p> <p>Detection is likely if industry and the public can be proactively engaged. As <i>O. nungara</i> has not established in all its potential European range, ongoing vigilance is required, and refresher messages should be disseminated to promote continued engagement.</p>	
<p>Methods to achieve eradication⁷</p>	<p>Eradication There are no reports in the literature about any attempts at eradication of <i>O. nungara</i>. For other flatworms (e.g. <i>A. triangulatus</i>), removal trapping (Cannon <i>et al.</i>, 1999; Blackshaw <i>et al.</i>, 1996), and soil removal and scorching (Murchie, 2017) have been suggested.</p>	<p>Scorching / soil removal Murchie (2017) considered that soil removal and scorching could eradicate invasive flatworms from a small area. However, such techniques have not been tested against flatworms and may not be effective if flatworms hide deep in the soil. Furthermore, any removed soil would also have to</p>	<p>Medium (no evidence in the literature of successful eradication of an invasive terrestrial flatworm)</p>

	<p>Eradication is likely only to be possible in a small, controlled area, following intensive treatments such as soil removal, chemical treatments, heat treatment and soil scorching (but these have not been attempted for alien flatworms). Once established in the wider environment, eradication of <i>O. nungara</i> is unlikely. Removal trapping was not successful for <i>A. triangulatus</i>, as shelter traps did not capture a sufficiently high proportion of the population and also it was very labour intensive.</p>	<p>be treated to eliminate flatworms therein, most likely with a form of heat treatment or freezing. Scorching and / or soil removal is only effective for very small areas (10s of m²) and at the very early stages of detection. Even so, the likely costs for small-scale eradication at a suspected nursery / garden site would be in the tens of thousands of euros if soil needed to be removed and monitoring implemented. Scorching and soil removal would have health and safety implications and need to be carried out by professional operators. The environmental impact would be highly localised. Stakeholders are generally likely to be supportive but may call for financial support to fund the eradication process or seek compensation for damaged produce and / or facilities. Eradication will be rapid if techniques are successful but these are untested.</p>	
<p>Methods to achieve management⁸</p>	<p>Management options There are no reports of any management methods being deployed against free-living <i>O. nungara</i>. In general, prevention of spread through the measures detailed in the ‘prevention of introduction’ section (above) would be valuable, particularly education and awareness, coupled with good biosecurity/phytosanitary measures and limitations on the movement of infested soil or plants.</p>	<p>Management options Management options for <i>O. nungara</i> are extremely limited and none have been used in the field. Chemical control will depend on identifying a suitable active ingredient and applying it in a way that minimises any effects on earthworms, slugs and snails. Even if this could be done, widespread use of pesticides in natural environments is likely to be met with public scrutiny and possible stakeholder rejection (e.g. organic nurseries) and could have other environmental consequences (e.g. non-target</p>	<p>Medium (little evidence of successful management techniques described in the literature)</p>

	<p>Trapping and hand collection of <i>A. triangulatus</i> have been used by domestic gardeners but the effectiveness of these techniques are unknown. Similarly, flooding and hand collection have been used to manage <i>Bipalium kewense</i> in earthworm beds in Australia (Winsor, 1998).</p> <p>Cultivation may physically damage terrestrial flatworms and could be used for management purposes within a horticultural or arable location. However, cultivation will also impact on earthworm and snail species, which are similarly vulnerable to physical damage.</p> <p>If native species are threatened by the presence of flatworms, species conservation measures may be required. For earthworms, this may entail methods to enhance their populations such as minimum-till cultivation (compare with above comments on cultivation) and application of farmyard manure. At the extreme level, rare and endemic species may need to be removed and captive breeding programmes initiated. Exclusion of predatory flatworms may be possible using physical or chemical barriers, but there is no information on this.</p>	<p>impacts, water pollution, etc.). In France, pesticides are restricted for personal use in gardens, so would not be a viable option in the most affected (to date) European country.</p> <p>Hand collection of flatworms may be possible within a limited area but is labour-intensive and gloves or forceps should also be used to prevent skin irritation or possible allergic reactions.</p> <p>It is probable that native predatory beetles and some birds will feed upon <i>O. nungara</i> in Europe and their activity should be encouraged through land management practices and judicious use of pesticides. However, terrestrial flatworms are generally distasteful and native predation may be insufficient to negate the flatworm's impacts. Protection and enhancement of earthworms and snail populations through conservation measures may mitigate some flatworm damage. For example, pasture earthworm species benefit from the application of farmyard manure, whilst endemic snail populations have been conserved through captive breeding programmes (Murray <i>et al.</i>, 1988) (albeit an extreme measure). Conservation measures are a long-term and costly commitment, although they would be a beneficial approach irrespective of the flatworm threat.</p>	
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	<p>Chemical control has not been used against terrestrial flatworms. No pesticides are approved for control and it is difficult to target flatworms on the soil, whilst avoiding impacts on non-target organisms. Fumigation may be a possibility for containerised plants providing phytotoxic effects were kept to a minimum; however, this has not been assessed. B. Boag quoted in Murchie (2017) reported that slug pellets (metaldehyde) had an impact on <i>A. triangulatus</i> sheltering under refuges on the soil surface. Veterinary anti-helminthics could potentially target invasive alien flatworms but this has not been tested and there would be significant regulatory hurdles to widespread environmental use.</p> <p>No specific predators, parasitoids or pathogens have been documented as potential biocontrol agents for <i>O. nungara</i>.</p>	<p>The large-scale management of such a cryptic and inaccessible pest species is impractical and unproven and would probably be limited to preventing further local spread. It is unlikely that a full-blown invasion could be managed effectively and the costs would be very high.</p>	
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Guidelines for Completing the Annex

1 Provide a brief summary description of the most cost-effective methods drawing on the reviews in the detailed assessments

2 Provide a description of the potential method. This should be based on the available key scientific evidence which should be gathered from sources including articles and reviews in technical and scientific journals, internet searches, online databases, grey literature and relevant books and personal communications from scientists, stakeholders, conservation practitioners and governmental bodies. This information should include a full bibliographic list detailing the literature and sources considered.

3 Provide an assessment of the likely cost and effectiveness of the method. Where information is available, consider the following range of questions, accepting that not all questions will be appropriate in all circumstances.

- How effective has this approach proven to be in the past or in an analogous situation?
- How publically acceptable is the approach likely to be?
- Over what period of time would this approach need to be applied to be effective?
- What is the direct cost of implementing this approach?
- How likely are the methods used in the approach to be available?
- How likely is it that relevant licences or other approvals to undertake the approach would be difficult to obtain?
- How likely is it that health and safety issues would prevent the use of this approach?
- How significant is the environmental harm caused by this approach?
- How significant is the economic harm caused by this approach?
- How significant is the social harm caused by this approach?
- How likely is it that the approach will be criticised on welfare grounds?
- How likely is it that the approach will be acceptable to other stakeholders?

Where available, factual information on the costs of specialist equipment, or case studies of management costs from across the Union or third countries should be provided. When describing case studies, if the information is available then provide both total cost and the area over which control was undertaken so that a cost per unit area might be derived. Where such quantitative information is not available, then any qualitative information from the literature is acceptable to help guide decision making. It is accepted that in the majority of cases the information required to assess the potential total cost of management at a member state level is unlikely to be available. This would normally require information on the extent and abundance of the species which is beyond the scope of this assessment. Assessors are not expected to extrapolate the potential total costs of management at a member state level, only to report on the information provided within the literature.

4 Provide an overall assessment of the confidence that can be applied to the information provided for this method. This confidence should relate to the quality of the available information using the guidance below. It should NOT relate to the confidence in the effectiveness of the method

The confidence scores are:

- **High:** Information comes from published material, or current practices based on expert experience applied in one of the EU countries or third country with similar environmental, economic and social conditions.
- **Medium:** Information comes from published data or expert opinion, but it is not commonly applied, or it is applied in regions that may be too different from Europe (e.g. tropical regions) to guarantee that the results will be transposable.
- **Low:** data are not published in reliable information sources and methods are not commonly practiced or are based solely on opinion; This is for example the case of a novel situation where there is little evidence on which to base an assessment.

If there are further factors beyond these that have determined the chosen level of confidence, then provide a brief written description to support the choice of the level of confidence.

5 Measures for preventing the species being introduced intentionally or unintentionally into the territory of a Member State (cf. Articles 7(1)(a), 7(2), 13, 15), i.e. preventing a species entering by blocking its pathways, including pre-border prevention and prevention of escape or release into the environment and secondary spread whenever relevant. Measures for preventing the species reproducing while in containment should be included, if appropriate. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

6 Measures to run an effective surveillance system for achieving an early detection of a new occurrence (cf. Article 16). This section assumes that the species is not currently present in a Member State, or part of a Member State's territory.

7 Measures to achieve eradication. Preferably, but not exclusively, used at an early stage of invasion, after an early detection of a new occurrence (cf. Article 17). Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

8 Measures to achieve management of the species once it has become widely spread within a Member State territory (cf. Article 19). These measures can be aimed at eradication, population control or containment of a population of the species. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

The development and completion of this template forms part of the EU project **No 07.0202/2020/834529/ETU/ENV.D.2** Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention.

Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention"
Contract No 07.0202/2020/834529/ETU/ENV.D.2¹

Name of organism: *Pycnonotus jocosus* (Linnaeus, 1758), red-whiskered bulbul

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Risk Assessment Area: The risk assessment area is the territory of the European Union 27 and the United Kingdom, excluding the EU-outermost regions.

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Acknowledgments: We thank Antonio Polo for sharing information on the species in Valencia province and Chris Feare for information on the eradication campaign in the Seychelles.

Date of completion: 15/10/2021

¹ This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA). A number of amendments have been introduced to ensure compliance with Regulation (EU) 1143/2014 on IAS and relevant legislation, including the Delegated Regulation (EU) 2018/968 of 30 April 2018, supplementing Regulation (EU) No 1143/2014 of the European Parliament and of the Council with regard to risk assessments in relation to invasive alien species (see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>).

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SECTION A – Organism Information and Screening

A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?

including the following elements:

- the taxonomic family, order and class to which the species belongs;
- the scientific name and author of the species, as well as a list of the most common synonym names;
- names used in commerce (if any)
- a list of the most common subspecies, lower taxa, varieties, breeds or hybrids

As a general rule, one risk assessment should be developed for a single species. However, there may be cases where it may be justified to develop one risk assessment covering more than one species (e.g. species belonging to the same genus with comparable or identical features and impact). It shall be clearly stated if the risk assessment covers more than one species, or if it excludes or only includes certain subspecies, lower taxa, hybrids, varieties or breeds (and if so, which subspecies, lower taxa, hybrids, varieties or breeds). Any such choice must be properly justified.

This risk assessment covers one species, the red-whiskered bulbul *Pycnonotus jocosus* (Linnaeus, 1758), sometimes also called crested bulbul, red-eared bulbul or Persian nightingale. *Lanius jocosus* is an older synonym. This medium-sized passerine songbird (class Aves, order Passeriformes) from Asia belongs to the bulbul family (Pycnonotidae).

Common names regularly used within the risk assessment area include bulbul Orfeo and bulbul orfeu (Spanish/Catalan), roodoorbuulbuul (Dutch), Rotohrbülbul (German), bulbul orphée, condé or merle de Maurice (French), tuta-de-faces-vermelhas (Portuguese), bulbul baffirossi, bulbul dai mustacchi rossi, bulbul mustacchi rossi (Italian).

Nine subspecies of the red-whiskered bulbul are currently recognized (Clements 2007), some of which were originally described as separate species (*P. j. fuscicaudatus* under the genus *Otocampsa*, *P. j. emeria* under the genus *Motacilla*, *P. j. pyrrhotis* and *P. j. monticola* under the genus *Ixos*) (Avibase, <https://avibase.bsc-eoc.org/>):

- *P. j. abuensis* (Whistler, 1931): north-western India
- *P. j. fuscicaudatus* (Gould, 1866): western and central India.
- *P. j. pyrrhotis* (Bonaparte, 1850): northern India and Nepal.
- *P. j. emeria* (Linnaeus, 1758): eastern India to south-western Thailand, Bangladesh and Myanmar.
- *P. j. whistleri* (Deignan, 1948): Andaman Islands.
- *P. j. monticola* (Horsfield, 1840): eastern Himalayas, north-eastern India, southern Tibet, northern Myanmar and southern China.

- *P. j. pattani* (Deignan, 1948): southern Myanmar, Thailand, northern peninsular Malaysian, Laos and southern Indochina.
- *P. j. jocosus* (Linnaeus, 1758): south-eastern China.
- *P. j. hainanensis* (Hachisuka, 1939): northern Vietnam and south-eastern China.

A number of hybrids with the red-whiskered bulbul have been identified: *P. jocosus* x *cafer*, *P. jocosus* x *melanicterus*, *P. jocosus* x *xanthorrhous*, *P. jocosus* x *xanthopygos*, *P. jocosus* x *leucotis*, *P. jocosus* x *leucogenis* (Avibase, <https://avibase.bsc-eoc.org/>). This risk assessment deals with *Pycnonotus jocosus* sensu lato (i.e. including all described subspecies). We do not exclude *P. jocosus* hybrids though information on impacts is lacking for hybrids.

A2. Provide information on the existence of other species that look very similar [that may be detected in the risk assessment area, either in the environment, in confinement or associated with a pathway of introduction]

Include both native and non-native species that could be confused with the species being assessed, including the following elements:

- other alien species with similar invasive characteristics, to be avoided as substitute species (in this case preparing a risk assessment for more than one species together may be considered);
- other alien species without similar invasive characteristics, potential substitute species;
- native species, potential misidentification and mis-targeting

The red-whiskered bulbul is a dark, medium-sized, rather slender bulbul species. It is almost unmistakable due to the combination of its conspicuous black head tuft, usually held vertically, the white and red cheeks and the red undertail-coverts. With a length of 18-20.5 cm the species is about the size of a song thrush *Turdus philomelos* or a European starling *Sturnus vulgaris*. Adults have a black head with an erect spiky crest and ear coverts have red whiskers behind the eyes above a white patch, separated from the white chin and throat by a narrow black malar (Brazil 2009). The throat is white and surrounded by extensions of the crest. The back, wings and tail are of a uniform dusky grey-brown colour but nape and neck-sides are black. The belly is whitish, with cream-coloured sides. The beak is black with the upper mandible somewhat curved and the eyes are dark brown. There is no clear sexual dimorphism between male and female. Juveniles have a shorter, browner crest, lack the red postocular patch and have a duller orange or bullfish-pink vent (Robson 2000). The red-whiskered bulbul has a pleasant song consisting of rich warbled phrases. Calls include high-pitched pips, a long buzzy note, and a sharp “*pik-pik-a-wew*” (Islam and Williams 2020). The vocalisation could potentially be confused with that of Iberian grey shrike (*Lanius meridionalis*) and great grey shrike (*L. excubitor*). These species also occur in Iberia but are not commonly found in the urban green areas (unless as rare winter visitors) around Valencia where red-whiskered bulbul is established (personal communication E. Murgui, September 2021).

Other species of *Pycnonotus* have been recorded in the risk assessment area, such as the brown bulbul (*P. barbatus*), the red-vented bulbul (*P. cafer*), and rarely the white-eared bulbul (*P. leucotis*), the Arabian bulbul (*P. xanthopygos*) and the bimaculate bulbul (*P. bimaculatus*) (Santos 2015). None of

these species have a conspicuous crest, nor do they have red and white cheeks (Mo 2015). The white-eared bulbul has white cheeks, but its caudal undertail-coverts are not red but yellow. The red-vented bulbul does have red caudal undertail-coverts and has a small crest but it does not have white cheeks. The other bulbul species have neither white cheeks nor red caudal undertail-coverts, which are mostly white or yellow. The brown-breasted bulbul *P. xanthorrous*, a species from China, Burma and northern Indochina which has not been observed in the risk assessment area, superficially resembles red-whiskered bulbul but equally lacks the red and white cheeks and its vent is ochre to yellow (Robson 2000).

In Australia, the Department of Primary Industries and Regional Development (2018) reported possible confusion with the native eastern whippbird *Psophodes olivaceus*. This species does however not occur in the risk assessment area and confusion with any crested native European bird is unlikely.

The only native bulbul species that somewhat resembles the red-whiskered bulbul and that therefore could be misidentified as such is the common bulbul (*P. barbatus*). This species is one of the commonest birds in Africa (including in the Spanish enclaves of Ceuta and Melilla, where it is common) in well-vegetated areas in dry landscapes such as gardens, orchards, and oases but is still very rare in the wild in the risk assessment area. In recent years (since 2013), common bulbul has bred in Tarifa (Cádiz, Andalusia, Spain) (van den Berg and Haas 2013). However, common bulbul is a rather dull, nondescript brown bird with a darker face and throat. The belly is pallid and the undertail white (north part of its range) or yellow (south part of its range) and is therefore unlikely to be confused with the red-whiskered bulbul.

The identification of hybrids which other Pycnonotus species might be more problematic. First and second generation hybrids often show intermediate characteristics, but further third and fourth generation hybrids acquired through further back crossing are often indistinguishable from pure-bred red-whiskered bulbul. Red-whiskered bulbuls are also sometimes cross-bred with stripe-throated bulbul *P. finlaysoni* and black-crested bulbul *Rubigula flaviventris* (McCarthy 2006; Techachoochert and Round 2013). Techachoochert and Round (2013) further report cross-breeding of red-whiskered bulbul with yellow-vented bulbul *P. goaivier* is common in Thailand. Hybrids are usually backcrosses with red-whiskered bulbuls from which they are almost indistinguishable (Techachoochert and Round 2013).

A3. Does a relevant earlier risk assessment exist? Give details of any previous risk assessment, including the final scores and its validity in relation to the risk assessment area.

No existing risk assessments with direct relevance for the risk assessment area were found. A scientific risk assessment conducted by the Department of Primary Industries and Regional Development, Western Australia and endorsed by the national Invasive Plants and Animals Committee, indicates that the red-whiskered bulbul poses a serious threat (the second highest of four categories) to Australia (Department of Primary Industries and Regional Development 2018). The species does not occur in Western Australia but has the legal status of a Prohibited Declared Pest species and may only be imported and kept subject to permits available to research organisations or similarly secure institutions. Part of the assessment showed that the climates of the bulbul's overseas range and areas of Australia are very similar. The current distribution of the species in Australia along

the east coast is in humid subtropical and oceanic climate (Köppen–Geiger climate classification Cfa and Cfb respectively, e.g. Sydney, see A5).

In Mexico, the red-whiskered bulbul was scored as a high risk species using the Método de Evaluación Rápida de Invasividad (MERI) scoring system (Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (2020). The species was officially listed as an invasive species for the country (Secretaría del Medio Ambiente y Recursos Naturales 2016).

Evans et al. (2016), in their global assessment of the impacts of alien birds using the Environmental Impact Classification for Alien Taxa (EICAT) protocol, classified the species as having a Major (MR) environmental impact with low confidence, with predation being the most important impact mechanism. Martin-Albarracín et al. (2015), in a global analysis of alien bird impact for 39 bird species, identified the red-whiskered bulbul as one of the three species with the highest global impact because of high local impact and a strong intrinsic ability to affect ecosystems. In this risk assessment the red-whiskered bulbul received a higher impact score than the related red-vented bulbul *P. cafer* for the impact criteria competition and interactions with other non-native organisms (for more details see Qu. 4.1).

A4. Where is the organism native?

including the following elements:

- an indication of the continent or part of a continent, climatic zone and habitat where the species is naturally occurring
- if applicable, indicate whether the species could naturally spread into the risk assessment area

The red-whiskered bulbul is native to tropical Asia. It has a very large range and occurs from Pakistan throughout India and southwest to China. Its natural range is from India to Southeast Asia and northeast into southern China (Guangdong, southern Jiangxi and southern Fujian) (Robson 2000; Brazil 2009). Islands and countries within the native range include: Andaman islands, Bangladesh, Bhutan, Cambodia, China, India, Laos, Myanmar, northern Malaysia, Nepal, Thailand and Vietnam (Hart 2020). According to BirdLife International (2017, 2021b) the species is least concern on the Red List but shows a decreasing population trend. Fishpool and Tobias (2017) report red-whiskered bulbul is in decline following local declines and extinctions owing to hunting and trapping pressure. For instance, in Thailand, Techachoochert and Round (2013) report the species is much in demand due to its sweet, chuckling song, and is possibly the most widely kept native cage-bird species in the country, but has nearly vanished from most of its Thai range due to the illegal trapping of wild birds for sale. Also, the practice of cross-breeding red-whiskered bulbul with yellow-vented bulbul *P. goaivier* presents a possible threat. Breeders believe the hybrids are more aggressive and sing more vigorously giving them an advantage in bird-singing competitions. The global population size has not been quantified, but the species is described as common in many areas, abundant in south and west India and very common in Hong Kong (Fishpool and Tobias 2017). National population sizes have been estimated at c.10,000-100,000 breeding pairs in China and c.100-10,000 introduced breeding pairs in Japan (Brazil 2009; BirdLife International 2017, 2021b).

The species occupies a range of lowland (< 2.000 m) habitats. Red-whiskered bulbul is a resident (non-migratory) species which is mostly closely associated with human habitation in native and introduced ranges (Islam and Williams 2000). It often occurs in wooded habitats (forest edges, mangrove, secondary forest), reed beds, near habitation, in villages, (sub)urban parks and gardens, orchards, scrubland and agricultural habitats where it usually occurs in pairs or small groups (Grimmet et al. 2001; Brazil 2009; Hart 2020). It tends to avoid densely vegetated areas such as continuous forest and their preferred habitat consists of well-watered, open wooded areas (Robson 2000). The species shows communal roosting behavior outside of the breeding season and was reported roosting in various species of trees (*Pinus*, *Casuarina*, *Ficus*) in Florida (Carleton and Owre 1975).

A5. What is the global non-native distribution of the organism outside the risk assessment area?

The red-whiskered bulbul has been introduced to many regions of the world as a cage bird and with ritual releases, including in Europe within the risk assessment area (Spain) and on the Canary Islands (Tenerife) outside the risk assessment area. It is widely kept as a cage bird and was introduced to many places, including Australia, Madagascar, Hawaii, Japan, the Seychelles, Indonesia, the Arabian Peninsula, Zimbabwe, South Africa, islands in the Indian Ocean and North America (California and Florida) (Dyer et al. 2017). It has established populations in Australia, Borneo, the Canary Islands, Hawaii, Hong Kong, Indonesia, Japan, Juan de Nova Island, southern Malaysia, Mauritius, Nicobar Islands, Oman, Réunion, Saudi Arabia, Singapore, Spain, Taiwan, the United Arab Emirates and the USA (Hart 2020 and references therein).

In **Taiwan** the species was first observed in 1985 with nesting documented since 2010 at two localities in Taipei City (Walther 2011).

On the **Canary Islands** a small population was established on Tenerife in the urban area Los Realejos (Barranco Godines) before some of those birds were removed in 2007 (Santos 2015). The species has only been observed on the island of Tenerife (Lorenzo 2010). The first observation was performed in 1997 in the locality of Las Casas de la Cumbrilla, in Anaga. Later, the species was recorded in Los Realejos, where it formed a breeding nucleus. Solitary individuals were observed here in 1998, in 2000 and in 2001, when a juvenile was accidentally captured, indicating reproduction. Further observations followed: two individuals in 2001, ten individuals in 2003, including a juvenile, and two individuals in 2003, including a juvenile, two in 2005, one of them carrying nest material. Around 2006 a population of more than 15 individuals was estimated in Los Realejos. Between 2006 and 2007 some control measures were carried out on this population (Santos 2015). The control action, commissioned by the Cabildo de Tenerife, succeeded in reducing the initial population from 15 individuals (using air rifles mostly but with one bird trapped in a trap with a female decoy – mist netting proved ineffective) to just three. It is thought that this nucleus has disappeared now.

The red-whiskered bulbul established in **Japan** (Hyogo, Tottori, and Kanagawa prefectures (National Institute for Environmental Studies 2017)) (Eguchi and Amano 2004a, b). The species is reported being scarce (i.e. between 100-10.000 breeding pairs) in Japan.

The red-whiskered bulbul has been reported from **South Africa** and **Zimbabwe** but is not currently considered established anywhere on continental Africa (Hart 2020).

The species has been introduced to several Indian Ocean islands: Mauritius, Reunion (France), Mayotte (Comoros), Juan de Nova (France) and Assumption (Seychelles). It has been a very successful invader on Mauritius and Réunion. On the **Mascarene Islands**, expansion appeared to be fast. Bulbuls were introduced in 1892 to northern Mauritius (Carié 1910), and 18 years later were abundant throughout the 1865 km² island (Clergeau and Mandon-Dalger 2001). Since then the red-whiskered bulbul became one of the most abundant birds on the island. On Assumption island (Seychelles) it was introduced in the 1970s and reached a population of over a thousand birds, but was recently successfully eradicated. Soon after the start of the Assumption eradication campaign, in early 2012, birds also invaded the neighbouring Aldabra Atoll (Bunbury et al. 2019). Both islands are however considered bulbul free again. Cruz and Reynolds (2019) and Bunbury et al. (2019) report 5279 red-whiskered bulbuls removed by 2014 from Assumption and a single bird from Aldabra. A combination of mist-netting and shooting was used to target the bulbuls over a 3-year period starting 2011-2012.

In **Réunion** it was introduced in 1972 (Barau 1978) and is now widely distributed in almost all parts of the 2515 km² island, occupying areas of native forest. In Nicobar, the species was introduced at the end of the 19th century on the island of Camorta, and was later introduced to other islands such as Nancowry, Trinkat, Katchall, Teressa and Car Nicobar (Mo 2015, Lever 2005).

In **Australia** there are four established populations on the east coast and the populations in Queensland and New South Wales are expanding (Department of Primary Industries and Regional Development 2018). These are all located in temperate climate zones of Australia. Interestingly, despite abundance in suburbia and colonisation of semi-rural districts (especially orchards), the red-whiskered bulbul also expanded its range further north and seemed not limited by low fruit abundance (Mo 2015). Peri-urban populations might act as bridgehead for invasion into other areas. In Sydney the species was introduced in the late 19th century. The red-whiskered bulbul became common and widespread in the suburban areas of Sydney in the 1920s with flocks of up to 100 birds (MacPherson 1921, 1923) and now occupies a large area, occurring particularly in peri-urban areas (Mo 2015). The species was introduced to Melbourne (Victoria) as early as the beginning of the 19th century, and to Coffs Harbour (New South Wales) and Mackay (Queensland) in the 1970s-80s. There are also records of introductions and breeding in other parts of the country (Mo 2015).

The species was introduced to the **United States** during the 1960s in the states of Florida and California. In Florida, the population could number several thousands of birds. Carleton and Owre (1975) provide a detailed account of the invasion history of the species in the United States. Red-whiskered bulbuls were first detected in Miami in 1960, when a few birds escaped while being transferred from one aviary to another; these birds were shipped to Miami from Calcutta, India. By fall 1961 they had spread about 56 km southwest of Miami and the first proof of reproduction was reported. In 1963, a flock of 23 birds was seen in Kendall, which increased to between 40 and 50 by 1964 (Banks and Laybourne 1968). During that first decade, Carleton (1971) estimated an annual increase of 30-40% in the total population. By winter of 1969-1970, total population was estimated at <250, and it was hypothesized that between 5 and 10 breeding pairs were the founders of this population, which occupied 8.2 km² of suburban Kendall (Carleton and Owre 1975). By 1973, the population had doubled to about 500 and continued to increase and expand in a southerly direction (Lever 1987). In California, where the species maintains only two small populations, red-whiskered bulbuls became established around 1968 from aviary escapees in Los Angeles at the Los Angeles Arboretum and Huntington Gardens. These birds were either deliberately or accidentally released (Hardy 1973, Lever 1987). Because this population increased substantially and became a threat to the

local citrus crop, the California Department of Agriculture initiated an eradication program that has been only partially successful (Long 1981).

In **Hawaii**, the red-whiskered bulbul was introduced to the island of Oahu in the 1960s and is now established in the southeast of the island, mainly in urban areas, favoured by the cultivation of exotic fruit trees such as papaya. In contrast to the Californian population population which grew in number but appears to remain limited in distribution, populations on Hawaii have undergone growth and range expansion, although the red-whiskered bulbul has shown a less dramatic increase in numbers and range than the red-vented bulbul (Islam and Williams 2000).

A6. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species been recorded and where is it established? The information needs be given separately for recorded (including casual or transient occurrences) and established occurrences.

“Established” means the process of an alien species successfully producing viable offspring with the likelihood of continued survival².

A6a. Recorded: List regions

A6b. Established: List regions

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

- Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea.

Comment on the sources of information on which the response is based and discuss any uncertainty in the response.

For delimitation of EU biogeographical regions please refer to <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2> (see also Annex VI).

For delimitation of EU marine regions and subregions consider the Marine Strategy Framework Directive areas; please refer to <https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions/technical-document/pdf> (see also Annex VI).

Response (6a - recorded): Mediterranean, Atlantic, Continental

The red-whiskered bulbul was recorded in the Mediterranean, Atlantic and Continental biogeographic regions with proof of these being escaped birds. Presumably a similar situation might occur in all of the other biogeographic regions.

² Convention on Biological Diversity, Decision VI/23

Response (6b - established): Mediterranean

The red-whiskered bulbul is known to be established in Spain in a river valley around Valencia in the Mediterranean biogeographic region where a breeding population of 100-150 birds exists (see A8 for more detail).

A7. In which biogeographic region(s) or marine subregion(s) in the risk assessment area could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A7a. Current climate: List regions

A7b. Future climate: List regions

With regard to EU biogeographic and marine (sub)regions, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (7a – current climate):

The species distribution model (Annex VIII) indicates suitable areas in the Mediterranean, Atlantic, Black Sea (and Anatolian) biogeographic regions under current climate. In the Mediterranean, the model predicts about 48% (26%-29%) of the surface area as suitable for establishment. For the Atlantic 9% (2%-50%) and for the Black Sea 8% (0%-26%) of the biogeographic region is predicted suitable under current climate. The Pannonian, Steppic, Continental, Alpine and Boreal biogeographic regions are not predicted to be at risk under current climate.

The ensemble model suggested that the climatic and environmental suitability for red-whiskered bulbul was most strongly determined by Annual mean temperature (Bio1), accounting for 41% of the variation explained, followed by Minimum temperature of the coldest month (Bio6) (21%), and Precipitation of the wettest month (Bio13) (9%). Annual precipitation (Bio12) and Climatic moisture index (CMI) all account for 7% of the variation, Mean temperature of the warmest quarter (Bio10) for 6%. The other variables included in the model had less explanatory power. Human influence index (HII) accounts for 5%; Precipitation of the driest month (Bio14) for 4% and Global tree cover (Tree) only explained about 1%. For more details, see Annex VIII.

Compared to the range of the red-vented bulbul *P. cafer*, the native distribution area of the red-whiskered bulbul is more extensive, especially across southeast Asia, and spans a bigger range of climate types including tropical, arid and temperate climate zones.

Response (7b – future climate):

Under future climate, the modeled suitability for establishment is predicted to increase in the Mediterranean biogeographic region from 48% (26%-69%) to 63% (39%-80%) and 67% (44%-85%) under RCP 2.6 and RCP 4.5 respectively by 2070. Note that there is considerable uncertainty on the predictions and potentially under climate change the suitable region in the Mediterranean could go up to 85% by 2070. In the Black Sea biogeographic region the proportion suitable increases from 8% (0%-26%) to 25% (4%-45%) and 32% (12%-55%) under both future emission scenarios with an upper limit of 55% of the region becoming suitable by 2070. In the Atlantic biogeographic region, where regular escaped birds are reported, the proportion of grid cells classified as suitable for establishment increases from 9% (5%-50%) to 20% (4%-78%) under RCP 2.6 and 25% (6%-85%) under RCP 4.5 by 2070. Also, here, there is considerable uncertainty on the predictions with a central estimate of 25%, but an upper range of 85% of the Atlantic biogeographic region becoming suitable by 2070. As for the other regions that were predicted unsuitable under current climate, only the Continental and Pannonian biogeographic regions are predicted to become suitable considering an upper estimate of 13% and 41% becoming suitable under RCP 4.5 by 2070, respectively (Annex VIII).

A8. In which EU Member States has the species been recorded and in which EU Member States has it established? List them with an indication of the timeline of observations. The information needs be given separately for recorded and established occurrences.

A8a. Recorded: List Member States

A8b. Established: List Member States

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden.

The description of the invasion history of the species shall include information on countries invaded and an indication of the timeline of the first observations, establishment and spread.

Response (8a - recorded):

The red-whiskered bulbul has been observed in several EU Member States that have well established birding communities. In **Germany**, a singing male was reported in July 1994 in the area of Lake Constance (Heine et al. 1999); a further two observations have been reported, a calling adult in Wedel along the Elbe near Hamburg in 2012 (D. Eichstedt, naturgucker.de) and in 2019 an adult male reported as a probable escape from Heidelberg Zoo (C. Stepf, naturgucker.de). In **Belgium**, there are several observations of individual birds since 1994 in at least nine locations, including an observation with photo in 2019 in Namur province (I. Volont, waarnemingen.be), with a maximum number of two birds seen together in an apple tree in Broechem (Antwerp) and Ukkel (resp. D. van Tulder and A. De

Broyer, waarnemingen.be). Sometimes birds hang around for some time, such as the bird in Uccle that was present from February 1998 until December 1999 and was often heard singing (A. De Broyer, waarnemingen.be). In **The Netherlands**, observations of at least 11 birds were reported through the online recording system waarneming.nl since 1990 across the country, mostly in (sub)urban areas, suggesting captive origin. Most of these birds were present for at least a few weeks and observed by several people. At least one bird survived the winter of 2005-2006 (B. van herk, waarneming.nl).

Additionally, the species has been observed in **Denmark**, the **United Kingdom** and **Switzerland** (Santos et al. 2007). In **Portugal**, an individual was recorded in Cascais in 2007 (T. Martins).

Outside the core population around Valencia, in **Spain**, isolated birds have also been reported in the province of Alicante and in Catalonia. In the province of Alicante, a bird was detected in 2003 in the town of Albir, in Altea. In 1993 a single bird was observed at the mouth of the river Tordera, between the provinces of Barcelona and Girona (Grupo de Aves Exóticas, unpublished). In 2008 a verified sighting was performed in a suburban area in the outskirts of Benidorm bordering the natural parc Serra Gelada (obs. Mathieu Woldhuis, observation.org), about 100 km south of the established population in the Turia river basin, and again in the same neighbourhood, in 2014, two escaped birds were reported (obs. R. Effern, observation.org).

In **Italy**, Andreotti et al. (2001) listed the species as a Category 4 species i.e. the red-whiskered bulbul has been recorded breeding in Italy but is not listed as a regular breeding bird in the country. This status is based on Brichetti and Massa (1998). There are however no recent sightings of the species on the recording platform ornitho.it. The species is not in the updated Italian bird checklist (Baccetti et al. 2020) and there are no recent sightings (personal communication E. Caprio, May 2021; A. Andreotti and L. Serra, June 2021).

Response (8b - established):

The red-whiskered bulbul is known to be established in **Spain** with one breeding population in a river valley near the city of Valencia, centred around the town of Paterna, where the species was originally introduced. Here, it occupies suburban habitats, generally residential areas with an abundance of parks and gardens. It is always found in the vicinity of a river valley, in its lower part, which it uses to disperse and expand its range. The red-whiskered bulbul established here shortly after the first observation of two birds in 2003 in the town of La Canyada (Paterna), in the lower Rio Turia basin area (Santos 2015; Polo-Aparisi and Polo-Aparisi 2021). Detailed impact studies or recent (post 2016) population estimates for Spain are lacking. The introduction of the species is probably due to the accidental escape or deliberate release from captivity. According to Santos (2015), the species is able to withstand natural population fluctuations, and its population is expanding.

In 2005 a group of twelve birds was observed in the rio Turia area, indicating breeding success of this core population. This was later confirmed by the presence of juveniles (Grupo de Aves Exóticas 2007). The red-whiskered bulbul then began to expand and progressively colonised other municipalities (Santos 2015). Heading west, the species was observed for the first time in San Antonio de Benagéber in 2004, in L'Elia in 2006, in Riba-roja de Túria in 2011, in Bétera in 2013 and in La Pobla de Vallbona in 2014. In an easterly direction, it was observed for the first time in Burjassot in 2012, in Mislata in 2013 and in Valencia in 2014. Currently red-whiskered bulbul can be found in La Cañada, La Vallesa and El Plantío (Paterna), Colinas de Sant Antoni (San Antonio de Benagéber), Entrepins, Los Almendros, Montesol y Mas del Carmen (L'Elia), Mas de Traver (Riba-roja de

Túria), Montesano (Bétera), Montecolorado and Vista Calderona (La Pobla de Vallbona), el Park of La Granja (Burjassot), the Military Hospital and the Park of La Canaleta (Mislata), and the Parque de Cabecera and the Botanical Gardens (Valencia). Despite this expansion, more numerous groups of birds, to a maximum of twelve birds, have only been recorded in the municipalities of Paterna, San Antonio de Benagéber and L'Eliana. Observations in other municipalities tend to correspond to solitary or paired individuals. It is very likely, however that in the future the species will continue to expand and colonise other localities along the Turia valley (Santos 2015). The recent breeding bird atlas of Valencia province (with data until 2016) describes the expansion in the Turia area from the first records in Paterna in 2003. Currently (up till 2016), during the breeding period the species was observed in six 5km² squares (UTM grid), during winter in seven 5km² squares, never in groups of more than 12 birds flocking together (Polo-Aparisi and Polo-Aparisi 2021). Its current distribution mostly involves (sub)urban areas where the birds nest and feed in private gardens and public green areas. The red-whiskered bulbul feeds here on a wide array of fruits and other plant resources, both exotic and native plant species (see also Qu. 4.3). It also consumes invertebrates. The breeding population has been estimated at 58 (range 26-130) breeding pairs and a wintering population of 258 (137-465) individuals. Polo-Aparisi and Polo-Aparisi (2021) state that the species will probably increase its range and population in the forthcoming years.

In Valencia city, the first records were made in 2013. Since then, the red-whiskered bulbul has been appearing regularly but never abundantly. Monthly surveys in Valencia city parks showed yearly records of maximum 8 birds (unpublished data E. Murgui). A small breeding population is now confined to a few urban parks in the west of the city (Parc de Benicalap, Parc de Polífil, Parc de Capçalera) which are relatively close to the river Turia that probably acts as a dispersal corridor. Currently, the population here is small (2-3 breeding pairs) and is not perceived as a threat (Murgui, in press). The birds were observed capturing invertebrates and consuming fruits of *Cotoneaster* spp., mastic *Pistacia lentiscus* and pomegranate *Punica granatum* (Murgui, in press).

Outside the Valencia area, there are several documented observations of red-whiskered bulbul elsewhere in Spain (see A8a).

A9. In which EU Member States could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A9a. Current climate: List Member States

A9b. Future climate: List Member States

With regard to EU Member States, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate

change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

(9a) Under current climate, suitable area for establishment of the red-vented bulbul is estimated to be present in Mediterranean Member States including Spain (35% (16%-53%) of the grid cells in the country predicted suitable), Portugal (96%, 86%-100%), Cyprus (100%, 75%-100%), Greece (49%, 29%-73%) and Italy (39%, 19%-65%). Suitable area is also predicted to a lesser extent in Ireland (20%), Croatia (13%) and France (10%) (Annex VIII). There is uncertainty on the predicted suitability under current climate in southwest Iberia, the eastern Mediterranean and the west coast of Ireland. Particularly in Iberia, some areas in southwest Iberia (Andalucia, Extremadura and nearby regions of Portugal) which have a mesomediterranean climate with hot, dry summers and cold, rainy winters, and some in northwest Iberia with colder climates (e.g. Galicia) seem less suitable to the red-whiskered bulbul (personal communication E. Murgui, September 2021) The presence of suitable area on Malta is likely, as similar conditions are present as in other Mediterranean Member States, but Malta was excluded from the distribution model because the Human Influence Index lacks coverage for the country.

(9b) Under foreseeable climate change conditions, by 2070, Portugal and Cyprus are still predicted 100% suitable for the establishment of the red-whiskered bulbul. In Greece and Italy the proportion suitable area for establishment is predicted to increase under RCP 2.6 and to almost double under RCP 4.5 to about 75%. In Spain, 44% and 47% of the area would be suitable under RCP 2.6 and RCP 4.5 respectively (but potentially going up to 75% of the territory under RCP 4.5), in Croatia 25%. In France, the suitable area for establishment would increase from 10% to 22% and 29% under RCP 2.6 and RCP 4.5 respectively. In Ireland these proportions could go up to 63% and 67% under RCP 2.6 and RCP 4.5 respectively yet with considerable uncertainty on the predictions (0%-100%). Also, under climate change, additional Member States suitable for establishment include Slovenia, Bulgaria and the United Kingdom (Annex VIII). There is some uncertainty on the predicted suitability under foreseeable climate change in southwest Iberia, the eastern Mediterranean and the west coast of Ireland (see also 9a).

A10. Is the organism known to be invasive (i.e. to threaten or adversely impact upon biodiversity and related ecosystem services) anywhere outside the risk assessment area?

The red-whiskered bulbul is known to be invasive on the Seychelles, Mauritius and on Hawaii. Environmental impacts are documented through the dispersal of seeds of invasive plants, interspecific competition with native birds and predation on reptiles, insects, spiders and other invertebrates (Hawaii Invasive Species Council 2017; Cottrell 2017). It is also a pest of agriculture and gardens, feeding on fruits, vegetables, flower buds and insects.

On the Nicobar Islands, competition by red-whiskered bulbul has been implicated in the decline of the endemic Nicobar bulbul *Ixos nicobariensis* together with habitat loss and (Sankaran 1998; BirdLife International 2021a). In Australia, the species may be aggressive to other bird species, either defensively (Wood 1999) or in competition for resources. Agonistic responses toward flocks of silvereye *Zosterops lateralis* and rosellas *Platycercus* spp. feeding on camphor laurel *Cinnamomum camphora* fruits in Sydney have been reported (MacPherson 1921, 1923). In Hawaii, red-whiskered bulbul chases native birds, competing with them for food and space (Hawaii Invasive Species Council 2017). In Mauritius, where the species was introduced in the late 1800s and became the most abundant bird species, the red-whiskered bulbul contributed to population declines of native white-eyes (*Zosterops*) and the endemic Mauritius bulbul *Hypsipetes olivaceus* through resource competition (Linnebjerg et al. 2010). Islam and Williams (2000) report that populations of geckos are severely depressed on Mauritius probably because of predation by bulbuls on young geckos. It also represents a threat to native invertebrates, and caused the disappearance of orb-web spiders (*Nephila inaurata*) by predation (Islam and Williams 2000). It facilitated the spread of invasive plant species such as *Ligustrum robustum*, *Lantana camara* and *Cordia interrupta*, which, prior to the introduction of the red-whiskered bulbul, were quiescent sleeper weeds (Linnebjerg et al. 2009, 2010). Linnebjerg et al. (2010) in an analysis of faecal pellets found the diet to consist primarily of fleshy fruits with seeds of ten plant species being present in 92% of all faecal pellets six of which were notorious invasive plant species on Mauritius (*Clidemia hirta*, *Ligustrum robustum*, *Litsea glutinosa*, *Psidium cattleianum*, *Rubus alceifolius* and *R. rosifolius*). Gut passage of seeds by the red-whiskered bulbul improved the germination success of the invasive plants *Clidemia hirta* (Linnebjerg et al. 2009), and *Schinus terebinthifolius* (Mandon-Dalger et al. 2004), on Mauritius and Réunion, respectively. In Hawaii, the red-whiskered bulbul spreads the seeds of invasive plants such as *Miconia calvescens*, ivy (*Hedera*), gourd and false kava (*Piper auritum*). Mo (2015) also identified the spread of exotic weeds as the main impact. In Florida, the red-whiskered bulbul's diet overlaps with the northern mockingbird *Mimus polyglottos* resulting in competition for mango, christmasberry, fig (*Ficus* spp.), day hessamine, lantana (*Lantana* spp.) and jasmine, especially during winter when insects are scarce (*Jasminum* spp.) (Carleton 1971; Gore and Doubilet 1976).

A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea

No information was found on invasiveness of the established population of red-whiskered bulbul in the Mediterranean biogeographic region within the risk assessment area (Valencia, Spain).

A12. In which EU Member States has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden.

The red-whiskered bulbul is established in one area in Spain (see A8b) and no information was found on its invasiveness there.

A13. Describe any known socio-economic benefits of the organism.

including the following elements:

- Description of known uses for the species, including a list and description of known uses in the risk assessment area and third countries, if relevant.
- Description of social and economic benefits deriving from those uses, including a description of the environmental, social and economic relevance of each of those uses and an indication of associated beneficiaries, quantitatively and/or qualitatively depending on what information is available.

If the information available is not sufficient to provide a description of those benefits for the entire risk assessment area, qualitative data or different case studies from across the risk assessment area or third countries shall be used, if available.

The red-whiskered bulbul is widely kept as a cage bird both in private collections and in zoos, and therefore represents economic, ornamental, sentimental, educational and aesthetic value as a pet, zoo animal and companion animal. Prices advertised by online shops range from 300-350 US\$ per bird (<https://www.softbillsforsale.com/sale/red-whiskered-bulbul.asp>). In their native range, red-whiskered bulbuls are also commonly bought and released for religious ceremonial purposes (prayer release i.e. religious practice in which captive wildlife are released as a demonstration of compassion and kindness in order to receive merit or good karma (Severinghaus and Chi 1999)) although no data could be found on the extent of this practice within the risk assessment area. Also, in the native range, red-whiskered bulbuls are used in bird singing competitions (Techachoochert and Round 2013). Mo (2015) mentions the species is appreciated as a predator of some invertebrate pests but without further details on species or regions.

SECTION B – Detailed assessment

Important instructions:

- In the case of lack of information the assessors are requested to use a standardized answer: “No information has been found.” In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable).
- With regard to the scoring of the likelihood of events or the magnitude of impacts see Annexes I and II.
- With regard to the confidence levels, see Annex III.
- Highlight the selected response score and confidence level in **bold** but keep the other scores in normal text (so that the selected score is evident in the final document).

1 PROBABILITY OF INTRODUCTION AND ENTRY

Important instructions:

- **Introduction** is the movement of the species into the risk assessment area (it may be either in captive conditions and/or in the environment, depending on the relevant pathways).
- **Entry** is the release/escape/arrival in the environment, i.e. occurrence in the wild
- Introduction and entry may coincide for species entering through pathways such as “corridor” or “unaided”, but it also may differ. If different, please consider all relevant pathways, both for the introduction into the risk assessment area and the entry in the environment.
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used (see Annex IV). For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document³ and the provided key to pathways⁴.
- For organisms which are already present (recorded or established) in the risk assessment area, the likelihood of introduction and entry should be scored as “very likely” by default.
- Repeated (independent) introductions and entries at separate locations in the risk assessment area should be considered here (see Qu. 1.7).

Qu. 1.1. List relevant pathways through which the organism could be introduced into the risk assessment area and/or enter into the environment. Where possible give details about the specific origins and end points of the pathways as well as a description of any associated commodities.

For each pathway answer questions 1.2 to 1.8 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 1.2a, 1.3a, etc. and then 1.2b, 1.3b etc. for the next pathway.

In this context a pathway is the route or mechanism of introduction and/or entry of the species.

The description of commodities with which the introduction of the species is generally associated shall include a list and description of commodities with an indication of associated risks (e.g. the

³ <https://circabc.europa.eu/sd/a/7e5f0bd4-34e8-4719-a2f7-c0cd7ec6a86e/2020-CBD-pathways-interpretation.pdf>

⁴ <https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf>

volume of trade; the likelihood of a commodity being contaminated or acting as vector).

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 1.2-1.9.

Pathway names:

- a. ESCAPE from confinement: Pet/aquarium/terrarium species (including live food for such species)
- b. ESCAPE from confinement: Botanical garden/zoo/aquaria (excluding domestic aquaria)
- c. RELEASE in nature: Other intentional release

Specifically, these pathways refer to (a.) pet escapes, (b.) escapes from zoos, and (c.) releases for religious purposes respectively.

For the congeneric species *P. cafer*, sightings of birds along coastlines of islands have led to the suggestion of hitchhikers on boats contributing to new introductions (Islam and Williams 2000). No such suggestions were made for the red-whiskered bulbul, so the pathway “TRANSPORT – stowaway: hitchhikers on ship/boat (excluding ballast water and hull fouling)” is not considered active in the Risk assessment area and is not dealt with here.

ESCAPE from confinement: Pet/aquarium/terrarium species (including live food for such species)

Qu. 1.2a. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)? --

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Red-whiskered bulbuls are intentionally introduced in the risk assessment area for the pet trade. Also their release is intentional. The escape of animals kept as pets, by itself, qualifies as unintentional.

Qu. 1.3a. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.

- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE		CONFIDENCE	
	very unlikely		low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

The red-whiskered bulbul is among the most popular bulbul species to be held as cagebirds by hobbyists. Wild caught bird trade (as opposed to captive bred) has been suspended in the EU since 2005, when a temporary ban on wild bird imports was installed to prevent the spread of avian influenza (Reino et al., 2017). The ban was made permanent in 2007 and considers all wild caught bird imports regardless of species' conservation status. This ban has been effective in reducing propagule pressure (Cardador et al., 2019). Given the European wild bird trade ban, the legal circuit of captive the red-whiskered bulbuls exclusively consists of animals bred and raised in confinement, which are known to have lower invasive potential than wild-caught birds due to changes in behavioral and physiological traits which lower their chances of successful establishment (Cabezas et al., 2013; Carrete and Tella 2015). Red-whiskered bulbuls are usually kept in pairs, not as solitary birds, nor in groups. The species is easily found on hobbyist websites for exchange (individuals for sale, or requested), with prices generally around 80-120 € per bird and 180 € to 400 € per pair (e.g. <https://www.softbillsforsale.com/sale/red-whiskered-bulbul.asp>, <https://www.prachtfinkenfreunde.de/>).

However, there are no official figures on the size of the captive population or trade volumes in the risk assessment area. There are also no formal figures on their risk of escaping, although of the 11 bird families considered, alien Pycnonotidae were found to be among the families with a significantly higher probability of escaping captivity than the overall escape rate in Taiwan (Shieh et al. 2006). Similar figures for Europe are lacking. Since the animals are usually kept in pairs, there is a fairly high chance of reproduction if the environment proves suitable.

Despite a lack in figures, outside the risk assessment area entry events of the red-whiskered bulbul are commonly attributed to escape or unauthorised release of privately owned cagebirds (Hardy 1973; Islam and Williams 2000; Eguchi and Amano 2004a,b; Clergeau and Mandon-Dalger 2001; Mo 2015; Cruz and Reynolds 2019). The same is true for the suspected origin of birds observed within the risk assessment area (Santos 2015; see Qu. A8. for an overview of observations).

Qu. 1.4a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Birds kept as pets are usually taken care of, with animal welfare and reproduction being of interest to the owner. There are specialist groups of hobbyists that specialize in the keeping of bulbuls. For example, in the Netherlands, the species is kept and bred by members of a bird breeders association specialized in insect and fruit-eating species who adopted a code of best practice for keeping red-vented bulbuls (NBvB 2014). It is known as a species which likes spacious aviaries with appropriate planting and it is mentioned that they are winter hardy. Also, they are known to be sociable birds which like the company of conspecifics or other tropical birds (NBvB 2014). We scored the likelihood and confidence lower in comparison with Qu. 1.4b (holdings in zoos), since holding conditions under private ownership are much more varied.

Qu. 1.5a. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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There are no known management practices for birds kept as pets. Control mechanisms should however be in place to ensure the adherence to legislation on animal welfare. In case of an infringement, authorities may decide to seize birds, for them to be re-homed or euthanised (both are considered moderately likely). There is however also the possibility of birds being illegally traded, for example on online platforms, with little or no control.

Qu. 1.6a. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: If the red-whiskered bulbul would escape from a privately owned cage or aviary, it is unlikely that this would go unnoticed as private owners are well aware of the number of birds they possess. Yet, in comparison with Qu. 1.6b (holdings in zoos), its likelihood is scored higher (moderately likely), and the confidence lower, since less control mechanisms are in place to ascertain discovery. It is also questionable if pet owners will (or can) take the necessary efforts to retrieve birds following an escape.

Qu. 1.7a. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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See Qu. 1.3c. As the red-whiskered bulbul is considered a fairly popular cagebird, it can be assumed that private holdings are widespread in the risk assessment area. Formal numbers are lacking but there is good observational evidence of regular and widespread escapes within the risk assessment area (see Qu. A8), hence the confidence on this response is medium.

Qu. 1.8a. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Worldwide, the entry of the red-whiskered bulbul into the environment is commonly attributed to escape (or unauthorised release) of cagebirds. The many observations of individual escaped birds illustrate this happens quite frequently across the risk assessment area (see A8a). As this is also the suggested pathway that led to the established population in Spain (Santos 2015), the likelihood is considered very likely (in accordance with the instructions). Because there is direct observational evidence of escapes across the risk assessment area we scored high confidence.

End of pathway assessment, repeat Qu. 1.2 to 1.8 as necessary using separate identifier.

ESCAPE from confinement: Botanical garden/zoo/aquaria (excluding domestic aquaria)

Qu. 1.2b. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Red-whiskered bulbuls are intentionally introduced in the risk assessment area to be put on display in zoos. s

Qu. 1.3b. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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It is difficult to assess the exact extent to which the red-whiskered bulbul is kept in zoological collections within the risk assessment area. Information provided by the database *zootierliste.de* (accessed 17 May 2021) shows that the species is present (or was recently present, considering some lag in website updates) in a considerable number of collections. In total, 67 holdings of red-whiskered bulbul (incl. *P. jocosus jocosus*) are mentioned, in Belgium (1), Denmark (4), Germany (30), France (3), Greece (1), the Netherlands (9), Austria (1), Poland (2), Slovakia (1), Spain (2, one of which is on Tenerife), Czechia (4), Hungary (1), and the United Kingdom (8). No information is available on the number of birds kept in other, non-EAZA (cf. <https://www.eaza.net/>) associated or private zoos, nor on trends in the captive population. By comparison, the red-vented bulbul, an aesthetically less

attractive species, is on display in a much lower (<10) number of EAZA zoos in the risk assessment area (*zootierliste.de*). There are examples of historic escapes from a collection where the red-whiskered bulbul was on display, for example in Miami where birds escaped from the Miami Rare Bird Farm, a working breeding farm as well as tourist attraction, in 1960 (Owre 1973; Kenward 2009). Nowadays, zoological collections on public display are set within tight legal frameworks (Council Directive 1999/22/EC of 29 March 1999 on the keeping of wild animals in zoos), with the risk of escape being of prime importance. Nonetheless, the risk of escapes can never be fully excluded as illustrated by the suspected escape from a German zoo in 2019 (see Qu. A8). As that risk is generally well-monitored by holding owners and public authorities, the likelihood for entry in the environment through escape from such collections can be considered unlikely, at least for EAZA associated zoos which are usually characterized by very high standards. The risk may however be higher for other facilities, like private backyards or illegal holdings (Cassey and Hogg 2015) and it is known that not all facilities are associated to EAZA.

Qu. 1.4b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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When birds are put on display in living collections, care is taken by holding owners to keep birds in good condition both during transport and storage. Holding would typically also include a possibility for reproduction as zoo keepers would actively encourage breeding to maintain a captive population for display.

Qu. 1.5b. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Management practices of birds in zoological collections are likely to increase the prospects of survival. Control mechanisms should be in place to ensure the adherence to legislation on animal display and welfare.

Qu. 1.6b. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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If the red-whiskered bulbul would escape from zoological collections, it is unlikely that this would go unnoticed. Although some time lag between the actual escape and its discovery may exist, the species is conspicuous, easy to identify, and the coverage by birdwatchers is fairly good so the species is unlikely to be unnoticed for a very long time. Also, zoos are likely to have to report on escapes (more than private people keeping birds as pets) (Council Directive 1999/22/EC of 29 March 1999 on the keeping of wild animals in zoos) and are known to sometimes initiate recapture efforts in case of accidental escapes. Such mechanisms might however not be adhered to by a lot of the non-official zoos that might not be up standard with the housing of birds. It is noteworthy that birds have a lower retrieval rate than escaped mammals and reptiles in such cases (Cassey and Hogg 2015).

Qu. 1.7b. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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See Qu. 1.3b. There is a considerable geographic spread of zoological facilities within the risk assessment area.

Qu. 1.8b. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Documented escapes of red-whiskered bulbul from zoological collections (on display to the wider public, as opposed to the next pathway) are scarce but do exist (e.g. in Germany, California, Florida, see A5, A8a). There are also numerous examples of other bird species escaping such facilities. For example, after storm damage in Planckendael zoo (Belgium) in february 2020, after which however most of the 45 escaped birds could be retrieved (this could be more difficult with small passerine birds like bulbuls). The risk of escape from zoological collections with high housing standards can be considered small. There are however many other types of facilities across the RA area with relatively poor standards. Also, Cassey and Hogg (2015) showed that compared to mammals and reptiles, bird escapes were significantly less likely to be retrieved, and more likely to remain undetected. Considering that both official and non-official holdings are relatively widespread (and considering that observations of escapes were regularly reported since 1990 (see A8a), even if they cannot unequivocally be attributed to escapes from zoos), the overall risk of at least one entry event happening within the risk assessment area within the next 10 years was scored likely, with medium confidence because there is direct observational evidence.

RELEASE in nature: Other intentional release (i.e. prayer release)

Qu. 1.2c. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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By definition, prayer release is intentionally performed in the belief that one can accrue merits by freeing captive animals into the wild (Severinghaus and Chi 1999). The importance of such releases as a pathway of introduction was stressed for a variety of taxa but especially for birds including bulbuls (Gilbert et al. 2012; Magellan 2019).

Qu. 1.3c. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent

establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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The ritual of animal release is regularly performed in Buddhist practice (Shiu and Stokes 2008). It is thus most prevalent in Asia, where the practice may to a limited extent also be performed by people that adhere to other religions. The species may be local or exotic, and bred or wild-caught. For Taiwan, *Pycnonotus sinensis* is known to be used in prayer release (Severinghaus and Chi 1999), and given that the red-whiskered bulbul is sold in shops (Su et al. 2014; 2016), it probably is too.

There is proof that prayer release is also performed by Buddhist groups in Western countries, with the local frequencies of release being as high as once per month (United States, Campbell et al. 2021; Canada and Australia, Shiu and Stokes 2008). However, the species pool used is very diverse (including invertebrates and fishes), with awareness on the environmental concerns and legal restrictions having grown considerably (Shiu and Stokes 2008).

No data could be found on the extent of prayer release within the risk assessment area, but given the above, the overall volume of entry is probably limited. In contrast to Asia, there are no supply chains dedicated to the practice. Therefore, for the red-whiskered bulbul in particular, entry into the European environment through this pathway is deemed unlikely, overall.

Qu. 1.4c. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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As prayer release is centered around the respect for lives of beings, care is taken by practitioners to keep captive birds alive and in good condition until the moment of release. The same would be expected from actors earlier in the supply chain (breeders, sellers), since the birds would be of commercial interest to them.

Qu. 1.5c. How likely is the organism to survive existing management practices before and

during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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There are no known management practices for birds subject to prayer release, maybe except for law enforcement measures on intended (illegal) release, in which case seized birds may become re-homed or euthanised depending on the authorities (both are considered moderately likely).

Qu. 1.6c. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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See Qu. 1.2a. As prayer release is intentional by definition, neither introduction nor entry would happen undetected, except for the theoretical scenario in which birds escape the cage environment prior to release.

Qu. 1.7c. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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There is no information on religious groups, notably Buddhist groups, in the risk assessment area that perform prayer release, let alone release involving the red-whiskered bulbul. Given that this religion is less prevalent, and given legal constraints, environmental awareness and the lack of supply chains dedicated to this purpose, the possible points of introduction for this pathway would anyhow be (very) limited in number in the risk assessment area.

Qu. 1.8c. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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In Eastern Asia, prayer release of *P. jocusus* poses a real risk (Severinghaus and Chi 1999; Su et al. 2014; Campbell et al. 2021). Although prayer release is known to have occurred in Western countries outside Europe, and may thus occur in the risk assessment area too, the likelihood for it involving this species is however deemed very small. For it to happen, the pathway would need to coincidentally bring together planned releases with the trade in red-whiskered bulbul.

Qu. 1.9. Estimate the overall likelihood of introduction into the risk assessment area or entry into the environment based on all pathways and specify if different in relevant biogeographical regions in current conditions.

Provide a thorough assessment of the risk of introduction in relevant biogeographical regions in current conditions: providing insight in to the risk of introduction into the risk assessment area.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Introduction into the risk assessment area has occurred (since the species is kept in zoos and as a pet bird), and the same is true for entry into the environment (given that the species is recorded in multiple countries, and locally established). The escape of pet birds from confinement is considered to be the prime pathway, in line with the species' invasion history outside of the risk assessment area. Further escapes or unauthorised releases are very likely to occur in the future, but the expected frequency is unclear. The contribution of prayer release is considered negligible, in contrast to the situation in Eastern Asia.

There is no evident geographical variation in holdings of red-whiskered bulbul (in zoos or as pets) throughout the risk assessment area. In terms of introduction and entry, we therefore consider all biogeographical regions to be equally prone. From the information available, it can be assumed that there are more red-whiskered bulbuls in zoos and collections than red-vented bulbuls. We scored very

likely with high confidence because the interpretation of information is straightforward and not controversial/contradictory (see A8).

Qu. 1.10. Estimate the overall likelihood of introduction into the risk assessment area or entry into the environment based on all pathways in foreseeable climate change conditions?

Thorough assessment of the risk of introduction in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of introduction (e.g. change in trade or user preferences)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely introduction within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Climate change is not expected to alter patterns of introduction in the risk assessment area, nor of entry into the environment (for any of the three pathways described). The response and its confidence are therefore scored equal to Qu. 1.9.

2 PROBABILITY OF ESTABLISHMENT

Important instructions:

- For organisms which are already established in parts of the risk assessment area or have previously been eradicated, the likelihood of establishment should be scored as “very likely” by default.
- Discuss the risk also for those parts of the risk assessment area, where the species is not yet established.

Qu. 2.1. How likely is it that the organism will be able to establish in the risk assessment area based on similarity of climatic and abiotic conditions in its distribution elsewhere in the world?

RESPONSE		CONFIDENCE	
	very unlikely		low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

See also Qu. A7 and Annex VIII. The red-whiskered bulbul is already established in Spain so suitable climatic and abiotic conditions are present in the risk assessment area. Using the climate matching methodology of Faulkner et al. (2014), and using the available validated distribution data (N = 7,208) of *P. cafer* in the native and invaded range gathered for the species distribution model (Annex VIII), we determined the degree (%) overlap with Köppen Geiger climate classification classes (Rubel and Kottek 2010; Beck et al. 2018) present in the risk assessment area. This shows that under current climate (2001-2025), there are similarly suitable climates for the red-whiskered bulbul in the risk assessment area, primarily humid subtropical climate (Cfa, 36% overlap), temperate oceanic climate (Cfb, 16% overlap), to a lesser extent other climates such as hot-summer Mediterranean climate (Csa, 3% overlap) which are present in a number of EU Member States (cf.

<https://www.plantmaps.com/koppen-climate-classification-map-europe.php>). This rough climate matching is refined and corroborated by the species distribution model (Annex VIII). Apart from climatological overlap, the species is associated with human influenced landscapes and can occupy a range of lowland habitats (forest edges, reed beds, gardens, orchards, scrubland, agricultural areas) (Hart 2020) which are not rare in Europe (see A4). The species distribution model (SDM), based on worldwide occurrence data, indicates suitable area in the Mediterranean, Black Sea and Atlantic biogeographic regions. The surface area suitable for establishment is about 48%, 8% and 9% respectively.

Qu. 2.2. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area? Consider if the organism specifically requires another species to complete its life cycle.

RESPONSE		CONFIDENCE	
	very isolated		low
	isolated		medium
	moderately widespread		high
	widespread		
	ubiquitous		

See Qu. A7, and Annex VIII. The SDM suggests that human influence on the environment is a poorer predictor for the occurrence of the red-whiskered bulbul than climate variables are. Nonetheless, the species is described as being associated with human habitation, preferring (semi)open vegetation (e.g. parks, gardens, orchards) in its native and introduced range (Islam and Williams 2000). Such (sub)urban landscapes are widespread in each of the biogeographic regions mentioned under Qu. 2.1.

The red-whiskered bulbul has a broad diet, consisting primarily of plants (petals, stamens, nectar, leaves, fleshy fruits), and secondarily of invertebrates (Islam and Williams 2000; Linnebjerg et al. 2009). Therefore, there is supposedly no dietary constraint for the species' establishment in the above biogeographic regions. But since no dietary studies have been performed within the risk assessment area, this conclusion comes with some uncertainty.

Qu. 2.3. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?

RESPONSE		CONFIDENCE	
	very unlikely		low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Following entry into the environment, the red-whiskered bulbul would probably compete for food (see Qu. 2.2.) and space (e.g. for nesting) with other bird species. Concerning food, however, the species' broad diet and the abundance of food items (e.g. leaves, fruits) would provide plenty of opportunity for niche differentiation with other omnivorous species.

The nests are open cups, and are generally well concealed in dense foliage. Height ranges described in literature are 0.6–2.4 m (Carleton and Owre, 1975), 1.5–3.6 m (van Riper et al., 1979), 0.9–3.8 m (Li et al., 2015) and 0.6–2.1 m (Khan and Naher, 2020). Li et al. (2015) found nests in 50 different plant species in China. Of the species mentioned by Islam and Williams (2000), *Nerium* (oleander) and *Hedera* (ivy) are widespread in the risk assessment area. House sparrow *Passer domesticus* has been frequently observed in association with the red-whiskered bulbul roosts in Florida, but without evident signs of direct competition (Islam and Williams 2000).

On Oahu (Hawaii), both the red-whiskered and the red-vented bulbul were introduced on year apart. Their population growth rates did not differ, but *P. cafer* became the more abundant species, which is attributed to a wider availability of dry habitats in favour of *P. cafer* (Williams and Giddings, 1984).

The existing literature suggests that the species is able to establish in new environments and to compete with a range of bird species there (see Qu. 4.1). Also, the current population in the risk assessment area established in a wet, riparian urban green area, which probably also attracts a good number of native songbirds. Yet, this did not hamper successful establishment. We therefore scored very likely with high confidence.

Qu. 2.4. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Predators: Potential predators (of relevance to the risk assessment area) of the red-whiskered bulbul are rats, cats, snakes (e.g. *Coluber* spp.), magpies (*Pica pica*), and birds of prey (e.g. *Falco* spp.; Carleton and Owre, 1975; Islam and Williams 2000; Li et al. 2015). Li et al. (2015) report relatively high rates of nest predation in the species’ native range in China. From a comparison of population expanse across tropical islands, Clergeau and Mandon-Dalger (2001) estimate predation to be less important as as constraint than inter-specific competition.

Parasites: Two ixodid tick species (*Haemaphysalis spinigera*, *H. wellingtoni*) were described on the red-whiskered bulbul from an Indian forest, both of which were highly abundant across the local pool of bird species (Rajagopalan, 1972). Furthermore, toxoplasmosis and three species of *Haemoproteus*, a blood parasite, have been detected (references within Islam and Williams, 2000).

Pathogens: The avian malaria parasite *Plasmodium (Novyella) jiangi* was first described from the red-whiskered bulbul (He and Huang, 1993), but its identity is doubtful (Valkiūnas and Iezhova 2018).

There is no reason to assume that predators, parasites or pathogens in the risk assessment area will cause a disproportionately high mortality during establishment of the red-whiskered bulbul. The species is already established in the risk assessment area (see A8b).

Qu. 2.5. How likely is the organism to establish despite existing management practices in the risk assessment area? Explain if existing management practices could facilitate establishment.

RESPONSE	very unlikely unlikely moderately likely	CONFIDENCE	low medium high
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	likely very likely		
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There are currently no dedicated management schemes (directed toward the species or its habitat) in place to prevent the red-whiskered bulbul from establishing. Hunting is unlikely to hamper establishment as hunting small passerines is a rather rare activity in the risk assessment area (especially also near human settlements). The species thrives in cultivated landscapes such as in agricultural areas or smaller scale vegetable gardens that can provide food. Birds are opportunistic in using natural and man-made nest material and can nest in hedges, shrubs and trees (Carleton and Owre 1975) therefore it is unlikely that commonly applied park and garden management is going to hamper successful establishment across the risk assessment area.

Qu. 2.6. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Red-whiskered bulbul have several (2-3) broods per year, a rather high (70-80% depending on the habitat) nesting success and low (10-17%) chick mortality in their native range (Hart 2020). Despite this, a well designed eradication campaign should be able to eradicate the red-whiskered bulbul in early stages of invasion, considering elements of visibility, the popularity of birdwatching (cf. citizen science), and the species' communal roosting behaviour which facilitates the mist-net catch along flight lines towards roosts. Also, the birds have a habit of vocalising from prominent perches which makes them conspicuous during eradication campaigns, for example when locating them for targeting of the last few birds through shooting campaigns (Bunbury et al., 2019). Different techniques have proven effective, such as mist-netting (Bunbury et al., 2019), trapping (Clergeau et al., 2002), and shooting (Clark, 1976). This is exemplified by successful campaigns in California (Clark, 1976), on Assumption and Aldabra (Seychelles; Bunbury et al. 2019), and on Tenerife (Cruz and Reynolds, 2019). Nonetheless, the time frame for (cost)effective eradication may be as short as five years following entry into the environment, as the species may rapidly spread in some situations (Clergeau and Mandon-Dalger, 2001). Considering these elements and examples of successful eradications, we scored moderately likely with medium confidence.

Qu. 2.7. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?

including the following elements:

- a list and description of the reproduction mechanisms of the species in relation to the environmental conditions in the risk assessment area

- an indication of the propagule pressure of the species (e.g. number of gametes, seeds, eggs or propagules, number of reproductive cycles per year) of each of those reproduction mechanisms in relation to the environmental conditions in the risk assessment area.
- If relevant, comment on the likelihood of establishment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in establishment whereas for others high propagule pressure (many thousands of individuals) may not.
- If relevant, comment on the adaptability of the organism to facilitate its establishment and if low genetic diversity in the founder population would have an influence on establishment.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Populations of the red-whiskered bulbul may develop from as little as a few breeding pairs (e.g. Pranty, 2010; Mo, 2015). A population could establish from just one single pair given that genetic diversity in founder birds is sufficiently high. For example, in Florida, the size of the founding population was reported small from “a few birds” (Fisk, 1966; Rand, 1980), “between 5 and 10 breeding pairs” (Carleton and Owre, 1975), or “five to ten” birds (Robertson and Woolfenden, 1992).

There is considerable variation in the species’ fecundity throughout its range. There is generally one brood per year, such as in China, with a mean clutch size of 2.5 eggs (Li et al., 2015). However, two and even three broods per year are described in parts of its native and non-native range, e.g. India and Hawaii (Islam and Williams, 2000; Mo, 2015; Li et al., 2015; and references therein). Clutch size generally varies between two and five eggs.

Since the species performs well close to human populations, with requirements for food and nesting that are easily fulfilled, its characteristics fit well with the environment it is most likely to enter in first. This makes survival at the very first stage likely. Climate (see Qu. 2.1.) and mortality events through competition (see Qu. 2.3.), predation or disease (see Qu. 2.4.) or human intervention (see Qu. 2.5. and 2.6.) subsequently are critical factors for initial population build-up. As stated above, only climate stands out as a structural constraint at that stage, whereas the others may prove a constraint only by their stochastic nature.

In general, the species shows behavioural flexibility, generalism (e.g. an opportunistic diet), has multiple broods throughout its lifespan and shows human commensalism (i.e. a propensity to living in close association with humans). These traits have been shown to link to invasion success in birds (Cassey et al. 2004; Callaghan et al. 2019; Sol et al. 2002).

Qu. 2.8. If the organism does not establish, then how likely is it that casual populations will continue to occur?

Consider, for example, a species which cannot reproduce in the risk assessment area, because of unsuitable climatic conditions or host plants, but is present because of recurring introduction, entry

and release events. This may also apply for long-living organisms.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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See section B1. Since the red-whiskered bulbul is kept as a popular pet bird, has a rather long expected lifespan of 15-25 years in captivity (Pangsuban and Thavarorith 2021), and is kept in zoological collections, there is a continuous risk of escape or unauthorised release. In the biogeographic regions that are not climatically suited (see Qu. 2.1.), casual records would be expected on a (close to) yearly basis as they currently already do (see Qu. A8a).

Qu. 2.9. Estimate the overall likelihood of establishment in the risk assessment area under current climatic conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under current climatic conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in current conditions: providing insight in the risk of establishment in (new areas in) the risk assessment area.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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The red-whiskered bulbul is already established in the risk assessment area (in Spain, see Qu. A8b). Despite some uncertainties on the model predictions in specific areas in the risk assessment area (see Qu. 9a), biogeographical regions predicted suitable for establishment are the Mediterranean (48% surface area), Black Sea (8%) and Atlantic (9%) biogeographic regions (see Qu. A7 and Annex VIII). This is exemplified by the presence of the established population near Valencia (Mediterranean, Qu. A8). Countries of the risk assessment area particularly at risk are Spain, Portugal, Cyprus, Greece, Italy and Malta (with France and Croatia to a lesser extent; see Qu. A9 and Annex VIII).

Competition (see Qu. 2.3.), predation or disease (see Qu. 2.4.) and current management (see Qu. 2.5. and 2.6.) are not considered constraints for establishment. They are expected to contribute to failed establishment in the first stages by chance only. In line with the guidance, as the species is already established in parts of the risk assessment area, the likelihood of establishment is scored as very likely.

Qu. 2.10. Estimate the overall likelihood of establishment in the risk assessment area under

foreseeable climate change conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under foreseeable climate change conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of establishment (e.g. increase in average winter temperature, increase in drought periods)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely establishment within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided.

However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Under foreseeable climate change conditions, the suitability of already-suitable biogeographic regions would increase, i.e. the Mediterranean ($\pm 60\%$ surface area), Black Sea ($\pm 25\%$), Atlantic ($\pm 20\%$) biogeographic regions (see Qu. A7 and Annex VIII). This results in the same countries particularly at risk (Spain, Portugal, Cyprus, Greece, Italy, Malta, France, Croatia, Ireland), with the addition of Slovenia, Bulgaria and the United Kingdom under foreseeable climate change conditions (see Qu. A9 and Annex VIII).

3 PROBABILITY OF SPREAD

Important instructions:

- Spread is defined as the expansion of the geographical distribution of an alien species within the risk assessment area.
- Repeated releases at separate locations do not represent continuous spread and should be considered in the probability of introduction and entry section (Qu. 1.7).

Qu. 3.1. How important is the expected spread of this organism within the risk assessment area by natural means? (List and comment on each of the mechanisms for natural spread.)

including the following elements:

- a list and description of the natural spread mechanisms of the species in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of spread discussed in relation to the species biology and the environmental conditions in the risk assessment area.

The description of spread patterns here refers to the CBD pathway category “Unaided (Natural Spread)”. It should include elements of the species life history and behavioural traits able to explain its ability to spread, including: reproduction or growth strategy, dispersal capacity, longevity, dietary requirements, environmental and climatic requirements, specialist or generalist characteristics.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Invasion histories within and outside the risk assessment area illustrate the red-whiskered bulbul is a mobile bird species with a capacity for natural spread (e.g. Clergeau and Mandon-Dalger, 2001; Mo, 2015; Santos 2015; Polo-Aparisi and Polo-Aparisi 2021). There is however considerable variation in reported spread rates for invasion histories outside the risk assessment area (Clergeau and Mandon-Dalger, 2001). Pranty (2010) describes the red-whiskered bulbul as “by far the most range-restricted exotic bird” established in Florida state, despite a 50-year presence. Also, in the risk assessment area, the established population around Valencia showed relatively moderate expansion from its original introduction point in 2003. In 15 years (2003-2019) the species has spread to only a few adjacent municipalities not very far from the original introduction area (Paterna). The spread has occurred mostly along the course of the river Turia which could act as a dispersal corridor, suggesting spread is influenced by landscape features. By 2016 it covered an area of seven 5km² squares (Polo-Aparisi and Polo-Aparisi 2021) (see Qu A8b). In contrast, the species has spread much faster elsewhere, as evidenced by patterns from Hawaii (Oahu) and the Mascarenes (Mauritius and Réunion). There, the red-whiskered bulbul colonized the entire area considered suitable within a few decades. This led Clergeau and Mandon-Dalger (2001) to estimate dispersion rates in continental areas to be about 3 km in ten years, but on islands, to be up to 30 km in ten years. Their data suggest that the rate of expansion is slower within the first five years after introduction. Clergeau and Mandon-Dalger (2001) note that the rate of spread on Réunion was most likely determined by habitat suitability, as bulbuls

first colonized the wet eastern coast, and then dry habitats and high altitudes (Mandon-Dalger et al. 1999). Within ten years of their initial introduction, bulbuls occupied ca 380 km². Although the speed of dispersal was not the same in all directions, they estimated an average dispersion rate of ca 6.2 km/yr during the first ten years, and 14.7 km/yr between 1985 and 1995. A limited spread rate overall has been attributed in part to the species' preference for communal roosting (Mo, 2015). The higher spread on islands has been suggested to be due to the smaller size of their bird communities, thus limiting competition (Clergeau and Mandon-Dalger, 2001).

Given contrasting evidence, and a limited insight into how local species pools and habitat suitability may constrain the spread rate of the red-whiskered bulbul within the risk assessment area, we estimate spread to be moderate, with a medium confidence since there is documented evidence available.

Qu. 3.2a. List and describe relevant pathways of spread other than "unaided". For each pathway answer questions 3.3 to 3.9 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 3.3a, 3.4a, etc. and then 3.3b, 3.4b etc. for the next pathway.

including the following elements:

- a list and description of pathways of spread with an indication of their importance and associated risks (e.g. the likelihood of spread in the risk assessment area, based on these pathways; likelihood of survival, or reproduction, or increase during transport and storage; ability and likelihood of transfer from the pathway to a suitable habitat or host) in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of spread for each pathway discussed in relation to the species biology and the environmental conditions in the risk assessment area.
- All relevant pathways of spread (except "Unaided (Natural Spread)", which is assessed in Qu. 3.1) should be considered. The classification of pathways developed by the Convention of Biological Diversity shall be used (see Annex IV).

Pathway name: Following entry into the environment through any of the pathways addressed under Section B1, and disregarding range expansion through multiple entries by the same pathways, spread is expected to occur only unaided, i.e. natural spread through bird flight. No indication for other spread pathways is found in literature.

Qu. 3.3a. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional	CONFIDENCE	low
	unintentional		medium high

N/A (see Qu. 3.2a.).

Qu. 3.4a. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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N/A (see Qu. 3.2a.).

Qu. 3.5a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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N/A (see Qu. 3.2a.).

Qu. 3.6a. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely	CONFIDENCE	low medium high
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	likely very likely		
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N/A (see Qu. 3.2a.).

Qu. 3.7a. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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N/A (see Qu. 3.2a.).

Qu. 3.8a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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N/A (see Qu. 3.2a.).

Qu. 3.9a. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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N/A (see Qu. 3.2a.).

Qu. 3.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?

RESPONSE	very easy easy with some difficulty difficult very difficult	CONFIDENCE	low medium high
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As spread is considered to be driven only by natural dispersal, efforts to contain the red-whiskered bulbul would translate into measures for early detection and rapid eradication around sites of entry. Some of the species' characteristics are favourable for such campaigns, e.g. its visibility, vocal nature, and communal roosting behaviour. Possible techniques include mist-netting (Bunbury et al., 2019), trapping (Clergeau et al., 2002), and shooting (Clark, 1976). Some campaigns have led to successful eradication (see Qu. 2.6.; Clark, 1976; Bunbury et al. 2019; Cruz and Reynolds, 2019).

Containment campaigns would thus be easy, in a relative sense to other introduced animal species. Yet, the human environment may also bring some drawbacks, such as public opposition or legal restrictions (e.g. on shooting). Given that the rate of spread can vary considerably (see Qu. 3.1.), the window of opportunity for rapid response campaigns is not so clear but would best be conceived as less than five years (Clergeau and Mandon-Dalger, 2001). We therefore classify the difficulty as moderate.

Qu. 3.11. Estimate the overall potential rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (indicate any key issues and provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in current conditions, providing insight in the risk of spread into (new areas in) the risk assessment area.

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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As spread is considered to be driven only by natural dispersal, the response is set as equal to that of Qu. 3.1. There is no established link between red-whiskered bulbul fecundity and climate, though the number of broods per year may be hypothesized to correlate with warmer regions and lower

seasonality (e.g. China versus India, Li et al., 2015; Florida versus Hawaii, Islam and Williams, 2001). If this is the case, the rate of spread may be higher in e.g. the Mediterranean biogeographic region as compared to the Atlantic biogeographic region.

Qu. 3.12. Estimate the overall potential rate of spread in relevant biogeographical regions in foreseeable climate change conditions (provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if rates of spread are likely slowed down or accelerated.

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Only through the hypothesized relation between fecundity and climate, under Qu. 3.11, would an increased rate of spread be expected under climate change conditions. Given foreseeable change, we nonetheless expect that the increase would be marginal (i.e. no consistent increase to two broods per year).

4 MAGNITUDE OF IMPACT

Important instructions:

- Questions 4.1-4.5 relate to biodiversity and ecosystem impacts, 4.6-4.8 to impacts on ecosystem services, 4.9-4.13 to economic impact, 4.14-4.15 to social and human health impact, and 4.16-4.18 to other impacts. These impacts can be interlinked, for example, a disease may cause impacts on biodiversity and/or ecosystem functioning that leads to impacts on ecosystem services and finally economic impacts. In such cases the assessor should try to note the different impacts where most appropriate, cross-referencing between questions when needed.
- Each set of questions starts with the impact elsewhere in the world, then considers impacts in the risk assessment area (=EU excluding outermost regions) separating known impacts to date (i.e. past and current impacts) from potential future impacts (including foreseeable climate change).
- Only negative impacts are considered in this section (socio-economic benefits are considered in Qu. A.7)
- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable).

Biodiversity and ecosystem impacts

Qu. 4.1. How important is the impact of the organism on biodiversity at all levels of organisation caused by the organism in its non-native range excluding the risk assessment area?

including the following elements:

- Biodiversity means the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems
- Impacted chemical, physical or structural characteristics and functioning of ecosystems

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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The red-whiskered bulbul impacts species and ecosystems through a number of impact mechanisms which are discussed below. Apart from impacts on individual species with documented cases of extinctions and declines in native species in the invaded range, the species can also exert ecosystem-level impacts through the facilitation of plant invasions and changes in species compositions in natural areas. As these changes are probably irreversible without severe management interventions and because of the documented local extinctions of native species, we scored major impact. This is in line with Evans et al. (2016) who classified the species as having a Major (MR) environmental impact

using the Environmental Impact Classification for Alien Taxa (EICAT) protocol with predation being the most severe impact mechanism (see also A.3). Martin-Albarracin et al. (2015), in a global analysis of alien bird impacts for 39 bird species, identified the red-whiskered bulbul as one of the three species with the highest global impact because of severe local impacts and a strong intrinsic ability to affect ecosystems. Note that in this risk assessment the red-whiskered bulbul received a higher impact score than the related red-vented bulbul *P. cafer* for the impact criteria competition and interactions with other non-native organisms based on the evidence provided in Carleton and Owre (1975) for Florida and the studies of Linnebjerg et al. (2009, 2010), Tassin et al. (2007) and Jones (1996) for Indian ocean populations. According to their scoring system used (an adapted scheme based on Kumschick and Nentwig (2010) and Blackburn et al. (2014), where 0 indicates no impact detected and 5 massive impact), the red-whiskered bulbul received a score of 4 on competition (i.e. *competition with many native species, several declining in population size, competition for food and/or space, behavioural changes in out-competed species*) and 5 on interactions with other non-native organisms (i.e. *Dispersal of seeds of non-native plants facilitates replacement or local extinction of one or several native species, and produces irreversible changes in community composition that would not have occurred in the absence of the species*) (Martin-Albarracin et al. 2015). Baker et al. (2014), in a global analysis of alien bird impacts, also report interference competition and predation as impact mechanisms for the species.

Impact mechanisms:

1: Frugivory (spreading the fruit/seeds of alien plants)

Red-whiskered bulbul are frugivorous and are notorious for spreading invasive weeds including *Lantana* spp., *Rubus* spp., *Phytolacca* spp., *Chrysanthemoides* spp. and *Ligustrum* spp. in Mediterranean parts of Australia and this is considered their biggest impact on ecosystems (Mo 2015). Of particular concern is the movement of the birds between plantations and natural forest areas which alters the plant species composition of natural areas (Linnebjerg 2009). Invasions of the species could therefore facilitate invasive plants (Traveset, 2006; Traveset and Richardson 2014; MacFarlane et al., 2016). On Mauritius, the red-whiskered bulbul facilitated the spread of invasive plant species like *Ligustrum robustum*, *Lantana camara* and *Cordia interrupta*, which, prior to the introduction of the red-whiskered bulbul, were sleeper weeds (Linnebjerg et al. 2009, 2010). Linnebjerg et al. (2010) in an analysis of faecal pellets found the diet to consist primarily of fleshy fruits with seeds of ten plant species being present in 92% of all faecal pellets six of which were notorious invasive plant species on Mauritius (*Clidemia hirta*, *Ligustrum robustum*, *Litsea glutinosa*, *Psidium cattleianum*, *Rubus alceifolius* and *R. rosifolius*). Gut passage of seeds by the red-whiskered bulbul improved the germination success of the invasive plants *Clidemia hirta* (Linnebjerg et al., 2009), and *Schinus terebinthifolius* (Mandon-Dalger et al., 2004), on Mauritius and Réunion, respectively. In Hawaii, the species spreads the seeds of invasive *Miconia calvescens*, ivy (*Hedera*), gourd and false kava (*Piper auritum*). On Réunion, involvement of the red-whiskered bulbul in facilitating plant invasions was reported by Clergeau and Mandon-Dalger (2001) and Mandon-Dalger et al. (2004). There, red-whiskered bulbul was implicated in invasion of Brazilian pepper tree *S. terebinthifolius* and the birds were suspected to form satellite foci of plant infestations. Although the authors note consequences of long distance dispersal of this invasive shrub through ornithochorie by red-whiskered bulbul for ecological restoration, they note that the recruitment of *S. terebinthifolius* is also dependent on the soaking of fruits in water to activate germination after deposition and early seedling development. In Australia, red-whiskered bulbul has been implicated in the establishment of African Olive *Olea europaea cuspidata* in western Sydney, which is considered an environmental weed in eastern

Australia (Cuneo and Leishman 2006). Other noxious weeds that red-whiskered bulbul was reported feeding on in Australia include Lantana *L. camara*, inkweed *Phytolacca octandra*, bitou bush *Chrysanthemoides monilifera* and Chinese privet *Ligustrum sinense* (see Mo 2015 and references therein).

2: Competition

In Florida, red-whiskered bulbul overlaps in diet with northern mockingbird *Mimus polyglottos* and competes with this native species for fruits of mango, christmasberry, fig (*Ficus* spp.), day hessamine, lantana (*Lantana* spp.) and jasmine (*Jasminum* spp.), especially during winter when insects are scarce (Carleton 1971; Gore and Doubilet 1976). In Hong Kong, where both species have long been abundant residents and co-occur in the same habitats and have similar food and environmental requirements, red-whiskered bulbul has replaced Chinese bulbul (*P. sinensis*) in urban and suburban contexts and caused the Chinese bulbul to retreat in more rural areas including mountains, mangroves and forests (Chan 2004). Chan (2004) hypothesizes red-whiskered bulbul is a better competitor for food and nesting sites in human-modified habitats. The same happened on the Nicobar islands where the species, after it was introduced from the Andaman islands in the late 1800s, displaced the endemic Nicobar bulbul (*Ixos nicobariensis*) (Sankaran 1998) which is now considered near threatened (Goyal 2020). In Sydney, agonistic responses toward flocks of silvereye *Zosterops lateralis* and rosellas *Platycercus* spp. feeding on camphor laurel *Cinnamomum camphora* fruits have been reported (MacPherson 1923). However, Mo (2015) states that concerns over interspecific competition would be most relevant to invasions of islands and that despite field observations, there is no evidence that interspecific competition is ecologically significant in Australia.

3: Hybridisation

Red-whiskered bulbuls have been hybridized with several other bulbul species in captivity to increase their qualities as a pet (McCarthy 2006; Techachoochert and Round 2013) (see A1, A2). Techachoochert and Round (2013) point out this practice might represent a genetic risk to wild native populations of the red-whiskered bulbul. It also highlights a potential risk of the species (or their hybrids) interbreeding with other native bulbul species (Hart 2020).

4: Predation

The Red-whiskered Bulbul preys on arthropods such as cicadas, flies, aphids (MacPherson 1921, 1923), ants, moths and their larvae (Chisholm 1933, Chaffer 1933) and spiders (Islam and Williams 2000). Xiaohua (1992) found arthropods comprising approximately 28% of the diet in southern China. It is also known to be a predator of smaller (or juvenile) vertebrates like geckos and lizards (Mo 2015). Islam and Williams (2000) report that populations of geckos severely declined on Mauritius probably because of predation by bulbuls on young geckos. There have also been instances of predation of chicks of other bird species, for instance on the Mascarene islands (Philippe and Mandon-Dalger 2001) and Assumption (Roberts 1988) although it was unclear whether these represented real predation events or rather aggressive interactions (Baker et al. 2014). Red-whiskered bulbuls are known to display intra- and interspecific territorial aggression year-round which intensifies during their nesting period (Hart 2020). The red-whiskered bulbul also represents a threat to native invertebrates, and caused the extinction of native orb-web spiders (*Nephila inaurata*) on Mauritius by predation (Islam and Williams 2000). Such impact could also have indirect effects on prey species or other native predators through cascading effects. On Hawaii, predation by red-whiskered bulbul on

larvae and adults of the iconic monarch butterfly *Danaus plexippus* (Lepidoptera: Nymphalidae) led to changes in the proportions of colour morphs in the population (Stimson and Kasuya 2000). Bulbuls are not deterred by the cardiac glycosides in the monarchs' tissues and can exert heavy predation on larvae feeding on their host plant milkweed (Stimson and Berman 1990). However, we could find no evidence of population level impacts on these species.

5: Pathogen transmission

Red-whiskered bulbuls are a potential reservoir of avian malaria (He and Huang 1993, Think et al. 2012) (see Qu 2.4, Qu. 4.16), which is a well-known threat to native forest birds, such as on Hawaii (Samuel et al. 2011). In India, red-whiskered bulbuls carry several species of birdlice (Arya et al. 2010, Saxena et al. 2012). Hart (2020) notes that it is likely that other parasites and diseases are hosted by red-whiskered bulbul and that given their interspecies roosting behaviour they could be important reservoirs of avian pathogens.

Qu. 4.2. How important is the current known impact of the organism on biodiversity at all levels of organisation (e.g. decline in native species, changes in native species communities, hybridisation) in the risk assessment area (include any past impact in your response)?

Discuss impacts that are currently occurring or are likely occurring or have occurred in the past in the risk assessment area. Where there is no direct evidence of impact in the risk assessment area (for example no studies have been conducted), evidence from outside of the risk assessment area can be used to infer impacts within the risk assessment area.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Although there is evidence of the red-whiskered bulbul foraging on non-native plants (see Qu 4.3), no studies have been found quantifying the impact of red-whiskered bulbul in the risk assessment area (Spain). Recent population estimates are lacking but in 2016 the population in Valencia was estimated at 100-150 individuals (Santos 2015). Since this established population is presumably still fairly small, impact can be assumed to be minor but with low confidence due to the lack of impact studies. Also, the Spanish population is likely interacting with relatively common native species. Although hybridization with the co-occurring non-native red-vented bulbul could theoretically occur in the risk assessment area, there are currently no native bulbul species in the area so impact would occur through other impact mechanisms (predation, frugivory, competition with native bird species).

Qu. 4.3. How important is the potential future impact of the organism on biodiversity at all levels of organisation likely to be in the risk assessment area?

See comment above. The potential future impact shall be assessed only for the risk assessment area. A potential increase in the distribution range due to climate change does not *per se* justify a higher impact score.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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1: Frugivory (spreading the fruit/seeds of alien plants)

In Spain, red-whiskered bulbuls have been reported feeding on seeds and fruits of native as well as non-native plant species e.g. kurrajong (*Brachychiton populneus*), fig (*Ficus carica*), strawberry tree (*Arbutus unedo*), loquat (*Eriobotrya japonica*), privet (*Ligustrum vulgare*), chinaberry tree (*Melia azedarach*), pomegranate (*Punica granatum*), Peruvian pepper (*Schinus molle*), date palm (*Phoenix dactylifera*), oleander (*Nerium oleander*) flowers, feijoa (*Acca sellowiana*) and yucca (*Yucca* sp.) (Santos 2015; Polo-Aparisi and Polo-Aparisi 2021). Spread of typical garden ornamentals by red-whiskered bulbul could be an issue in the Mediterranean considering the habitat preference of the species for gardens and man-made habitat, for example *Trachycarpus fortunei*, *Mahonia aquifolium*, non-native *Ribes* sp., *Parthenocissus* sp., *Cotoneaster* sp., *Rosa* sp., *Elaeagnus* sp., *Ziziphus jujuba*, *Morus* sp., *Pittosporum* sp., *Myoporum* sp., *Mirabilis jalapa*, *Opuntia* sp., *Lycium* sp., *Lonicera* sp., *Aralia* sp. and *Hedera* sp. With regards to IAS of Union concern (Union list as it stands in April 2021), *Persicaria perfoliata* is the only listed species that produces berry-like fruits (personal communication G. Brundu, 23/10/2019). This Union list species has however not been found across the EU by 2017 (Tsiamis et al. 2017). American pokeweed (*Phytolacca americana*) and oriental bittersweet (*Celastrus orbiculatus*) are relevant to mention as candidate species proposed for uptake on the Union List in 2022. Both species occur in open, disturbed habitats and are popular with berry eating birds (McDonnell et al., 1984; Beringen et al., 2017). It should be noted that these alien plants can also be spread by native birds (e.g. thrushes) and there is currently no information suggesting red-whiskered bulbul would do this to a greater extent.

It is well known that island ecosystems are especially sensitive to the impacts of invasive alien species because of high levels of endemism (e.g. Tershy et al., 2015; Bellard et al., 2016). The Mediterranean basin is particularly vulnerable to invasive alien plant invasions because its climatic conditions potentially allow the establishment of sub-tropical and tropical plant species (Lloret et al., 2005; Vila et al., 2006, 2008; Hulme et al., 2008; Lambdon et al., 2008; Brunel et al., 2010; Brundu 2015). A number of established and emerging invasive alien plants for Mediterranean countries produce fleshy fruits and could therefore potentially be spread by birds such as the red-whiskered bulbul in the risk assessment area (cf. Gosper et al., 2005; Spotswood et al., 2012, 2013). American pokeweed (*Phytolacca americana*) and Indian pokeweed (*P. acinosa*) produce fleshy purple berries that are spread by birds (McDonnell et al., 1984). Other examples of (potentially) invasive alien plants that might be spread by red-whiskered bulbuls in the risk assessment area include Brazilian peppertree (*Schinus terebinthifolius*), a commonly planted ornamental in the Mediterranean which is reported to

be spread by the red-vented bulbul (Thibault et al., 2018), silver-leaved nightshade (*Solanum elaeagnifolium*), a common agricultural weed (Brunel et al., 2010), sticky nightshade (*S. sisymbriifolium*), a weed of pastures and irrigated crops, roseleaf bramble (*Rubus rosifolius*), Chinese privet (*Ligustrum sinense*) and garden privet (*L. ovalifolium*) (Tanner 2017). Some of these species typically occur in human-modified areas such as parks, gardens and ruderal terrains and red-whiskered bulbul is currently also confined to this kind of habitat.

2: Competition

Impact on native bird species could occur through competition for food or space including harassment of native birds (mostly passerine birds, see Qu 4.1) by (groups of) red-whiskered bulbul, which is reported as an aggressive species in the invaded range through territorial interactions. Where the species occurs in urban, human influenced landscapes, most of the passerine species it would compete with are relatively common (e.g. blackcap *Sylvia atricapilla*, Sardinian warbler *S. melanocephala*, common blackbird *Turdus merula*) and the presence of the bulbul is expected to lead mostly to niche contraction rather than declines or extinctions, at least on larger land masses. Competition for food would likely occur with bird species that exploit similar resources such as species of Turdidae, Sylviidae and Sturnidae that occur in urban, wasteland and farmland areas. Also, several common species with broad diets such as great tit (*Parus major*), greenfinch (*Chloris chloris*) or wood pigeon (*Columba palumbus*) could be impacted yet the population level impact on these very common species would probably be small. However, based on evidence elsewhere in the invaded range (see Qu 4.1), the impact on small populations on islands might be severe.

3: Hybridisation

Hybridisation is unlikely to be an issue, as the only native species, common bulbul (*P. barbatus*), is currently still a very rare bird in the risk assessment area yet might increase its distribution with climate change.

4: Predation

The Red-whiskered Bulbul might have an impact on arthropods such as cicadas, flies, aphids, ants, moths and their larvae and spiders (see Qu 4.1). In Valencia the species was observed catching insects in flight on trees (Santos 2015; Polo-Aparisi and Polo-Aparisi 2021). It is also known to be a predator of smaller (or juvenile) vertebrate prey like geckos and lizards and possibly chicks of songbirds. Depending on the population size and the subsequent levels of predation exerted on populations, this could represent a problem for small populations of rare prey species of red-whiskered bulbul. Populations on islands (including isolated habitats which can be relatively species-poor), could be severely impacted should bulbuls establish and develop a feeding preference for such species. In extreme cases this could also lead to (local) extinctions (cf. the disappearance of *Nephila* orb-web spiders on Mauritius, see A10 and Qu 4.1). To exemplify a few sensitive receptors of conservation concern, we compiled a list of lizards and geckos that could potentially be predated upon using Speybroeck et al. (2016) and data compiled on native reptiles on Mediterranean islands by Ficetola et al. (2014). A lot of those have restricted, endemic ranges within Mediterranean islands. These include: Greek Algyroides (*Algyroides moreoticus*) (endemic), Dalmatian Algyroides (*A. nigropunctatus*) and Peloponnese slow worm (*Anguis cephallonica*) on Ionian islands; Sicilian wall lizard (*Podarcis waglerianus*) (endemic) on Sicily and the threatened Aeolian wall lizard (*Podarcis raffoneae*) (endemic) on some smaller Aeolian islets; Milos wall lizard (*Podarcis milensis*) (endemic), Balkan

green lizard (*Lacerta trilineata hansschweizeri*) (endemic subspecies), Skyros wall lizard (*Podarcis gaigeae*) (endemic) and Erhard's wall lizard (*Podarcis erhardii*) on the Aegean islands; Cretan wall lizard (*Podarcis cretensis*) (endemic), Kotschy's gecko (*Mediodactylus kotschyi bartoni*) (endemic subspecies), Balkan green lizard (*L. trilineata polylepidota*) (endemic subspecies) and Pori wall lizard (*Podarcis levendis*) (endemic) on Crete; Pygmy algyroides (*A. fitzingeri*) (endemic), Tyrrhenian wall lizard (*Podarcis tiliguerta*) (endemic) and European leaf-toad gecko (*Euleptes europaea*) (endemic) on the Thyrrenian islands; Ibiza wall lizard (*Podarcis pityusensis*) (endemic) and Lilford's wall lizard (*Podarcis lilfordi*) (endemic) on the Balearic islands; Kotschy's gecko (*Mediodactylus kotschyi*) on Cyprus and the eastern Mediterranean. Likewise, the list of insects (Lepidoptera, Coleoptera, Orthoptera) that could be predated upon by bulbuls is very long. Considering documented predation on monarch butterfly (*Danaus plexippus*) on Hawaii (see Q 5.1), this species could also be impacted in southern Spain where it is established yet rare (Gil 2006; Lafranchis 2004). Also similar species of Nymphalidae (and other families such as Papilionidae) with conspicuous colours and/or conspicuous larvae could be impacted in Spain and the Mediterranean, such as the African queen (*Danaus chrysippus*) but documented information is lacking for the risk assessment area. Because there is evidence of local extinctions, and because the species has a wide ecological amplitude which is not confined to urban or rural habitats, in line with current assessments of alien bird impacts (e.g. Martin-Albarracin et al. 2015, Evans et al. 2016), we scored major but with low confidence because impacts are inferred from other areas.

Qu. 4.4. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism currently in the risk assessment area?

including the following elements:

- native species impacted, including red list species, endemic species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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We found no documented impact information for the population around Valencia. Currently, in the risk assessment area, there is no evidence that red-whiskered bulbul occurs or is spreading in high conservation value habitats. The part of the Rio Turia basin in Valencia, where the species currently has its main stronghold in only a small part of the lower course of the river (but seems to be spreading, see A8a), is an important suburban green space for the city with cultural value (e.g. the Garden of the

Turia which was developed over the old course of the river close to the river's mouth) and flood defence functions provided by an artificial canal developed after the severe floods in 1950. Although the area is not a NATURA2000 site, it is a part of the Turia Natural Park, which encompasses 4,680 ha of protected land along the river, with valuable Mediterranean riparian forest, stands of Aleppo pine (*Pinus halepensis*), Mediterranean scrub and typical Valencian agricultural landscapes rich in biodiversity and traditional values (<https://www.valenciaturisme.org/en/parquenatural/parque-natural-del-rio-turia/>). As the species is currently limited in distribution and numbers, and confined to only a small part of the Rio Turia basin, we scored minor impact. The confidence on this response is low in the absence of studies on the subject.

Qu. 4.5. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.3 and 4.4.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Based on what is known of its ecological amplitude and habitat characteristics, the red-whiskered bulbul could potentially establish and spread in a variety of habitats, mostly (peri)urban habitats as is the case Valencia, but the species is known to cross the barrier between man-made and natural (forest) areas. Currently, the red-whiskered bulbul rarely occurs outside urban areas in the risk assessment area, and most of the landscape around the currently invaded area consists of irrigated orange grove and row crop farmland which are probably mostly unsuitable for the species. More inland areas consist of dry cultivation areas (olive, almond, carob trees, vineyards) and natural habitats consisting of dry treeless shrubland and pine woods where the red-whiskered bulbul would probably also have a hard time finding food (pers. comm. E. Murgui, September 2021). However, in other parts of the risk assessment area, the landscape context might be different. In (sub)urban and rural-agricultural areas, impact (e.g. through competition) would occur on rather common species (see Qu. 5.3). However, if the species becomes more widespread, also conservation value habitats could be invaded where the bulbuls could affect species of concern. Moreover, they could also have more systemic, irreversible ecosystem-level effects through the spread of invasive plant species from ruderal to natural areas (see Qu. 4.1). Habitats protected by the Habitats Directive which could potentially be invaded and impacted upon through seed dispersal of invasive alien plants include sub-Mediterranean and temperate scrub, Mediterranean arborescent matorral (maquis), Mediterranean sclerophyllous forests (e.g. wild olive woodland, cork-oak forests), garrigue (also known as phrygana in the eastern Mediterranean) and maquis shrubland, which is a complex of several possible vegetation types but characterized by densely growing evergreen shrubs. Several of these vegetation types have unique representation as specific habitats of the Habitats Directive on islands in the Mediterranean (e.g. Tyrrhenian islands, Ionian islands, Cyprus, Malta) (based on European Commission 2013). The

Mediterranean scrub biome is also home to a number of breeding birds that could be affected by the red-whiskered bulbul through competition for nesting space and food. These include a range of songbirds (shrikes, warblers, buntings etc.) and include many species protected by the Birds Directive as well as species listed as vulnerable on the IUCN Red List e.g. Iberian grey shrike (*Lanius meridionalis*), bunting species (*Emberiza spp.*) or commoner species of similar habitats like European stonechat (*Saxicola rubicola*). Other vertebrate species that could potentially be affected include geckos and small lizards, many of which are endemic or have very restricted ranges and are listed on the Annexes II and IV of the EU Habitats Directive (see Qu 4.3).

We scored moderate impact because, although the species is known to invade natural areas, presumably it is still primarily bound to human-influenced landscape. Confidence is low because most of the assumed future impacts on conservation values are inferred from cases outside the risk assessment area.

Ecosystem Services impacts

Qu. 4.6. How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?

- For a list of services use the CICES classification V5.1 provided in Annex V.
- Impacts on ecosystem services build on the observed impacts on biodiversity (habitat, species, genetic, functional) but focus exclusively on reflecting these changes in relation to their links with socio-economic well-being.
- Quantitative data should be provided whenever available and references duly reported.

RESPONSE		CONFIDENCE	
	minimal		low
	minor		medium
	moderate		high
	major		
	massive		

1 / Provisioning ecosystem services: red-whiskered bulbul may have an impact on provisioning services such as cultivated terrestrial plants grown for nutritional purposes and as ornamentals and also on wild plants used for nutrition. The species is considered a pest for horticultural and agricultural produce outside its native range, especially in gardens and orchards where it impacts flowers, fruits and vegetables. It is a reported nuisance species in gardens causing damage to fruits such as peas, figs and strawberries (MacPherson 1923, Mo 2015), but also to the buds and ripening fruit of orchard crops, including guava, mango, orange, papaya, plum, raspberry, strawberry and flowers including orchids, seedlings, vegetables and ripening crops such as coffee and pea (Department of Primary Industries and Regional Development 2018). It also causes crop failure in soft-fruit and citrus orchards (Chaffer 1933), as it has in California (Islam and Williams 2000) and on Hawaii (Williams and Giddings 1984). Apart from this direct economic impact, the species impacts by spreading weeds into natural areas and the human environment (see Qu 4.1 - frugivory).

2 / Cultural ecosystem services: impacts on cultural ecosystem services may include disturbance of the heritage of isolated island ecosystems. In particular, changes in vegetation structure and species composition could be caused by seed dispersal of invasive weedy plant species (see Qu. 4.1). This may make landscapes less attractive for recreation and wildlife watching, and impact the qualities of ecosystems with cultural importance.

We scored this as moderate since impacts on provisioning services represent measurable, temporary, local (but probably reversible) effects, although loss of agricultural production might locally be irreversible. We scored confidence as medium because there is evidence from the invaded range of both types of impacts to ecosystem services.

Qu. 4.7. How important is the impact of the organism on provisioning, regulating, and cultural services currently in the different biogeographic regions or marine sub-regions where the species has established in the risk assessment area (include any past impact in your response)?

- See guidance to Qu. 4.6.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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No information was found on reported damages in the Valencia area where the red-whiskered bulbul is established. However, it has been reported feeding on seeds and fruits of native as well as non-native plant species there including species of nutritional and ornamental value (Santos 2015) (see Qu. 4.3). Therefore, and because the population is increasing (see A8a), it is reasonable to assume there is an actual impact on provisioning ecosystem services in that area but it is unmeasurable, hence minor impact. As no information has been found on the area where it is currently established (Valencia), the confidence on this assessment is low.

Qu. 4.8. How important is the impact of the organism on provisioning, regulating, and cultural services likely to be in the different biogeographic regions or marine sub-regions where the species can establish in the risk assessment area in the future?

- See guidance to Qu. 4.6.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Wherever it could potentially establish in the risk assessment area (see Annex VIII), the red-whiskered bulbul could impact upon several ecosystem services (Annex V), including:

- Provisioning - Biomass - Cultivated terrestrial plants - Cultivated terrestrial plants grown for nutritional purposes
- Regulation and Maintenance - Regulation of physical, chemical, biological conditions - Lifecycle maintenance, habitat and gene pool protection - Seed dispersal
- Cultural - Direct, in-situ and outdoor interactions with living systems that depend on presence in the environmental setting - Physical and experiential interactions with the natural environment - Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through passive or observational interactions
- Cultural - Direct, in-situ and outdoor interactions with living systems that depend on presence in the environmental setting - Physical and experiential interactions with the natural environment - Intellectual and representative interactions with natural environment - Characteristics of living systems that enable aesthetic experiences
- Cultural - Indirect, remote, often indoor interactions with living systems that do not require presence in the environmental setting - Other biotic characteristics that have a non-use value - Characteristics or features of living systems that have an option or bequest value

Impacts would be comparable to other areas outside the native range (see Qu 4.6). Besides crop damage (e.g. olives, grapes, figs, peaches) or ornamental plant damage in the endangered area (Annex VIII), the red-whiskered bulbul also impacts seed dispersal and could act as a vector for seeds of invasive plant species causing changes in ecosystem structure and species composition of ecosystems; it could also lead to local extinctions of endemic species on islands, including habitat islands on the mainland (for examples see Qu. 4.3 and Qu. 4.4). This could impact on culturally valued elements of ecosystems (structure, species composition etc.) that make them attractive to ecotourism, recreation, wildlife watching, or that have cultural and intrinsic value, particularly on Mediterranean islands.

Economic impacts

Qu. 4.9. How great is the overall economic cost caused by the organism within its current area of distribution (excluding the risk assessment area), including both costs of / loss due to damage and the cost of current management.

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the world these should be reported here. The assessment of the potential costs of / loss due to damage shall describe those costs quantitatively and/or qualitatively depending on what information is available. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage. As far as possible, it would be useful to separate costs of / loss due to the organism from costs of current management.

RESPONSE		CONFIDENCE	
	minimal		low
	minor		medium
	moderate		high
	major		
	massive		

The fruit diet of the red-whiskered bulbul may have direct economic impacts, by causing production loss of cultivated plants, or indirect impacts, by spreading weed species into the human environment that are problematic.

Regarding the first (damage by herbivory), van Riper et al. (1979) reported consumption of fruits of the following species with a commercial interest from Hawaii: date palm (*Phoenix dactylifera*), papaya (*Carica papaya*), mango (*Mangifera indica*), autograph tree (*Clusia rosea*), figs (*Ficus*), loquat (*Eriobotrya japonica*), avocado (*Persea americana*), octopus tree (*Schefflera actinophylla*), and mock orange (*Murraya exotica*). Largely the same species are mentioned for Florida, where also citrus is a suggested food item (Carleton and Owre, 1975). For Australia, reference is made to damage to guava, peas, figs, strawberries and other soft fruits (MacPherson, 1923; Chaffer 1933). The species is also reported to damage agriculture in Japan (National Institute for Environmental Studies, 2017). In accordance with such observations, the red-whiskered bulbul is widely regarded as an agricultural threat to fruit orchards, vegetable gardens, and flower nurseries in introduced and native ranges (references in Islam and Williams, 2000). However, some debate exists on the extent to which the red-whiskered bulbul is responsible for the initial damage to larger fruits. Van Riper et al. (1979) noted that birds pierced the skin of the fruits, except for mature avocados, yet also noted that prior skin tearing from fruit ripening cannot be excluded. From limited experimentation and surveys with growers, although citrus (*Citrus* spp.) and mango (*Mangifera indica*) are readily consumed by the birds, Carleton and Owre (1975) concluded that the red-whiskered bulbul only damages fruits secondarily. On the internet, video footage can be found of red-whiskered bulbul damaging and eating white and red grapes and clearly causing the primary damage to these soft fruits (e.g. https://www.naturefootage.com/video-clips/ASHA170208_0012/the-red-whiskered-bulbuls-pycnonotus-jocosus-landed-on-the-branch). Overall no studies were found that provide formal, quantitative data on production losses. Mo (2015) ranks the species' importance as an agricultural pest to be more limited in Australia as compared to e.g. Common starling (*Sturnus vulgaris*).

Regarding the second (damage by weed dispersal), the red-whiskered bulbul can disperse many weeds that are a concern to agriculture or human settlements. These have been dealt with in Qu. 4.1.

As the species is a recognized agricultural pest species in many parts of the world and there are many, although mostly anecdotal, records of economic damages (many more probably remain unreported), we assume yearly damages can easily mount up to more than € 1,00,000 per year and scored moderate with low confidence.

Qu. 4.10. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism currently in the risk assessment area (include any past costs in your response)?

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the EU these should be reported here. Assessment of the potential costs of damage on human health, safety, and the economy, including the cost of non-action. A full economic assessment at EU scale might not be possible, but qualitative data or different case studies from across the EU (or third countries if relevant) may provide useful information to inform decision making. In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable). Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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No studies were found that quantify the economic impact of the red-whiskered bulbul in its currently established population within the risk assessment area (Spain). Since this population is presumably still fairly small (estimated at 100-150 birds in 2015 but recent estimates are lacking, see Santos 2015), the current impact is assumed to be minimal with low confidence.

Qu. 4.11. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.10.

RESPONSE	minimal minor moderate	CONFIDENCE	low medium high
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	major massive		
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Given uncertainty on the extent to which the species is the causative agent of direct damage to fruits, future economic damage is expected to be limited to private (garden) or public (park) areas. In gardens the species might impact small agricultural produce of vegetables and be perceived as a nuisance species, such as in other areas of the world (e.g. Hawaii) (see Qu 4.9). Indirect economic damage, through dispersal of weeds, is harder to assess.

In a recent horizon scan for future problematic invaders in Cyprus, the red-whiskered bulbul was identified as a top ranking species in terms of future economic impacts, which is linked to favourable conditions for establishment and the presence of economically important sensitive receptor crops like citrus and grapes (Peyton et al. 2019, 2020). Spain is an important producer of fresh fruit (e.g. strawberries) and vegetables (<https://wits.worldbank.org/>), with the area of Valencia where the species is currently established as an important citrus region. More widely, the European Union produces a wide range of fruit, berries and nuts. In 2019, the EU-27 produced 13.7 million tonnes of pome fruit (apples, pears and quinces), 7.3 million tonnes of stone fruit (peaches, nectarines, apricots, cherries, plums, sloes and medlar), 2.5 million tonnes of sub-tropical and tropical fruits (such as figs, kiwis, avocados and bananas), 0.6 million tonnes of berries (excluding strawberries), and 1.1 million tonnes of nuts. In addition, the EU also produced 10.6 million tonnes of citrus fruit (such as oranges, satsumas, clementines, mandarins, lemons, limes and grapefruit) in 2019 (source: eurostat). Spain and Italy are the key producers of fruit and large parts of their territory are within the endangered area for invasion by red-whiskered bulbul (see Annex VIII). Grapes are of particular concern since red-whiskered bulbuls might find suitable habitat in vineyards and could cause damages to the wine industry.

We scored moderate because although potentially impacted crops are widely present in the risk assessment area and therefore crop production could be impacted, research has shown red-whiskered bulbuls mostly induce damages secondarily and rely on other species to break open the skin of the fruit with the exception of some fruits such as citrus, grapes and mango that are readily consumed (Carlton and Owre, 1975). Also, growers can implement risk management strategies to reduce bird damages using netting, growing indoors or using bird repellents (these however, represent a cost). Confidence is low because most of the documented cases of damage to crops are anecdotal and there is no direct evidence of damages for the risk assessment area.

Qu. 4.12. How great are the economic costs / losses associated with managing this organism currently in the risk assessment area (include any past costs in your response)?

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable).

RESPONSE	minimal minor	CONFIDENCE	low medium
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	moderate major massive		high
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The species is not under post-border management in Spain. However, the species is listed on the Spanish catalogue of invasive alien species (Royal Decree 630/2013 and its modifications 216/2019). This implies the species can not be kept, transported or traded (Article 7). Transition measures oblige owners to report the possession of birds acquired prior to their incorporation on this list to the competent authorities (by January 2022). There are also provisions on border controls, early warning systems and management actions which in practice have to be implemented by the regional administrations, water authorities and national parks. As the implementation of these preventive actions requires human resources and capacity, this surely represents a cost. We therefore scored minor but with low confidence because this actual cost is not documented.

Qu. 4.13. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.12.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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In a future situation where the suitable area for establishment within the risk assessment area increases (see Qu. 2.10.) and the species becomes widespread over that area (see Qu. 3.12.), the overall cost of management could become substantial. For comparison, on a small island like Assumption (11.6km²), a total of 5297 birds were removed with mist netting accounting for 80% of captures and shooting the majority of the rest (Seychelles; Bunbury et al. 2019) (see also Qu. 2.6). This eradication, after some initial desktop work for planning, took three years (2012-2014) and involved a team of between 3 and 30 people (Uranie 2015). Other documented eradications include the removal of a single bird from Aldabra (Bunbury et al. 2019), seven birds from Fuerteventura (Canary Islands) (Saavedra & Reynolds, 2019) and 75 birds in California (Clark, 1976). None of these actions was documented in terms of cost, but Saavedra & Reynolds (2019) provide a long list of activities requiring human and financial resources during such interventions. As a comparison, Parkes et al. (2006) estimated that the costs to achieve eradication of common myna (*Acridotheres tristis*) from Mangaia (Cook Islands, 5180 ha) with appropriate levels of monitoring would be about NZ\$100,000 (c. € 55,000), with 80% of that budget needed for preparation and training and surveillance, including detecting surviving birds. However, here, the method proposed was toxic baiting.

Given that effective management measures exist (see Qu. 2.6), and that these are relatively easy and cost-efficient to apply, but considering the cost of eradication campaigns on other bird species, we estimate that in a worst case scenario the management costs could easily exceed 100,000€ and

therefore scored moderate. Since data on costs from the literature are not species specific, confidence is low.

Social and human health impacts

Qu. 4.14. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism for the risk assessment area and for third countries, if relevant (e.g. with similar eco-climatic conditions).

The description of the known impact and the assessment of potential future impact on human health, safety and the economy, shall, if relevant, include information on

- illnesses, allergies or other affections to humans that may derive directly or indirectly from a species;
- damages provoked directly or indirectly by a species with consequences for the safety of people, property or infrastructure;
- direct or indirect disruption of, or other consequences for, an economic or social activity due to the presence of a species.

Social and human health impacts can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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No social or human health impact has been described for the red-whiskered bulbul so far. Red-whiskered bulbul, like many other bird species, is a potential host and reservoir of avian influenza (see Q4.1). Thinh et al. (2012), out of 16 birds tested in Cuc Phuong National Park (northern Vietnam, 2007), describe one virus antibody-positive individual for the H9 hemagglutinin subtype, a low pathogenic avian influenza virus subtype widespread in nature in many species of wild waterfowl and shorebirds worldwide (Alexander, 2000). Influenza A viruses (IAVs) of the H9 subtype are enzootic in Asia, the Middle East, and parts of North and Central Africa, where they cause significant economic losses to the poultry industry (In Europe, vaccination has been adopted to prevent reoccurrences especially in turkeys in Germany and Italy (Capua and Alexander 2009)). Some strains of H9N2 viruses have been linked to zoonotic episodes of mild respiratory diseases (Carnaccini and Perez, 2020). H9N2 viruses are considered of pandemic concern by the World Health Organization (WHO). Another bulbul species, the black-capped bulbul *P. melanicterus*, tested positive for the H5 subtype. Thinh et al. (2012) also point out that sociality (bulbuls often occur in small groups and display communal roosting behaviour) is thought to enhance pathogen transmission among animals, in part because transmission is density-dependent. Because there is no evidence that the red-whiskered bulbul was directly implicated in transmission of highly pathogenic influenza and because poultry farming in the risk assessment area could locally be affected but the impact mitigated through vaccination campaigns, we scored minor.

Qu. 4.15. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism in the future for the risk assessment area.

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. In this case, no score and confidence should be given and the standardized “score” is N/A (not applicable).

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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No changes to the response in Qu. 4.14. are expected under future conditions.

Other impacts

Qu. 4.16. How important is the organism in facilitating other damaging organisms (e.g. diseases) as food source, a host, a symbiont or a vector etc.?

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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A limited number of ectoparasites (ticks; Rajagopalan, 1972) and endoparasites (*Haemoproteus*; Islam and Williams, 2000) have been described, none of which are exclusively or disproportionately linked to the red-whiskered bulbul. The avian malaria parasite *Plasmodium (Novyella) jiangi* is an exception, as it was newly described from the species (He and Huang, 1993). However, the identity of this taxon is doubtful (Valkiūnas and Iezhova 2018) (see also Qu. 2.4).

Qu. 4.17. How important might other impacts not already covered by previous questions be resulting from introduction of the organism?

RESPONSE	N/A minimal minor	CONFIDENCE	low medium high
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	moderate major massive		
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No other possible impacts were found.

Qu. 4.18. How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area?

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Potential predators described in literature, and of relevance to the risk assessment area, are rats, cats, snakes (*Coluber* spp.), and birds of prey (*Falco* spp.; Carleton and Owre, 1975; Islam and Williams 2000; Li et al. 2015) (see also Qu. 2.4). Specific predators that occur in the suitable area, and that thrive well near human settlements, may thus be House cat (*Felis catus*), Brown rat (*Rattus norvegicus*), Black rat (*Rattus rattus*), Barn owl (*Tyto alba*), Sparrowhawk (*Accipiter nisus*), Ladder snake (*Zamenis scalaris*), Stone marten (*Martes foina*), or any related species with a similar feeding ecology. However, neither predators, parasites nor pathogens in the risk assessment area are expected to constrain the expected impacts of the red-whiskered bulbul in the risk assessment area.

Qu. 4.19. Estimate the overall impact in the risk assessment area under current climate conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, in current conditions.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Under current conditions and should the species firmly establish and spread in natural areas, the species could exert a major impact on biodiversity and ecosystem services through the spread of invasive alien plants and associated changes in natural species compositions, predation, competition

and to a lesser extent pathogen transmission (see Qu 4.2). This could especially be the case on islands with small populations of endemic species. The endangered area for the red-whiskered bulbul covers the Mediterranean, Black sea, Atlantic (and Anatolian) biogeographic regions (A2.1, A2.9, A2.10, Annex VIII).

Qu. 4.20. Estimate the overall impact in the risk assessment area in foreseeable climate change conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, under future conditions.

See also guidance to Qu. 4.3.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Under future climate, the suitable region for establishment in the risk assessment area is predicted to almost double in the Mediterranean biogeographic region and to increase in the other biogeographic regions (Black Sea). Also, in the Atlantic biogeographic region, where regular escaped birds are reported, the suitability for establishment increases slightly (see also Qu 2.10, Annex VIII).

RISK SUMMARIES			
	RESPONSE	CONFIDENCE	COMMENT
Summarise Introduction and Entry*	very unlikely unlikely moderately likely likely very likely	low medium high	The red-whiskered bulbul is present in zoos and private collections in multiple countries geographically spread within the risk assessment area, presumably in good numbers. It is the most popular bulbul species with specialised breeders and bird keepers although quantitative data on size and trends in captive population are lacking for the risk assessment area. The many confirmed observations of escaped birds across the risk assessment area confirm that continued escapes (and/or releases) are very likely.
Summarise Establishment*	very unlikely unlikely moderately likely	low medium high	The species is already established in the risk assessment area (Spain) and this population is increasing

	likely very likely		in abundance and spreading. Several biogeographic regions are vulnerable to invasion by red-whiskered bulbul. Predators or pathogens are unlikely to prevent successful establishment and the proportion of the area suitable for establishment is expected to increase under foreseeable climatic conditions.
Summarise Spread*	very slowly slowly moderately rapidly very rapidly	low medium high	Population growth rates and geographic spread of red-whiskered bulbul outside the risk assessment area vary, with island populations showing rapid spread and mainland populations often showing longer lag times. The current established population in the risk assessment area is spreading since its introduction about two decades ago; this area however provides continuous suitable habitat to the species. Climate change could foster reproduction which might positively enhance dispersal and spread.
Summarise Impact*	minimal minor moderate major massive	low medium high	The red-whiskered bulbul can impact on native species and ecosystems through competition, frugivory and spreading of invasive alien plants, predation on (in)vertebrates and pathogen transmission. It is also a reported pest species impacting on garden plants, fruits and vegetables. Although the current impact in the risk assessment area is moderate, there is sufficient evidence elsewhere of ecosystem level effects through the spread of invasive plants from the urban to the natural (mostly forest) biome, competitive exclusion of other bird species and local extinction by predation.
Conclusion of the risk assessment (overall risk)	low moderate high	low medium high	Considering the multi-faceted potential ecological and economic impacts of the red-whiskered bulbul, its large native range in line with broad climatic and environmental amplitudes, the suitable area for establishment

			<p>which spans multiple biogeographic regions and countries, the presence of sensitive island biota, ecosystem-level effects and documented competitive exclusions and local extinctions of native species the overall risk is scored high. This score is in line with several environmental impact assessments of alien birds using established scoring protocols. There is an urgent need to provide evidence of impacts in the risk assessment area.</p>
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*in current climate conditions and in foreseeable future climate conditions

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Distribution Summary

Please answer as follows:

- Yes if recorded, established or invasive
- if not recorded, established or invasive
- ? Unknown; data deficient

The columns refer to the answers to Questions A5 to A12 under Section A.

For data on marine species at the Member State level, delete Member States that have no marine borders. In all other cases, provide answers for all columns.

Member States and the United Kingdom

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Austria	-	-	-	-	-
Belgium	Yes	-	-	-	-
Bulgaria	-	-	-	Yes	-
Croatia	-	-	Yes	Yes	-
Cyprus	-	-	Yes	Yes	-
Czech Republic	-	-	-	-	-
Denmark	Yes	-	-	-	-
Estonia	-	-	-	-	-
Finland	-	-	-	-	-
France	?	-	Yes	Yes	-
Germany	Yes	-	-	-	-
Greece	-	-	Yes	Yes	-
Hungary	-	-	-	-	-
Ireland	-	-	Yes	Yes	-
Italy	Yes	-	Yes	Yes	-
Latvia	-	-	-	-	-
Lithuania	-	-	-	-	-
Luxembourg	-	-	-	-	-
Malta	-	-	Yes	Yes	-
Netherlands	Yes	-	-	-	-
Poland	-	-	-	-	-
Portugal	-	-	Yes	Yes	-
Romania	-	-	-	-	-
Slovakia	-	-	-	-	-
Slovenia	-	-	-	Yes	-
Spain	Yes	Yes	Yes	Yes	?
Sweden	-	-	-	-	-
United Kingdom	Yes	-	-	Yes	-

Biogeographical regions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Alpine	-	-	-	-	-
Atlantic	Yes	-	Yes	Yes	-
Black Sea	-	-	Yes	Yes	-
Boreal	-	-	-	-	-
Continental	Yes	-	-	?	-
Mediterranean	Yes	Yes	Yes	Yes	?
Pannonian	-	-	-	?	-
Steppic	-	-	-	?	-

ANNEX I Scoring of Likelihoods of Events

(taken from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Description	Frequency
Very unlikely	This sort of event is theoretically possible, but is never known to have occurred and is not expected to occur	1 in 10,000 years
Unlikely	This sort of event has occurred somewhere at least once in the last millenium	1 in 1,000 years
Moderately likely	This sort of event has occurred somewhere at least once in the last century	1 in 100 years
Likely	This sort of event has happened on several occasions elsewhere, or on at least once in the last decade	1 in 10 years
Very likely	This sort of event happens continually and would be expected to occur	Once a year

ANNEX II Scoring of Magnitude of Impacts

(modified from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Biodiversity and ecosystem impact	Ecosystem Services impact	Economic impact (Monetary loss and response costs per year)	Social and human health impact, and other impacts
	<i>Question 5.1-5</i>	<i>Question 5.6-8</i>	<i>Question 5.9-13</i>	<i>Question 5.14-18</i>
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected ⁵	Up to 10,000 Euro	No social disruption. Local, mild, short-term reversible effects to individuals.
Minor	Some ecosystem impact, reversible changes, localised	Local and temporary, reversible effects to one or few services	10,000-100,000 Euro	Significant concern expressed at local level. Mild short-term reversible effects to identifiable groups, localised.
Moderate	Measureable long-term damage to populations and ecosystem, but reversible; little spread, no extinction	Measureable, temporary, local and reversible effects on one or several services	100,000-1,000,000 Euro	Temporary changes to normal activities at local level. Minor irreversible effects and/or larger numbers covered by reversible effects, localised.
Major	Long-term irreversible ecosystem change, spreading beyond local area	Local and irreversible or widespread and reversible effects on one / several services	1,000,000-10,000,000 Euro	Some permanent change of activity locally, concern expressed over wider area. Significant irreversible effects locally or reversible effects over large area.
Massive	Widespread, long-term population loss or extinction, affecting several species with serious ecosystem effects	Widespread and irreversible effects on one / several services	Above 10,000,000 Euro	Long-term social change, significant loss of employment, migration from affected area. Widespread, severe, long-term, irreversible health effects.

⁵ Not to be confused with “no impact”.

ANNEX III Scoring of Confidence Levels

(modified from Bacher et al. 2017)

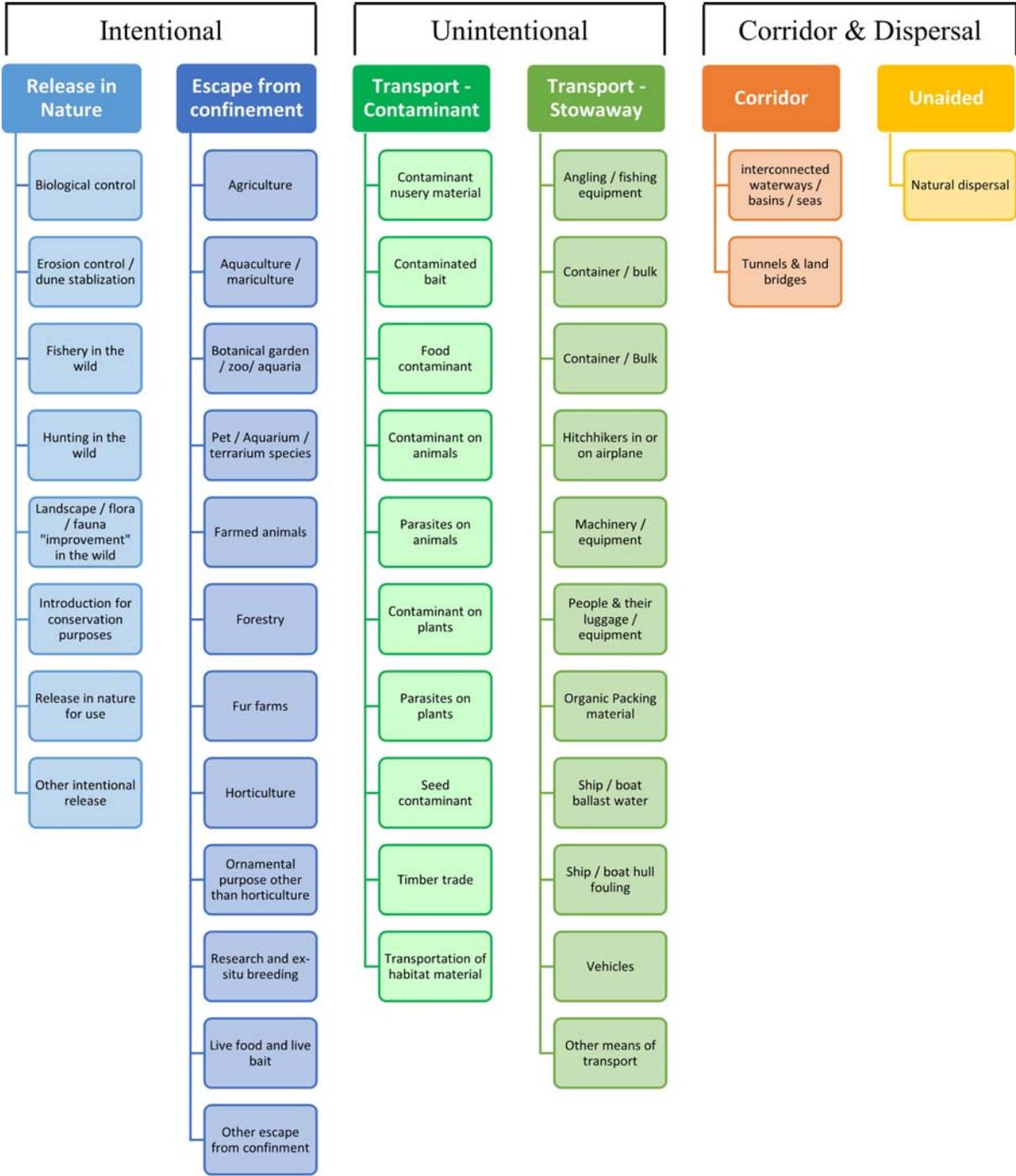
Each answer provided in the risk assessment must include an assessment of the level of confidence attached to that answer, reflecting the possibility that information needed for the answer is not available or is insufficient or available but conflicting.

The responses in the risk assessment should clearly support the choice of the confidence level.

Confidence level	Description
Low	There is no direct observational evidence to support the assessment, e.g. only inferred data have been used as supporting evidence <i>and/or</i> Impacts are recorded at a spatial scale which is unlikely to be relevant to the assessment area <i>and/or</i> Evidence is poor and difficult to interpret, e.g. because it is strongly ambiguous <i>and/or</i> The information sources are considered to be of low quality or contain information that is unreliable.
Medium	There is some direct observational evidence to support the assessment, but some information is inferred <i>and/or</i> Impacts are recorded at a small spatial scale, but rescaling of the data to relevant scales of the assessment area is considered reliable, or to embrace little uncertainty <i>and/or</i> The interpretation of the data is to some extent ambiguous or contradictory.
High	There is direct relevant observational evidence to support the assessment (including causality) <i>and</i> Impacts are recorded at a comparable scale <i>and/or</i> There are reliable/good quality data sources on impacts of the taxa <i>and</i> The interpretation of data/information is straightforward <i>and/or</i> Data/information are not controversial or contradictory.

ANNEX IV CBD pathway categorisation scheme

Overview of CBD pathway categorisation scheme showing how the 44 pathways relate to the six main pathway categories. All of the pathways can be broadly classified into 1) those that involve intentional transport (blue), 2) those in which the taxa are unintentionally transported (green) and 3) those where taxa moved between regions without direct transportation by humans and/or via artificial corridors (orange and yellow). **Note that the pathways in the category “Escape from confinement” can be considered intentional for the introduction into the risk assessment area and unintentional for the entry into the environment.**



ANNEX V Ecosystem services classification (CICES V5.1, simplified) and examples

For the purposes of this risk assessment, please feel free to use what seems as the most appropriate category / level / combination of impact (Section – Division – Group), reflecting information available.

Section	Division	Group	Examples (i.e. relevant CICES “classes”)
Provisioning	Biomass	Cultivated <i>terrestrial</i> plants	Cultivated terrestrial plants (including fungi, algae) grown for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from cultivated plants, fungi, algae and bacteria for direct use or processing (excluding genetic materials); Cultivated plants (including fungi, algae) grown as a <u>source of energy</u> <i>Example: negative impacts of non-native organisms to crops, orchards, timber etc.</i>
		Cultivated <i>aquatic</i> plants	Plants cultivated by in- situ aquaculture grown for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from in-situ aquaculture for direct use or processing (excluding genetic materials); Plants cultivated by in- situ aquaculture grown as an <u>energy source</u> . <i>Example: negative impacts of non-native organisms to aquatic plants cultivated for nutrition, gardening etc. purposes.</i>
		Reared animals	Animals reared for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from reared animals for direct use or processing (excluding genetic materials); Animals reared to provide <u>energy</u> (including mechanical) <i>Example: negative impacts of non-native organisms to livestock</i>
		Reared <i>aquatic</i> animals	Animals reared by in-situ aquaculture for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from animals grown by in-situ aquaculture for direct use or processing (excluding genetic materials); Animals reared by in-situ aquaculture as an <u>energy source</u> <i>Example: negative impacts of non-native organisms to fish farming</i>
		Wild plants (terrestrial and aquatic)	Wild plants (terrestrial and aquatic, including fungi, algae) used for <u>nutrition</u> ; <u>Fibres and other materials</u> from wild plants for direct use or processing (excluding genetic materials); Wild plants (terrestrial and aquatic, including fungi, algae) used as a <u>source of energy</u> <i>Example: reduction in the availability of wild plants (e.g. wild berries, ornamentals) due to non-native organisms (competition, spread of disease etc.)</i>
		Wild animals (terrestrial and aquatic)	Wild animals (terrestrial and aquatic) used for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from wild animals for direct use or processing (excluding genetic materials); Wild animals (terrestrial and aquatic) used as a <u>source of energy</u>

			<i>Example: reduction in the availability of wild animals (e.g. fish stocks, game) due to non-native organisms (competition, predations, spread of disease etc.)</i>
	Genetic material from all biota	Genetic material from plants, algae or fungi	Seeds, spores and other plant materials collected for maintaining or establishing a population; Higher and lower plants (whole organisms) used to <u>breed new strains or varieties</u> ; Individual genes extracted from higher and lower plants for the design and construction of new biological entities <i>Example: negative impacts of non-native organisms due to interbreeding</i>
		Genetic material from animals	Animal material collected for the purposes of maintaining or establishing a population; Wild animals (whole organisms) used to breed new strains or varieties; Individual genes extracted from organisms for the design and construction of new biological entities <i>Example: negative impacts of non-native organisms due to interbreeding</i>
	Water ⁶	Surface water used for nutrition, materials or energy	Surface water for <u>drinking</u> ; Surface water used as a material (<u>non-drinking purposes</u>); Freshwater surface water, coastal and marine water used as an <u>energy source</u> <i>Example: loss of access to surface water due to spread of non-native organisms</i>
		Ground water for used for nutrition, materials or energy	Ground (and subsurface) water for <u>drinking</u> ; Ground water (and subsurface) used as a material (<u>non-drinking purposes</u>); Ground water (and subsurface) used as an <u>energy source</u> <i>Example: reduced availability of ground water due to spread of non-native organisms and associated increase of ground water consumption by vegetation.</i>
Regulation & Maintenance	Transformation of biochemical or physical inputs to ecosystems	Mediation of wastes or toxic substances of anthropogenic origin by living processes	<u>Bio-remediation</u> by micro-organisms, algae, plants, and animals; <u>Filtration/sequestration/storage/accumulation</u> by micro-organisms, algae, plants, and animals <i>Example: changes caused by non-native organisms to ecosystem functioning and ability to filtrate etc. waste or toxics</i>
		Mediation of nuisances of anthropogenic origin	<u>Smell reduction; noise attenuation; visual screening</u> (e.g. by means of green infrastructure) <i>Example: changes caused by non-native organisms to ecosystem structure, leading to reduced ability to mediate nuisances.</i>
	Regulation of physical, chemical, biological conditions	Baseline flows and extreme event regulation	Control of <u>erosion</u> rates; Buffering and attenuation of <u>mass movement</u> ; <u>Hydrological cycle and water flow regulation</u> (Including flood control, and coastal protection); <u>Wind</u> protection; <u>Fire</u> protection <i>Example: changes caused by non-native organisms to ecosystem functioning or structure leading to, for example, destabilisation of soil, increased risk or intensity of wild fires</i>

⁶ Note: in the CICES classification provisioning of water is considered as an abiotic service whereas the rest of ecosystem services listed here are considered biotic.

			etc.
		Lifecycle maintenance, habitat and gene pool protection	<p>Pollination (or 'gamete' dispersal in a marine context); <u>Seed dispersal</u>; Maintaining <u>nursery populations and habitats</u> (Including gene pool protection)</p> <p><i>Example: changes caused by non-native organisms to the abundance and/or distribution of wild pollinators; changes to the availability / quality of nursery habitats for fisheries</i></p>
		Pest and disease control	<p>Pest control; Disease control</p> <p><i>Example: changes caused by non-native organisms to the abundance and/or distribution of pests</i></p>
		Soil quality regulation	<p><u>Weathering processes</u> and their effect on soil quality; <u>Decomposition and fixing processes</u> and their effect on soil quality</p> <p><i>Example: changes caused by non-native organisms to vegetation structure and/or soil fauna leading to reduced soil quality</i></p>
		Water conditions	<p>Regulation of the <u>chemical condition</u> of freshwaters by living processes; Regulation of the chemical condition of salt waters by living processes</p> <p><i>Example: changes caused by non-native organisms to buffer strips along water courses that remove nutrients in runoff and/or fish communities that regulate the resilience and resistance of water bodies to eutrophication</i></p>
		Atmospheric composition and conditions	<p>Regulation of <u>chemical composition</u> of atmosphere and oceans; Regulation of <u>temperature and humidity</u>, including ventilation and transpiration</p> <p><i>Example: changes caused by non-native organisms to ecosystems' ability to sequester carbon and/or evaporative cooling (e.g. by urban trees)</i></p>
Cultural	Direct, in-situ and outdoor interactions with living systems that depend on presence in the environmental setting	Physical and experiential interactions with natural environment	<p>Characteristics of living systems that that enable activities promoting health, recuperation or enjoyment through <u>active or immersive interactions</u>;</p> <p>Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through <u>passive or observational interactions</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that make it attractive for recreation, wild life watching etc.</i></p>
		Intellectual and representative interactions with natural environment	<p>Characteristics of living systems that enable <u>scientific investigation</u> or the creation of traditional ecological knowledge;</p> <p>Characteristics of living systems that enable <u>education and training</u>;</p> <p>Characteristics of living systems that are resonant in terms of <u>culture or heritage</u>;</p> <p>Characteristics of living systems that enable <u>aesthetic experiences</u></p>

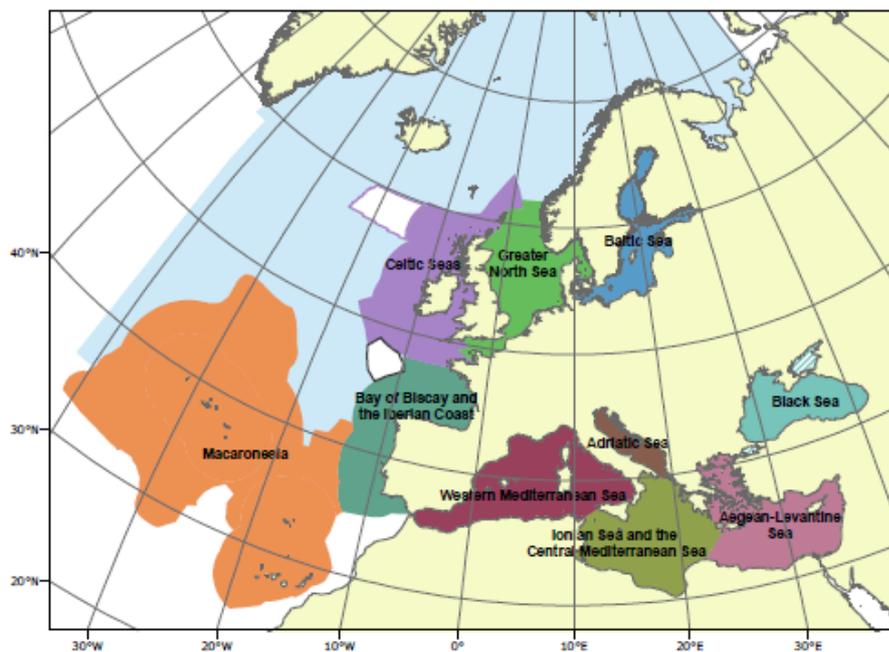
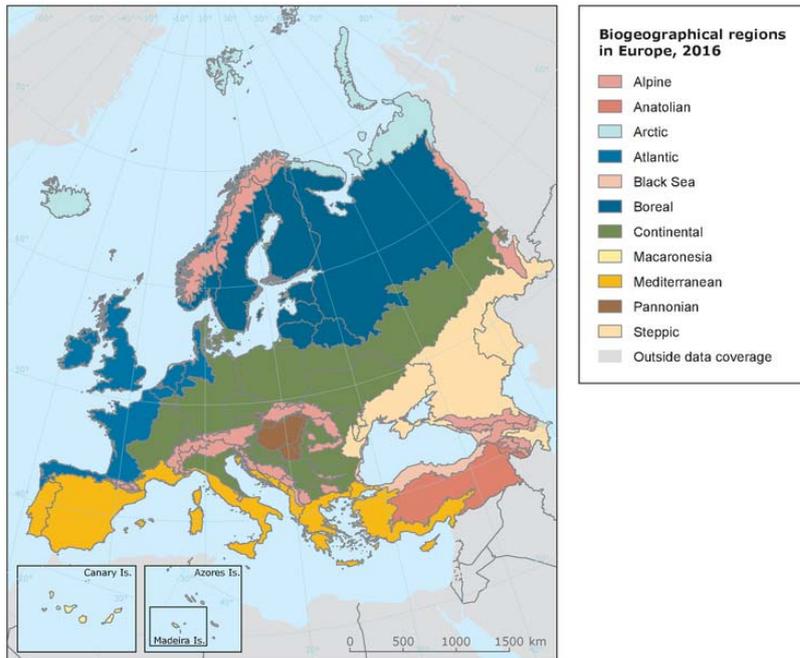
			<i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have cultural importance</i>
	Indirect, remote, often indoor interactions with living systems that do not require presence in the environmental setting	Spiritual, symbolic and other interactions with natural environment	Elements of living systems that have <u>symbolic meaning</u> ; Elements of living systems that have <u>sacred or religious meaning</u> ; Elements of living systems used for <u>entertainment or representation</u> <i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have sacred or religious meaning</i>
		Other biotic characteristics that have a non-use value	Characteristics or features of living systems that have an <u>existence value</u> ; Characteristics or features of living systems that have an <u>option or bequest value</u> <i>Example: changes caused by non-native organisms to ecosystems designated as wilderness areas, habitats of endangered species etc.</i>

ANNEX VI EU Biogeographic Regions and MSFD Subregions

See <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2> ,
http://ec.europa.eu/environment/nature/natura2000/biogeog_regions/

and

<https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions-1/technical-document/pdf>



ANNEX VII Delegated Regulation (EU) 2018/968 of 30 April 2018

see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>

ANNEX VIII Projection of environmental suitability for the establishment of the red-whiskered bulbul *Pycnonotus jocosus* in Europe

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15 June 2021 (version 3)

Aim

To project the suitability for potential establishment of the red-whiskered bulbul *Pycnonotus jocosus* in Europe, under current and predicted future climatic conditions.

Data for modelling

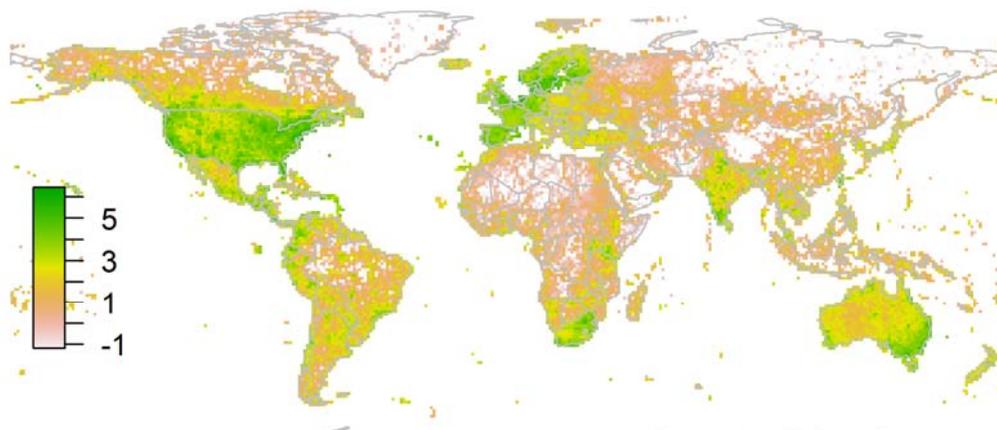
Species occurrence data were obtained from the Global Biodiversity Information Facility (GBIF) (291092 records), the Biodiversity Information Serving Our Nation database (BISON) (10458 records), iNaturalist (3915 records), the Integrated Digitized Biocollections (iDigBio) (38 records), the VertNet databases (30 records), and additional records from the risk assessment team. We scrutinised occurrence records from regions where the species is not known to be established and removed any dubious records or where the georeferencing was too imprecise (e.g. records referenced to a country or island centroid) or outside of the coverage of the predictor layers (e.g. small island or coastal occurrences). The remaining records were gridded at a 0.25 x 0.25 degree resolution for modelling, yielding 2307 grid cells with occurrences (Figure 1a). As a proxy for recording effort, the density of Aves records held by GBIF was also compiled on the same grid (Figure 1b).

Figure 1. (a) Occurrence records obtained for the red-whiskered bulbul and used in the modelling, showing native and invaded distributions. (b) The recording density of Aves on GBIF, which was used as a proxy for recording effort.

(a) Species distribution used in modelling



(b) Estimated recording effort (log10-scaled)



Climate data were selected from the 'Bioclim' variables contained within the WorldClim database (Hijmans et al., 2005), originally at 5 arcminute resolution (0.083 x 0.083 degrees of longitude/latitude) and aggregated to a 0.25 x 0.25 degree grid for use in the model.

Based on the biology of the red-whiskered bulbul, the following climate variables were used in the modelling:

- Annual mean temperature (Bio1)
- Minimum temperature of the coldest month (Bio6)
- Mean temperature of the warmest quarter (Bio10)
- Annual precipitation (Bio12)
- Precipitation of the wettest month (Bio13)
- Precipitation of the driest month (Bio14)

- Climatic moisture index (CMI): ratio of mean annual precipitation to potential evapotranspiration, log+1 transformed. For its calculation, monthly potential evapotranspirations were estimated from the WorldClim monthly temperature data and solar radiation using the simple method of Zomer et al. (2008) which is based on the Hargreaves evapotranspiration equation (Hargreaves, 1994).

To estimate the effect of climate change on the potential distribution, equivalent modelled future climate conditions for the 2070s under the Representative Concentration Pathways (RCP) 2.6 and 4.5 were also obtained. These represent low and medium emissions scenarios, respectively. The above variables were obtained as averages of outputs of eight Global Climate Models (BCC-CSM1-1, CCSM4, GISS-E2-R, HadGEM2-AO, IPSL-CM5A-LR, MIROC-ESM, MRI-CGCM3, NorESM1-M), downscaled and calibrated against the WorldClim baseline (http://www.worldclim.org/cmip5_5m).

The following habitat layers were also used:

- Tree cover (Tree): This was estimated from the MODerate-resolution Imaging Spectroradiometer (MODIS) satellite continuous tree cover raster product, produced by the Global Land Cover Facility (<http://glcf.umd.edu/data/vcf/>). The raw product contains the percentage cover by trees in each 0.002083 x 0.002083 degree grid cell. We aggregated this to the mean cover in our 0.25 x 0.25 degree grid cells.
- Human influence index (HII): As many non-native invasive species associate with anthropogenically disturbed habitats. We used the Global Human Influence Index Dataset of the Last of the Wild Project (Wildlife Conservation Society - WCS & Center for International Earth Science Information Network - CIESIN - Columbia University, 2005), which is developed from nine global data layers covering human population pressure (population density), human land use and infrastructure (built-up areas, nighttime lights, land use/land cover) and human access (coastlines, roads, railroads, navigable rivers). The index ranges between 0 and 1 and was ln+1 transformed for the modelling to improve normality.

Species distribution model

A presence-background (presence-only) ensemble modelling strategy was employed using the BIOMOD2 R package version 3.4.6 (Thuiller et al., 2020, Thuiller et al., 2009). These models contrast the environment at the species' occurrence locations against a random sample of the global background environmental conditions (often termed 'pseudo-absences') in order to characterise and project suitability for occurrence. This approach has been developed for distributions that are in equilibrium with the environment. Because invasive species' distributions are not at equilibrium and subject to dispersal constraints at a global scale, we took care to minimise the inclusion of locations suitable for the species but where it has not been able to disperse to (Chapman et al. 2019). Therefore the background sampling region included:

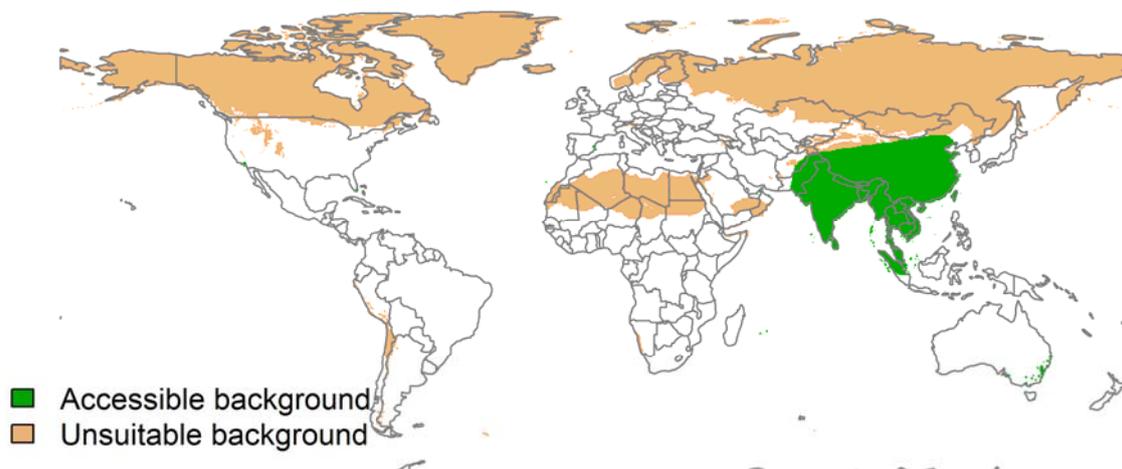
- The area accessible by native red-whiskered bulbul populations, in which the species is likely to have had sufficient time to disperse to all locations. Based on presumed maximum dispersal distances, the accessible region was defined as a 400km buffer around the native range occurrences; AND
- A 30km buffer around the non-native occurrences, encompassing regions likely to have had high propagule pressure for introduction by humans and/or dispersal of the species; AND
- Regions where we have an *a priori* expectation of high unsuitability for the species so that absence is assumed irrespective of dispersal constraints (see Figure 2). The following rules were applied to define a region expected to be highly unsuitable for the red-whiskered bulbul at the spatial scale of the model:

- Annual mean temperature (Bio1) < 4°C
- Annual precipitation (Bio12) < 55mm

Altogether, only 0.3% of occurrence grid cells were located in the unsuitable background region.

Within the unsuitable background region, 10 samples of 5000 randomly sampled grid cells were obtained. In the accessible background (comprising the accessible areas around native and non-native occurrences as detailed above), the same number of pseudo-absence samples were drawn as there were presence records (2307), weighting the sampling by a proxy for recording effort (Figure 2).

Figure 2. The background from which pseudo-absence samples were taken in the modelling of the red-whiskered bulbul. Samples were taken from a 400km buffer around the native range and a 30km buffer around non-native occurrences (together forming the accessible background), and from areas expected to be highly unsuitable for the species (the unsuitable background region). Samples from the accessible background were weighted by a proxy for recording effort (Figure 1(b)).



Each dataset (i.e. combination of the presences and the individual background samples) was randomly split into 80% for model training and 20% for model evaluation. With each training dataset, seven statistical algorithms were fitted with the default BIOMOD2 settings and rescaled using logistic regression, except where specified below:

- Generalised linear model (GLM)
- Generalised boosting model (GBM)
- Generalised additive model (GAM) with a maximum of four degrees of freedom per smoothing spline
- Artificial neural network (ANN)
- Multivariate adaptive regression splines (MARS)
- Random forest (RF)
- Maxent

Since the total background sample was larger than the number of occurrences, prevalence fitting weights were applied to give equal overall importance to the occurrences and the background. Normalised variable importance was assessed and variable response functions were produced using BIOMOD2's default procedure.

Model predictive performance was assessed by the following three measures:

- AUC, the area under the receiver operating characteristic curve (Fielding & Bell 1997). Predictions of presence-absence models can be compared with a subset of records set aside for model evaluation (here 20%) by constructing a confusion matrix with the number of true positive, false positive, false negative and true negative cases. For models generating non-dichotomous scores (as here) a threshold can be applied to transform the scores into a dichotomous set of presence-absence predictions. Two measures that can be derived from the confusion matrix are sensitivity (the proportion of observed presences that are predicted as such, quantifying omission errors), and specificity (the proportion of observed absences that are predicted as such, quantifying commission errors). A receiver operating characteristic (ROC) curve can be constructed by using all possible thresholds to classify the scores into confusion matrices, obtaining sensitivity and specificity for each matrix, and plotting sensitivity against the corresponding proportion of false positives (equal to 1 - specificity). The use of all possible thresholds avoids the need for a selection of a single threshold, which is often arbitrary, and allows appreciation of the trade-off between sensitivity and specificity. The area under the ROC curve (AUC) is often used as a single threshold-independent measure for model performance (Manel, Williams & Ormerod 2001). AUC is the probability that a randomly selected presence has a higher model-predicted suitability than a randomly selected absence (Allouche et al. 2006).
- Cohen's Kappa (Cohen 1960). This measure corrects the overall accuracy of model predictions (ratio of the sum of true presences plus true absences to the total number of records) by the accuracy expected to occur by chance. The kappa statistic ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random. Advantages of kappa are its simplicity, the fact that both commission and omission errors are accounted for in one parameter, and its relative tolerance to zero values in the confusion matrix (Manel, Williams & Ormerod 2001). However, Kappa has been criticised for being sensitive to prevalence (the proportion of sites in which the species was recorded as present) and may therefore be inappropriate for comparisons of model accuracy between species or regions (McPherson, Jetz & Rogers 2004, Allouche et al. 2006).
- TSS, the true skill statistic (Allouche et al. 2006). TSS is defined as sensitivity + specificity - 1, and corrects for Kappa's dependency on prevalence. TSS compares the number of correct forecasts, minus those attributable to random guessing, to that of a hypothetical set of perfect forecasts. Like kappa, TSS takes into account both omission and commission errors, and success as a result of random guessing, and ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random (Allouche et al. 2006).

An ensemble model was created by first rejecting poorly performing algorithms with relatively extreme low AUC values and then averaging the predictions of the remaining algorithms, weighted by their AUC. To identify poorly performing algorithms, AUC values were converted into modified z-scores based on their difference to the median and the median absolute deviation across all algorithms (Iglewicz & Hoaglin 1993). Algorithms with $z < -2$ were rejected. In this way, ensemble projections were made for each dataset and then averaged to give an overall suitability, as well as its standard deviation.

Projections were classified into suitable and unsuitable regions using a "lowest presence threshold" (Pearson et al. 2007), setting the cut-off as the lowest value at which 98% of all presence records are classified correctly under the current climate (here 0.29). In order to express the sensitivity of classifications to the choice of this threshold, thresholds at which 95% and 99% of records are

classified correctly (here 0.42 and 0.2 respectively) were used in the calculation of error bars in Figs. 9 and 10 below in addition to taking account of uncertainty in the projections themselves.

We also produced a limiting factor map for Europe following Elith et al. (2010). For this, projections were made separately with each individual variable fixed at a near-optimal value. These were chosen as the median values at the occurrence grid cells. Then, the most strongly limiting factors were identified as the ones resulting in the highest increase in suitability in each grid cell.

Results

The ensemble model suggested that suitability for the red-whiskered bulbul was most strongly determined by Annual mean temperature (Bio1), accounting for 40.5% of variation explained, followed by Minimum temperature of the coldest month (Bio6) (20.8%), Precipitation of the wettest month (Bio13) (8.7%), Annual precipitation (Bio12) (7.2%), Climatic moisture index (CMI) (6.8%), Mean temperature of the warmest quarter (Bio10) (6.2%), Human influence index (HII) (4.6%), Precipitation of the driest month (Bio14) (4.2%) and Global tree cover (Tree) (1%) (Table 1, Figure 3).

Table 1. Summary of the cross-validation predictive performance (AUC, Kappa, TSS) and variable importance of the fitted model algorithms and the ensemble (AUC-weighted average of the best performing algorithms). Results are the average from models fitted to 10 different background samples of the data.

Algorithm	AUC	Kappa	TSS	Used in the ensemble	Variable importance (%)								
					Annual mean temperature (Bio1)	Minimum temperature of the coldest month (Bio6)	Precipitation of the wettest month (Bio13)	Annual precipitation (Bio12)	Climatic moisture index (CMI)	Mean temperature of the warmest quarter (Bio10)	Human influence index (HII)	Precipitation of the driest month (Bio14)	Global tree cover (Tree)
GLM	0.895	0.561	0.703	yes	54	5	2	9	14	6	5	4	0
GAM	0.896	0.561	0.701	yes	57	5	4	2	12	7	8	4	1
GBM	0.897	0.571	0.699	yes	25	43	24	1	1	1	1	4	0
ANN	0.905	0.584	0.705	yes	25	24	11	12	2	17	6	3	1
MARS	0.894	0.561	0.703	yes	56	8	2	17	8	4	0	4	0
RF	0.832	0.514	0.682	no	20	19	22	6	6	6	7	10	4
Maxent	0.892	0.557	0.694	yes	27	39	8	3	4	2	8	5	4
Ensemble	0.903	0.576	0.703		41	21	9	7	7	6	5	4	1

Figure 3. Partial response plots from the fitted models. Thin coloured lines show responses from the algorithms in the ensemble, while the thick black line is their ensemble. In each plot, other model variables are held at their median value in the training data. Some of the divergence among algorithms is because of their different treatment of interactions among variables.

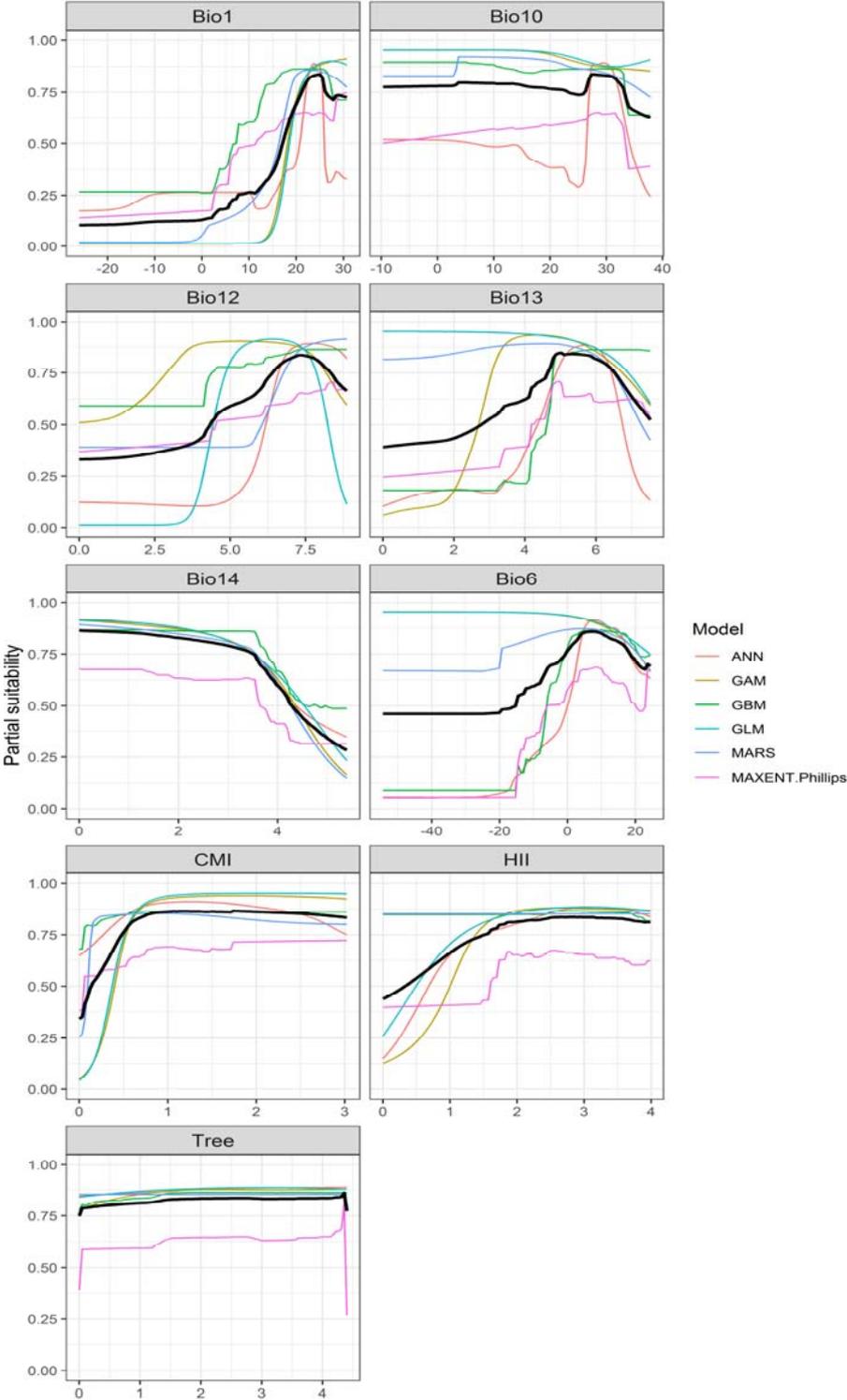


Figure 4. (a) Projected global suitability for the establishment of the red-whiskered bulbul in the current climate. For visualisation, the projection has been aggregated to a 0.5 x 0.5 degree resolution, by taking the maximum suitability of constituent higher resolution grid cells. Values > 0.29 are suitable for the species, with 98% of global presence records above this threshold. Values below 0.29 indicate lower relative suitability. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

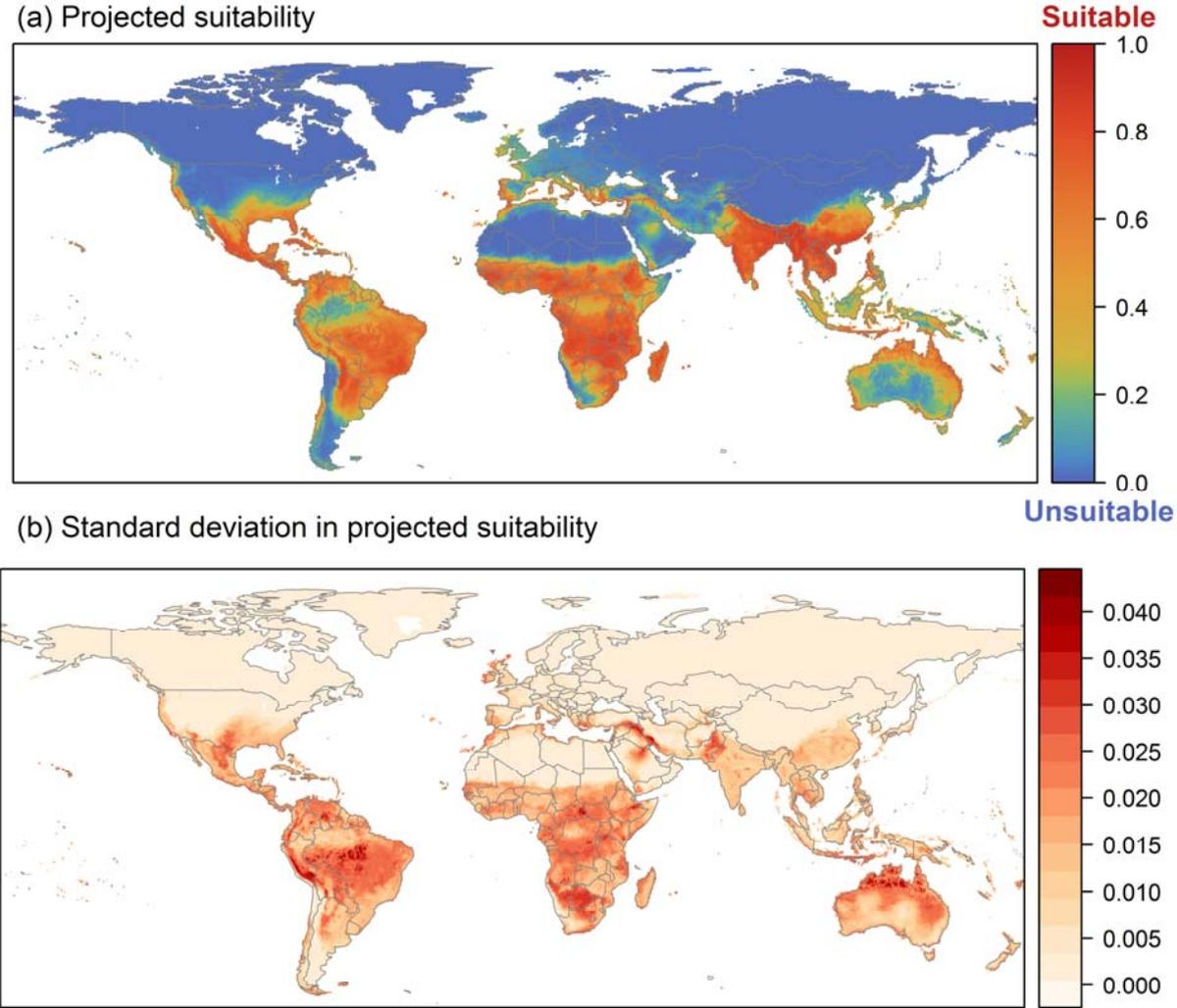


Figure 5. (a) Projected current suitability for the establishment of the red-whiskered bulbul in Europe and the Mediterranean region. Values > 0.29 are suitable for the species, with 98% of global presence records above this threshold. Values below 0.29 indicate lower relative suitability. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

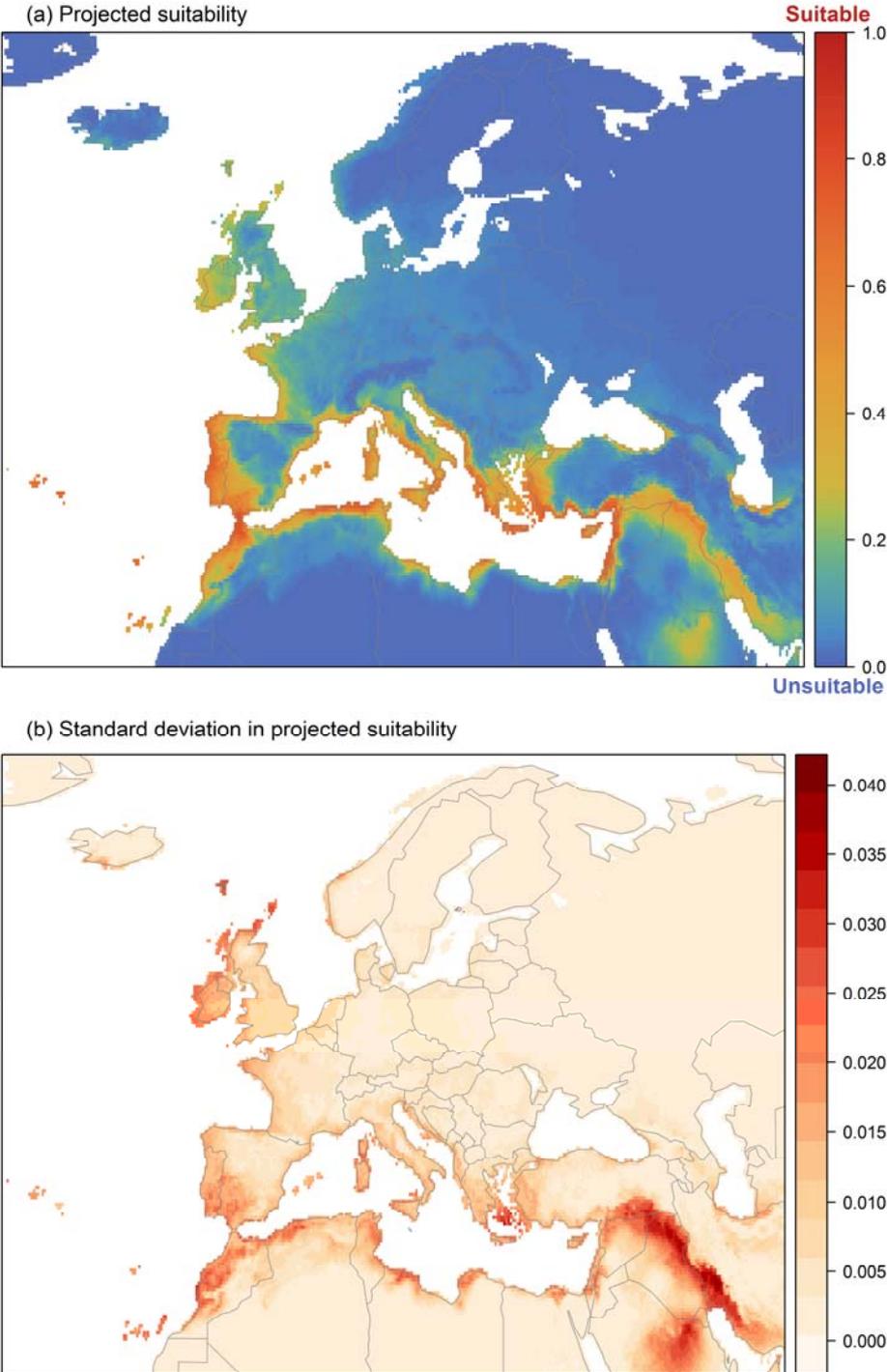


Figure 6. The most strongly limiting factors for the establishment of the red-whiskered bulbul estimated by the model in Europe and the Mediterranean region in current climatic conditions.

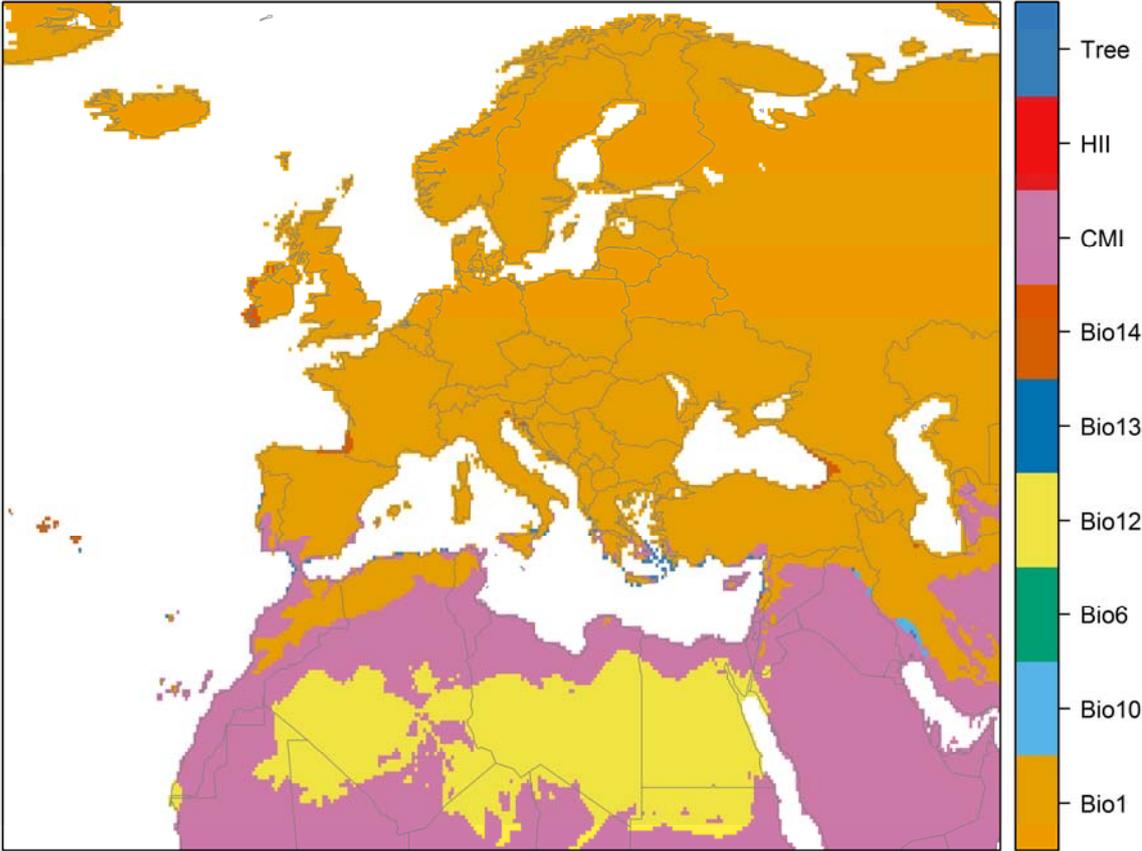


Figure 7. (a) Projected suitability for the establishment of the red-whiskered bulbul in Europe and the Mediterranean region in the 2070s under climate change scenario RCP2.6. Values > 0.29 are suitable for the species, with 98% of global presence records above this threshold under current climate. Values below 0.29 indicate lower relative suitability. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

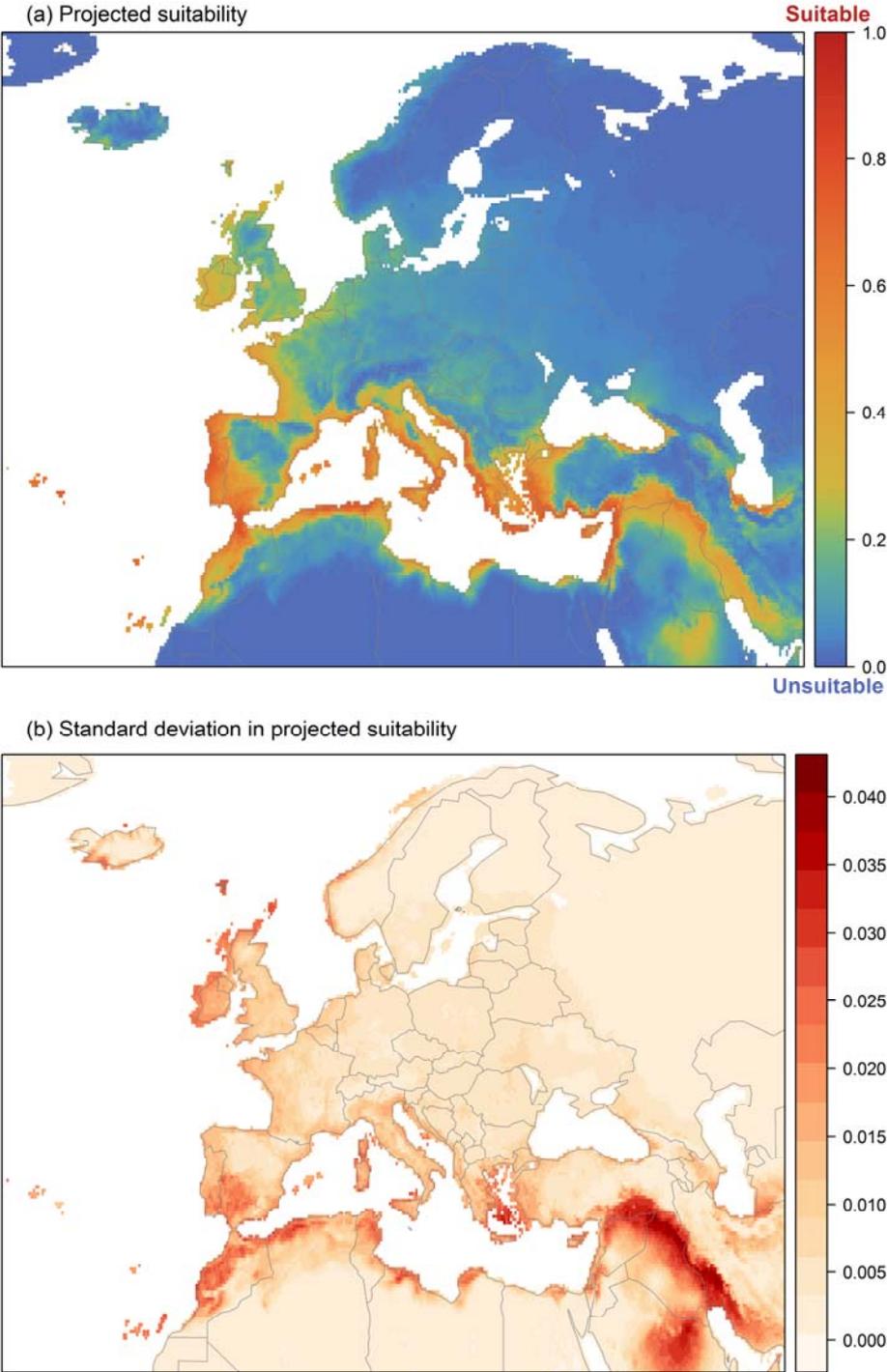


Figure 8. (a) Projected suitability for the establishment of the red-whiskered bulbul in Europe and the Mediterranean region in the 2070s under climate change scenario RCP4.5. Values > 0.29 are suitable for the species, with 98% of global presence records above this threshold under current climate. Values below 0.29 indicate lower relative suitability. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

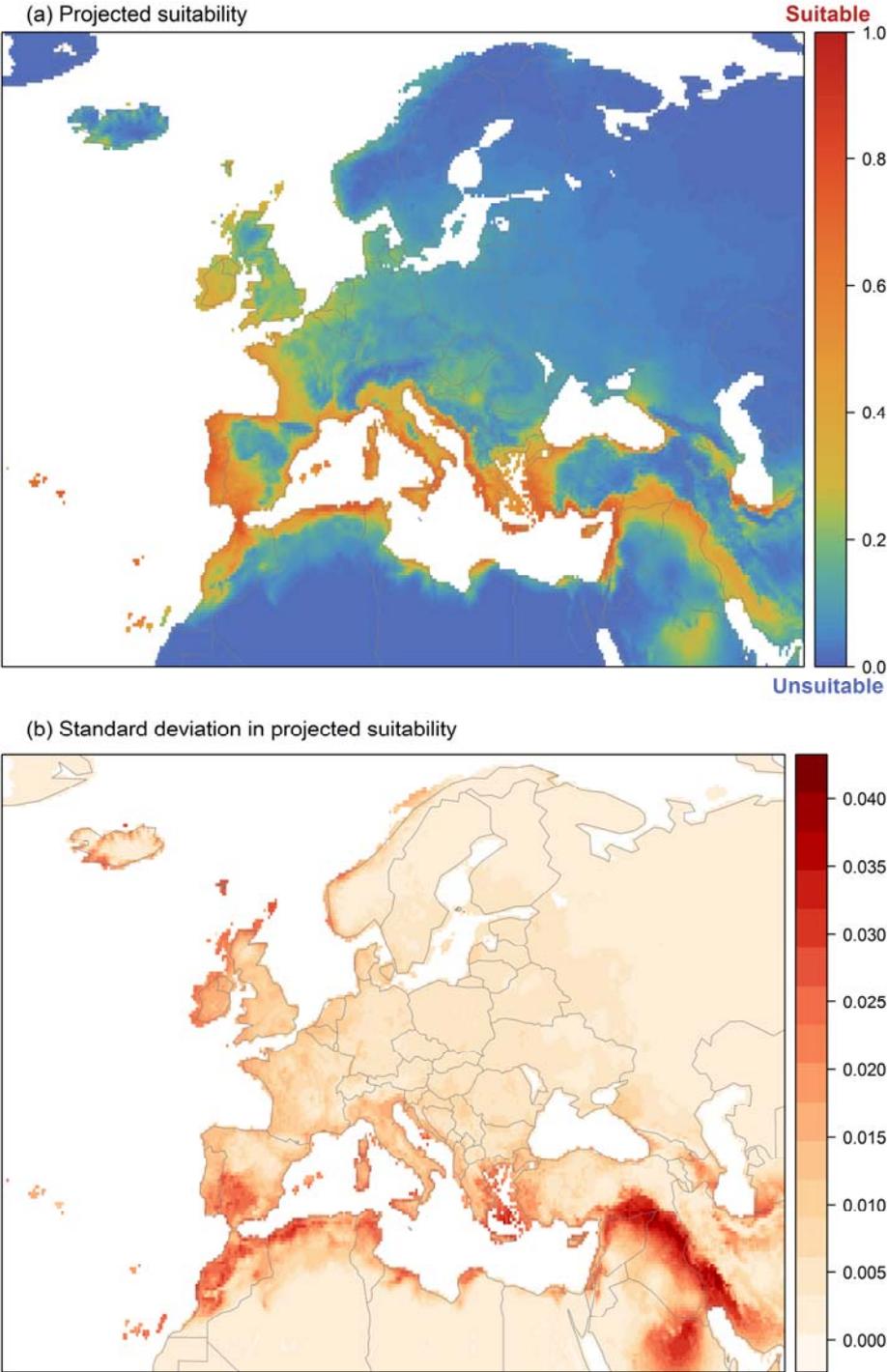


Figure 9. Variation in projected suitability for the establishment of the red-whiskered bulbul among Biogeographical Regions of Europe (<https://www.eea.europa.eu/data-and-maps/data/biogeographical-regions-europe-3>). The bar plots show the proportion of grid cells in each region classified as suitable (with values > 0.29) in the current climate and projected climate for the 2070s under two RCP emissions scenarios. Error bars indicate uncertainty due to both the choice of classification threshold (cf. p.5/6) and uncertainty in the projections themselves (cf. part (b) of Figs. 5,7,8). The location of each region is also shown. The Arctic and Macaronesian regions are not part of the study area, but are included for completeness.

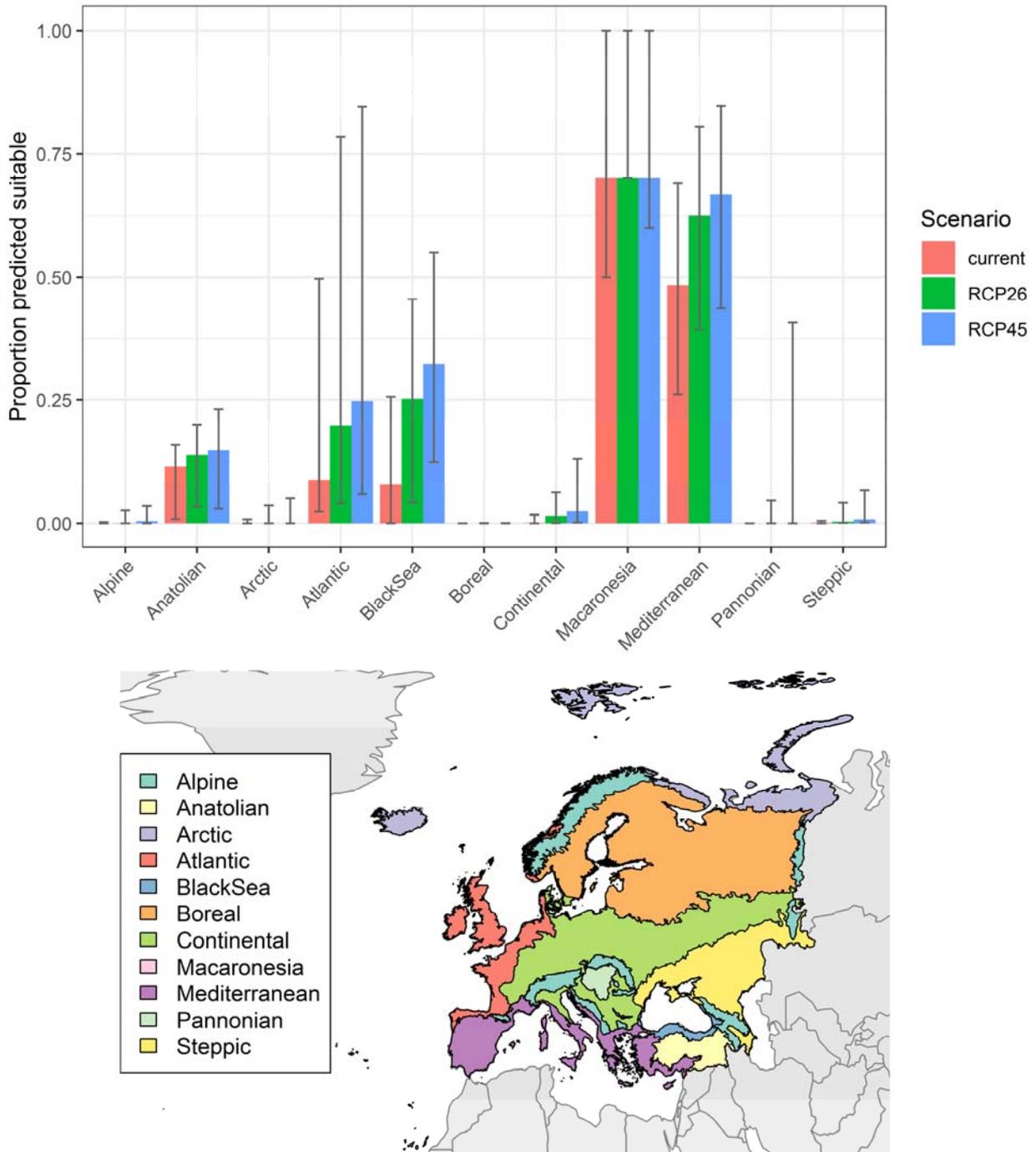


Table 2. Variation in projected suitability for the establishment of the red-whiskered bulbul among Biogeographical regions of Europe (numerical values of Figure 9 above). The numbers are the proportion of grid cells in each region classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. The Arctic and Macaronesian biogeographical regions are not part of the study area, but are included for completeness.

	current climate			2070s RCP2.6			2070s RCP4.5		
	lower	central estimate	upper	lower	central estimate	upper	lower	central estimate	upper
Alpine	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.03
Anatolian	0.01	0.12	0.16	0.03	0.14	0.20	0.03	0.15	0.23
Arctic	0.00	0.00	0.01	0.00	0.00	0.04	0.00	0.00	0.05
Atlantic	0.02	0.09	0.50	0.04	0.20	0.78	0.06	0.25	0.85
Black Sea	0.00	0.08	0.26	0.04	0.25	0.45	0.12	0.32	0.55
Boreal	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Continental	0.00	0.00	0.02	0.00	0.01	0.06	0.00	0.02	0.13
Macaronesia	0.50	0.70	1.00	0.70	0.70	1.00	0.60	0.70	1.00
Mediterranean	0.26	0.48	0.69	0.39	0.63	0.80	0.44	0.67	0.85
Pannonian	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.41
Steppic	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.01	0.07

Figure 10. Variation in projected suitability for the establishment of the red-whiskered bulbul among European Union countries and the UK. The bar plots show the proportion of grid cells in each country classified as suitable (with values > 0.29) in the current climate and projected climate for the 2070s under two RCP emissions scenarios. Error bars indicate uncertainty due to both the choice of classification threshold (cf. p.5/6) and uncertainty in the projections themselves (cf. part (b) of Figs. 5,7,8). Malta has been excluded because the Human Influence Index dataset lacks coverage for Malta.

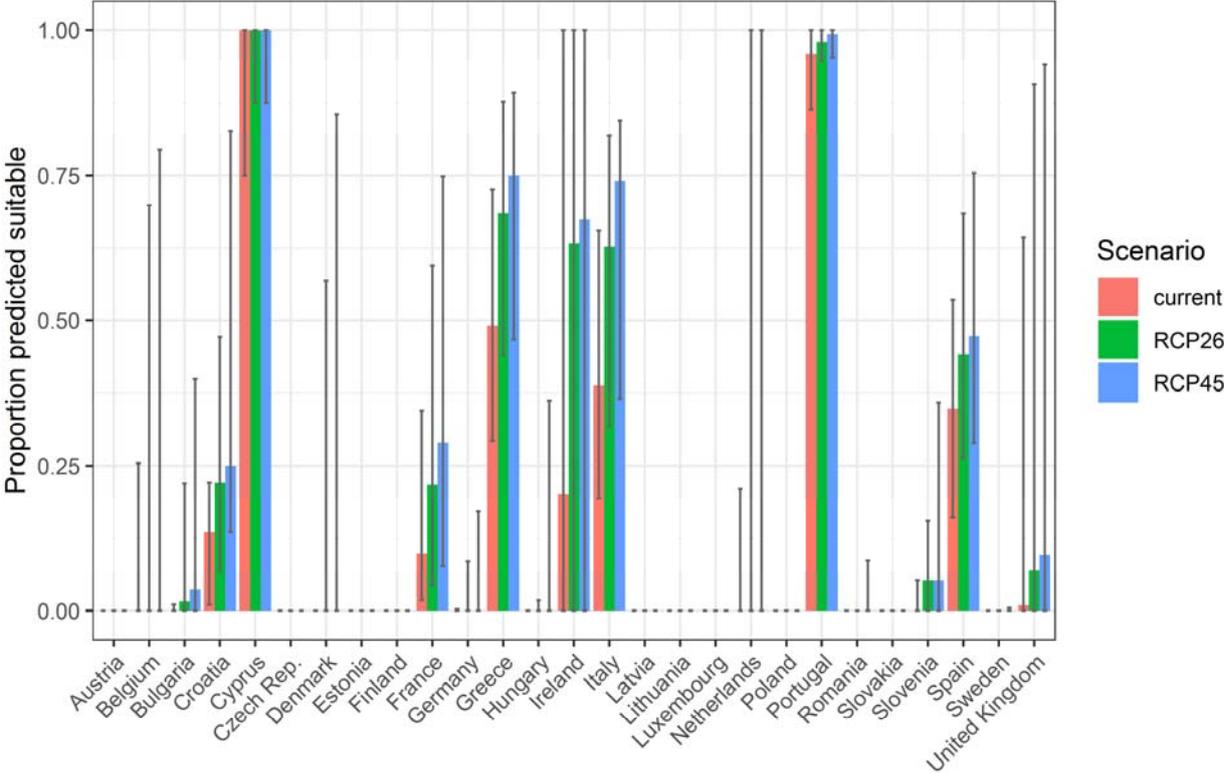


Table 3. Variation in projected suitability for the establishment of the red-whiskered bulbul among European Union countries and the UK (numerical values of Figure 10 above). The numbers are the proportion of grid cells in each country classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios.

	current climate			2070s RCP2.6			2070s RCP4.5		
	lower	central estimate	upper	lower	central estimate	upper	lower	central estimate	upper
Austria	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Belgium	0.00	0.00	0.25	0.00	0.00	0.70	0.00	0.00	0.79
Bulgaria	0.00	0.00	0.01	0.00	0.02	0.22	0.00	0.04	0.40
Croatia	0.01	0.13	0.22	0.07	0.22	0.47	0.13	0.25	0.83
Cyprus	0.75	1.00	1.00	0.88	1.00	1.00	0.88	1.00	1.00
Czech Rep.	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Denmark	0.00	0.00	0.00	0.00	0.00	0.57	0.00	0.00	0.86
Estonia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Finland	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
France	0.02	0.10	0.34	0.04	0.22	0.59	0.08	0.29	0.75
Germany	0.00	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.17
Greece	0.29	0.49	0.73	0.44	0.68	0.88	0.47	0.75	0.89
Hungary	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.36
Ireland	0.00	0.20	1.00	0.00	0.63	1.00	0.00	0.67	1.00
Italy	0.19	0.39	0.65	0.32	0.63	0.82	0.36	0.74	0.84
Latvia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Lithuania	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Luxembourg	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Netherlands	0.00	0.00	0.21	0.00	0.00	1.00	0.00	0.00	1.00
Poland	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Portugal	0.86	0.96	1.00	0.95	0.98	1.00	0.95	0.99	1.00
Romania	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09

Slovakia	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Slovenia	0.00	0.00	0.05	0.00	0.05	0.15	0.00	0.05	0.36
Spain	0.16	0.35	0.53	0.26	0.44	0.68	0.29	0.47	0.75
Sweden	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
UK	0.00	0.01	0.64	0.00	0.07	0.91	0.00	0.10	0.94

Caveats to the modelling

To remove spatial recording biases, the selection of the background sample from the accessible background was weighted by the density of Aves records on the Global Biodiversity Information Facility (GBIF). While this is preferable to not accounting for recording bias at all, it may not provide the perfect measure of recording bias.

There was substantial variation among modelling algorithms in the partial response plots (Figure 3). In part this will reflect their different treatment of interactions among variables. Since partial plots are made with other variables held at their median, there may be values of a particular variable at which this does not provide a realistic combination of variables to predict from.

Other variables potentially affecting the distribution of the species, such as types of land cover other than trees were not included in the model.

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Annex with evidence on measures and their implementation cost and cost-effectiveness

Species (scientific name)	<i>Pycnonotus jocosus</i>
Species (common name)	Red-whiskered Bulbul
Author(s)	Peter Robertson
Reviewer	Thomas Evans, Enrique Murgui
Date Completed	15/10/2021

Summary¹

Highlight of measures that provide the most cost-effective options to prevent the introduction, achieve early detection, rapidly eradicate and manage the species, including significant gaps in information or knowledge to identify cost-effective measures.

Red-whiskered Bulbuls have been introduced through a variety of pathways, including the pet trade and escapes from captivity. Control of these pathways and public awareness raising for this species and invasive alien species in general provides the most cost-effective method of ensuring this species does not establish more widely in Europe.

This species is highly visible and not readily confused with any native European species. Given the popularity of birdwatching, early sightings are likely to arise through birdwatching reports and these provide a source of citizen science to help record this species.

Red-whiskered Bulbuls have been successfully controlled in a variety of eradication and management programmes in their introduced ranges. These programmes have typically involved a combination of mist-netting, shooting and the use of traps containing decoy birds. Given the communal roosting behaviour of this species, mist nets near the roost have been used successfully, as have searches based on their loud and distinctive calls followed by shooting. Details of suitable trap designs are also publicly available, traps containing decoy birds are particularly effective. A variety of toxins have been used to control this and similar species, but there are no currently approved avian toxins suitable for use in the EU.

A combination of mist-netting, trapping and shooting has been used to successfully eradicate this species from islands in the Seychelles, to remove small populations from Tenerife and California while the same methods are used to control established populations and their spread in Australia, Florida and Reunion. This species is often associated with human modified habitats which may aid its control as it often forms isolated populations. Eradication will be most cost effective if started quickly after reports of an established population are first received. Public concern over the control of this species may be raised, particularly if proposing to use lethal methods in urban or semi-urban environments.

This species can damage crops, particularly fruits but also plants themselves. A variety of chemical deterrents have proven to reduce foraging by similar species under experimental conditions but have not been widely used in practice. Netting may also reduce crop damage in some circumstances.

Consideration of the risk of damage when siting new orchards has been proposed as a method of reducing future conflicts.

Detailed assessment			
	Description of measures²	Assessment of implementation cost and cost-effectiveness (per measure)³	Level of confidence⁴
Methods to achieve prevention of intentional and unintentional introduction⁵			
Managing pathways	Red-whiskered Bulbuls have been introduced to new areas, probably through a variety of pathways, including the pet trade, and escapes from confinement (CABI 2019). The adoption and enforcement of appropriate legislation and codes of best practice to reduce the risks posed by these pathways should reduce the probability of further introductions.	Authorities in Australia provide advice to prevent the escape of this species, stating that it is essential that when kept, the Red-whiskered Bulbul is maintained in secure (preferably double-doored) enclosures. Unwanted birds should be surrendered to the authorities or a responsible organisation, not released into the wild (Government of Western Australia 2017). The USDA prohibits the importation of the species to the USA and its territories (CABI 2019).	High. Managing pathways is widely recognised as an effective approach to limit the establishment of new species and has already been implemented for this species
Methods to achieve early detection⁶			
Citizen science	Red-whiskered Bulbuls are a highly visible species often found in association with human activity. Encouraging rapid reporting of new incursions increases the likely success of rapid response before the species can become established.	Citizen-science species occurrence datasets are increasingly recognized as a valid tool for monitoring the occurrence and spread of invasive species across large spatial and temporal scales (Roy et al., 2015). They are dependent on citizen-scientists who collect and upload data, typically from 'opportunistic sampling' with no underlying scientific survey design (Boakes et al., 2010) which can limit the conclusions that can be drawn from these	

		<p>data (Isaac et al., 2014). Most parts of north-west Europe have an extensive network of volunteer observers although this is less true of southern and especially eastern Europe (Boakes et al., 2010). Nevertheless the focus on native species, which is typical for monitoring activities carried out by bird-watchers, may lead to disregard the presence of non-native species, and consequently to a delay in detecting a new presence of this non native species. However, this naturalist community also provides an opportunity for developing an effective surveillance system. Unstructured citizen-science data do not reliably allow the estimation of species abundance or population trends (Kamp et al., 2016), yet in an early-warning scenario it is likely sufficient to know where a species is establishing, and these data limitations are thus of a lesser concern.</p> <p>Where the risk is considered high, more targeted approaches may be applied, actively encouraging reports from the public. In Australia, where the species has already formed local populations, the public is asked to report any members of the species in the wild where it has not been seen before (Government of Western Australia, 2017). In the 1980s, the Hawaii Department of Land and Natural Resources distributed brochures and flyers to the public requesting that any sightings be reported (CABI 2019).</p>	
Raising awareness	Raising public awareness of the risks posed by invasive alien species in general and Red-whiskered Bulbuls in particular. This can include the production of targeted publicity and identification material.	<p>A number of information sources and sheets are available on-line which can assist with the production of new material to raise public awareness. The Hawaii Invasive Species Council provide a handout describing the identification of the species and key information and links to other on-line sources.</p> <p>https://dlnr.hawaii.gov/hisc/info/invasive-species-profiles/red-whiskered-bulbul/</p>	High. This method already features in the management of this species in some areas
Methods to achieve eradication⁷		A number of successful eradications of Red-whiskered Bulbuls have been reported. Feare and Fries-Linnebjerg (2012). Uranie (2015) and Bunbury et al. (2019) report on the successful	

		<p>eradication of this species from Assumption (11.6km²) and the Aldabra complex of islands (155km²) in the Seychelles. A total of 5297 birds were removed from Assumption, with mist netting accounting for 80% of captures and shooting the majority of the rest. Red-whiskered bulbuls roosted communally in the early stages of the project and could be targeted with mist-nets along their flight lines towards roosts, which maximised the mist-net catch. Nocturnal shooting at the roost may have led to roost desertion, decreasing the effectiveness of mist-netting (Feare and Fries-Linnebjerg 2012). Later in the eradication, their habit of vocalising from prominent perches meant that they could be reliably located from several hundred metres away, which greatly helped in the search for and targeting of the last few birds. One bird was removed from Aldabra by mist-netting. These programmes were part of a wider eradication that included other invasive bird species, so data on effort needs to be treated with caution, but the eradications took three years and involved a team of between 3 and 30 people (Uranie 2015). A small population of seven individuals have also been eradicated from Tenerife in the Canary Islands using a combination of trapping, mist netting and shooting (Cruz and Reynolds 2019). In California, Clark (1976) reports the eradication of a Red-whiskered Bulbul population including the removal of 75 individuals. This describes stuffed decoy birds surrounded by speakers playing Red-whiskered Bulbul calls being used to lure birds within range for shooting.</p> <p>Introduced populations of Red-whiskered Bulbuls in areas such as Florida (Carleton and Owre 1975, Pranty 2010) and near Sydney in Australia (Mo 2015) have remained concentrated in areas associated with human activity, forming 'island' populations in a larger landscape. It has been suggested that this may make their eradication more feasible than is the case for more widely dispersed species in continental landscapes (Mo 2015). This</p>	
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		<p>feature of their distribution may also help limit their spread, authorities in Australia have exterminated reported individuals appearing in new areas, effectively preventing new population establishment (Barrington 1985; Paton 1985). This contrasts with the situation on Reunion where the species is widespread, although the reasons for such differences remain unclear (Clergeau et al. 2002).</p> <p>Birds are popular with the public, and opposition to the control of this species may be anticipated, particularly in urban or semi-urban areas. In, particular the use of lethal methods such as shooting, or different forms of trapping may attract particular concern.</p>	
<p>Trapping</p>	<p>A wide variety of designs are available for funnel entry or walk-in traps for birds.</p> <p>Successful trap designs for Red-whiskered Bulbuls often include the use of a decoy animal or any form of wire-netting trap. The decoy birds are usually in a separate chamber and must be provided with shade, food and water (Sharp and Saunders 2004). The decoys serve to attract other birds towards the trap. Decoy birds can be used in most trap types if suitably modified. Traps must be monitored at least on a daily basis and any caught birds removed.</p> <p>Clergeau et al. (2002) describe the use of a multi-capture decoy trap on Reunion. This circular wire cage is 65cm in diameter and 20cm high, split into 8 quadrants each with a</p>	<p>Traps, in particular decoy traps, are an effective method to catch Red-whiskered Bulbuls and have been successfully used to support control programmes and eradications including the Seychelles, Reunion and Tenerife (Clergeau et al. 2002, Bunbury et al. 2019, Cruz and Reynolds 2019).</p> <p>Clergeau et al. (2002) describe the use of multi-capture decoy traps to catch Red-whiskered Bulbuls on low-lying agricultural land in Reunion. Cages baited with fruit were placed at a height of 2m, mainly within orchards and achieved capture rates of between 1.9 and 11.5 birds per cage per week. A study using 91 cage traps in three Reunion villages captured 2837 birds, with the quality of the captive decoy and the location of the trap being the main determinants of success (Georger, 2000, Amiot et al. 2007).</p> <p>Any control of a vertebrate is likely to attract some opposition, but live capture traps have been widely used in bird control programmes, for example control of the Myna, with the active cooperation of the public (Feare and Cruz 2009, Pham and van Son 2009).</p> <p>The use of decoy animals brings added complications, and potential welfare concerns. Decoys require food, water, perches, space to stretch and shelter when in the trap and care is needed</p>	<p>High - Decoy traps are widely recognised as a successful method for capturing Red-whiskered Bulbuls and have been the used to support successful eradications.</p>

	<p>separate spring-loaded entrance. In the centre of the trap is a 25cm wide enclosure for captive decoy. Most literature on suitable trap designs relates to Mynas and will probably also be suitable to catch Red-whiskered Bulbuls. However, placing traps on raised platforms has been reported as the favoured method to catch Red-whiskered Bulbuls (Clergeau et al. 2002) compared to being set on the ground for Mynas. Pham and van Son (2009), Saavedra (2010) and Feare et al. (2017) describe a variety of trap designs for use on Mynas. A wide range of myna traps based on these designs are commercially available and described on-line (search term 'myna trap'). Captured birds can be humanely dispatched. Information on the humane dispatch of Mynas should also be applicable to Red-whiskered Bulbuls. The Pacific Island Initiative (2013) have published best practice guidelines for euthanising Common Myna. Myna trap designs have also been produced which enclose the trap in a canvas sleeve which can then be flooded with CO₂ to humanely kill captured birds</p>	<p>to ensure their welfare.</p> <p>Traps are simple to manufacture and the skills should be available locally after adequate training. A range of effective designs have been published and similar traps for use on Mynas are commercially available online. The main costs of their use are manpower for setting and checking. Experience of trap placement is required for their effective use and this can take time to develop. The use of decoy birds and traps may be covered by local Member State regulations. Local authorities should be asked for authorisation before their use.</p> <p>Traps are likely to also catch other birds and mammals. Care is needed to avoid non-target captures, for example through trap siting and the appropriate choice of baits. Regular checking and the release of non-target species can reduce the risks posed. Traps are a relatively safe and benign method of capture. Local authorities and regulations should be consulted before using a rtrap in a particular region. If used appropriately they do not pose particular health and safety, environmental, economic or social risks (although they can be mis-used to capture other protected species).</p>	
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	(Tidemann 2005, described in Pierce 2005).		
Shooting	<p>Shooting can be an effective method to remove Red-whiskered Bulbuls. It is highly selective and, if used by experienced personnel, provides a humane method of despatch. It is labour intensive but has been used effectively to remove small numbers of birds during the early stages of establishment (Clark 1975), and remove dispersed birds during the latter stages of wider eradications (Bunbury et al. 2019).</p>	<p>Clark (1975) report the use of shooting as the main method used to remove at least 75 Red-whiskered Bulbuls as part of an eradication programme in California. This used recorded Red-whiskered Bulbul calls with a high degree of success to lure the birds to within shooting range or to elicit a call so they could be located. Combining this with a stuffed bird mounted on a tall pole, with speakers playing the birds' call, was also successful in luring red-whiskered Bulbuls to within shooting range. Shooting used a combination of shotguns, pellet rifles, and sling shot. Shooting has also been used in the control of this species on Assumption Island in the Seychelles, where it accounted for the removal of around 20% of the population and was particularly useful in the latter stages of the eradication (Bunbury et al. 2019). Given that the birds often perch prominently on vegetation, together with their loud and readily identifiable calls, makes the use of shooting particularly effective to remove widely dispersed individuals during the latter stages of wider eradications. However, care is needed if other bird species are attracted to the calls.</p> <p>The use of different firearms is heavily regulated and the details vary between member states. These are likely to restrict the nature of the weapon, the requirements for the operator and the times and locations where they may be used. The humaneness and effectiveness of the method is likely to be dependent on the skill and training of the operative. Local authorities must be consulted before their use.</p> <p>The use of firearms brings risks to health and safety which need to be managed. Its use in public places is likely to bring opposition and raise particular concerns which may limit its use. The use of lead projectiles has been restricted in some areas due to</p>	<p>High -Shooting has been used to remove small populations of Red-whiskered Bulbuls and to assist in the eradication of larger populations.</p>

		environmental concerns, although non-toxic alternatives are available.	
Mist-nets	Mist nets are fine polyester or nylon nets which are suspended between two upright poles, requiring continual monitoring and expert handling of caught birds (Sharp and Saunders, 2004).	<p>Mist nets are commonly used for the live-capture of birds for ringing. Appropriate equipment and experienced personnel should be locally available. Experience and training is required to safely extract birds from the nets and a number of EU Member States run approval and licensing schemes for their use. The use of mist nets may be covered by local member state regulations. Local authorities should be consulted before their use.</p> <p>The use of mist nets provided the main control method for the eradication of Red-whiskered Bulbuls from Assumption and Aldabra islands in the Seychelles. Bunbury et al. (2019) report that 80% of the 5279 birds removed were caught in mist nets. Nets placed on flight lines and around the communal roosts of this species proved particularly effective. Mist nets also contributed to the removal of seven birds from Tenerife (Cruz and Reynolds 2019).</p> <p>Mist nets are unselective and are likely to catch birds of a range of species. However, trained personnel should be able to extract and release any non-target species caught. Mist nets need to be used under constant supervision to quickly remove captured birds, reducing their cost-effectiveness compared to other methods, although they can be very effective at sites where birds concentrate, such as communal roosts. Mist nets are widely used for bird ringing, their use poses few risks to health and safety, the environment, economy or society.</p>	High – mist nets have proven effective for the capture of this species in a number of successful eradications, particularly when used around communal roosts.
Use of toxins	Spurr and Eason (1999) review the available avicides worldwide. The two most widely used compounds are Starlicide (©DCR-1339, 3-chloro-p-toluidine hydrochloride) (Ramey et al., 1994, ACVM, 2002), and the stupifactant	<p>The lack of approved compounds effectively prevents the use of toxicants for bird control in the EU. Consequently these methods are not considered in great detail in this review. Starlicide is not currently approved for use in the EU.</p> <p>In the past, Alphachloralose has been approved as an avicide and was used in a number of EU states (Ridpath et al., 1961, Spurr and Eason, 1999). However, it is not currently registered for use</p>	High – There is an existing literature on the use and effectiveness of these compounds and examples of their use on other Bulbul

	<p>Alphachloralose ((R)-1,2-0-(2,2,2-trichloroethylidene)-α-D-glucofuranose) (Nelson, 1994; Thearle, 1969).</p> <p>Cruz and Reynolds (2019) report that Starlicide has been an effective toxicant used against Bulbuls in American Samoa. However, no products are currently approved for use in the EU.</p>	<p>as an avicide in the EU. It is currently approved for use against rodents (EU Directive 98/8/EC 2008).</p> <p>Although toxins can be an effective method of bird control, there are significant concerns regarding their non-target effects and public acceptability. Farmers on the Mascarene Islands are reported to have used use bird lime and pesticides to try and limit populations of Red-whiskered Bulbuls, although this also has an impact on non-target birds (Clergeau and Mandon-Dalger, 2001).</p>	<p>species in areas where their use has been approved.</p>
<p>Methods to achieve management⁸</p>	<p>All of the methods described to support eradication can also be used to manage existing Red-whiskered Bulbul populations.</p>	<p>See above.</p>	<p>See above.</p>
<p>Protecting fruit and plants from damage.</p>	<p>To reduce damage to commercial fruit crops, it may be possible to apply chemical feeding deterrents. Avery (2003) reviews the methods and use of chemical bird deterrents. A cage test conducted in Hawaii on bird repellent showed that Ziram, Methiocarb and Methyl anthranilate reduced the consumption of treated papaya mash by the similar Red-vented Bulbul (Cummings et al. 1994). In an open-field test, the same authors showed that Methiocarb significantly reduced damage on orchids. Netting of valuable crops can also be used. Thibault et al. (2019) describe netting to protect tomato plants</p>	<p>Netting and the use of chemical deterrents have not been widely used to protect fruits from damage by this species, most evidence arises from scientific studies rather than their practical application. The economics of their use remain undetermined and there is only limited data available from which to assess the seriousness of any crop damage. The use of netting brings a range of issues related to practicality as nets need regular maintenance and are susceptible to damage. Nets may limit access to the crops for other species with potentially wider impacts. Chemical deterrents require licensing before use. The use of nets is likely to be acceptable to the general public, the use of chemical deterrents may raise concern if the fruits are destined for human consumption.</p>	<p>Medium – These methods have been used to reduce damage by other bird species, but there is no information related to Red-whiskered Bulbuls.</p>

	from damage by the similar Red-vented Bulbul.		
Risk mapping	The combination of mapping species extent, the risks of damage and the location of vulnerable resources to inform spatial decision making.	On Réunion, a multi-party working group was established to explore management practices for the widely established population of Red-whiskered Bulbuls rather than simply undertaking untargeted control. These include using planning decisions to limit their spread (for example decisions on the locations of new orchards), producing risk maps, categorising vulnerability (sanitary, social, economic and ecological) to a population of the birds, producing maps of overall vulnerability, and correlating these with maps of potential habitats for Red-whiskered Bulbuls. A comprehensive risk map was then developed to help guide control programmes (Clergeau et al., 2006).	Medium. This approach has been applied to this species, but evidence of its effectiveness is limited

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Guidelines for Completing the Annex

1 Provide a brief summary description of the most cost-effective methods drawing on the reviews in the detailed assessments

2 Provide a description of the potential method. This should be based on the available key scientific evidence which should be gathered from sources including articles and reviews in technical and scientific journals, internet searches, online databases, grey literature and relevant books and personal communications from scientists, stakeholders, conservation practitioners and governmental bodies. This information should include a full bibliographic list detailing the literature and sources considered.

3 Provide an assessment of the likely cost and effectiveness of the method. Where information is available, consider the following range of questions, accepting that not all questions will be appropriate in all circumstances.

- How effective has this approach proven to be in the past or in an analogous situation?
- How publically acceptable is the approach likely to be?
- Over what period of time would this approach need to be applied to be effective?
- What is the direct cost of implementing this approach?
- How likely are the methods used in the approach to be available?
- How likely is it that relevant licences or other approvals to undertake the approach would be difficult to obtain?
- How likely is it that health and safety issues would prevent the use of this approach?
- How significant is the environmental harm caused by this approach?
- How significant is the economic harm caused by this approach?
- How significant is the social harm caused by this approach?
- How likely is it that the approach will be criticised on welfare grounds?
- How likely is it that the approach will be acceptable to other stakeholders?

Where available, factual information on the costs of specialist equipment, or case studies of management costs from across the Union or third countries should be provided. When describing case studies, if the information is available then provide both total cost and the area over which control was undertaken so that a cost per unit area might be derived. Where such quantitative information is not available, then any qualitative information from the literature is acceptable to help guide decision making. It is accepted that in the majority of cases the information required to assess the potential total cost of management at a member state level is unlikely to be available. This would normally require information on the extent and abundance of the species which is beyond the scope of this assessment. Assessors are not expected to extrapolate the potential total costs of management at a member state level, only to report on the information provided within the literature.

4 Provide an overall assessment of the confidence that can be applied to the information provided for this method. This confidence should relate to the quality of the available information using the guidance below. It should NOT relate to the confidence in the effectiveness of the method

The confidence scores are:

- **High:** Information comes from published material, or current practices based on expert experience applied in one of the EU countries or third country with similar environmental, economic and social conditions.
- **Medium:** Information comes from published data or expert opinion, but it is not commonly applied, or it is applied in regions that may be too different from Europe (e.g. tropical regions) to guarantee that the results will be transposable.
- **Low:** data are not published in reliable information sources and methods are not commonly practiced or are based solely on opinion; This is for example the case of a novel situation where there is little evidence on which to base an assessment.

If there are further factors beyond these that have determined the chosen level of confidence, then provide a brief written description to support the choice of the level of confidence.

5 Measures for preventing the species being introduced intentionally or unintentionally into the territory of a Member State (cf. Articles 7(1)(a), 7(2), 13, 15), i.e. preventing a species entering by blocking its pathways, including pre-border prevention and prevention of escape or release into the environment and secondary spread whenever relevant. Measures for preventing the species reproducing while in containment should be included, if appropriate. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

6 Measures to run an effective surveillance system for achieving an early detection of a new occurrence (cf. Article 16). This section assumes that the species is not currently present in a Member State, or part of a Member State's territory.

7 Measures to achieve eradication. Preferably, but not exclusively, used at an early stage of invasion, after an early detection of a new occurrence (cf. Article 17). Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

8 Measures to achieve management of the species once it has become widely spread within a Member State territory (cf. Article 19). These measures can be aimed at eradication, population control or containment of a population of the species. Consider all methods that might be applied, including any that have not proven useful. Provide a description of each method in turn, using separate rows to consider each method.

The development and completion of this template forms part of the EU project **No 07.0202/2020/834529/ETU/ENV.D.2** Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention.

Revised Risk Assessments on

**Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention"
Contract No 07.0202/2019/812602/ETU/ENV.D.2¹**

Name of organism: *Asterias amurensis* Lutken, 1871

Author(s) of the assessment: Marika Galanidi, Ustun Energy Engineering LTD, Izmir, Turkey

Risk Assessment Area: The risk assessment area is the territory of the European Union 27 and the United Kingdom, excluding the EU-outermost regions.

Peer review 1: Jack Sewell, The Marine Biological Association, UK

Peer review 2: Frances Lucy, Centre for Environmental Research Innovation and Sustainability CERIS, Dept of Environmental Science, Institute of Technology Sligo, Ireland

Date of completion: 15/10/2020

Date of revision: 15/10/2021

¹ This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA). A number of amendments have been introduced to ensure compliance with Regulation (EU) 1143/2014 on IAS and relevant legislation, including the Delegated Regulation (EU) 2018/968 of 30 April 2018, supplementing Regulation (EU) No 1143/2014 of the European Parliament and of the Council with regard to risk assessments in relation to invasive alien species (see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>).

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SECTION A – Organism Information and Screening

A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?

including the following elements:

- the taxonomic family, order and class to which the species belongs;
- the scientific name and author of the species, as well as a list of the most common synonym names;
- names used in commerce (if any)
- a list of the most common subspecies, lower taxa, varieties, breeds or hybrids

As a general rule, one risk assessment should be developed for a single species. However, there may be cases where it may be justified to develop one risk assessment covering more than one species (e.g. species belonging to the same genus with comparable or identical features and impact). It shall be clearly stated if the risk assessment covers more than one species, or if it excludes or only includes certain subspecies, lower taxa, hybrids, varieties or breeds (and if so, which subspecies, lower taxa, hybrids, varieties or breeds). Any such choice must be properly justified.

Response: This risk assessment covers one species: *Asterias amurensis* Lutken, 1871

Subspecies/varieties: *Asterias amurensis f. amurensis* Lutken, 1871
Asterias amurensis f. robusta Djakonov, 1950

Both subspecies are covered in the Risk Assessment.

Phylum: Echinodermata, **Class:** Stelleroidea, **Order:** Forcipulatida, **Family:** Asteroiidae

Synonyms: *Allasterias migrata* Sladen, 1879
Allasterias rathbuni var. nortonensis Verrill, 1909 †
Asteracanthion rubens var. migratum Doderlein, 1879
Asterias acervispinis Djakonov, 1950
Asterias amurensis f. acervispinis Djakonov, 1950
Asterias amurensis f. flabellifera Djakonov, 1950
Asterias amurensis f. gracilispinis Djakonov, 1950
Asterias amurensis f. latissima Djakonov, 1950
Asterias flabellifera Djakonov, 1950
Asterias gracilispinis Djakonov, 1950
Asterias latissima Djakonov, 1950
Asterias pectinata Brandt, 1835 (Synonym according to Fisher (1930))
Asterias rubens var. migratum Sladen, 1879
Parasterias albertensis Verrill, 1914 (Synonym according to Fisher (1930))

Common names: North Pacific sea star (EN); Japanese sea star (EN); Japanese starfish (EN); purple-orange sea star (EN); northern Pacific sea star (EN); flatbottom sea star (EN); Nordpazifischer Seestern (DE)

A2. Provide information on the existence of other species that look very similar [that may be detected in the risk assessment area, either in the environment, in confinement or associated with a pathway of introduction]

Include both native and non-native species that could be confused with the species being assessed, including the following elements:

- other alien species with similar invasive characteristics, to be avoided as substitute species (in this case preparing a risk assessment for more than one species together may be considered);
- other alien species without similar invasive characteristics, potential substitute species;
- native species, potential misidentification and mis-targeting

Response:

Asterias amurensis is a starfish that can grow up to 50cm in diameter. It is yellow with red and purple pigmentation on its five arms, and a small central disk. The undersides are completely yellow and arms are unevenly covered with small, jagged-edged spines. It shows a wide range of colours on its dorsal side: orange to yellow, sometimes red and purple. These spines line the groove in which the tube feet lie, and join up at the mouth in a fan-like shape (MPSC, 2015).

Similar species: According to Verrill (1914) it most resembles the species *Asterias forbesi* (North-west Atlantic) and *A. rubens* from the north-east Atlantic, which is native to the RA area. It is distinguished by its lack of interactinal plates and the evenly reticulated arrangement of the dorsal plates.

Fisher (1930) notes the following: “Differing from *A. rubens* in having, when adult, conspicuously broader rays, more numerous, usual shorter abactinal spinelets; 5 to 10 channeled or scoop-shaped superomarginal spines instead of two to five more or less terete, tapered, cylindrical, or clavate ones; in having a more nearly plane actinal surface (sharply defined by the rather thin edge of the ray, bearing a chevaux-defrise of superomarginal spines), and by having a broader, flatter actinal interradial region; intermarginal spines frequently present.

In more simple terms, a number of ‘photo-ID features’ could be used in combination to differentiate between *A. rubens* and *A. amurensis*.

- *Asterias rubens* appears to consistently display a straight row of spines along the centre of the dorsal surface of each arm. In *A. amurensis* this central, upper row is almost always irregularly arranged, waved or ‘zig-zagging’.
- In orange specimens of *A. amurensis* arm tips often appear to have been ‘dipped in purple paint’. *Asterias rubens* do not appear to display this colour pattern.
- In purple specimens of *A. Amurensis* the tip region of the arms is more densely purple than rest of arm and usually with white, yellow or pale patches/ blotches around the spines towards the central disk. *Asterias rubens* is more uniformly coloured along the length of the arms and is lacking pale blotches around the spines.

Asterias amurensis is very variable. While large specimens all have the same general appearance and are easily recognized, there is considerable diversity in the number, robustness and form of the spines.

The breadth of the ray increases with age in such a way as to alter very materially the appearance of the animals. Quite immature specimens are therefore unlike the adults in general appearance.”

Besides the congeneric *A. rubens*, two other, common to the RA area, starfish species present some similarities with *A. amurensis* but should not be difficult to differentiate. These are:

- *Leptasterias mueleri*: this species has a ‘rosette of spines on the dorsal surface rather than single spines. The central row of dorsal spines is arranged in a straight line, while the arm tips are white/ pale in purple specimens. This species is sometimes confused with young *A. rubens* but the common features are different in *A. amurensis* .
- *Marthasterias glacialis* often has purple tips to arms but has conspicuous knobs and large thorn-like spines arranged in straight lines and should not be mistaken for *A. amurensis*. It is also a larger species (up to 80cm across but usually smaller) and usually has a pale blue colouration, although it can also be grey, brown or white.

A number of starfish species exist in the aquarium trade (e.g. *Echinaster (Echinaster) sepositus* (Retzius, 1783), which is also native to the RA area) but they are distinctly different from *A. amurensis* and *Asterias* species in general.

A3. Does a relevant earlier risk assessment exist? Give details of any previous risk assessment, including the final scores and its validity in relation to the risk assessment area.

Response: Extensive work has been carried out in Australia, where the species is considered invasive since the 1990s (see McEnnulty et al., 2001). It is widely viewed as one of the most serious invasive marine pests in Australia (Hayes et al. 2005) and considerable scientific effort has been devoted to the prevention of its spread and its management (e.g. Goggin, 1998; Joint SCC/SCFA, 1999; Hayes et al., 2004; Bax et al., 2006; Aquenal, 2008; MPSC, 2015).

Asterias amurensis is on New Zealand’s register of unwanted and notifiable marine organisms (<http://www.environmentguide.org.nz/issues/marine/marine-biosecurity/invasive-marine-species/>).

The species was assessed with the CMIST screening-level risk assessment tool for NIS for Pacific North America in relation to the Japanese Tsunami Marine Debris JTMD (Therriault et al., 2018), and was one of the highest scoring species. Higher scores for the species may be expected in Pacific N. America, due to the higher proximity to donor regions and an already confirmed introduction event with JTMD.

It is included in the MSFD UK priority surveillance species list (version 1 – Stebbing et al., 2015) and was assessed with the MI-ISK protocol by Townhill et al. (2017) for the UK, where it was one of the highest scoring species (5th highest score out of 20, driven by high likelihood of spread and impact). The last two studies are directly relevant to the RA area (particularly Atlantic Europe), in terms of climatic conditions but also risk of introduction and spread.

Included in the marine species Horizon Scanning exercise carried out under the auspices of JRC (Tsiamis et al., 2020), and scored medium (weighed score of 31), identified as posing a threat to the NE Atlantic.

Considered as a “door knocker species” in the Nordic region (covering Sweden, Norway, Denmark, Finland, Estonia, Lithuania, Latvia, Iceland and the Faroe Islands) but was not risk assessed by the NOBANIS Horizon Scanning exercise (NOBANIS, 2015).

Assessed as a potential invader of medium impact by the Irish Horizon Scanning exercise (O’Flynn et al., 2014).

A4. Where is the organism native?

including the following elements:

- an indication of the continent or part of a continent, climatic zone and habitat where the species is naturally occurring
- if applicable, indicate whether the species could naturally spread into the risk assessment area



Figure 1: Occurrence records of *A. amurensis* in the native (red) and invaded (blue) range. Data points retrieved from the literature, biodiversity databases and screened as explained in A4-A6 and the modelling Annex (Annex IX). Map created by Bjorn Beckmann.

Response: *Asterias amurensis* is one of the most common northern Pacific starfish species, native to the cold-temperate, coastal waters of Korea, Russia, Japan (Lee et al. 2004) and Northern China (Li et al., 2018). It is common in the Eastern Bering Sea from the eastern Chukchi Sea to the Gulf of Alaska and British Columbia (Smith & Armistead 2014 and references therein), where it is considered cryptogenic (McLoughlin & Bax, 1993, cited in Talman et al., 1999). *Asterias amurensis* inhabits a variety of coastal habitats, from muddy, sandy, to coarser and more consolidated bottoms (Smith & Armistead, 2014), to kelp beds (Won et al., 2013) and rocky sheltered areas of intertidal zones (Aquenal, 2008). In its native range it is typically found at depths down to approximately 100m (Gabaev, 2018) but more commonly below 40-50m (Hatanaka & Kosaka, 1959; D’yakonov, 1968).

A5. What is the global non-native distribution of the organism outside the risk assessment area?

Response: Outside its native distribution, the species has been introduced to Tasmania and Victoria, in temperate Australia. In Tasmania it is believed to have been introduced as early as 1982 but was only correctly identified in 1992 (see Dunstan & Bax, 2007). The population in the Derwent estuary (Tasmania) was estimated in 1994 to be more than 30 million individuals (Goggin 1998). Today the species can be found all along the south and east coast of Tasmania (MPSC, 2015). In Port Phillip Bay, Victoria, it first appeared in 1995 with a few adult individuals, while spawning was confirmed in 1997 (Parry & Cohen, 2001). Within 3 years of confirmed establishment the population had reached ≈165 million individuals (Parry et al., 2004).

In Australia, it is predominantly found on shallow (<25m) soft sediment habitats and reefs, mostly limited to protected embayments and estuaries (Richardson et al., 2016; NIMPIS, 2020), as well as artificial structures, such as wharves and piers (Aqenal, 2008). It is not clear whether the narrower range of colonized habitats in the invaded range vs the native range (see previous question) is due to different habitat availability/distribution, time since colonization or other selection factors.

In Alaskan waters, where the species is considered cryptogenic, it occurs at depths down to 200m (Smith & Armistead, 2014 and references therein).

Note: Global Biodiversity Information Facility (<http://www.gbif.org>, occurrence data: https://www.gbif.org/occurrence/search?taxon_key=5187508) contains a number of records based on non-validated citizen science observations from South-East Asia (Indonesia, Thailand, Philippines), southern USA (Gulf of Mexico, California) as well as various European locations (see question A6 for details). These are considered questionable (see also Byrne et al., 2016), are not included in the peer reviewed literature on the non-native range of the species and were ignored for the purposes of this Risk Assessment.

A6. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species been recorded and where is it established? The information needs to be given separately for recorded (including casual or transient occurrences) and established occurrences. “Established” means the process of an alien species successfully producing viable offspring with the likelihood of continued survival².

A6a. Recorded: List regions

A6b. Established: List regions

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

- Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea.

² Convention on Biological Diversity, Decision VI/23

Comment on the sources of information on which the response is based and discuss any uncertainty in the response.

For delimitation of EU biogeographical regions please refer to <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2> (see also Annex VI).

For delimitation of EU marine regions and subregions consider the Marine Strategy Framework Directive areas; please refer to <https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions/technical-document/pdf> (see also Annex VI).

Response (6a): None

Response (6b): None

Note: as above (A5), GBIF and OBIS records based on non-validated citizen science observations (besides, no photograph was supplied) were not taken into account in the answer to A6. For the RA area, these refer to records from Greece, Portugal, Spain, France, the UK, the Netherlands, corresponding to the marine regions North-East Atlantic and Mediterranean Sea. The presence of the species is not confirmed in the peer-reviewed literature (e.g. Tsiamis et al., 2020), in the European Alien Species Information Network (EASIN) or in national biodiversity databases, e.g. the NBN Atlas for the UK (<https://nbnatlas.org/>), ELNAIS for Greece (<https://elnais.hcmr.gr/>), Netherlands Soortenregister for the Netherlands (<https://www.nederlandsesoorten.nl/>), thus it is considered extremely unlikely that these observations belong to *A. amurensis*.

A7. In which biogeographic region(s) or marine subregion(s) in the risk assessment area could the species establish in the future under current climate and under foreseeable climate change? The information needs to be given separately for current climate and under foreseeable climate change conditions.

A7a. Current climate: List regions

A7b. Future climate: List regions

With regard to EU biogeographic and marine (sub)regions, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (7a): The response is based on combining physiological tolerances and the results from the distribution modeling (see Qu 2.1, Qu 2.9 and Annex VIII & IX for details). For purposes of mapping and assessing the risk of establishment, the following tolerance limits were defined:

- Average surface temperature of the warmest month < 20°C
- Average surface temperature of the coldest month > 5°C
- Minimum surface salinity > 20psu

Baltic Sea: unlikely, high confidence

Greater North Sea: likely, medium confidence

Celtic Seas: likely, medium confidence

Bay of Biscay and the Iberian coast: likely, medium confidence

Mediterranean Sea: moderately likely, low confidence

Black Sea: unlikely, high confidence (BUT moderately likely with medium confidence ONLY for the Sea of Marmara, which is outside the RA Area)

In the Black Sea and the Baltic Sea, establishment of *A. amurensis* will be prevented by low salinities (Black Sea range of 14-19 psu for surface salinity – Baltic Sea beyond the Kattegat <15 psu). The species requires salinities above 22 psu for spawning, fertilization, and embryonic development (i.e. salinity near the seabed) and above 20 psu for complete larval development, i.e. surface salinity (Kashenko, 2005). The only exception here is the Sea of Marmara (part of the Black Sea), which exhibits surface salinities > 20 psu and fully marine bottom salinities (Beşiktepe et al., 1994) and can potentially support establishment of *A. amurensis*. Establishment in the Mediterranean Sea is expected to be limited by high temperatures to the northern, cooler basins.

Response (7b): The response is based on combining physiological tolerances and the results from the distribution modeling (see Qu 2.1, Qu 2.9 and Annex VIII & IX for details). Aspects of climate change most likely to affect future distribution were considered as an increase in minimum and maximum Sea Surface Temperatures (SST). The methodology for the developed models is described in Annex IX and considers scenarios RCP 2.6 and RCP 4.5 by 2070. See also Qu. 2.10.

Baltic Sea: unlikely, high confidence

Greater North Sea: likely, medium confidence

Celtic Seas: likely, medium confidence

Bay of Biscay and the Iberian coast: moderately likely, medium confidence

Mediterranean Sea: unlikely, low confidence

Black Sea: unlikely, high confidence (Sea of Marmara: moderately likely, medium confidence)

The species distribution model predicted a very small reduction in projected suitability for *A. amurensis* for all EU marine subregions under future climate change and a small northward shift of the overall suitable area for the species. Suitable conditions in the Mediterranean are likely to become even more restricted spatially and temporally (Annex VIII – Figure 2), rendering establishment in this marine subregion unlikely. The possibility of physiological adaptations and/or introductions from southern Japan, where the sea surface temperature of the warmest month can exceed 27 C, drives the low confidence of this assessment. In the Bay of Biscay and the Iberian coast suitable conditions for establishment are expected to last for shorter time periods in the future, hence the lower likelihood score. In the Greater North Sea, an increase in sea temperature will offer suitable conditions for

spawning and larval development for prolonged periods in the south and east of the region, extending into the winter months, which under current conditions are too cold for the survival of larvae (see Annex VIII – Figure 1).

A8. In which EU Member States has the species been recorded and in which EU Member States has it established? List them with an indication of the timeline of observations. The information needs be given separately for recorded and established occurrences.

A8a. Recorded: List Member States

A8b. Established: List Member States

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden

The description of the invasion history of the species shall include information on countries invaded and an indication of the timeline of the first observations, establishment and spread.

Response (8a): None

Response (8b): None

See also comment in Qu. A6

A9. In which EU Member States could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A9a. Current climate: List Member States

A9b. Future climate: List Member States

With regard to EU Member States, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (9a): Belgium, Croatia, Denmark, France, Germany, Greece, Ireland, Italy, Netherlands, Portugal, Slovenia, Spain, Sweden; and the United Kingdom

Response (9b): Belgium, Denmark, France, Germany, Ireland, Netherlands, Portugal, Spain, Sweden; and the United Kingdom

The response to 9b is based on the RCP4.5 scenario for the period 2050/2070. The aspect of climate change most likely to affect the organism's ability to establish is an increase in winter sea surface temperatures. Higher winter temperatures in the Mediterranean Sea will exceed the species' upper limit for larval survival/development, particularly throughout the southern basin, while the same change will favour larvae for longer periods in the eastern North Sea. (see Annexes VIII & IX for details on modelling and future climate conditions in the RA area).

A10. Is the organism known to be invasive (i.e. to threaten or adversely impact upon biodiversity and related ecosystem services) anywhere outside the risk assessment area?

Response: for details and references see Qu. 4.1.

In Australia, *Asterias amurensis* reached very high densities (up to 24 individuals/m²) and developed massive populations within a relatively short time of its establishment (Byrne et al. 1997b; Grannum et al. 1996) in the two main invaded areas, where it has become the dominant benthic invertebrate predator. By the late 1990s, the adult population in Derwent was estimated at 30 million sea stars, and in Port Phillip Bay at 165 million individuals by 2003 (Parry et al., 2004).

With its voracious appetite and preference for bivalve prey, it has caused drastic population declines of (commercially important) native bivalve species and severely reduced the recruitment of juvenile bivalves to the benthos (Ross et al., 2002; 2004). It has been implicated in population declines of fish species, with which it competes for food (Parry & Hirst, 2016) and was considered responsible for the precipitous decline of the endemic fish species *Brachionichthys hirsutus* (Barrett et al., 1996, Ross et al., 1999), which was subsequently considered critically endangered. The mechanism was hypothesized to involve predation by *A. amurensis* on stalked ascidians, which form the primary spawning substrate of *B. hirsutus*, and subsequent reduction of key habitat availability (Bruce & Green, 1998; Green et al. 2012), although direct evidence for this is lacking.

A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea

Response: None. *Asterias amurensis* is not present in the risk assessment area.

A12. In which EU Member States has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, United Kingdom

Response: None. *Asterias amurensis* is not present in the risk assessment area.

A13. Describe any known socio-economic benefits of the organism.

including the following elements:

- Description of known uses for the species, including a list and description of known uses in the risk assessment area and third countries, if relevant.
- Description of social and economic benefits deriving from those uses, including a description of the environmental, social and economic relevance of each of those uses and an indication of associated beneficiaries, quantitatively and/or qualitatively depending on what information is available.

If the information available is not sufficient to provide a description of those benefits for the entire risk assessment area, qualitative data or different case studies from across the risk assessment area or third countries shall be used, if available.

Response:

Species of the genus *Asterias* have been used in the past in their native range (e.g. Japan, Canada and the USA), albeit to a limited extent, for the production of fish meal, compost or fertiliser (McEnulty et al., 2001; Barkhouse et al., 2007 and references therein). For example, in Japan, where the large amounts of *A. amurensis* by-catch from fisheries and aquaculture activities constitute a waste disposal problem, the species has been used to produce plant growth promoting compost (Line, 1994; Ishii et al., 2007). In Denmark, a starfish meal processing plant was inaugurated in 2019, utilising starfish *A. rubens* caught in an important mussel cultivation area, intended for animal feed. Similar uses can be envisaged for *A. amurensis*, in case the species is introduced to the RA area.

Preliminary research work has been conducted with *A. amurensis* in China, in order to obtain saponin (Hwang et al., 2011) and collagen (Hao and Li, 1999) from viscera or body wall for biotechnological purposes. In another area, the gametes of *A. amurensis* have been used in the assessment of marine environmental quality in Japan (Yu, 1998; Yu et al., 1998; Lee and Choi, 2003).

It is listed as a medicinal species in Asia (Goya & Youngsung, 2017), primarily in relation to its use in homeopathy.

SECTION B – Detailed assessment

Important instructions:

- In the case of lack of information the assessors are requested to use a standardized answer: “No information has been found.”
- With regard to the scoring of the likelihood of events or the magnitude of impacts see Annexes I and II.
- With regard to the confidence levels, see Annex III.
- Highlight the selected response score and confidence level in **bold** but keep the other scores in normal text (so that the selected score is evident in the final document).

1 PROBABILITY OF INTRODUCTION AND ENTRY

Important instructions:

- **Introduction** is the movement of the species into the risk assessment area (it may be either in captive conditions and/or in the environment, depending on the relevant pathways).
- **Entry** is the release/escape/arrival in the environment, i.e. occurrence in the wild
- Introduction and entry may coincide for species entering through pathways such as “corridor” or “unaided”, but it also may differ. If different, please consider all relevant pathways, both for the introduction into the risk assessment area and the entry in the environment.
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used (see Annex IV). For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document³ and the provided key to pathways⁴.
- For organisms which are already present (recorded or established) in the risk assessment area, the likelihood of introduction and entry should be scored as “very likely” by default.
- Repeated (independent) introductions and entries at separate locations in the risk assessment area should be considered here (see Qu. 1.7).

Qu. 1.1. List relevant pathways through which the organism could be introduced into the risk assessment area and/or enter into the environment. Where possible give details about the specific origins and end points of the pathways as well as a description of any associated commodities.

For each pathway answer questions 1.2 to 1.7 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 1.2a, 1.3a, etc. and then 1.2b, 1.3b etc. for the next pathway.

In this context a pathway is the route or mechanism of introduction and/or entry of the species.

³ <https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf>

⁴ <https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf>

The description of commodities with which the introduction of the species is generally associated shall include a list and description of commodities with an indication of associated risks (e.g. the volume of trade; the likelihood of a commodity being contaminated or acting as vector).

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 1.2-1.9.

Pathway name:

a) TRANSPORT-STOWAWAY (ship/boat ballast water)

Asterias amurensis is believed to have been introduced to Tasmanian waters from central Japan via ballast water discharge (Ward & Andrew, 1995). Based on a thorough evaluation of alternative pathways (Hayes et al., 2004), ballast water transfer is still considered the most plausible pathway of introduction of this species to Tasmania, as well as its subsequent spread to Victoria, mainland Australia (Dunstan & Bax, 2008).

b) TRANSPORT-STOWAWAY Hitchhikers on ship/boat (excluding ship/boat hull fouling)

This pathway covers specifically cases where species are transported with boat/ship in locations other than ballast water and hull fouling (namely where water is held or collected within the hull, such as sea chests, bilge water and within the hull itself – IUCN, 2017). While it is unlikely that starfish would remain attached to vessels when underway for any length of time, their translocation is possible in sheltered niche areas (Dommissie & Hough, 2004; MAF, 2011). For example, *Asterias amurensis* was observed in the sea-chest of a commercial vessel operating between the Derwent Estuary, Tasmania, and Port Phillip Bay in Australia (Thresher, pers. comm in Dommissie & Hough, 2004). Additionally, *A. amurensis* DNA was found in biofouling samples collected from internal surfaces of fishing/recreational vessels offering further support that the species can be translocated via this pathway (Hayes et al., 2004).

c) TRANSPORT-CONTAMINANT Contaminant on animals (except parasites, species transported by host/vector)

Asterias amurensis is a notorious pest in shellfish aquaculture, both in the native and the invaded range, and is known to settle on and infest oyster trays, mussel lines and scallop spat collectors, as well as salmon cages, on-shore and offshore abalone facilities and other fish-farming equipment (e.g. Gabaev, 2018; Dommissie & Hough, 2004). Even though introduction via this pathway is not documented for the species anywhere in the invaded range, it is considered possible that infested consignments of commercial bivalve species intended for aquaculture can transport it unintentionally, as a contaminant, into the RA area.

d) TRANSPORT-STOWAWAY (ship/boat hull fouling)

Asterias amurensis DNA was found in biofouling samples collected from the external surfaces of fishing and recreational vessel hulls examined in Australia, in the absence of *A. amurensis* larvae in the surrounding water, offering support that the species can be translocated via vessel fouling (Hayes et al., 2004). However, it is unlikely that starfish would remain attached to vessels when underway for any length of time (Dommissie & Hough, 2004; MAF, 2011), such that this pathway is not considered a risk for primary introductions from existing native and invasive populations and is instead discussed in conjunction with the pathway “Hitchhikers on ship/boat” only in the Risk of Spread section.

a) **TRANSPORT-STOWAWAY (ship/boat ballast water)**

Qu. 1.2a. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: It can be stated with high certainty that this pathway is unintentional. See categorization of pathways in Annex IV and guidance notes in the beginning of this section.

Qu. 1.3a. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: This will depend on the larval densities in the donor location, the time of the year (i.e. if uptake of ballast water coincides with the peak spawning period), as well as the densities of the adult population, which will affect the reproductive output. The highest larval densities ever recorded in the Derwent estuary, Tasmania were 1000/m³ (Sutton & Greene, 2002), other studies however reported much lower densities during spawning season, in the range of 50/m³ in areas with smaller adult populations (Dommissse & Hough, 2004). By the late 1990s, the adult population in Derwent was estimated at 30 million sea stars, and in Port Phillip Bay at 165 million individuals by 2003 (Parry et al., 2004). During outbreak events in China, adult *A. amurensis* densities can reach 300ind./m² (Li et

al., 2018), although much lower densities have been reported for outbreaks in Japan (i.e. 6 ind/m² – Nojima et al., 1986) and similar densities of 7 ind/m² are maintained throughout the year in Australia (MPSC, 2015); with a reproductive output of up to 19 million eggs per female (Buttermore et al., 1994), there is a very high potential for extremely high larval densities in the donor region.

According to Kaluza et al. (2010), the ports of Japan, Korea and northern China are well connected in terms of trading with the ports of the Mediterranean and Atlantic Europe (especially the Le Havre – Hamburg range) with thousands of journeys per year through the Suez Canal. With increasing sea surface temperatures (SST) and extended ice melting periods in the Arctic, some of this shipping traffic is expected to be diverted to the Northern Sea Route (Arctic passage), which is currently being used by a relatively small number of commercial vessels on a “trial” basis (Fernandez et al. 2014). However, an annual increase in traffic volume of 20% is expected over the next 25 years (Miller & Ruiz, 2014; Rosenhaim et al. 2019). Less maritime traffic takes place between temperate Australasia and the RA area (see Annex VIII for a more detailed analysis).

The lowest estimates of the volumes of ballast water taken-up, transferred and discharged into world oceans each year are around 10 billion tonnes (Interwies & Khuchua, 2017), whereas just one cubic metre of ballast water may contain from 21 up to 50,000 zooplankton specimens (Locke et al., 1991, 1993; Gollasch, 1997) and a heavy bulk carrier can carry up to more than 130,000 tonnes of ballast water (GloBallast, 2009). It is thus evident that high numbers of planktonic propagules of *A. amurensis* are likely to enter the RA area via ballast water and this remains true in the event of a possible eradication.

Qu. 1.4a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Pelagic larval duration (PLD) of *A. amurensis* larvae can range between 50 and 120 days at temperatures between 19°C and 10°C respectively (Bruce et al., 1995 and references therein), while a journey from the north-west Pacific to the RA area lasts approximately 6 weeks (information retrieved from shipping companies’ itinerary pages). Despite the high PLD of the species, there is high uncertainty regarding the likelihood of survival of its larvae in ballast water when travelling through tropical regions. Ballast water temperature follows the temperature of the surrounding water with 1-2 days delay (Gollasch et al., 2000; Lenz et al., 2018). In tropical seas sea surface temperature can reach values of >30 °C in the summer and as high as 27-28 °C in the winter, far above the optimal temperature for survival of *A. amurensis* larvae as surmised by the literature (see e.g. Qu. 2.7). Nevertheless, under laboratory conditions, larvae have survived temperatures of 27.9 °C for up to 6 days but died within hours at 31.3 °C (Sagara & Ino, 1954). The dominant hypothesis about the introduction pathway to Australia is ballast water, presumably due to the sheer volume transported with international shipping and the number of larvae that could be carried in it. Even if this is indeed

the case and *A. amurensis* larvae have already survived one such trip, the time travelled through warm waters between the native region and Australia is presumably much shorter than the respective time spent in the tropics while traveling from the west Pacific to the RA area.

For vessels navigating the Northern Sea Route in the future, the transit time can be reduced by e.g. approximately 10 days for a journey between Japan and Rotterdam (ABS, 2013). Lower temperatures in the Arctic will extend larval development duration, while shorter trips will potentially increase likelihood of survival for the larvae. Reproduction is clearly not possible during ballast transport of larvae, since this would require mature adults to be present in ballast sediments and furthermore to be diverting resources to gamete production and maturation in a stressful and food poor environment.

The overall likelihood of survival with current shipping routes is assessed as moderate with low confidence.

Qu. 1.5a. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The International Maritime Organization (IMO) Ballast Water Management Convention (BWMC) entered into force in September 2017. It requires ships in international traffic to apply ballast water management measures, in particular:

- ballast water exchange in open seas, away from coastal areas (D-1 standard for an interim period)
- fulfil a certain discharge standard (D-2 standard according to the ship specific application schedule phased in up to 8 September 2024). D-2 standard requires the installation of a certified ballast water treatment device, which enables sterilization to avoid transfers of ballast water mediated species.

Ballast Water Exchange (BWE) is currently practiced and requires ships to exchange a minimum of 95% ballast water volume whenever possible at least 200 nautical miles (nm) from the nearest land and in water depths of at least 200 metres. When this is not possible, the BWE shall be conducted at least 50 nm from the nearest land and in waters at least 200 metres in depth (David et al., 2007 and BWMC Guideline 6). Even though BWE can reduce the concentration of live organisms in ballast by 80–95% (Ruiz & Reid 2007), its application has severe limitations, primarily dependant on shipping patterns of a port (e.g., shipping routes, length of voyages) and local specifics i.e., distance from nearest shore, water depth (David et al., 2007), particularly for EU Seas where these conditions are often not possible to meet. Also, organisms may still remain in the volume of ballast not exchanged and in ballast sediment, or BWE may not be possible due to weather conditions or other safety restrictions. The survival of zooplanktonic organisms (including *A. amurensis*) is thus not unlikely when only BWE measure are implemented.

As a result, ballast water treatment has been deemed necessary, such that ships shall discharge (in relation to the organism size range of interest for *A. amurensis*): less than 10 viable organisms per cubic metre greater than or equal to 50 micrometres in minimum dimension (IMO D-2 standard).

Ballast water treatment options include mechanical (filtration, separation), physical (heat treatment, ozone, UV light) and chemical methods (biocides). Efficiencies of various technologies utilised for ballast water treatment are reviewed in Tsolaki & Diamadopoulos (2010) and can vary with treatment method but the application of many combined methods (e.g. Filtration+UV or Hydroclone+chemical disinfectant) can decrease live zooplankton to undetectable levels, practically diminishing propagule pressure. As such, the survival of *A. amurensis* larvae in ballast water with full implementation of the D-2 standard (i.e. after 2024) is considered unlikely.

With some of the global fleet already compliant with the D2 standard, the chances of larval survival are currently assessed as moderate. Full implementation of the BWMC by 2024, despite its current operational challenges, is expected to reduce the likelihood of survival before the Arctic route becomes relevant for the species.

Qu. 1.6a. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: After September 2017, with the BWMC coming into effect and gradually being implemented, detection of larval stages in ballast water during Port State Control inspections may be possible. According to Resolution MEPC.252(67), if initial inspections of ballast water samples indicate non-compliance with the D-2 standard, detailed inspections will be carried out. eDNA methodologies are rapidly becoming one of the fastest and most cost-efficient tools for the detection of NIS⁵ in introduction water samples (Darling & Frederick, 2018; Borrell et al., 2017; Koziol et al., 2019) and have proven suitable for the detection of *A. amurensis* larvae specifically in port water samples as well as water samples from the internal spaces of small vessels (Hayes et al., 2004). In fact, due to their similarities with closely related species and the lack of taxonomic expertise, genetic methods are the only way to unequivocally identify *A. amurensis* larvae (Bruce et al., 1995). However, full implementation of the BWMC is not anticipated until 2024. Until then, the likelihood that *A. amurensis* will enter the RA area undetected in ballast waters remains high, particularly since eDNA methodologies are not yet widely used. On the other hand, if the species is targeted through a national monitoring programme with molecular methods, larvae in water samples from ports/harbours can be currently detected.

⁵ NIS: non-indigenous species, term used in the Marine Strategy Framework Directive, synonym of “alien species” as used in the framework of Regulation (EU) 1143/2014

Qu. 1.7a. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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Response: In the Mediterranean Sea, potential recipient ports are relatively evenly spaced but slightly more concentrated along the central and western Mediterranean, with a small number in Malta, southern Italy and Spain acting as the main transshipment hubs (Rodrigue, 2020). Conversely, in Atlantic Europe, the main ports of entry are located within a more confined region, i.e. both coasts of the English Channel, but principally the Le Havre-Hamburg range along the north coast of continental Europe.

Qu. 1.8a. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Asterias amurensis* is assumed to have been transported from central Japan to Tasmania as larvae in ballast water. The species has a very high reproductive output and can reach high densities during outbreak events, both at the seabed but also in surface waters during peak spawning periods. Despite the long PLD of the species, a number of conditions need to be met for the successful introduction of viable propagules from possible donor regions into the RA area, most importantly uptake of a sufficient number of larvae (i.e. during spawning) and transportation during the winter months, which would offer the best chances of survival during passage from tropical waters.

Out of the two main donor regions, north-western Pacific ports in Japan, Korea and China have the highest maritime connectivity with potential recipient ports in the RA area although some shipping traffic exists with southern Australia as well. Overall, the likelihood of introduction in the RA area as larvae in ballast water is judged as moderate but this applies to Atlantic Europe and primarily the Greater North Sea region. Concerning the Mediterranean Sea, considering that the primary shipping route from Asia, as well as the main transshipment hubs are located along the southern basins (Rodrigue, 2020), successful introduction and entry are considered far less likely in the Mediterranean parts of the RA area. The same applies for the Black Sea and the Baltic Sea. Concerning Arctic shipping routes, these are currently only used on a “trial” and seasonal basis with significant associated challenges and are expected to become more widely available around mid-century (Melia et al., 2017). While the increased use of the Arctic passage in the future is expected to increase the potential for introduction of *A. amurensis* (and other NIS) via ballast water, this will be of concern

further into the future, by which time it will be largely ameliorated by full implementation of the BWMC.

b) TRANSPORT-STOWAWAY Hitchhikers on ship/boat (excluding ship/boat hull fouling)

Qu. 1.2b. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional	CONFIDENCE	low
	unintentional		medium
			high

Response: It can be stated with high certainty that this pathway is unintentional. See categorization of pathways in Annex IV and guidance notes in the beginning of this section.

Qu. 1.3b. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely	CONFIDENCE	low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response: The translocation of starfish is documented in sheltered niche areas of both large commercial and small recreational vessels (Dommissé & Hough, 2004; MAF, 2011). For example, *Asterias amurensis* was observed in the sea-chest of a commercial vessel operating between the Derwent Estuary, Tasmania, and Port Phillip Bay in Australia (Thresher, pers. comm in Dommissé & Hough, 2004). Additionally, *A. amurensis* DNA was found in biofouling samples collected from the internal surfaces of fishing and recreational vessel hulls, offering further support that the species can be translocated via this pathway (Hayes et al., 2004).

At this point it should be considered that DNA detected with meta-barcoding of a fouling sample may come from non-viable propagules or parts of organisms present in the stomachs of filter feeders or entrained within the fouling community. In this particular case however, fouling samples tested positive for *A. amurensis* DNA in the absence of *A. amurensis* larvae in the surrounding water, which was concurrently tested, as they were collected outside the species spawning season (Hayes et al., 2004). This does not necessarily mean that the detected DNA corresponds to viable propagules and indeed recent advances in environmental monitoring of marine pests are suggesting the combined use of eDNA and eRNA for a more accurate representation of viable communities (Pochon et al., 2017).

While it is certainly possible for the species to settle on the internal surfaces of ship/boat hulls and be transported over long distances as part of a fouling community, the likelihood of large number being transported via this pathway is not very high. Only a few such incidents are reported in the literature (see also Frey et al., 2014 for other asteroid species), and the number of individuals detected is small. On the other hand, the amount of maritime traffic between possible donor and recipient regions is very high (see Qu. 1.3a), considering both large merchant vessels as well as leisure craft. Based on the above, reinvasion after eradication is possible. Entry into the environment would require dislodgment of fouling material or active movement by the adults/juveniles. Alternatively, propagules could enter the environment in the form of gametes by at least a pair of *A. amurensis*, an event which is not considered very likely.

Qu. 1.4b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: If entrained in areas of the vessel with low food availability, *Asterias* species are capable of surviving prolonged periods of starvation, up to several weeks (St-Pierre & Gagnon, 2015) or even months (Vevers, 1949). If, on the other hand, starfish are transported in sea chests, where heavy fouling can accumulate (i.e. providing food resources, primarily in the form of bivalves), not only survival but also growth and maturation may be possible. For *A. amurensis*, this is attested by the survival and translocation of live individuals on debris from the Tohoku tsunami of 2011 from Japan to the west coast of North America almost a year later (Carlton et al., 2017; 2018). Reproduction would require the presence of at least a pair of adults and would be unlikely to happen under starvation conditions, when the species would mobilise its energy reserves for survival (Aguera & Byrne, 2018).

Qu. 1.5b. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Fouling organisms such as *A. amurensis* can be transported for weeks or months in mature fouling communities or sea chests. Implementing practices to control and manage biofouling can greatly assist in reducing the risk of the transfer of invasive marine species.

While the International Convention on the Control of Harmful Anti-Fouling Systems on Ships, 2001 (AFS Convention) addresses anti-fouling systems on ships, its focus is on the prevention of adverse impacts from the use of anti-fouling systems and the biocides they may contain, rather than preventing the transfer of invasive aquatic species.

Guidelines for the control and management of ships' biofouling to minimize the transfer of invasive aquatic species were adopted on 15 July 2011 [RESOLUTION MEPC.207(62)] The Biofouling Guidelines represent a decisive step towards reducing the transfer of invasive aquatic species by ships. Especially vessel cleaning during dry-docking in a shipyard generates a very low biosecurity risk because the debris is sent to local deposit and residue water from cleaning is collected (Bohn et al., 2016). Moreover, maintenance during dry-docking involves the re-application of anti-fouling paint, which seems to be efficient for up to 1-1.5 years – thereafter heavy fouling can start occurring (Sylvester et al., 2011; Frey et al., 2014). Nevertheless, dry-docking frequency is determined by performance (fuel consumption below a certain threshold) and can range from 0.5-5 years (Bohn et al., 2016).

On the other hand, in-water cleaning (IWC) of hulls (used as an additional tool, in-between dry-dock cleaning), especially without capturing the biofouling debris, might represent a higher risk of introducing NIS relative to land based cleaning in dry-docks with land based waste disposal because physical disturbance of the fouling communities may trigger the release of propagules or viable gametes (Hopkins & Forrest, 2008). This is especially significant with *A. amurensis* as regeneration following fragmentation may result in the proliferation of individuals. High migratory distance (reported as at least 10-15 m/day in Japan – Miyoshi et al., 2018) and survival at depth mean that even offshore activity may pose a threat.

There has been a proliferation of new IWC technologies in the past decade (e.g. <https://www.ecosubsea.com/>, <http://econetsaustralia.com/>) that capture debris and render it non-viable through e.g. UV treatment, although such systems sometimes fail to contain all of the removed debris (for reviews see Zabin et al., 2016; Scianni & Georgiades, 2019).

The suite of measures described above can prove effective against *A. amurensis* and other fouling organisms, if fully implemented, although sea-chests would still remain higher risk areas and may require more frequent in-water treatment. However, anti-fouling practices are not legally required, and can be financially costly, making it likely that a number of vessels traveling between contaminated and uncontaminated marinas and ports will not have been treated, motivating a likely score for this question. Even though there does not appear to be any comprehensive analysis of the compliance levels to the MEPC 2011 biofouling guidelines, a considerable reduction in the arrival of high-risk vessels was observed in New Zealand, after biofouling management measures became mandatory (Hayes et al., 2019).

Although (as with physical hull cleaning), antifouling is not currently a legal requirement, there is potential that treatments with biocidal compounds may prove an effective method of controlling fouling and reduce the likelihood of introduction and spread.

Qu. 1.6b. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species is unlikely to be detected upon introduction, unless thorough inspections of fouling communities in sea chests and internal hull surfaces are carried out, which is currently not a routine practice. Even then, because of the small size of newly settled individuals, the likelihood of detection via visual inspections remains low.

In order to reach GES targets with reference to Descriptor D2, most EU states are already designing or implementing national/regional NIS-targeted monitoring programmes. Monitoring should focus on introduction hotspots (e.g. ports, marinas, aquaculture plots) and this will increase the detectability of *A. amurensis* entering the RA area through hull fouling, particularly if molecular methods are employed (Hayes et al., 2004).

Qu. 1.7b. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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Response: Besides the big ports, which are relevant for large commercial vessels and are described in Qu. 1.7a, marinas for recreational vessels also constitute possible points of introduction and these are much more widespread throughout the RA area. There are over 4500 marinas in Europe, of which the largest percentage is small facilities (EC, 2017), but there are some that receive leisure craft from ports outside the EU (e.g. Gittenberger et al., 2017). Additionally, there are numerous open mooring and anchoring areas for recreational vessels, particularly in touristic hotspots, attracting high numbers of vessels often during the summer months.

Qu. 1.8b. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Asterias amurensis* are capable of settling on ships/boat internal surfaces and sea chests and surviving over the time period it takes for a journey from the possible donor regions to the RA area, especially considering that management measures for this pathway are not mandatory. Maritime traffic between the west Pacific and EU ports and harbours is generally high but the number of individuals transported via this pathway and entering the environment is unlikely to be high. Thus, introduction with ships/boats fouling is considered to be moderately likely.

c) TRANSPORT-CONTAMINANT Contaminant on animals (except parasites, species transported by host/vector) mariculture

Qu. 1.2c. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: It can be stated with high certainty that this pathway is unintentional. See categorization of pathways in Annex IV and guidance notes in the beginning of this section.

Even though introduction via this pathway is not documented for the species anywhere in the invaded range, it is considered possible that infested consignments of commercial bivalve species intended for aquaculture can transport it unintentionally, as a contaminant, into the RA area.

Qu. 1.3c. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication

- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *A. amurensis* on cultured molluscs can display high densities, e.g. in the order of 10 ind/m² on suspended scallop spat collectors (Gabaev, 2018 - for more details see Qu 4.9). The main commercial bivalve species, with which *A. amurensis* could be accidentally imported into the RA area are the oyster *Magallana gigas* and the clam *Ruditapes philippinarum*, both of which constitute preferred prey for the starfish in its native range, as well as settlement habitat in the case of *M. gigas* (see also Qu 1.5c). However, shellfish imports from countries outside the EU are not very common and are prohibited in many cases, if not requiring strict quarantine procedures.

Available information in the peer reviewed literature suggests that bivalve culture in Europe is largely dependent on harvesting/collecting wild seed from nearby locations to aquaculture plots, bivalve seed from hatcheries to a smaller extent and, when necessary, imports of seed from other EU countries (Muehlbauer et al., 2014, Robert et al., 2013; Occhipinti-Ambrogi et al., 2016; Marchini et al., 2016). Small quantities of bivalves and other cultured molluscs may still be imported from non-EU countries (e.g. small quantities of oysters *C. gigas*, up to 3-4 tonnes per year, were directly imported from Japan and Korea into the Netherlands between 2004 and 2008 – Haydar & Wolff, 2011), but more extensive information on bivalve imports from countries outside the EU could not be found. The non-native abalone species *Haliotis discus hannai*, originally imported from Japan, is cultivated in Ireland (Hannon et al., 2013) and Spain (<http://abalonbygma.com/en/abalone-exclusive-seafood/>, see also Cook, 2014), where current production takes place in closed systems and relies on local hatcheries for seed. If more stock was imported from the native range, it would be subjected to the stringent controls of COUNCIL REGULATION (EC) No 708/2007 (see Qu.1.5c), such that this species is not considered to pose additional risks for new introductions of *A. amurensis* into the RA area.

Qu. 1.4c. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Reproduction would not be possible considering the short duration of bivalve transfers. However, survival is very likely, given the abundance of suitable prey species and the conditions under which shellfish consignments are transported, which may actually enhance the likelihood of survival of contaminant species, as well by providing moisture and protection from harsher conditions (Minchin, 2007; Curtis et al., 2015).

Qu. 1.5c. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: COUNCIL REGULATION (EC) No 708/2007 concerning use of alien and locally absent species in aquaculture defines the procedures to be followed that minimise the risk of introducing non-target alien species accompanying commercial shellfish spat and stocks. It requires a permit procedure, involving risk assessment for the non-target species and a quarantine period for the translocated stock.

The bivalves *Crassostrea gigas* (now *Magallana gigas*) and *Ruditapes philippinarum*, listed in Annex IV, constitute exceptions and can be moved without any risk assessment or quarantine; however local/national legislation exists that can limit the translocation possibilities of species like *M. gigas*, e.g. see WG-AS & Gittenberger (2018) for the trilateral Wadden Sea area. Additionally, if the import region is a Natura2000 area, regulations can be much stricter as they aim to protect the conservation objectives of the protected area first.

Other initiatives have produced codes of conduct for the transfer of bivalve seed/stock at the national/regional level, such as the ICES Code of Practice on the Introductions and Transfers of Marine Organisms 2005 (ICES, 2005).

The implementation of EC regulation 708/2007 (EC 2007) introduces a high biosecurity level for most bivalve transfers from areas outside the EU, that has already proven to be effective in preventing new introductions of marine alien species (Katsanevakis et al., 2013; Zenetos, 2019). However, the exemption of these two species, which both constitute common prey items of *A. amurensis* in its native (Kim, 1969) and invaded range (Dommissse & Hough, 2004), means that *M. gigas* and *R. philippinarum* consignments potentially infested with *A. amurensis* would not be subjected to mandatory control measures before being released into the wild, unless stricter national/regional regulations apply, thus increasing the risk of introduction of the species. On the other hand, shellfish growers are aware of the risks posed to bivalve stock by the introduction of *Asterias* species and would be expected to adhere to codes of conduct for thorough inspections and other additional measures if necessary.

Qu. 1.6c. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: In situations where regular inspection of stock is a normal part of shellfish farm operations, *Asterias amurensis* can and has been detected on bivalve stock as early as recently settled juveniles of 10-15mm diameter, on e.g. mussel lines (Garnham, 1998; Martin & Proctor, 2000). Thus, early detection is not impossible, as long as there is awareness of the risk of introduction of the species and the ability to discriminate it from native species. However, *A. amurensis* is very variable in appearance, closely resembles the native common starfish *A. rubens* and even more so at the juvenile stage (See Qu. A2). Thus, inspections during aquaculture operations by unsuspecting/untrained staff could easily misidentify it.

Qu. 1.7c. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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Response: Concerning the two species that are exempted from the EC regulation 708/2007, i.e. *M. gigas* and *R. philippinarum*, their cultivation is widespread throughout the RA area, particularly that of *M. gigas*. *Magallana gigas* is extensively cultivated, particularly in Atlantic Europe (Muehlbauer et al., 2014) but also in some of the Mediterranean countries (Greece, Italy, Mediterranean France and Spain) to a smaller extent (Rodrigues et al., 2015). *Ruditapes philippinarum* is cultivated mainly in Italy, Spain, Portugal, France, Ireland and the UK, where the species has naturalized and developed substantial wild populations, which provide the seed for culture operations, alongside hatcheries (Moura et al., 2017). Other non-native bivalve species (potential vectors of *A. amurensis*) intended for aquaculture would be subjected to the Regulation stipulations for strict inspections, quarantine, etc, making introduction highly unlikely – This follows from Qu. 1.5c. Besides, these two are the main species that fulfil both conditions, i.e. have been/are still occasionally imported for aquaculture into the RA area and can act as vectors of *A. amurensis* as they constitute prey species and/or settlement habitat.

Qu. 1.8c. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species may be introduced as pest, primarily on imported oysters *M. gigas* and clams *R. philippinarum* from the North West Pacific. Available literature suggests that shellfish imports from countries outside the EU are generally limited in the past couple of decades and well regulated. The risk of introduction is associated with a few species listed in Annex IV of Council Regulation (EC) No 708/2007 if stricter local/regional regulations are not in place (see Qu. 1.5c) and with illegal/unreported transfers. Even for the exempted species of Annex IV however, the amount imported from outside the EU is at levels low enough to render this pathway unlikely.

Qu. 1.9. Estimate the overall likelihood of introduction into the risk assessment area or entry into the environment based on all pathways and specify if different in relevant biogeographical regions in current conditions.

Provide a thorough assessment of the risk of introduction in relevant biogeographical regions in current conditions: providing insight in to the risk of introduction into the risk assessment area.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Baltic Sea: unlikely, high confidence

Greater North Sea: moderately likely, medium confidence

Celtic Seas: moderately likely, medium confidence

Bay of Biscay and the Iberian coast: moderately likely, low confidence

Mediterranean Sea: moderately likely, medium confidence

Black Sea: unlikely, high confidence

The most likely pathways of introduction of *A. amurensis* in the RA area are through shipping vectors. While ballast water can potentially transport a large number of larvae, a number of conditions need to be met for the successful introduction of viable propagules from possible donor regions into the RA area, primarily uptake and transportation during thermally suitable times of the year. Moreover, the increasing implementation of the BWMC D2 standard will gradually reduce even further the survival probability of *A. amurensis* larvae entrained in ballast water.

Entrainment in ships' sea chest and other hull internal surfaces is a plausible mode of transport but less likely to be responsible for a large number of propagules entering the RA area. Based on the maritime traffic between potential source regions and EU ports, introduction and entry of *A. amurensis* larvae is considered most likely in the Greater North Sea region (with important transshipment hubs) and less likely in the Mediterranean Sea, where most shipping traffic nowadays is concentrated along its southern parts.

Qu. 1.10. Estimate the overall likelihood of introduction into the risk assessment area or entry into the environment based on all pathways in foreseeable climate change conditions?

Thorough assessment of the risk of introduction in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of introduction (e.g. change in trade or user preferences)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely introduction within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The response is based on the RCP 4.5 scenario for 2070.

Baltic Sea: unlikely, medium confidence

Greater North Sea: moderately likely, medium confidence

Celtic Seas: moderately likely, medium confidence

Bay of Biscay and the Iberian coast: moderately likely, low confidence

Mediterranean Sea: moderately likely, low confidence

Black Sea: unlikely, high confidence

A reduction in the extent and duration of ice-cover at northern latitudes under future climate conditions is likely to make the Northern Sea Route (via the Arctic Ocean) more attractive to global shipping and possibly add new ports to the potential entry points for *A. amurensis*, although big operational challenges are still associated with this (Hansen et al., 2016). Even if this happens

however, the main recipient area in the RA area where introduction is likely will remain the Greater North Sea. Increased traffic in Siberian harbours however may facilitate a stepping stone movement of *A. amurensis* larvae throughout Arctic waters towards the North-East Atlantic.

At the same time, heat waves, can cause mass mortality of aquaculture bivalves, leading to increased shellfish transfers to replete the stocks (Rodrigues et al., 2015), and this is more likely to happen in the Mediterranean Sea. More shellfish movements may be associated with a higher risk of introduction if the stocks/seed originate from areas outside the EU with a high risk of contamination with *A. amurensis* and the necessary precautions are not taken.

2 PROBABILITY OF ESTABLISHMENT

Important instructions:

- For organisms which are already established in parts of the risk assessment area or have previously been eradicated, the likelihood of establishment should be scored as “very likely” by default.
- Discuss the risk also for those parts of the risk assessment area, where the species is not yet established.

Qu. 2.1. How likely is it that the organism will be able to establish in the risk assessment area based on similarity of climatic and abiotic conditions in its distribution elsewhere in the world?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Asterias amurensis* is an arctic-boreal species that can survive a wide range of temperatures. In the northwestern Sea of Japan (Russia), the species overwinters under negative temperatures of -1.6 to -1.9°C, and in shallow-water bays in summer it lives at temperatures of up to 28-30°C (Gabaev, 2018 and references therein). In Alaskan waters *A. amurensis* can withstand sub-zero temperatures for weeks and it reproduces in the summer months (see also Qu. 2.7). *Asterias amurensis*, in its native range, spawns primarily in late winter-early spring, at optimal temperatures between 5°C and 17°C (Li et al., 2018) but is reported to spawn at temperatures of up to 23°C (Novikova, 1978). This upper temperature limit to spawning comes in contrast with all other published literature derived both from field studies as well as laboratory experiments (e.g. Kashenko, 2005; Lee et al., 2004; Sutton & Bruce, 1996; Byrne et al., 2016), which indicates that normal larval development can occur at a maximum temperature of 20°C.

These thermal tolerances are depicted in the global distribution of the species, combined with temperature data, retrieved from BIO-ORACLE (Assis et al., 2018, URL: <http://www.bio-oracle.org/>) and MARSPEC (Sbrocco & Barber 2013), URL: <http://www.esapubs.org/archive/ecol/E094/086/metadata.php>).

Concerning its salinity requirements, Kashenko (2003) suggests a minimum value of 22 psu for the survival of adult *A. amurensis*, based on laboratory experiments. Kashenko (2005) reports a similar tolerance to salinity during embryonic development, which is arrested at 22 psu. Other, pelagic, larval stages are able to survive salinities at an absolute minimum of 18 psu but exhibit higher survivorship above 20 psu (Kashenko, 2005). Thus, salinity limitations are expected to prevent establishment of the species in the Black Sea (surface salinities in the range of 14-19 psu), except for the Sea of Marmara with salinities > 20 psu and the Baltic Sea, except at the very entrance of the western Baltic.

For purposes of mapping and assessing risk of establishment, the following tolerance limits were defined:

- Average surface temperature of the warmest month < 20°C
- Average surface temperature of the coldest month > 5°C
- Minimum surface salinity > 20psu

Considering the above, the species is very likely to find suitable abiotic conditions for establishment throughout most of Atlantic Europe, with the exception of the Bay of Biscay, where sea surface temperatures are generally higher than 20°C in the summer months but cooler between October and June. In the Mediterranean Sea however, there are two short time windows for larval development, in April-May and November-December, depending on the donor region of larvae being Japan/Korea or Australia. Propagules arriving to the Mediterranean from China in the winter months are still likely to find suitable temperatures for development and settlement upon release. Thus, the likelihood of establishment in the Mediterranean Sea is considered as moderate (See Annexes VIII & IX for more details).

Qu. 2.2. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area? Consider if the organism specifically requires another species to complete its life cycle.

RESPONSE	very isolated isolated moderately widespread widespread ubiquitous	CONFIDENCE	low medium high
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Response: *Asterias amurensis* inhabits a variety of coastal habitats, from muddy, sandy, to coarser and more consolidated bottoms (Smith & Armistead, 2014), to kelp beds (Won et al., 2013) and rocky sheltered areas of intertidal zones (Aquenal, 2008). In its native range it is typically found at depths down to approximately 100m (Gabaev, 2018) but more commonly below 40-50m (Hatanaka & Kosaka, 1959; D'yakonov, 1968), while in Alaskan waters it occurs at depths down to 200m (Smith & Armistead, 2014 and references therein). Some of these habitats, such as kelp beds and the rocky intertidal, are more widespread in Atlantic Europe, but the sublittoral soft and mixed sediment habitats are practically ubiquitous throughout the shallow waters (down to 200m) of all EU Seas.

The species is well known to aggregate over shellfish grounds (mostly bivalve molluscs), natural or cultivated (e.g. Paik et al., 2005; Gabaev, 2018), aquaculture equipment, such as suspended bivalve spat collectors, mussel lines and oyster trays (Dommissse & Hough, 2004; Gabaev et al., 2005) and piers/wharves (Aquenal, 2008). Such habitats are also widespread in the RA area, where extensive bivalve cultivation (and harvesting) takes place (Muehlbauer et al., 2014).

The organism does not require another species to complete its life cycle.

Qu. 2.3. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: In the RA area, *A. amurensis* is likely to face competition by asteroid species with a similar trophic position and functional role in the ecosystem, i.e. *A. rubens*, *Marthasterias glacialis*, as well as crab species with a similar dietary preference for bivalves, e.g. *Carcinus maenas*, *C. aestuarii*. The outcome of such interactions is difficult to predict, it is known however that subtidal starfish aggregations can consist of more than one species of asteroids, e.g. *A. rubens* and *M. glacialis* occurring together in Brittany (France) and sharing bivalve resources (Guillou, 1996) or *Leptasterias polaris* and *A. rubens* (referred to with the synonym *A. vulgaris*) in the Gulf of Saint Lawrence (North America), preying on *Mytilus edulis* (Gaymer & Himmelman, 2002). Often the outcome of such interactions is resource partitioning by prey size or depth zone, rather than competitive exclusion (Gaymer et al., 2002). As an example, in Japan, *A. amurensis* dominates the shallow waters of Sendai Bay to be gradually replaced beyond the depth of 50 m by the congeneric *A. nippon* (now *Distolasterias nippon*) (Hatanaka & Kosaka, 1959). Conversely, in Port Phillip Bay, Australia, *A. amurensis* is mostly found in water deeper than 15 m and *Coscinasterias muricata* in the shallow parts of the bay (Parry, 2015).

Even more informative is the case of co-existence of *A. rubens* with *A. forbesi* (the third boreal, closely related species of the genus *Asterias*) in parts of their range in the north-west Atlantic, where the two species display high overlap in space (geographic and bathymetric), time, mean body size, diet composition and mean prey size (Menge, 1979). Menge (1979) suggested that the two species in this region compete only infrequently and their population dynamics were controlled by storms, disease, competition and prey patchiness, in order of importance.

As regards competition with other taxonomic groups, the interaction between *C. maenas* and *A. amurensis* in Australia, where they are both introduced, appears to be one of resource competition, resulting in partitioning of bivalves according to size between predators, with *A. amurensis* consuming the large bivalves and *C. maenas* the small ones (Ross et al., 2004). On the other hand, competitive interactions with predatory crabs for bivalve prey can result in sub-lethal injuries to arm tips of *A. amurensis* (Ling & Johnson, 2013), which are more pronounced in juvenile starfish.

Qu. 2.4. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely	CONFIDENCE	low medium high
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	likely very likely		
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Response: *Asterias amurensis*, like many asteroid species, is generally considered to be a terminal consumer in food webs; it does however have some documented predators. In its native range the species is consumed by the starfish *Solaster paxillatus* and the red king crab *Paralithodes camtschaticus* (Gabaev, 2018). In Australia, predation by the native starfish *Coscinasterias muricata* is suspected to be responsible for high post-settlement mortality of *A. amurensis* juveniles in Port Phillip Bay (Parry & Cohen 2001), while in laboratory experiments *C. muricata* was identified as the most efficient predator of *A. amurensis* among a suite of species examined (Botsios 2001, in Parry 2015). Nevertheless, further research is required to determine whether such native predators have the potential to influence *A. amurensis* establishment and densities in the field (Aquenal, 2008). On the other hand, in Australia, Ling & Johnson (2013) observed a high-incidence of sub-lethal injuries to *A. amurensis* by the native spider crab *Leptomithrax gaimardi*, occurring in large aggregations, and suggested that seasonal aggregations of the large spider crab can have strong but localized effects on *A. amurensis*.

Regarding the RA area, native starfish predators of the congeneric *A. rubens* may be able to exert some predation pressure on *A. amurensis*, particularly at the post-settlement, juvenile stage. Such predators include the species *Crossaster papposus* (Castilla, 1972), the specialized echinoderm predators *Luidia ciliaris* and *L. sarsi*, which are known to control *A. rubens* populations (Guillou, 1996), possibly native species of the genus *Coscinasterias*, such as *C. tenuispina*, but also large predatory crab species. It is noteworthy that the native predator *P. camtschaticus* is introduced and invasive to Norway and currently spreading to the south and west, while smaller related stone crabs (e.g of the genus *Lithodes*, which is known to prey on asteroid species – Fuhrmann et al., 2017), may fulfil a similar role. The potential of predation by these species to limit *A. amurensis* populations is unknown, but judging from the establishment of the species in Australia in the presence of native predators, it is thought that establishment is unlikely to be prevented.

With respect to parasites and pathogens, male *A. amurensis* are liable to gonad parasitisation by the ciliate parasite *Orchitophrya stellarum* (Byrne et al., 1997a), which causes complete atrophy of the testes, leading to castration. In Japan, infestation rates were as high as 100% in some populations (Byrne et al., 1997a). This has implications for recruitment of the species, as it decreases the concentration of male gametes and, consequently, the chances of successful external fertilisation. *Orchitophrya stellarum* has a circumboreal distribution and is known to parasitize a number of asteroid species, including the native to the RA area *A. rubens*, albeit with a lower prevalence (Jangoux, 1987). It has also been found in asteroids from the Mediterranean Sea (Febvre et al., 1981 in Jangoux, 1987). Since this ciliate infests mature males, it is considered unlikely that it will affect the potential for establishment by larval propagules, it can however affect the following generations and adults entering the RA area by other vectors.

Sea star wasting disease (SSWD) can cause mass mortality events in asteroid species, accounting for strong population declines and shifts in the demography of the affected species. SSWD is an umbrella term used to describe a suit of clinical signs starting with the appearance of skin lesions which may enlarge over time to allow internal organs to protrude, leading to the animal's death (Hewson et al., 2019). While clinical signs are similar for different affected species, the etiologies vary and can range from abiotic factors to pathogens or combinations of both (Hewson et al., 2018). Such events have become more wide-spread and prevalent in the last decade but are unpredictable. Asteroid wasting

events have been documented for *A. amurensis* both in the native area (China) and in Australia (Hewson et al., 2019) but also for different species in European waters (Thorpe & Spencer, 2000; Staehli et al., 2009).

Qu. 2.5. How likely is the organism to establish despite existing management practices in the risk assessment area? Explain if existing management practices could facilitate establishment.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: While Ballast Water Exchange (BWE) and Ballast Water Treatment (BWT) can reduce propagule pressure and, consequently, the rate of establishment (see Q1.5a for details), these management practices are not always possible or yet in effect. On the other hand, bivalve transportations for aquaculture purposes (which constitute a pathway of spread) offer suitable habitats to *A. amurensis* in the form of the aquaculture plots themselves and, thus, facilitate establishment. Moreover, seed harvesting devices and seed relaying offer favourable substrates and conditions for settlement and growth, enhancing establishment potential. Management practices during aquaculture operations (such as the inspection of stock and removal of predators or the removal of fouling species from seed/spat before relaying to on-grow sites) can reduce establishment potential. On the other hand, management of the natural environment (e.g. MPAs with no-take zones), is likely to limit early detection and removal by stakeholders, affording some protection to early populations.

Qu. 2.6. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Eradication may be attempted at an early stage of invasion by manual removal with divers and/or snorkeling, i.e. targeting juveniles and adults. If the population has not yet reached sexual maturity the chances of local eradication are relatively high (see Millers, 2015; Richardson et al., 2016 and references therein for an overview of known eradication campaigns of *A. amurensis* in Australia). In the case of reproducing populations however, the very high fecundity and long pelagic larval duration of the species (see following question) would provide a high number of propagules able to disperse over long distances and negate eradication efforts. Poorly planned and executed removal

campaigns, involving chopping and fragmentation of adults may have the opposite effect, by increasing the reproducing population (see following question for details).

Size at maturity for *A. amurensis* (i.e. distance from the center of the disc to the arm tip) is reported to vary between 46-55 mm (Ino et al., 1955; Hatanaka and Kosaka 1959; Li et al., 2018) and is generally reached at one year of age. The fact that the species takes a year and grows relatively large before maturity increases the likelihood of detection before a reproductive population develops and can facilitate eradication campaigns (Crombie et al., 2007; Millers, 2015).

Qu. 2.7. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?

including the following elements:

- a list and description of the reproduction mechanisms of the species in relation to the environmental conditions in the risk assessment area
- an indication of the propagule pressure of the species (e.g. number of gametes, seeds, eggs or propagules, number of reproductive cycles per year) of each of those reproduction mechanisms in relation to the environmental conditions in the risk assessment area.
- If relevant, comment on the likelihood of establishment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in establishment whereas for others high propagule pressure (many thousands of individuals) may not.
- If relevant, comment on the adaptability of the organism to facilitate its establishment and if low genetic diversity in the founder population would have an influence on establishment.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Asterias amurensis is a dioecious species (i.e. sexes are separate with the male and female reproductive organs being in different individuals) with external fertilization and an annual reproductive cycle (Novikova, 1978), although damaged individuals can reproduce asexually by the regeneration of arms attached to a portion of the central disc (Ward & Andrew, 1995). One-year old females produce approximately 0.4 – 2.8 million eggs and two-year old 5.3 – 15.5 million (Hatanaka & Kosaka, 1959).

Spawning is reported at different times in different locations, ranging from late January/February to July (e.g. Akkeshi Bay, Hokkaido, Japan) and at temperatures ranging from 5 °C to 23 °C (Li et al., 2018; Hatanaka & Kosaka, 1959 and references therein). Spawning takes place primarily in February-April, at temperatures between 5°C and 17°C (Lee et al., 2004; Yu et al., 1998; Kim, 1968; Hatanaka & Kosaka 1959; Kashenko, 2005), although in Alaska the species spawns in the summer (Smith & Armistead, 2014). In some locations however there are two spawning events; such is the case in China, where spawning takes place in October-November (coinciding well with the lower bottom

water temperature (14.29°C) and again in March-May (Li et al., 2018) and also in Peter the Great Bay, Russia, with spawning events in June-July and in September, at temperatures of 17°C and 23°C, respectively (Novikova, 1978). In the invaded range, *A. amurensis* spawns during the austral winter, at similar degrees of latitude, but in the southern hemisphere, indicating photoperiodic regulation of gametogenesis, as well as modulation by temperature (Smith & Armistead, 2014). The adaptability of the species with respect to spawning periods and temperatures is very likely to facilitate establishment in the RA area, where suitable temperatures for reproduction are encountered throughout much of its extent at different times of the year (see Annex VIII).

Fertilised eggs are demersal and develop into swimming and feeding larvae (termed bipinnariae) within 60h to 120h of fertilization depending on water temperature (20°C and 10°C respectively) (Lee et al., 2004). Larval duration in the plankton also varies with temperature, as well as with feeding conditions, and can range between 50 and 120 days at temperatures between 19°C and 10°C respectively (Bruce et al., 1995 and references therein). In the native range (Korea-Japan), settlement occurs during June-July, while early juveniles appear during August-September (Paik et al., 2005).

Early Tasmanian populations, sampled in 1994, showed a considerable loss of genetic diversity compared to native Russian and Japanese populations – in the order of 30-40% - indicating a small number of initial colonisers and a genetic bottleneck (Ward & Andrew, 1995). Despite the above, the species was a very successful invader in Tasmanian and later on in Victorian waters.

Qu. 2.8. If the organism does not establish, then how likely is it that casual populations will continue to occur?

Consider, for example, a species which cannot reproduce in the risk assessment area, because of unsuitable climatic conditions or host plants, but is present because of recurring introduction, entry and release events. This may also apply for long-living organisms.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Considering that the most likely life stage of *A. amurensis* to enter the RA area is the planktonic larva, with the narrowest abiotic requirements, if the environmental conditions they encounter upon entry are unfavourable for survival, they are unlikely to establish or form casual populations. Casual populations may occur in the case of the arrival of adults/juveniles as hitchhikers on vessels, and this vector is unlikely to transport *A. amurensis* in large numbers or frequently (see Risk of Introduction section). Thus, the occurrence of casual populations is deemed unlikely.

Qu. 2.9. Estimate the overall likelihood of establishment in the risk assessment area under current climatic conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under current climatic conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in current conditions: providing insight in the risk of establishment in (new areas in) the risk assessment area.

RESPONSE		CONFIDENCE	
	very unlikely		low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response: The response is based on combining physiological tolerances and the results from the distribution modeling (see Qu 2.1, Qu 2.9 and Annex VIII & IX for details). The reader is encouraged to consult Annex VIII in particular for a full explanation of the rationale.

Baltic Sea: unlikely, high confidence

Greater North Sea: likely, medium confidence

Celtic Seas: likely, medium confidence

Bay of Biscay and the Iberian coast: likely, medium confidence

Mediterranean Sea: moderately likely, low confidence

Black Sea: unlikely, high confidence (Sea of Marmara: moderately likely, medium confidence)

The likelihood of a successful establishment of *A. amurensis* in the RA area will be largely determined by a combination of source region, arrival time (i.e. time of the year) and abiotic conditions of the recipient region when the first larvae arrive. The survival and settlement of the first introduced larvae is likely to be of high importance for initial establishment, since, once this happens, the species has demonstrated its adaptability to local conditions by changing the seasonality of its reproductive cycle (see the Australian invasion, also spawning periods throughout its native range).

Considering spawning times in the donor regions, travel duration and arrival times (see Annex VIII), the larvae of the species are very likely to find suitable abiotic conditions for establishment during the whole year throughout most of Atlantic Europe, with the exception of the Bay of Biscay, where sea surface temperatures are generally higher than 20°C in the summer months but cooler between October and June. In the Mediterranean Sea however, there are two short time windows for larval development, in April-May and November-December, depending on the donor region of larvae being Japan/Korea or Australia. Propagules arriving to the Mediterranean from China in the winter months are still likely to find suitable temperatures for development and settlement upon release. Thus, the likelihood of establishment in the Mediterranean Sea is considered as moderate. Furthermore, if it is adults rather than larvae that first arrive, temperatures at the seabed are generally lower in the summer months due to stratification and this would favour establishment even in areas with thermally marginal surface waters, as long as there is a sufficient window for spawning and dispersal.

In the Black Sea and the Baltic Sea, establishment of *A. amurensis* will be prevented by low salinities (Black Sea range of 14-19 psu for surface salinity – Baltic Sea <15 psu). The species requires salinities above 22 psu for spawning, fertilization, and embryonic development (i.e. salinity near the seabed) and above 20 psu for complete larval development, i.e. surface salinity (Kashenko, 2005).

Biotic interactions with native species and in particular with the con-generic *A. rubens*, which is very similar ecologically and functionally, have the potential to alter the likelihood of establishment but this is very difficult to predict.

Qu. 2.10. Estimate the overall likelihood of establishment in the risk assessment area under foreseeable climate change conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under foreseeable climate change conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of establishment (e.g. increase in average winter temperature, increase in drought periods)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely establishment within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The response is based on the RCP4.5 scenario for the period 2050/2070. Aspects of climate change most likely to affect future distribution were considered as an increase in minimum and maximum Sea Surface Temperatures (SST). The methodology for the isotherm approach is described in Annex VIII and for the developed models in Annex IX. See also Qu. A7.

- Baltic Sea: unlikely, high confidence
- Greater North Sea: likely, medium confidence
- Celtic Seas: likely, medium confidence
- Bay of Biscay and the Iberian coast: moderately likely, medium confidence
- Mediterranean Sea: unlikely, low confidence
- Black Sea: unlikely, high confidence (Sea of Marmara: moderately likely, medium confidence)

The species distribution model predicted a very small reduction in projected suitability for *A. amurensis* for all EU marine subregions under future climate change and a small northward shift of the overall suitable area for the species. This largely agrees with previous modeling work, carried out by Townhill et al. (2017), which predicted a slight poleward shift in the projected suitability for *A. amurensis* along the continental shelf of Atlantic Europe in the order of 40-50 km. According to the isotherm approach, the warming of the Mediterranean Sea but also the Bay of Biscay and the Iberian coast, is likely to further restrict the potential for establishment of *A. amurensis* in these regions.

3 PROBABILITY OF SPREAD

Important instructions:

- Spread is defined as the expansion of the geographical distribution of an alien species within the risk assessment area.
- Repeated releases at separate locations do not represent continuous spread and should be considered in the probability of introduction and entry section (Qu. 1.7).

Qu. 3.1. How important is the expected spread of this organism within the risk assessment area by natural means? (List and comment on each of the mechanisms for natural spread.)

including the following elements:

- a list and description of the natural spread mechanisms of the species in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of spread discussed in relation to the species biology and the environmental conditions in the risk assessment area.

The description of spread patterns here refers to the CBD pathway category “Unaided (Natural Spread)”. It should include elements of the species life history and behavioural traits able to explain its ability to spread, including: reproduction or growth strategy, dispersal capacity, longevity, dietary requirements, environmental and climatic requirements, specialist or generalist characteristics.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Natural larval dispersal: *Asterias amurensis* is a highly fecund species (up to 19 million eggs per female) with pelagic larvae that can stay in the water column for up to 120 days, depending on the temperature (Bruce et al., 1995 – see also Qu. 2.7). It has the potential to develop dense populations, which increases the fertilization success, since the species is a broadcast spawner, and the ability to reproduce at different times of the year between temperatures of 5°C and 20°C. In Atlantic Europe, the thermal requirements for successful larval development are met throughout most of the year, except for the winter months in the western North Sea and the summer months in the Bay of Biscay (see Annex VIII and Qu. 2.1). This can lead to a significant potential for natural dispersal, depending of course on local/regional hydrodynamic regimes. In the Mediterranean Sea, higher sea surface temperatures will limit both the period with conditions suitable for development (November to May – See Annex VIII), as well as the duration of larval development, which can be as low as 50-60 days at 19°C (Bruce et al., 1995). Even at these temperatures, 2 months of natural dispersal with oceanic currents would be sufficient for a considerable spread.

Rafting: Drifting macroalgae are known to support entire faunal assemblages and transport them over long distances, in the range of 100s of kilometers, with starfish species having been sampled on numerous occasions from such natural rafting objects (Thiel & Gutow, 2005; Fraser et al., 2011).

Besides translocated adults or juveniles, competent larvae of *A. amurensis* could also settle on already drifting macroalgae or other natural objects (Byrne et al., 2016), enhancing the potential for spread through this mechanism. Additionally, the species has the potential for 'ballooning' (filling body cavity with air and using currents to migrate or settle on floating objects) which has led to 'swarming' in Tokyo Bay (Kurata et al., 1954, cited in Nelson et al., 2016) and may enhance the potential for spread via rafting or natural dispersal.

Qu. 3.2a. List and describe relevant pathways of spread other than "unaided". For each pathway answer questions 4.3 to 4.9 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 4.3a, 4.4a, etc. and then 4.3b, 4.4b etc. for the next pathway.

including the following elements:

- a list and description of pathways of spread with an indication of their importance and associated risks (e.g. the likelihood of spread in the risk assessment area, based on these pathways; likelihood of survival, or reproduction, or increase during transport and storage; ability and likelihood of transfer from the pathway to a suitable habitat or host) in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of spread for each pathway discussed in relation to the species biology and the environmental conditions in the risk assessment area.
- All relevant pathways of spread (except "Unaided (Natural Spread)", which is assessed in Qu. 4.1) should be considered. The classification of pathways developed by the Convention of Biological Diversity shall be used (see Annex IV).

Pathway names:

- a) TRANSPORT-STOWAWAY (ship/boat ballast water)
The evidence base in the Australian invaded range suggests that *Asterias amurensis* was introduced to Tasmanian waters from central Japan via ballast water discharge (Ward & Andrew, 1995). Based on a thorough evaluation of alternative pathways (Hayes et al., 2004), ballast water transfer is still considered the most plausible pathway of introduction of this species to Tasmania, as well as its subsequent spread to Victoria, mainland Australia (Dunstan & Bax, 2008). With respect to the RA area, movement of vessels between ports within its boundaries is less restricted and ballast water regulations do not apply to short journeys within states. This means that the potential of ballast waters transport of larvae to act as a vector of spread is very significant.
- b) TRANSPORT-STOWAWAY (Hitchhikers on ship/boat & ship/boat hull fouling)
While it is unlikely that starfish would remain attached to vessels when underway for any length of time, their translocation is possible in sheltered niche areas (Dommissé & Hough, 2004; MAF, 2011). For example, *Asterias amurensis* was observed in the sea-chest of a commercial vessel operating between the Derwent Estuary, Tasmania, and Port Phillip Bay in Australia (Thresher, pers. comm in Dommissé & Hough, 2004). Additionally, *A. amurensis* DNA was found in biofouling samples collected from 3 fishing and recreational vessel hulls, both from internal and external surfaces, offering further evidence that the species can be translocated via vessel fouling (Hayes et al., 2004).
- c) TRANSPORT-CONTAMINANT Contaminant on animals (except parasites, species transported by host/vector)

Asterias amurensis is a notorious pest in shellfish aquaculture, both in the native and the invaded range, and is known to settle on and infest oyster trays, mussel lines and scallop spat collectors, as well as salmon cages, on-shore and offshore abalone facilities and other fish-farming equipment (e.g. Gabaev, 2018; Dommissse & Hough, 2004). Spread via this pathway is not documented for the species in the invaded range, it is considered however that infested consignments of commercial bivalve species intended for aquaculture can transport it unintentionally, as a contaminant, throughout the RA area.

d) **TRANSPORT-STOWAWAY (angling/fishing equipment)**

In both the native and invaded range, *A. amurensis* is caught, often in high quantities, as by-catch of demersal and bivalve fisheries (e.g. Hatanaka & Kosaka, 1959, Aquenal, 2008, Smith & Armistead, 2014), both by bottom towed gear as well as by longlines and hooks by professional and recreational fishermen (Parry et al., 2003). Since the species is not targeted as a commercial catch, it is discarded by the fishermen (but see potential waste disposal solutions in A13 and later in this section). Depending on discard practices and disposal of by-catch, entrainment in fishing/angling equipment poses a high risk of unintentionally translocating the species.

e) **TRANSPORT-STOWAWAY (machinery/equipment & floating debris)**

This pathway includes a diverse array of vectors of different sizes and mobility, such as mobile drilling rigs, energy extraction devices, moorings and buoys, as well as equipment dislodged by storms or damaged structures and debris lost at sea and drifting (e.g. aquaculture equipment, pontoons, pier debris, etc.). Such structures are often heavily fouled with mature fouling communities (Nall, 2015; Astudillo et al., 2009), which can provide both settlement space and abundant food resources for *A. amurensis*. Some of them enter and stay in ports for extended periods of time (e.g. tidal and wave energy devices – Loxton et al., 2017b), or are in frequent contact with offshore support vessels and can thus enhance the connectivity network for propagules of the species.

a) **TRANSPORT-STOWAWAY (ship/boat ballast water)**

Qu. 3.3a. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional	CONFIDENCE	low
	unintentional		medium
			high

Response: It can be stated with high certainty that this pathway is unintentional. There is no doubt that uptake of larvae in ballast water is accidental. See categorization of pathways in Annex IV and guidance notes in the beginning of this section.

Qu. 3.4a. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: For reproductive output and ship ballast volume & potential larval concentration, see Qu. 1.3a. With respect to spread of the organism within the EU, transshipment operations constitute the main maritime traffic that will act as the vector for spread. Important transshipment hubs are situated along the southern Mediterranean (serving the rest of the Mediterranean and the Black Sea) and the Le Havre-Hamburg range, serving the UK, the Baltic and Scandinavia (Notteboom et al., 2013). Considering that *A. amurensis* larvae are favoured by water temperatures encountered in many parts of the RA area, it is considered likely that sufficient numbers can be transferred with ballast water along this pathway. Finally, in many parts of the European Seas, ballast in passenger ferries is also a potential vector mainly due to high traffic volume potential. Due to the routes taken, measures described in the ballast water convention cannot easily be followed (exchange at depth and distance from shore) so the risk of depositing propagules at suitable locations is higher.

Qu. 3.5a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Short Sea shipping routes/trips within EU ports in particular are shorter than international shipping trips from Asia and Australia (where introduction events may originate from), such that survival in ballast water during passage along this pathway is much more likely. Most importantly, sea surface temperatures (which determine ballast water temperature) are generally within the optimal range

for *A. amurensis* larval survival (i.e. 5-20 °C), particularly in Atlantic Europe. In the Mediterranean favourable thermal conditions are restricted to the autumn and winter months (see Annex VIII). This is also the case for ferry routes between many European locations.

Qu. 3.6a. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

The International Maritime Organization (IMO) Ballast Water Management Convention (BWMC) entered into force in September 2017. It requires ships in international traffic to apply ballast water management measures, in particular:

- ballast water exchange in open seas, away from coastal areas (D-1 standard for an interim period)
- fulfil a certain discharge standard (D-2 standard according to the ship specific application schedule phased in up to 8 September 2024). D-2 standard requires the installation of a certified ballast water treatment device, which enables sterilization to avoid transfers of ballast water mediated species.

Ballast Water Exchange (BWE) is currently practiced and requires ships to exchange a minimum of 95% ballast water volume whenever possible at least 200 nautical miles (nm) from the nearest land and in water depths of at least 200 metres. When this is not possible, the BWE shall be conducted at least 50 nm from the nearest land and in waters at least 200 metres in depth (David et al., 2007 and BWMC Guideline 6). Even though BWE can reduce the concentration of live organisms in ballast by 80–95% (Ruiz & Reid 2007), its application has severe limitations, primarily dependant on shipping patterns of a port (e.g., shipping routes, length of voyages) and local specifics i.e., distance from nearest shore, water depth (David et al., 2007), particularly for EU Seas where these conditions are often not met. Also, organisms may still remain in the volume of ballast not exchanged, or BWE may not be possible due to weather conditions or other safety restrictions. The survival of zooplanktonic organisms (including *A. amurensis*) is thus not unlikely when only BWE measure are implemented.

BWE for EU short sea shipping routes is usually restricted to the second criterion of at least 50 nm from the nearest land and in waters at least 200 metres in depth in the Mediterranean Sea and is often not even feasible within these limits in northern European Seas (David et al., 2007), such that ballast water exchange is not likely to be effective in preventing the spread of *A. amurensis* (and other organisms potentially transferred in ballast water) within European Seas.

As a result, ballast water treatment has been deemed necessary, such that ships shall discharge (in relation to the organism size range of interest for *A. amurensis*): less than 10 viable organisms per cubic metre greater than or equal to 50 micrometres in minimum dimension (IMO D-2 standard).

Ballast water treatment options include mechanical (filtration, separation), physical (heat treatment, ozone, UV light) and chemical methods (biocides). Efficiencies of various technologies utilised for ballast water treatment are reviewed in Tsolaki & Diamadopoulos (2010) and can vary with treatment method but the application of many combined methods (e.g. Filtration+UV or Hydroclone+chemical disinfectant) can decrease live zooplankton to undetectable levels, practically diminishing propagule pressure. As such, the survival of *A. amurensis* larvae in ballast water with full implementation of the D-2 standard (i.e. after 2024) is considered unlikely. Until then (i.e. currently), planktonic propagules of the species are likely to survive in ballast water.

It is worth noting that, even after full implementation of D2, exemptions can be granted by member states on ‘low risk’ routes (Stuer-Lauridsen et al., 2018) and it is possible that e.g. internal routes by passenger ferries etc may fall into this. If *A. amurensis* arrives undetected at a port included in this exemption it may have chance to spread to others before detection and interception is possible.

Qu. 3.7a. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: After September 2017, with the BWMC coming into effect and gradually being implemented, detection of larval stages in ballast water during Port State Control inspections may be possible. According to Resolution MEPC.252(67), if initial inspections of ballast water samples indicate non-compliance with the D-2 standard, detailed inspections will be carried out. eDNA methodologies are rapidly becoming one of the fastest and most cost-efficient tools for the detection of NIS⁶ in introduction water samples (Darling & Frederick, 2018; Borrell et al., 2017; Koziol et al., 2019) and have proven suitable for the detection of *A. amurensis* larvae specifically in port water samples as well as water samples from the internal spaces of small vessels (Hayes et al., 2004). However, full implementation of the BWMC is not anticipated until 2024. Until then, the likelihood that *A. amurensis* will enter the RA area undetected in ballast waters remains high. On the other hand, if the species is targeted through a national monitoring programme with molecular methods, larvae in water samples from ports/harbours can be currently detected.

Qu. 3.8a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely	CONFIDENCE	low medium
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⁶ NIS: non-indigenous species, term used in the Marine Strategy Framework Directive, synonym of “alien species” as used in the framework of Regulation (EU) 1143/2014

	moderately likely likely very likely		high
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Response: If ballast water exchange occurs in open seas rather than in coastal areas, transfer of planktonic larvae to suitable substrate will be hampered. If, however, untreated ballast water is released in ports, estuaries or other coastal areas, then establishment will be dependent on availability of suitable habitat. Considering a) the breadth of habitat that characterizes the species; b) the wide distribution of such habitats in the RA area and c) the invasion history of the species in Australia, which is closely connected with ports and estuaries, there is a high likelihood that *A. amurensis* can transfer to a suitable habitat after release with ballast water.

Qu. 3.9a. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area. (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: Given the amount of maritime traffic between ports and harbours of the RA area (thousands of journeys per year), the ballast volume of commercial vessels (10^4 - 10^5 tonnes) and the potentially high densities of both adult *A. amurensis* on the seabed as well as larvae in surface waters, the potential rate of spread of the species via ballast water is considerable but mainly in Atlantic Europe (see also Risk of Establishment). Due to the more limited extent and duration of favourable environmental conditions in other marine regions/subregions of the RA area, the likelihood that dense populations will develop there (and hence the corresponding larval density in the seawater in ports and harbours) is lower.

b) TRANSPORT-STOWAWAY (ship/boat hull fouling)

Qu. 3.3b. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: There is direct evidence that *A. amurensis* can travel in the sea chests of large commercial vessels and that it can settle on external and internal surfaces of small recreational (and professional) craft (Hayes et al., 2004 – see also Qu 1.3b). Moreover, it is possible that larvae may be transported in sea water systems, live wells or other deck basins of recreational craft or other small commercial craft (MPSC, 2015). In these cases, the entrainment of the species on ships/boats hulls and niche areas is accidental.

Qu. 3.4b. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: While the maritime traffic by large commercial vessels as well as smaller professional and recreational craft is very high, only a few incidents of entrainment on hulls have been reported in the literature, and the number of individuals detected was small. For example, *Asterias amurensis* was observed in the sea-chest of a commercial vessel operating between the Derwent Estuary, Tasmania, and Port Phillip Bay in Australia (Thresher, pers. comm in Dommissie & Hough, 2004). Additionally, *A. amurensis* DNA was found in biofouling samples collected from 3 fishing and recreational vessel hulls, both from internal and external surfaces (Hayes et al., 2004). This does not necessarily mean the presence of viable propagules (as discussed in Qu. 1.3b) but is nevertheless an indication that passive or active settlement may be taking place. Considering that travel durations within the RA area are much shorter compared to journeys from the native region, particularly for small craft, it is conceivable that larvae or juveniles sheltering among protected spaces in a fouling community (e.g. barnacle or bivalve shells) can remain entrained and be translocated.

Qu. 3.5b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely	CONFIDENCE	low
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	unlikely moderately likely likely very likely		medium high
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Response: If entrained in areas of the vessel with low food availability, *Asterias* species are capable of surviving prolonged periods of starvation, up to several weeks (St-Pierre & Gagnon, 2015) or even months (Vevers, 1949). If, on the other hand, starfish are transported in sea chests, where heavy fouling can accumulate (i.e. providing food resources, primarily in the form of bivalves), not only survival but also growth and maturation may be possible. For *A. amurensis*, this is attested by the survival and translocation of live individuals on debris from the Tohoku tsunami of 2011 from Japan to the west coast of North America almost a year later (Carlton et al., 2017; 2018). Reproduction would require the presence of at least a pair of adults and would be unlikely to happen if starvation conditions are reached (e.g. depletion of resources in sea chests), when the species would mobilise its energy reserves for survival (Aguera & Byrne, 2018). On the other hand, poorly maintained vessels (largely recreational) are a particular risk if moored for long periods of time and moved between marinas occasionally because they have the potential to harbour reproducing populations in stable, sheltered conditions for suitable periods of time to enable reproduction to occur.

Qu. 3.6b. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Even though there are methods to prevent and minimize fouling on vessels hulls, these management measures are not mandatory in the RA area and can be expensive and time consuming, which may create a barrier to implementation. Thus, they are not carried out at a frequency and prevalence sufficient to prevent the entrainment and translocation of species like *A. amurensis* via ships hulls and niche areas (see also Qu 1.5b).

Qu. 3.7b. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species is unlikely to be detected upon introduction, unless thorough inspections of hull fouling communities are carried out, which is currently not a routine practice. Even then, because the species can also settle on internal surfaces of vessels (Hayes et al., 2004), the likelihood of detection via visual inspections remains low.

In order to reach GES targets with reference to Descriptor D2, most EU states are already designing or implementing national/regional NIS-targeted monitoring programmes. Monitoring should focus on introduction hotspots (e.g. ports, marinas, aquaculture plots) and this will increase the detectability of *A. amurensis* entering the RA area through hull fouling, particularly if molecular methods are employed (Hayes et al., 2004).

Qu. 3.8b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Entry into the environment would require dislodgment of fouling material or active movement by the adults/juveniles. Alternatively, propagules could enter the environment in the form of gametes by at least a pair of *A. amurensis*, an event which is not considered very likely. In any case, ports and harbours provide abundant suitable habitats for colonization for *A. amurensis*, which a) is a habitat generalist and b) develops high densities on wharves and piers, due to their high food availability (Aguenal, 2008).

Qu. 3.9b. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area. (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: Despite direct evidence that *A. amurensis* can settle on external and internal surfaces of vessels and can be transported via this pathway, the translocation risk is likely to be smaller compared to that associated with the ballast water of large commercial vessels (Hayes et al., 2004), primarily due to the smaller number of individuals potentially transported via hull fouling at one time. Additionally, the environmental limitations for establishment that apply in the different marine subregions of the RA

area (see Annex VIII) will also make entrainment and transport more likely in the North-East Atlantic (as in 3.9a).

c) **TRANSPORT-CONTAMINANT Contaminant on animals** (except parasites, species transported by host/vector)

Qu. 3.3c. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: It can be stated with high certainty that this pathway is unintentional. See categorization of pathways in Annex IV and guidance notes in the beginning of this section.

Spread via this pathway is not documented for the species in the invaded range, it is considered however that infested consignments of commercial bivalve species intended for aquaculture can transport it unintentionally, as a contaminant, throughout the RA area.

Qu. 3.4c. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Asterias amurensis*, like many of its congenics, is a notorious pest of shellfish aquaculture both in its native and the invaded range. It is known to infest and predate on scallops, oysters, mussels, clams and abalone (see Qu. 4.9 for details and references) and could easily spread within the RA area with routine bivalve transfer operations. All countries along the European Atlantic and the

Mediterranean coasts involved in the cultivation of bivalves are currently conducting transfer activities (Muehlbauer et al., 2014; Occhipinti-Ambrogi et al., 2016; Marchini et al., 2014; Rodrigues et al., 2015). These activities include transfers at all life stages, from field sites to wild fishery sites or from field to culture sites, from nearshore to onshore facilities or from nearshore wild bottom beds to offshore hanging cultivation devices (Muehlbauer et al., 2014).

Asterias amurensis can achieve very high densities when food resources are abundant, from 300 ind/m² during outbreaks in China (Li et al., 2018) to a more modest but still considerable 6 ind/m² in Japan (Nojima et al. 1986) and 7 ind/m² around shellfish beds and aquaculture facilities in Australia (MPSC, 2015). It also settles on spat collecting devices (see Qu 4.9 for details), which poses an even higher risk of spread, due to the high volume of spat transfers and the small size of the starfish at this stage.

Additionally, *A. amurensis* can reproduce asexually by regeneration of its arms, as long as part of the central disc is attached (see Qu. 2.7), thus, even a very small number of adult individuals could potentially lead to successful spread via this pathway if e.g. damaged during dredging operations to harvest the transferred material.

An additional means of transport in the invaded range was considered to be salmon cages, which can be heavily fouled with bivalves and act as settlement surfaces for starfish (Dommissé & Hough, 2004). Nevertheless, in a survey of aquaculture operators, there were no *A. amurensis* (or any native starfish species) reported from salmon cages and it was thought that application of anti-fouling on fish cages reduces the attachment of bivalves and hence settling starfish (Dommissé & Hough, 2004).

Qu. 3.5c. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely	CONFIDENCE	low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response: *Asterias amurensis* is a broadcast spawner so it will not reproduce during transport, neither will it increase within the small duration of such operations (within the RA area). It is very likely to survive though, as the shellfish themselves and the methods used to contain them during transport may actually enhance the likelihood of survival of contaminant species by providing moisture and protection from harsher conditions (Minchin, 2007).

Qu. 3.6c. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely	CONFIDENCE	low
	unlikely		medium
	moderately likely		high

	likely very likely		
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Response: Management practices relevant to this pathway fall into two categories.

The first one is legislation regarding aquaculture transfers. At the EU level, COUNCIL REGULATION (EC) No 708/2007 concerning use of alien and locally absent species in aquaculture defines the procedures to be followed that minimise the risk of introducing non-target alien species accompanying commercial shellfish spat and stocks. It requires a permit procedure, involving risk assessment for the non-target species and a quarantine period for the translocated stock. Importantly, in relation to spread within the RA area, the regulation does not apply to movements of locally absent species within the Member States (i.e. in this case cultivated native species of bivalves) “except for cases where, on the basis of scientific advice, there are grounds for foreseeing environmental threats due to the translocation, Art. 2 para. 2.” Additionally, the bivalves *M. gigas* and *R. philippinarum*, listed in Annex IV, which are common prey species for *A. amurensis*, constitute exceptions and can be moved without any risk assessment or quarantine. However local/national legislation exists that can limit the translocation possibilities of species like *M. gigas*, e.g. see WG-AS & Gittenberger (2018) for the trilateral Wadden Sea area. Moreover, if the import region is a Natura2000 area, regulations can be much stricter as they aim to protect the conservation objectives of the protected area first. In general, restrictions on transfers based on the risk associated with the source areas is an effective management method, as long as extensive and up-to-date data on the distribution of the high-risk NIS are available.

The second category of measures are standard industry practices to minimize predator infestations, in this case commonly employed in the RA area to reduce predation by the native starfish *A. rubens*. There is a variety of methods, depending on the cultivated bivalve species and type of cultivation (bottom or suspended culture) that include manual removal (e.g. handpicking from suspended lines), immersion in chemical solutions or freshwater, removal by traps or specialized towed gear (see McEnnulty et al., 2001 and Barkhouse et al., 2007). The efficiency of these methods is variable but would allow opportunities for spread.

Qu. 3.7c. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: If the species transfers at the juvenile stage, recently settled on bivalve spat, it is likely to go undetected due to its small size (<10mm ray length, Davenport & McLoughlin, 1993). Perfunctory visual inspections during bivalve aquaculture operations are likely to miss small size individuals. Moreover, juvenile/young *A. amurensis* could easily be misidentified for the native *A. rubens*, which is also a common shellfish culture pest and frequently encountered during transfer operations. See Qu. A2 for a comparison between *A. rubens* and *A. amurensis*.

Qu. 3.8c. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: If *A. amurensis* infested bivalve seed/stock is relayed on cultivation plots without any prior management measure, the likelihood of transfer to other suitable habitats (the cultivation plots themselves are suitable habitats) is very high. These plots are often situated in coastal areas in close proximity to suitable natural habitat to which individuals may spread. The high mobility of the adults (Miyoshi et al., 2018) augments this mechanism of spread.

Qu. 3.9c. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area. (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: Bivalve transfers are a likely mechanism of entraining and translocating *A. amurensis* in the RA area, particularly considering that suitable environmental conditions for the establishment of new populations are widespread in areas where a high volume of transfer operations takes place (i.e. throughout shallow coastal areas of Atlantic Europe, as well as the northern Adriatic, northern Aegean and the western Mediterranean).

Taking into account the degree of regulation and management in the industry and the fact that in many cases transfers are predominantly conducted within Member States, spread to distant locations through this pathway may be less important than spread through ship-mediated pathways.

d) TRANSPORT-STOWAWAY (fishing/angling equipment)

Qu. 3.3d. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: In both the native and invaded range, *A. amurensis* is caught, often in high quantities, as by-catch of demersal and bivalve fisheries (e.g. Hatanaka & Kosaka, 1959, Aquenal, 2008, Smith & Armistead, 2014), both by bottom towed gear as well as by longlines and hooks by professional and recreational fishermen (Parry et al., 2003). Since the species is not targeted as a commercial catch, it is discarded by the fishermen (but see potential waste disposal solutions in A13 and later in this section). Depending on discard practices and disposal of by-catch, entrapment in fishing/angling equipment poses a high risk of unintentionally translocating the species. Additionally, starfish may be entrained in wet wells or damp crevices on the deck (Kinloch et al., 2003).

Qu. 3.4d. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: This will depend on the densities the species has achieved where fishing operations take place, the type of fishing gear used and the discard practices. Gear that is submerged for a long time, comes into contact with the seabed and is unselective (gill and trawl nets, dredges, traps and pots) is ranked the most likely to entrain and spread marine pests and particularly *A. amurensis* (Hayes 2002; Kinloch et al., 2003). Considering that *A. amurensis* can dominate the by-catch of demersal fisheries (see Qu 4.9 for details) and how widespread these operations are in the RA area, there is a high risk that a sufficient number of individuals to establish a new population can spread via this pathway, especially if the species is discarded at locations far away from the point of capture. Since this is a continuously active pathway of spread, reinvasion after eradication is also possible, unless preventative measures are taken.

Qu. 3.5d. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Asterias* species generally display relatively high survival rates when discarded, unless heavily damaged during fishing operations (e.g. for *A. rubens* see Bergmann & Moore, 2001; Ramsay et al., 2001; Revill, 2012 for review). If it remains entangled in wet fishing equipment or damp crevices on the deck, it also has high chances of survival until the fishing gear is submerged again or the vessel returns to harbour. Furthermore, even damaged individuals can regenerate and have the capability to reproduce after translocation, even though they cannot reproduce along the pathway, being broadcast spawners. Some fishing practices fragment individuals and this will increase the number of viable propagules.

Qu. 3.6d. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: There are currently no management practices addressing starfish by-catch during fishing operations and their discarding at sea. Handling of by-catch can be variable and depends on fishing gear and practices, such that survival of the species is generally considered likely during fishing operations. The Common Fisheries Policy (CFP) of 2013, which aims at gradually eliminating the practice of discarding through the introduction of the landing obligation by 2019, only applies to regulated commercial species.

Qu. 3.7d. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: If *A. amurensis* is caught together with native species in high quantities, as is often the case during fishing operations, it would be easy to miss on the deck, as by-catch is not given much attention. Additionally, juveniles and even adults may be overlooked or mistaken as “unusual looking” native *A. rubens* by untrained individuals (see Qu. 3.7c).

Qu. 3.8d. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely	CONFIDENCE	low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response: This will depend again on discard practices, fishing operations (e.g. if the gear is used multiple times during the same fishing trip before being cleaned and stored) and where the species has been entrained. If discarded at sea, it is very likely that individuals will survive and be released over suitable depths and substrates.

Qu. 3.9d. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area. (please provide quantitative data where possible).

RESPONSE	very slowly	CONFIDENCE	low
	slowly		medium
	moderately		high
	rapidly		
	very rapidly		

Response: Fishing/angling equipment is considered a high risk vector for the translocation and spread of *A. amurensis*, mainly due to the high numbers of individuals that can potentially be entrained and survive transport but also due to the fact that fishing operations are so widespread throughout the RA area. Nevertheless, the geographic spread of the species via this route would be relatively limited, hence a moderately rapid rate of spread may be expected.

e) TRANSPORT-STOWAWAY (machinery/equipment & floating debris)

Qu. 3.3e. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: It can be stated with high certainty that this pathway is unintentional. See categorization of pathways in Annex IV and guidance notes in the beginning of this section.

This pathway includes a diverse array of vectors of different sizes and mobility, such as mobile drilling rigs, energy extraction devices, moorings and buoys, as well as equipment dislodged by storms or damaged structures and debris lost at sea and drifting (e.g. aquaculture equipment, pontoons, pier debris, etc.). Such structures are often heavily fouled with mature fouling communities (Nall, 2015; Astudillo et al., 2009), which provide both settlement space and abundant food resources for *A. amurensis*. Some of them enter and stay in ports for extended periods of time (e.g. tidal and wave energy devices – Loxton et al., 2017b), or are in frequent contact with offshore support vessels and can thus enhance the connectivity network for propagules of the species.

Qu. 3.4e. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: This is difficult to predict as it will depend on the density of nearby populations, the larval density and hydrodynamic patterns in the vicinity of the different structures, the biofouling communities that develop on them and their frequency of movement between locations. As corroborating information, it is worth noting that the native starfish *A. rubens* is frequent and abundant in the subtidal zone of both fixed windmill pilings (de Mesel et al., 2015), floating, mobile wave energy devices (Nall, 2015), as well as navigational buoys (Orton & Fraser, 1930). Thus, even though

it is possible that significant numbers of *A. amurensis* may be found on mobile structures (should the species invade the RA area), particularly juveniles and young individuals, it is less likely that extensive spread will occur during the course of one year.

As far as smaller, dislodged equipment and debris are concerned, these may be the carriers of denser aggregations of *A. amurensis*. It is well documented that the starfish heavily settles and fouls e.g. aquaculture cages and piers (see previous sections for details), that are often damaged due to poor maintenance or extreme weather and lost at sea. This may increase spread sporadically and unpredictably over longer distances as this lighter material can drift for considerable distances depending on local hydrodynamic conditions.

Qu. 3.5e. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: If *A. amurensis* find sufficient food resources on the submerged part of these structures (which is often the case, due to extensive fouling), they are very likely to survive, grow and even reproduce, if they remain undisturbed and attached for long enough (maturation in *A. amurensis* occurs at approximately 1 year – see Qu. 2.7), especially under the favourable environmental conditions of Atlantic Europe. Moreover, when such equipment/machinery is transferred between locations it is usually towed at low speeds (e.g. typically around 2 knots for a mobile drilling rig – Kinloch et al., 2003), which reduces the likelihood of dislodgement of the starfish.

Moreover, *A. amurensis* as part of fouling communities on floating debris also has high chances of survival. The most characteristic and extreme example is that of the translocation of live individuals on pier debris from the Tohoku tsunami of 2011 from Japan to the west coast of North America almost a year later (Carlton et al., 2017; 2018).

Qu. 3.6e. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Floating/mobile marine devices have different challenges to ships/boats when it comes to fouling. Because this category covers a variety of objects with different purposes and operational characteristics, there are no overall standards for anti-fouling, like in the shipping sector. Nevertheless, anti-fouling coatings/paints can be used on moorings, buoys and sensors, and mechanical removal of

fouling communities is part of the maintenance procedure for marine renewable energy (MRE) devices (Loxton et al., 2017a; Venkatesan et al., 2017). With anti-fouling measures, the same limitations apply as for vessels, paints are effective for a period of 1.5-2 years, after which heavy fouling can start developing. As such, they would not affect the survival of *A. amurensis* on objects remaining at sea for long periods. In the case of mechanical removal (e.g. in-water cleaning), survival during spread would depend on the method employed, the disposal of the generated waste and whether cleaning takes place before a device is moved to another location.

Qu. 3.7c. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: This will depend on the level of inspection of the fouling communities during maintenance or before transportation of the machinery/equipment. Considering that taxonomic characterization of the attached biota is not a priority in these cases and given the similarity of *A. amurensis* with the native common starfish *A. rubens*, the likelihood that the introduced species will remain undetected seems high. See also Qu. 1.6b, 1.6c, 3.7c.

With regards to floating debris, all natural sink areas can receive floating debris (Rech et al., 2016) such that rafting *A. amurensis* could potentially get stranded all along the risk assessment area, lowering the chances of detection away from monitoring hotspots.

Qu. 3.8e. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Asterias amurensis* utilizes a wide range of habitats in relatively shallow coastal areas (see Qu. 2.2 for details), where machinery/equipment is most commonly deployed. Moreover, ports and harbours, where such equipment is also transferred and stored, provide additional artificial habitats, highly suitable for colonization due to the high food availability they present. For example, in the Derwent river estuary, Tasmania, *A. amurensis* near wharf structures were present in higher densities and had larger gonads compared to *Asterias* occurring on natural substrates (Ling, 2000; Aquenal, 2008). It is also important to note that many of the structures in question are anchored or connected by other means to the seabed, providing a bridge or direct physical link over which developed starfish can

move between the structure and the seabed to forage or search for suitable habitat and conspecifics. Finally, spawning of individuals living as part of these fouling communities would release planktonic propagules to the water column at locations with conditions suitable for development.

Asterias amurensis attached to floating debris is also likely to transfer to suitable habitats as the drifting material approaches the shore; moreover, its wide depth range and relatively high adult mobility can potentially expand the spatial scale of suitable habitats around rafting communities.

Qu. 3.9e. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area. (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: Mobile machinery and equipment has a high likelihood to act as a settlement surface for the larvae of *A. amurensis*, as well as adult individuals if it comes in contact with the seabed or port/harbour structures that can host high densities of the species. The rate of spread however is not expected to be high, as these objects generally spend long periods at sea and are moved only infrequently.

On the other hand, a moderate rate of spread may be expected for *A. amurensis* rafting on dislodged and drifting objects, such as aquaculture gear and damaged structures/pieces, given the affinity of the species for such objects. Increased storminess resulting from climate change has the potential to increase this risk even further.

Qu. 3.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?

RESPONSE	very easy easy with some difficulty difficult very difficult	CONFIDENCE	low medium high
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Response: Naturally dispersing organisms are very difficult to contain, especially species such as *A. amurensis*, with high fecundity, long pelagic larval duration and the capability to develop extremely dense populations in a very short time (See Qu. 2.7 for the biological characteristics of the species and A5 for its invasion history in Australia). This was exemplified in Australia, where despite removal

efforts, the species spread from Tasmania to Port Phillip Bay in Victoria and from there to adjacent bays within a relatively short period of time (Millers, 2015; MPSC, 2015)

Qu. 3.11. Estimate the overall potential rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (indicate any key issues and provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in current conditions, providing insight in the risk of spread into (new areas in) the risk assessment area.

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: Unaided dispersal and multiple pathways of human-aided spread create a considerable potential for spread of *A. amurensis* in the north-east Atlantic. Mature females are highly fecund, producing between 0.5 and 15.5 million eggs per year (see Qu. 2.7 for details). The species has long-lived planktonic larvae, whose physiological requirements are met during prolonged periods in this region; additionally, it can spread rapidly via ballast water and can also be translocated with hull fouling, bivalve consignments, and fishing equipment, although at a more moderate rate with the latter pathways. In the Mediterranean Sea, if the species is introduced and establishes, its distribution is likely to be more disjunct in the cooler, northern basins of this marine region, due to less favourable environmental conditions for larval development. Natural dispersal is expected to be more limited and human-aided spread will likely proceed at a more moderate rate due to the smaller number of potential nodes.

Qu. 3.12. Estimate the overall potential rate of spread in relevant biogeographical regions in foreseeable climate change conditions (provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if rates of spread are likely slowed down or accelerated.

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: The rate of spread is not expected to change significantly in Atlantic Europe under foreseeable climate change conditions, therefore a rapid rate of spread may still be expected.

Higher frequency and severity of storms can increase the amount of time vessels have to spend in port, increasing the likelihood of entrainment (Galil et al., 2019); it may also increase the likelihood that equipment with mature fouling communities (e.g. buoys, cages) can be detached from docks, marinas and aquaculture facilities.

In the Mediterranean Sea, an overall increase in sea surface temperatures will limit even more the potential for natural dispersal. At the same time, heat waves, can cause mass mortality of aquaculture bivalves, leading to increased shellfish transfers to replete the stocks (Rodrigues et al., 2015). More shellfish movements may be associated with a higher risk of introduction if the stocks/seed originate from areas with a high risk of contamination with *A. amurensis* and the necessary precautions are not taken.

4 MAGNITUDE OF IMPACT

Important instructions:

- Questions 5.1-5.5 relate to biodiversity and ecosystem impacts, 5.6-5.8 to impacts on ecosystem services, 5.9-5.13 to economic impact, 5.14-5.15 to social and human health impact, and 5.16-5.18 to other impacts. These impacts can be interlinked, for example, a disease may cause impacts on biodiversity and/or ecosystem functioning that leads to impacts on ecosystem services and finally economic impacts. In such cases the assessor should try to note the different impacts where most appropriate, cross-referencing between questions when needed.
- Each set of questions starts with the impact elsewhere in the world, then considers impacts in the risk assessment area (=EU excluding outermost regions) separating known impacts to date (i.e. past and current impacts) from potential future impacts (including foreseeable climate change).
- Only negative impacts are considered in this section (socio-economic benefits are considered in Qu. A.7)
- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

Biodiversity and ecosystem impacts

Qu. 4.1. How important is the impact of the organism on biodiversity at all levels of organisation caused by the organism in its non-native range excluding the risk assessment area?

including the following elements:

- Biodiversity means the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems
- impacted chemical, physical or structural characteristics and functioning of ecosystems

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

NOTE: Some information is given for the functional role of the organism in its native range as a point of reference for impacts in the invaded range. It is acknowledged that these effects are not directly transferable to the invaded range and they are not taken into account when assessing the impact score for this question.

Asterias amurensis is an opportunistic generalist predator with a preference for bivalves (Lockhart & Ritz, 2001). It feeds on a variety of infaunal and epifaunal species including mollusks, ascidians,

bryozoans, sponges, crustaceans, polychaetes, fish and echinoderms (Hatanaka & Kosaka, 1959; Fukuyama and Oliver, 1985), as well as scavenging on carrion (Smith & Armistead, 2014 and references therein). In common with other *Asterias* species, *A. amurensis* in its native range undergoes periodic population density fluctuations (Gabaev, 2018) or “outbreak-like” population increases (Uthicke et al., 2009). Outbreak events are associated with heavy predation effects on wild and cultured bivalve populations (see Qu. 4.9)

They also compete for food with native (commercial) flatfish species (main food items are small crustaceans, lamellibranchs and small fish) Hatanaka & Kosaka (1959).

In Australia, *Asterias amurensis* reached densities up to 24 individuals/m² over a relatively short time (10 yrs) after its introduction (Byrne et al. 1997a, Grannum et al. 1996). These are densities far higher than those reported during outbreak events in its native range (e.g. 6 individuals/m² during an outbreak in Japan – Nojima et al., 1986). The starfish also has the ability to maintain high densities when food availability is high (MPSC, 2015). It has thus become the dominant benthic invertebrate predator in the two main invaded areas (Port Phillip Bay, Victoria and Derwent Estuary, Tasmania) with massive populations (See also Qu. A5, Qu. 1.3a for quantitative information).

The species was demonstrated to dramatically reduce the recruitment of native bivalves. In a manipulative experiment in Tasmania, densities of juveniles of the bivalve *Fulvia tenuicostata* were reduced by ca. 15 fold (from 580 to 35/m²) in the presence of sea stars at background densities relative to the treatment without sea stars, effectively arresting a massive settlement event (Ross et al., 2002). Predation by the starfish was also responsible for drastic population declines of adult *F. tenuicostata*, as well as another bivalve species *Katelysia rhytiphora* (Ross et al., 2004).

Asterias amurensis has been implicated in the dangerous decline in the 1980s of populations of the Tasmanian endemic spotted handfish, *Brachionichthys hirsutus* (Barrett et al., 1996, Ross et al., 1999), which was subsequently considered critically endangered. The mechanism was hypothesized to involve predation by *A. amurensis* on stalked ascidians, which form the primary spawning substrate of *B. hirsutus*, and subsequent reduction of key habitat availability (Bruce & Green, 1998; Green et al. 2012), although direct evidence of this is lacking (Green et al., 2012). Spotted handfish had already suffered historical habitat degradation due to scallop dredging, mooring disturbances and coastal urbanization (Wong et al., 2018). It is a species strictly restricted to the Derwent estuary, with low dispersal capability due to the lack of a planktonic larval stage, and genetic isolation between local populations. The species is vulnerable to stochastic processes that can lead to serial local extinctions such that continued conservation action (i.e. deployment of artificial spawning habitats and captive breeding program) is considered essential for its survival. (Stuart-Smith et al., 2020). In this context, the cumulative effect of *A. amurensis* on its habitat and subsequent survival can be considered a major impact.

Asterias amurensis has also been suggested to cause large changes to fish populations through competition for food (Parry & Hirst, 2016). During its peak abundance in Port Phillip Bay, *A. amurensis* biomass was equivalent to 56% of total fish biomass in the deep region of the bay, where sharp declines of 3 demersal fish species were observed. These species have a strong dietary overlap with *A. amurensis*, with bivalves and polychaetes being their preferred prey items (Parry & Hirst, 2016).

Qu. 4.2. How important is the current known impact of the organism on biodiversity at all levels of organisation (e.g. decline in native species, changes in native species communities, hybridisation) in the risk assessment area (include any past impact in your response)?

Discuss impacts that are currently occurring or are likely occurring or have occurred in the past in the risk assessment area. Where there is no direct evidence of impact in the risk assessment area (for example no studies have been conducted), evidence from outside of the risk assessment area can be used to infer impacts within the risk assessment area.

RESPONSE	N/A	CONFIDENCE	high
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Response: There is no evidence to confirm that the species is currently present in the RA area.

Qu. 4.3. How important is the potential future impact of the organism on biodiversity at all levels of organisation likely to be in the risk assessment area?

See comment above. The potential future impact shall be assessed only for the risk assessment area.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: In the risk assessment area, the functional and ecological role that *A. amurensis* plays in benthic communities is fulfilled by the native, con-specific and closely related *A. rubens*. This is the case in Atlantic Europe, where *A. amurensis* is more likely to be introduced, establish and spread. Like *A. amurensis* in the north-west Pacific, *A. rubens* is also a keystone benthic predator (Casties et al., 2015), that undergoes periodic population outbreaks (Uthicke et al., 2009), during which the species can develop extensive aggregations with high densities (“feeding fronts”) and decimate bivalve beds (Hancock, 1955; Gallagher et al., 2008). Thus, the potential biodiversity impacts of *A. amurensis* will depend to a large extent on its interaction with *A. rubens* and the density/distribution it achieves in relation to its sister species. The literature suggests that asteroid species can often co-exist, either sharing or partitioning prey resources (e.g. in terms of prey size) or by partitioning the habitat (for an extensive discussion see Qu. 2.3). *Asterias rubens* in particular has been shown to only infrequently compete with *A. forbesi* in parts of their range in the north-west Atlantic (Menge, 1979), where it is suggested that their dynamics are controlled by environmental stochasticity and prey patchiness. A higher tolerance of *A. rubens* larvae to slightly lower temperatures (down to 2 °C - Benitez Villalobos et al. 2006) and salinities (locally as low as 14-15 psu - Casties et al., 2015) may give it a competitive advantage at higher latitudes. While difficult to predict, it is hypothesised that, as long as there is sufficient prey availability and favourable conditions for successful recruitment, biotic interactions with *A. rubens* will not significantly affect the potential of *A. amurensis* to cause population declines of native species (mainly bivalves) in the RA area, such that moderate impacts may be expected, similar to elsewhere in the invaded range (Qu. 4.1). On the other hand, impacts of *A. rubens*

population outbreaks on native species may be exacerbated by simultaneous or staggered outbreaks of *A. amurensis* and this may limit system recovery from natural events.

With regards to hybridisation, within the genus *Asterias*, hybridization has been documented between *A. rubens* and *A. forbesi* in the laboratory, producing viable, fertile adults (Harper & Hart, 2005) but is limited in wild populations of the north-west Atlantic (Gulf of Maine to Nova Scotia), where the two species are sympatric (Harper & Hart, 2007). Considering the close phylogenetic relationships between all three species (Wares, 2001), hybridization between *A. rubens* and *A. amurensis* may be possible.

Qu. 4.4. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism currently in the risk assessment area?

including the following elements:

- native species impacted, including red list species, endemic species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	N/A	CONFIDENCE	high
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Response: There is no evidence to confirm that the species is currently present in the RA area.

Qu. 4.5. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.4.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: A list of habitats of conservation concern that may be threatened by *A. amurensis* through predation is as follows:

Mussel beds on infralittoral rock are part of the wider Reef NATURA-1170 habitat type (Annex I of the Habitats Directive). The habitat is also part of the sublittoral rocky seabeds and kelp forests (code 11.24), listed as endangered in the Resolution no. 4 of the Council of Bern Convention (1996) (Salomidi et al., 2012). They are under severe threat from *A. amurensis* which can obliterate mussel bed populations (Gallagher et al., 2008).

Mussel beds on circalittoral rock (EUNIS A4.24).

Sublittoral mussel beds on sediment (EUNIS A5.62). Within the Habitats Directive, this biotope can be protected as Reefs (habitat type 1170).

Modiolus modiolus (horse mussel) beds/reefs are considered a type of Annex I biogenic reef habitat, an OSPAR listed habitat and a Habitat of Principal Importance across the UK (Morris, 2015). They significantly modify the underlying habitat and provide substratum and refuge for a wide variety of species, including commercially important bivalves (Baxter et al., 2011). Despite their sparse and patchy distribution and the somewhat intermittent recruitment, dense reefs are generally thought to be very stable in the long term (Holt et al., 1998; Rees, 2009). They are subject to predation by *A. rubens* (Tillin & Tyler-Walters, 2015) and could come under threat by *A. amurensis* as well. Predation by starfish (and crabs) is thought to be particularly important for the survival of juveniles, however it is suspected that survival is greatly enhanced by juveniles living within the mass of adult's byssus threads where predators cannot easily get them (Holt et al., 1998; Rees, 2009). Thus, irreversible impacts by *A. amurensis* are considered unlikely.

Sabellaria spp. reefs are listed as protected habitats under Annex I of the Habitats Directive and as a threatened and/or declining priority habitat by OSPAR (OSPAR, 2008). They provide topographically complex structures with varied microhabitats which can support high levels of biodiversity (NRW, 2019). Native starfish *A. rubens* are commonly found preying on these habitats but due to the lack of knowledge on prey-predator interactions with the reef building worms and the associated fauna, the level of risk cannot be assessed (Gibb et al., 2014). *Asterias amurensis* is known to feed on tube-building polychaetes (e.g. *Lagis koreni*, in Smith & Armistead, 2014) such that it could constitute a threat to *Sabellaria* reefs as well, however there is large uncertainty associated with this assessment. Nevertheless, the starfish has the potential to impoverish the diversity of these reef habitats by preying on the erect epifauna they support.

Ostrea edulis beds on shallow sublittoral muddy mixed sediment are part of the wider Reef NATURA-1170 habitat type (Annex I of the Habitats Directive). They are included in the European Red List of Habitats as Critically Endangered (EC 2016). These habitats provide important ecosystem functions, as described in Qu 4.7 and 4.9 (namely high biodiversity and productivity, nutrient cycling and benthic-pelagic coupling, increased water quality, etc.), which are under direct threat by *A. amurensis* predation. In the Mediterranean Sea, both mussel beds (*M. galloprovincialis*) and native oyster beds (*Ostrea edulis*) are included in the European Red List of Habitats as Vulnerable (EC 2016). Infralittoral mussel beds are of conservation concern (Near Threatened to Critically Endangered) across the regional seas. Nevertheless, *A. amurensis* is not expected to develop very high densities on a wide scale throughout the Mediterranean, such that habitats of conservation concern are not considered under very serious risk.

Loss of both infaunal and epifaunal bivalve populations has the potential to affect the overwintering success of protected diving seabirds such as common eider (*Somateria mollissima*) and common scoter (*Melanitta nigra*), which depend upon these prey resources for their survival (Bräger et al., 1995).

Ecosystem Services impacts

Qu. 4.6. How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?

- For a list of services use the CICES classification V5.1 provided in Annex V.
- Impacts on ecosystem services build on the observed impacts on biodiversity (habitat, species, genetic, functional) but focus exclusively on reflecting these changes in relation to their links with socio-economic well-being.
- Quantitative data should be provided whenever available and references duly reported.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Provisioning services: Biomass – Reared aquatic animals & Wild animals: *Asterias amurensis* affects ecosystem services primarily through its negative impacts on wild and cultivated bivalve populations, as well as on commercial fisheries. Loss of food provisioning services could constitute a major impact but at the local scale, since the species heavily affects many different cultured species, including scallops, oysters and mussels, wild harvested species of clams and cockles but also interferes with trawl and long-line fisheries as by-catch (see Qu 4.1 & 4.9).

Regulation & maintenance services: Regulation of physical, chemical, biological conditions: Additionally, it is thought likely that it affects other ecological processes, such as nutrient cycling, by heavily reducing infaunal populations (Joint SCC/SCFA, 1999), and regulating and maintenance services through water quality regulation (bivalve suspension feeders remove particulate organic matter, reduce nitrogen load and improve water quality (Salomidi et al., 2012).

Cultural services: Direct, in-situ and outdoor interactions – Physical and experiential: Finally, it may be affecting the amenity and recreational value of the infested areas by reducing recreational harvests (MPSC, 2015). However, such impacts have not been quantified or assessed in the invaded range.

Qu. 4.7. How important is the impact of the organism on provisioning, regulating, and cultural services currently in the different biogeographic regions or marine sub-regions where the species has established in the risk assessment area (include any past impact in your response)?

- See guidance to Qu. 4.6.

RESPONSE	N/A	CONFIDENCE	
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Response: There is no evidence to confirm that the species is currently present in the RA area.

Qu. 4.8. How important is the impact of the organism on provisioning, regulating, and cultural services likely to be in the different biogeographic regions or marine sub-regions where the species can establish in the risk assessment area in the future?

- See guidance to Qu. 4.6.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: The establishment and development of dense population of *A. amurensis* in the RA area has the potential to severely impact food provisioning and regulating services through predation, as described in Qu. 4.6.

Provisioning services: Biomass – Reared aquatic animals & Wild animals: Mussel, oyster beds and reefs, cockle and clam beds are present throughout the regional seas (with similar structure and function albeit different species and community composition) and support both commercial and recreational harvests.

Regulation & maintenance services: Regulation of physical, chemical, biological conditions & lifecycle maintenance: Filter feeding bivalves and the biogenic structures they create offer valuable regulating and maintenance services in three ways. 1) Through water quality regulation (bivalve suspension feeders remove particulate organic matter, reduce nitrogen load and improve water quality (Salomidi et al., 2012), 2) through water flow regulation and coastal protection - three-dimensional structures dissipate wave energy (Boström et al., 2011; Scyphers et al., 2011) - and 3) through lifecycle maintenance processes by providing important feeding and nursery habitats (references in Scyphers et al., 2011 for oysters, for mussels see Salomidi et al., 2012; Gundersen et al., 2017).

Predation on these organisms will impact on these ecosystem services.

Cultural services: Direct, in-situ and outdoor interactions – Physical and experiential: Cultural services in the form of recreation, tourism and the degradation of important/symbolic habitats may also be impacted (Katsanevakis et al., 2014; Gundersen et al., 2017).

Economic impacts

Qu. 4.9. How great is the overall economic cost caused by the organism within its current area of distribution (excluding the risk assessment area), including both costs of / loss due to damage and the cost of current management.

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the world these should be reported here. The assessment of the potential costs of / loss due to damage shall describe those costs quantitatively and/or qualitatively depending on what information is available. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage. As far as possible, it would be useful to separate costs of / loss due to the organism from costs of current management.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Population outbreaks of *A. amurensis* in its native range are known to cause significant economic damages to fisheries and aquaculture, particularly of bivalve species, such as scallops, oysters, clams and mussels. For example, the damage on the marketable shellfishes caused by the starfish in Tokyo Bay in 1954 amounted to as much as 400 million yen (Kim, 1968), while the outbreak that occurred in the coastal waters of Qingdao, China in 2006-2007, caused economic losses up to 1.5 million US dollars in mariculture of scallop, abalone, and clams (Zhou and Wang, 2008 in Li et al., 2018). Besides predation on the seabed, the species also settles on spat collectors, where a high abundance of juvenile *A. amurensis* (with a mean value of about 10 ind./m²), can result in 95% mortality of juvenile scallops *Mizuhopecten yessoensis* (as *Patinopecten yessoensis*) (Gabaev, 1987, cited in Gabaev, 2018).

Additionally, it is a nuisance to trawl fisheries, increasing the by-catch and damaging nets (Nojima et al., 1986), while at the same time creating a waste disposal problem. In Hokkaido, Japan, the amount of starfish removed from aquaculture and fishing grounds and disposed as waste on land is estimated to be about 15,000 tons per year (Ishii et al., 2007). In the Gulf of Alaska and the Bering Sea, *A. amurensis* also constitutes a large part of the by-catch and damages trawl nets (D. Urban, pers. comm in Smith & Armistead, 2014).

In the invaded range, large numbers of *Asterias amurensis* adults and juveniles have been found on scallop spat collector bags and suspended 'grow out' cages, mussel long-lines and oyster trays (Dommissie & Hough, 2004 and references therein). It is believed that, in all cases, settlement from plankton directly onto the different gears used is the most likely origin of the sea stars (Martin and Proctor 2000). In Tasmania, losses of commercial scallop spat over a settlement season due to *A. amurensis* predation were reported to be as high as 50% (S. Crawford pers. comm. in Hutson et al., 2005) or even 100%, according to one scallop farmer (Dommissie & Hough, 2004), resulting in an overall economic loss of 1 million AUD to the industry in 2000 (Aquenal, 2008). Additionally, in 2006, 25 tonnes of *A. amurensis* were caught as by-catch by commercial scallop fishermen on the east coast of Tasmania (Aquenal, 2008). *A. amurensis* heavily reduces the densities and potential for recruitment of wild populations of commercially important clams and cockles (Ross et al., 2002; 2004). Commercial long line fishermen are also affected in Victoria, Australia, where in areas of high infestation they report significant losses of bait and the majority of their hooks catching *A. amurensis* (Parry et al., 2003).

Qu. 4.10. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism currently in the risk assessment area (include any past costs in your response)?

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the EU these should be reported here. Assessment of the potential costs of damage on human

health, safety, and the economy, including the cost of non-action. A full economic assessment at EU scale might not be possible, but qualitative data or different case studies from across the EU (or third countries if relevant) may provide useful information to inform decision making. In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	N/A	CONFIDENCE	high
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Response: There is no evidence to confirm that the species is currently present in the RA area.

Qu. 4.11. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.10.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Throughout the world, predation by starfish is considered one of the primary causes of mortality of cultivated bivalves, such that prevention/mitigation measures are standard practice for shellfish growers (e.g. see Barkhouse et al., 2007; Calderwood et al., 2016; Kamermans & Capelle, 2019). During outbreak events, *Asterias* species can eliminate entire mussel beds (e.g. Gallagher et al., 2008; Paul-Burke & Burke, 2013) and threaten local resources and industries (Magnesen & Redmond, 2012). Considering the high abundances and predation mortality rates *A. amurensis* can achieve within cultivated bivalves, both on the seabed and on suspended culture means, such as lines, trays, spat collectors (see Qu. 4.9), it has the potential to cause major economic losses to the shellfish culture industry. For reference, predation mortality by *A. amurensis* on scallop spat collectors can result in 95% mortality of juvenile scallops in northern Russia and up to 50-100% in Tasmania (Qu. 4.9). Significant resources are at risk throughout the RA area, including mussel, oyster, scallops and other cultivated molluscs, as well as harvested wild populations of cockles and clams. Considering that such bivalve resources are already under predation pressure by the native *A. rubens*, additional and/or prolonged predation impacts by *A. amurensis* can exacerbate the economic losses.

Qu. 4.12. How great are the economic costs / losses associated with managing this organism currently in the risk assessment area (include any past costs in your response)?

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	N/A	CONFIDENCE	high
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Response: There is no evidence to confirm that the species is currently present in the RA area.

Qu. 4.13. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.12.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Costs related to shipping (ballasts/fouling) will be borne by the shipping companies. Significant costs can be associated with the ratification of relevant legislation, e.g. the Ballast Water Management Convention, in ensuring its compliance, related to planning, monitoring, enforcement and capacity-building. These costs however are not specific to *A. amurensis* and will prevent/reduce the introduction of a wide range of species carried by ballast water.

Significant costs are anticipated if the shellfish aquaculture sector is heavily impacted but overall estimates will depend on the extent and intensity of infestation. Shellfish growers routinely employ a variety of measures to combat predation by *Asterias* species (for a review see Barkhouse et al., 2007). These range from removals with towed gear (e.g. starfish mops, dredges) or traps, manual removal by hand, chemical methods, with estimated costs in the range of 10^3 - 10^4 USD per application (Barkhouse et al., 2007). These are costs that will need to be applied on a yearly basis, where the species is established and has attained high population densities. Cost estimates for the subsequent disposal of the starfish waste could not be found but need to be considered. On the other hand, potential commercial exploitation of the harvested populations (as outlined in Qu. A13) could reduce the negative costs associated with disposal. A ban of imports or restrictions in the movement of shellfish seed/stock from infected areas could have potentially significant economic implications for shellfish producers (but the alternative of allowing the risk of introduction may be even more harmful, i.e. cost of inaction – see Qu. 4.9 & 4.11).

If eradication is attempted at an early stage of invasion, estimates from a successful eradication in Australia indicate costs in the range of 500.000 AUD for a small and shallow area (<1ha and shallower than 15m) over a period of one year (Crombie et al., 2007). Population removal from artificial structures in the Derwent estuary (Australia) by divers has been estimated to incur a cost of approximately 250K AUD per year (Aquenal, 2008), while monitoring costs for this species alone were calculated in the range of 10^4 - 10^5 AUD per year (Aquenal, 2008). These are examples of costs

related to different management measures, which may need to be used in combination or be repeated depending on the stage of the invasion.

For long-established and dense populations (e.g. in Port Phillip Bay, Australia), Bax et al. (2006) examined the possibility of genetic control methods and arrived at a rough cost estimate >20 million AUD for research, infrastructure and operational costs. This management option however has not materialised and was expected to be met with public concerns and legislative obstacles in Australia (Aquenal, 2008), such that the potential costs were not taken into account for the score of this question.

Social and human health impacts

Qu. 4.14. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism for the risk assessment area and for third countries, if relevant (e.g. with similar eco-climatic conditions).

The description of the known impact and the assessment of potential future impact on human health, safety and the economy, shall, if relevant, include information on

- illnesses, allergies or other affections to humans that may derive directly or indirectly from a species;
- damages provoked directly or indirectly by a species with consequences for the safety of people, property or infrastructure;
- direct or indirect disruption of, or other consequences for, an economic or social activity due to the presence of a species.

Social and human health impacts can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE		CONFIDENCE	
	minimal		low
	minor		medium
	moderate		high
	major		
	massive		

Response: *Asterias amurensis* can accumulate paralytic shellfish toxins (Asakawa et al., 1997), and considering its (potential) uses for the production of fish meal, compost or fertiliser (McEnulty et al., 2001; Barkhouse et al., 2007) there may be concerns for indirect effects on human health. It is expected however that most of the toxin will be leached out or destroyed during the processing steps (e.g. cooking and sterilization are known to remove up to 90% of PSP toxins – EFSA, 2009 and references therein) or diluted by the other non-toxic components such that the danger to human health will be minimized. No information was found on human PSP intoxications via this indirect pathway and the metabolic routes of the toxins can vary considerably between different species (Vilarinho et al., 2018) such that there is high uncertainty associated with this assessment.

On the other hand, through its effects on recreational activities (i.e. fishing, harvest of bivalves – see Qu. 4.6 & 4.9) and the aesthetic values of the marine environment, it could potentially impact tourism and the amenity value of coastal areas (Aquenal, 2008).

Qu. 4.15. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism in the future for the risk assessment area.

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: As in Qu. 4.14. Minor social and health impacts may be anticipated in the RA area in the future.

Other impacts

Qu. 4.16. How important is the organism in facilitating other damaging organisms (e.g. diseases) as food source, a host, a symbiont or a vector etc.?

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Male *A. amurensis* are liable to gonad parasitisation by the ciliate parasite *Orchitophrya stellarum* (Byrne et al., 1997a), which causes complete atrophy of the testes, leading to castration. The same ciliate species infests native asteroids in the RA area, i.e. *A. rubens* in Atlantic Europe and *Sclerasterias richardi* in the Mediterranean Sea but with a lower prevalence (Jangoux, 1987). It was hypothesized that the high infestation rates in native Japan were due to the fact that *A. amurensis* was a new host for *O. stellarum* there (Byrne et al., 1997a). It is possible that the establishment of dense populations of *A. amurensis* can mediate an increase in *O. stellarum* infestations of other asteroids but high uncertainty is associated with this assessment.

Besides the risks posed for native species, *O. stellarum* is highly infectious and pathogenic to blue crabs *Callinectes sapidus* (Miller et al., 2013), another introduced species to European waters, which has already gained economic importance (Mancinelli et al., 2017).

Qu. 4.17. How important might other impacts not already covered by previous questions be resulting from introduction of the organism?

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No other impacts were found.

Qu. 4.18. How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area?

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Biotic interactions of *A. amurensis* with other species in its native and invaded range, as well as the RA area are discussed in Qu. 2.3, 2.4 & 4.3, 4.16. In Australia, the possibility for natural control has been suggested in relation to the parasite *O. stellarum* (see above) but also to the native starfish *Coscinasterias muricata* (Parry, 2015), which predate on *A. amurensis* juveniles and small adults. Possible predators of *A. amurensis* (e.g. native starfish and crab species) were also identified in Qu. 2.4, however the potential of predation by these species to limit *A. amurensis* populations is unknown. Judging from the establishment of extremely dense populations in Australia in the presence of native predators and competitors, it is thought that the impacts of the species are unlikely to be significantly moderated by natural control. On the other hand, *O. stellarum* is present and a parasite of native *A. rubens* in the RA area with the potential to infect *A. amurensis* with high prevalence rates (Byrne et al., 1997a), should it invade the RA area, hence the score of this question is accompanied by low confidence.

Qu. 4.19. Estimate the overall impact in the risk assessment area under current climate conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, in current conditions.

RESPONSE	minimal minor moderate major	CONFIDENCE	low medium high
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	massive		
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Response: *Asterias amurensis*, like other asteroid species, is a benthic keystone predator with a propensity for population outbreaks that can have strong impacts on local (mainly) bivalve populations. In its invaded Australian range, it quickly developed massive populations and also maintained high densities after the first population boom. The ability to behave in a similar manner in the RA area will depend to some extent on its interaction with its sister species, the native *A. rubens*, which is very similar functionally and ecologically. Driven by examples in the literature and assuming that the two species can co-exist, *A. amurensis* has the potential to cause moderate environmental impacts in the RA area, and primarily the North East Atlantic region, through predation and, to a lesser extent, competition. Thus, population declines (locally very severe, and likely stochastic) of native species of mussels, oysters, scallops and clams may be expected, as well as declines in erect macrofauna and habitat forming polychaetes and demersal fish species that utilise the same prey resources as the starfish. Impacts on bivalve populations, although likely to be widespread, are not anticipated to be irreversible. Bivalves are broadcast spawners with high dispersal potential and relatively short generation times. Furthermore, native bivalve species are widespread and generally well connected, able to offset sudden population losses or bad recruitment years.

Mussel and oyster beds/reefs, which are listed as endangered habitats and fulfil an important ecological role are anticipated to come under threat. Of these, the most vulnerable to predation by *A. amurensis* are the *M. modiolus* reefs and the diverse fauna they support. Where infaunal bivalves are impacted, this may have implications for sediment processes and nutrient cycling, while declines in epifaunal, filtering bivalves will affect water quality regulating services but such impacts are likely to be transient. Food provisioning services through declines in commercial and recreational harvests will also be affected. The economic impacts of *A. amurensis* are likely to be even more serious (major) due to its demonstrated ability elsewhere in the invaded range to cause heavy mortalities of commercially harvested in the wild and cultivated bivalve species and, most importantly, their spat/seed. These impacts may be readily mitigated, as similar native starfish predators are routinely removed from bivalve culture plots and devices, but enhanced management practices for additional starfish populations (and possibly at different times of the year if recruitment seasons for native and alien starfish species do not exactly overlap) will entail additional significant costs. Other fisheries sectors, e.g. demersal trawling and long-lining may also suffer by increased by-catch and loss of catch.

Qu. 4.20. Estimate the overall impact in the risk assessment area in foreseeable climate change conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, under future conditions.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Due to the higher tolerance of *A. amurensis* for higher temperatures compared to *A. rubens*, both at the larval and the adult stage, a future warming of the seas is likely to change the dynamics of the two species in favour of the invader and push *A. rubens* northwards. In this case it may be expected that *A. amurensis* solely assumes the role of the keystone benthic predator in areas no longer suitable for *A. rubens* but the ecological and functional niche will not remain empty. The severity of the impacts is not likely to change in Atlantic Europe, the Mediterranean Sea however will present *A. amurensis* with even less favourable environmental conditions and for shorter periods of time, lowering the risk of strong recruitment, population outbreaks and associated predation impacts, such that native species, habitats and resources will be at lower risk.

RISK SUMMARIES			
	RESPONSE	CONFIDENCE	COMMENT
Summarise Introduction and Entry*	very unlikely unlikely moderately likely likely very likely	low medium high	<p>The most likely pathways of introduction of <i>A. amurensis</i> in the RA area are through shipping vectors. While ballast water can potentially transport a large number of larvae, a number of conditions need to be met for the successful introduction of viable propagules from possible donor regions into the RA area, primarily uptake and transportation during thermally suitable times of the year. Moreover, the increasing implementation of the BWMC D2 standard will gradually reduce even further the survival probability of <i>A. amurensis</i> larvae entrained in ballast water.</p> <p>Entrainment in ships' sea chests and hull internal surfaces is a plausible mode of transport but less likely to be responsible for a large number of propagules entering the RA area. Based on the maritime traffic between potential source regions and EU ports, introduction and entry of <i>A. amurensis</i> larvae is considered more likely in the Greater North Sea region and less likely in the Mediterranean Sea. New Arctic Ocean shipping routes will not change the main recipient areas.</p>
Summarise Establishment*	very unlikely unlikely moderately likely likely very likely	low medium high	<p>Taking into account spawning times in the donor regions, travel duration and arrival times at the different parts of the RA area, the species is very likely to find suitable abiotic conditions for establishment during most of the year throughout most of Atlantic Europe, with the exception of the Bay of Biscay. Likelihood of establishment in the Mediterranean Sea is considered as moderate. Under future climate change, a small northward shift of the overall suitable area for the species is predicted, while the Mediterranean is expected to become widely unsuitable for establishment due to high water temperatures.</p>
Summarise Spread*	very slowly slowly moderately rapidly very rapidly	low medium high	<p>Unaided dispersal and multiple pathways of human-aided spread create a considerable potential for spread of <i>A. amurensis</i> in the north-east Atlantic. The species has long-lived planktonic larvae,</p>

			whose physiological requirements are met during prolonged periods in this region; additionally, it can spread rapidly via ballast water and can also be translocated with hull fouling, in sea chests, bivalve consignments, and fishing equipment.
Summarise Impact*	minimal minor moderate major massive	low medium high	<i>Asterias amurensis</i> has the potential to cause moderate environmental impacts in the RA area, and primarily the North East Atlantic region, through predation and, to a lesser extent, competition. Population declines (locally very severe) of native species of mussels, oysters, scallops and clams may be expected, as well as declines in demersal fish species and protected bird populations that utilise the same prey resources as the starfish. Mussel and oyster beds/reefs, which are listed as endangered habitats and fulfil an important ecological role are anticipated to come under threat. Where infaunal bivalves are impacted, this may have implications for sediment processes and nutrient cycling, while declines in epifaunal, filtering bivalves will affect water quality regulating services. Food provisioning services through declines in commercial and recreational harvests will also be affected. The economic impacts of <i>A. amurensis</i> are likely to be major due to its demonstrated ability elsewhere in the invaded range to cause heavy mortalities of commercially used bivalve species.
Conclusion of the risk assessment (overall risk)	low moderate high	low medium high	<i>Asterias amurensis</i> is a keystone predator of benthic invertebrates, mainly bivalve molluscs, with a well-studied invasion history in Australia, where it was most likely introduced via ballast water. The North-east Atlantic is the most likely recipient region and offers highly suitable conditions for establishment and possibilities for rapid spread. The species has the propensity for massive population outbreaks, which can be very persistent in the invaded range, and can cause severe population declines of benthic bivalve and fish species. It constitutes a serious threat to bivalve fisheries and the aquaculture industry.

*in current climate conditions and in foreseeable future climate conditions

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Distribution Summary

Please answer as follows:

- Yes if recorded, established or invasive
- if not recorded, established or invasive
- ? Unknown; data deficient

The columns refer to the answers to Questions A5 to A12 under Section A.

For data on marine species at the Member State level, delete Member States that have no marine borders.

In all other cases, provide answers for all columns.

Member States and the United Kingdom

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Belgium	-	-	YES	YES	-
Bulgaria	-	-	-	-	-
Croatia	-	-	YES	-	-
Cyprus	-	-	-	-	-
Denmark	-	-	YES	YES	-
Estonia	-	-	-	-	-
Finland	-	-	-	-	-
France	-	-	YES	YES	-
Germany	-	-	YES	YES	-
Greece	-	-	YES	-	-
Ireland	-	-	YES	YES	-
Italy	-	-	YES	-	-
Latvia	-	-	-	-	-
Lithuania	-	-	-	-	-
Malta	-	-	-	-	-
Netherlands	-	-	YES	YES	-
Poland	-	-	-	-	-
Portugal	-	-	YES	YES	-
Romania	-	-	-	-	-
Slovenia	-	-	YES	YES	-
Spain	-	-	YES	YES	-
Sweden	-	-	YES	YES	-
United Kingdom	-	-	YES	YES	-

Marine regions and subregions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Baltic Sea	-	-	-	-	-
Black Sea	-	-	-	-	-
North-east Atlantic Ocean	-	-	YES	YES	-
Bay of Biscay and the Iberian Coast	-	-	YES	YES	-
Celtic Sea	-	-	YES	YES	-
Greater North Sea	-	-	YES	YES	-
Mediterranean Sea	-	-	YES	-	-
Adriatic Sea	-	-	YES	-	-
Aegean-Levantine Sea	-	-	YES	-	-
Ionian Sea and the Central Mediterranean Sea	-	-	YES	-	-
Western Mediterranean Sea	-	-	YES	-	-

ANNEX I Scoring of Likelihoods of Events

(taken from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Description	Frequency
Very unlikely	This sort of event is theoretically possible, but is never known to have occurred and is not expected to occur	1 in 10,000 years
Unlikely	This sort of event has occurred somewhere at least once in the last millenium	1 in 1,000 years
Modeately likely	This sort of event has occurred somewhere at least once in the last century	1 in 100 years
Likely	This sort of event has happened on several occasions elsewhere, or on at least once in the last decade	1 in 10 years
Very likely	This sort of event happens continually and would be expected to occur	Once a year

ANNEX II Scoring of Magnitude of Impacts

(modified from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Biodiversity and ecosystem impact	Ecosystem Services impact	Economic impact (Monetary loss and response costs per year)	Social and human health impact, and other impacts
	<i>Question 5.1-5</i>	<i>Question 5.6-8</i>	<i>Question 5.9-13</i>	<i>Question 5.14-18</i>
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected ⁷	Up to 10,000 Euro	No social disruption. Local, mild, short-term reversible effects to individuals.
Minor	Some ecosystem impact, reversible changes, localised	Local and temporary, reversible effects to one or few services	10,000-100,000 Euro	Significant concern expressed at local level. Mild short-term reversible effects to identifiable groups, localised.
Moderate	Measureable long-term damage to populations and ecosystem, but reversible; little spread, no extinction	Measureable, temporary, local and reversible effects on one or several services	100,000-1,000,000 Euro	Temporary changes to normal activities at local level. Minor irreversible effects and/or larger numbers covered by reversible effects, localised.
Major	Long-term irreversible ecosystem change, spreading beyond local area	Local and irreversible or widespread and reversible effects on one / several services	1,000,000-10,000,000 Euro	Some permanent change of activity locally, concern expressed over wider area. Significant irreversible effects locally or reversible effects over large area.
Massive	Widespread, long-term population loss or extinction, affecting several species with serious ecosystem effects	Widespread and irreversible effects on one / several services	Above 10,000,000 Euro	Long-term social change, significant loss of employment, migration from affected area. Widespread, severe, long-term, irreversible health effects.

⁷ Not to be confused with “no impact”.

ANNEX III Scoring of Confidence Levels

(modified from Bacher et al. 2017)

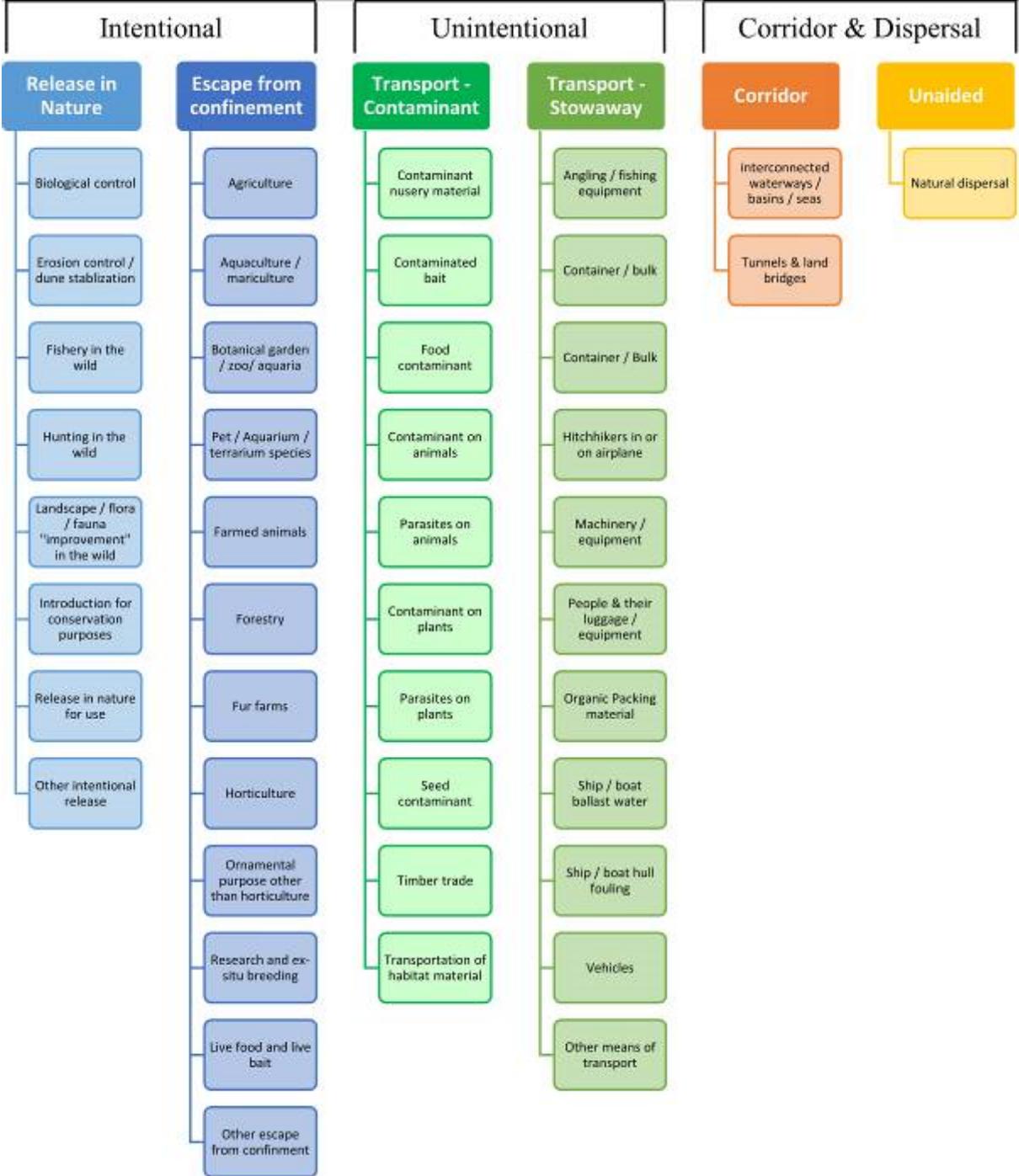
Each answer provided in the risk assessment must include an assessment of the level of confidence attached to that answer, reflecting the possibility that information needed for the answer is not available or is insufficient or available but conflicting.

The responses in the risk assessment should clearly support the choice of the confidence level.

Confidence level	Description
Low	There is no direct observational evidence to support the assessment, e.g. only inferred data have been used as supporting evidence <i>and/or</i> Impacts are recorded at a spatial scale which is unlikely to be relevant to the assessment area <i>and/or</i> Evidence is poor and difficult to interpret, e.g. because it is strongly ambiguous <i>and/or</i> The information sources are considered to be of low quality or contain information that is unreliable.
Medium	There is some direct observational evidence to support the assessment, but some information is inferred <i>and/or</i> Impacts are recorded at a small spatial scale, but rescaling of the data to relevant scales of the assessment area is considered reliable, or to embrace little uncertainty <i>and/or</i> The interpretation of the data is to some extent ambiguous or contradictory.
High	There is direct relevant observational evidence to support the assessment (including causality) <i>and</i> Impacts are recorded at a comparable scale <i>and/or</i> There are reliable/good quality data sources on impacts of the taxa <i>and</i> The interpretation of data/information is straightforward <i>and/or</i> Data/information are not controversial or contradictory.

ANNEX IV CBD pathway categorisation scheme

Overview of CBD pathway categorisation scheme showing how the 44 pathways relate to the six main pathway categories. All of the pathways can be broadly classified into 1) those that involve intentional transport (blue), 2) those in which the taxa are unintentionally transported (green) and 3) those where taxa moved between regions without direct transportation by humans and/or via artificial corridors (orange and yellow). **Note that the pathways in the category “Escape from confinement” can be considered intentional for the introduction into the risk assessment area and unintentional for the entry into the environment.**



ANNEX V Ecosystem services classification (CICES V5.1, simplified) and examples

For the purposes of this risk assessment, please feel free to use what seems as the most appropriate category / level / combination of impact (Section – Division – Group), reflecting information available.

Section	Division	Group	Examples (i.e. relevant CICES “classes”)
Provisioning	Biomass	Cultivated <i>terrestrial</i> plants	<p>Cultivated terrestrial plants (including fungi, algae) grown for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from cultivated plants, fungi, algae and bacteria for direct use or processing (excluding genetic materials); Cultivated plants (including fungi, algae) grown as a <u>source of energy</u></p> <p><i>Example: negative impacts of non-native organisms to crops, orchards, timber etc.</i></p>
		Cultivated <i>aquatic</i> plants	<p>Plants cultivated by in- situ aquaculture grown for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from in-situ aquaculture for direct use or processing (excluding genetic materials); Plants cultivated by in- situ aquaculture grown as an <u>energy source</u>.</p> <p><i>Example: negative impacts of non-native organisms to aquatic plants cultivated for nutrition, gardening etc. purposes.</i></p>
		Reared animals	<p>Animals reared for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from reared animals for direct use or processing (excluding genetic materials); Animals reared to provide <u>energy</u> (including mechanical)</p> <p><i>Example: negative impacts of non-native organisms to livestock</i></p>
		Reared <i>aquatic</i> animals	<p>Animals reared by in-situ aquaculture for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from animals grown by in-situ aquaculture for direct use or processing (excluding genetic materials); Animals reared by in-situ aquaculture as an <u>energy source</u></p> <p><i>Example: negative impacts of non-native organisms to fish farming</i></p>
		Wild plants (terrestrial and aquatic)	<p>Wild plants (terrestrial and aquatic, including fungi, algae) used for <u>nutrition</u>; <u>Fibres and other materials</u> from wild plants for direct use or processing (excluding genetic materials); Wild plants (terrestrial and aquatic, including fungi, algae) used as a <u>source of energy</u></p> <p><i>Example: reduction in the availability of wild plants (e.g. wild berries, ornamentals) due to non-native organisms (competition, spread of disease etc.)</i></p>
		Wild animals (terrestrial and aquatic)	<p>Wild animals (terrestrial and aquatic) used for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from wild animals for direct use or processing (excluding genetic materials); Wild animals (terrestrial and aquatic) used as a <u>source of energy</u></p>

			<i>Example: reduction in the availability of wild animals (e.g. fish stocks, game) due to non-native organisms (competition, predations, spread of disease etc.)</i>
	Genetic material from all biota	Genetic material from plants, algae or fungi	<p><u>Seeds, spores and other plant materials</u> collected for maintaining or establishing a population; Higher and lower plants (whole organisms) used to <u>breed new strains or varieties</u>; Individual genes extracted from higher and lower plants for the <u>design and construction of new biological entities</u></p> <p><i>Example: negative impacts of non-native organisms due to interbreeding</i></p>
		Genetic material from animals	<p>Animal material collected for the purposes of maintaining or establishing a population; Wild animals (whole organisms) used to breed new strains or varieties; Individual genes extracted from organisms for the design and construction of new biological entities</p> <p><i>Example: negative impacts of non-native organisms due to interbreeding</i></p>
	Water⁸	Surface water used for nutrition, materials or energy	<p>Surface water for <u>drinking</u>; Surface water used as a material (<u>non-drinking purposes</u>); Freshwater surface water, coastal and marine water used as an <u>energy source</u></p> <p><i>Example: loss of access to surface water due to spread of non-native organisms</i></p>
		Ground water for used for nutrition, materials or energy	<p>Ground (and subsurface) water for <u>drinking</u>; Ground water (and subsurface) used as a material (<u>non-drinking purposes</u>); Ground water (and subsurface) used as an <u>energy source</u></p> <p><i>Example: reduced availability of ground water due to spread of non-native organisms and associated increase of ground water consumption by vegetation.</i></p>
Regulation & Maintenance	Transformation of biochemical or physical inputs to ecosystems	Mediation of wastes or toxic substances of anthropogenic origin by living processes	<p><u>Bio-remediation</u> by micro-organisms, algae, plants, and animals; <u>Filtration/sequestration/storage/accumulation</u> by micro-organisms, algae, plants, and animals</p> <p><i>Example: changes caused by non-native organisms to ecosystem functioning and ability to filtrate etc. waste or toxics</i></p>
		Mediation of nuisances of anthropogenic origin	<p><u>Smell reduction</u>; <u>noise attenuation</u>; <u>visual screening</u> (e.g. by means of green infrastructure)</p> <p><i>Example: changes caused by non-native organisms to ecosystem structure, leading to reduced ability to mediate nuisances.</i></p>
	Regulation of physical, chemical, biological conditions	Baseline flows and extreme event regulation	<p>Control of <u>erosion</u> rates; Buffering and attenuation of <u>mass movement</u>; <u>Hydrological cycle and water flow regulation</u> (Including flood control, and coastal protection); <u>Wind</u> protection; <u>Fire</u> protection</p>

⁸ Note: in the CICES classification provisioning of water is considered as an abiotic service whereas the rest of ecosystem services listed here are considered biotic.

			<p><i>Example: changes caused by non-native organisms to ecosystem functioning or structure leading to, for example, destabilisation of soil, increased risk or intensity of wild fires etc.</i></p>
		<p>Lifecycle maintenance, habitat and gene pool protection</p>	<p>Pollination (or 'gamete' dispersal in a marine context); <u>Seed dispersal</u>; Maintaining <u>nursery populations and habitats</u> (Including gene pool protection)</p> <p><i>Example: changes caused by non-native organisms to the abundance and/or distribution of wild pollinators; changes to the availability / quality of nursery habitats for fisheries</i></p>
		<p>Pest and disease control</p>	<p>Pest control; Disease control</p> <p><i>Example: changes caused by non-native organisms to the abundance and/or distribution of pests</i></p>
		<p>Soil quality regulation</p>	<p><u>Weathering processes</u> and their effect on soil quality; <u>Decomposition and fixing processes</u> and their effect on soil quality</p> <p><i>Example: changes caused by non-native organisms to vegetation structure and/or soil fauna leading to reduced soil quality</i></p>
		<p>Water conditions</p>	<p>Regulation of the <u>chemical condition</u> of freshwaters by living processes; Regulation of the chemical condition of salt waters by living processes</p> <p><i>Example: changes caused by non-native organisms to buffer strips along water courses that remove nutrients in runoff and/or fish communities that regulate the resilience and resistance of water bodies to eutrophication</i></p>
		<p>Atmospheric composition and conditions</p>	<p>Regulation of <u>chemical composition</u> of atmosphere and oceans; Regulation of <u>temperature and humidity</u>, including ventilation and transpiration</p> <p><i>Example: changes caused by non-native organisms to ecosystems' ability to sequester carbon and/or evaporative cooling (e.g. by urban trees)</i></p>
Cultural	Direct, in-situ and outdoor interactions with living systems that depend on presence in the environmental setting	<p>Physical and experiential interactions with natural environment</p>	<p>Characteristics of living systems that that enable activities promoting health, recuperation or enjoyment through <u>active or immersive interactions</u>;</p> <p>Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through <u>passive or observational interactions</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that make it attractive for recreation, wild life watching etc.</i></p>
		<p>Intellectual and representative interactions with natural environment</p>	<p>Characteristics of living systems that enable <u>scientific investigation</u> or the creation of traditional ecological knowledge;</p> <p>Characteristics of living systems that enable <u>education and training</u>;</p> <p>Characteristics of living systems that are resonant in terms of <u>culture or heritage</u>;</p> <p>Characteristics of living systems that enable <u>aesthetic experiences</u></p>

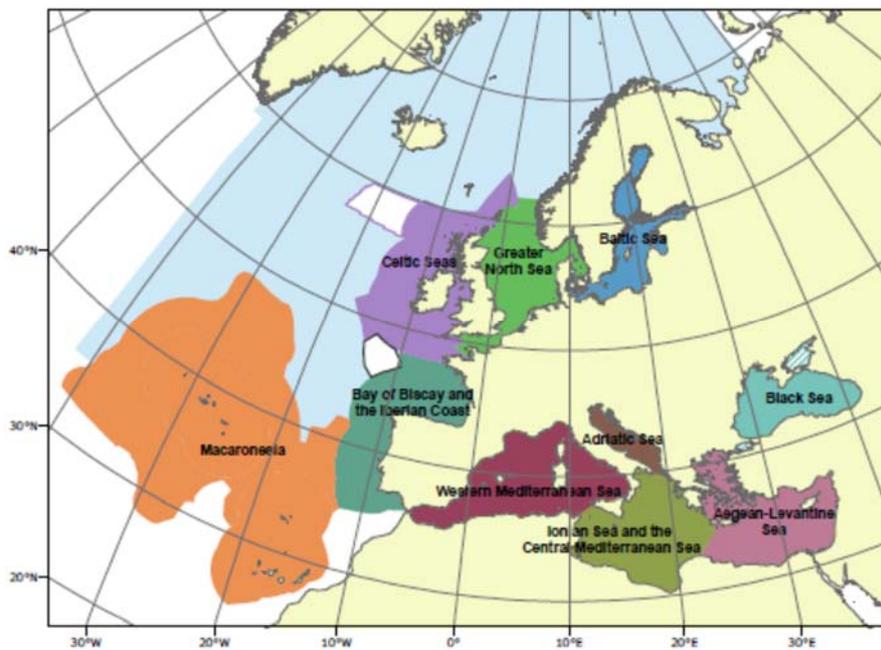
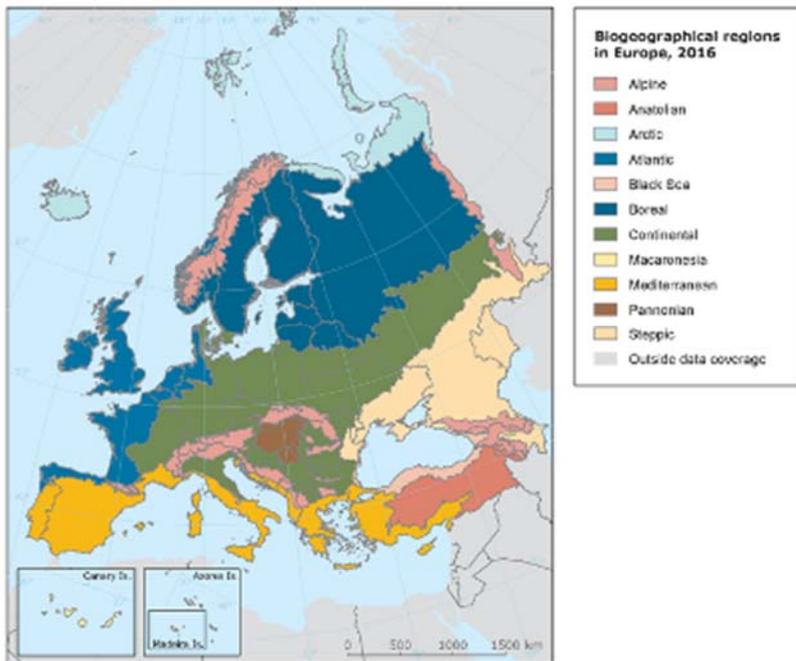
			<i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have cultural importance</i>
	Indirect, remote, often indoor interactions with living systems that do not require presence in the environmental setting	Spiritual, symbolic and other interactions with natural environment	<p>Elements of living systems that have <u>symbolic meaning</u>;</p> <p>Elements of living systems that have <u>sacred or religious meaning</u>;</p> <p>Elements of living systems used for <u>entertainment or representation</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have sacred or religious meaning</i></p>
		Other biotic characteristics that have a non-use value	<p>Characteristics or features of living systems that have an <u>existence value</u>;</p> <p>Characteristics or features of living systems that have an <u>option or bequest value</u></p> <p><i>Example: changes caused by non-native organisms to ecosystems designated as wilderness areas, habitats of endangered species etc.</i></p>

ANNEX VI EU Biogeographic Regions and MSFD Subregions

See <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2>,
http://ec.europa.eu/environment/nature/natura2000/biogeog_regions/

and

<https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions-1/technical-document/pdf>



ANNEX VII Delegated Regulation (EU) 2018/968 of 30 April 2018

see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>

ANNEX VIII Larval biology/ecology of *A. amurensis* and invasion risk through ballast water

Marika Galanidi (text and analysis specifically developed for the purposes of this risk assessment)

Spawning for *A. amurensis* is reported at different times in different locations, ranging from late January/February to July (e.g. Akkeshi Bay, Hokkaido, Japan) and at temperatures ranging from 5 °C to 23 °C. In Japan and Korea, spawning takes place primarily in February-April, at temperatures between 5°C and 20°C (Paik et al., 2005; Lee et al., 2004; Yu et al., 1998; Kim, 1968; Hatanaka & Kosaka, 1959; Kashenko, 2005). In Peter the Great Bay, Russia and Alaska the species has shifted its spawning period to the summer months (June-September) and in China it is reported to reproduce between October and January (Li et al., 2018; Zhang et al., 2014). In some locations however there are two spawning events; such is the case in Yantai, China, where spawning takes place in October-November (coinciding well with the lower bottom water temperature (14.29°C) and again in March-May (Li et al., 2018) and also in Peter the Great Bay, Russia, with spawning events in June-July and in September, at temperatures of 17°C and 23°C, respectively (Novikova, 1978). This upper temperature limit to spawning comes in contrast with all other published literature derived both from field studies as well as laboratory experiments (e.g. Kashenko, 2005; Lee et al., 2004; Sutton & Bruce, 1996; Byrne et al., 2016), which indicate that normal larval development can occur at a **maximum temperature of 20°C**. This was consequently regarded as the maximum temperature threshold for establishment, with the **minimum temperature threshold set at 5°C**. In the invaded range, *A. amurensis* spawns during the austral winter from July to October, at similar degrees of latitude and a similar temperature range (i.e. 8-14°C, Byrne et al., 2016), but in the southern hemisphere, indicating photoperiodic regulation of gametogenesis, as well as modulation by temperature (Smith & Armistead, 2014). Fertilised eggs are demersal and develop into swimming and feeding larvae (termed bipinnariae) within 60h to 120h of fertilization depending on water temperature (20°C and 10°C respectively) (Lee et al., 2004). Larval duration in the plankton also varies with temperature, as well as with feeding conditions, and can range between 50 and 120 days at temperatures between 19°C and 10°C respectively (Bruce et al., 1995 and references therein).

Larvae taken up in ballast water at possible source regions are estimated to arrive at release locations in the RA area after a travel period of approximately 6 weeks. It can be seen from the following table that larvae can, theoretically, arrive at the RA area at any time of the year (this without taking into account the frequency of connections between source area ports and arrival ports).

Source region	Spawning period	Temperature	Estimated arrival	Ecoregion
Japan-Korea	February-May	5-20°C	April-July	NW Pacific
Russia-Alaska	June-September	7-??	August-November	NW/NE Pacific
Australia	July-October	8-14°C	September-December	Temperate Australasia
China	October-January	5-14.3°C	December-March	NW Pacific

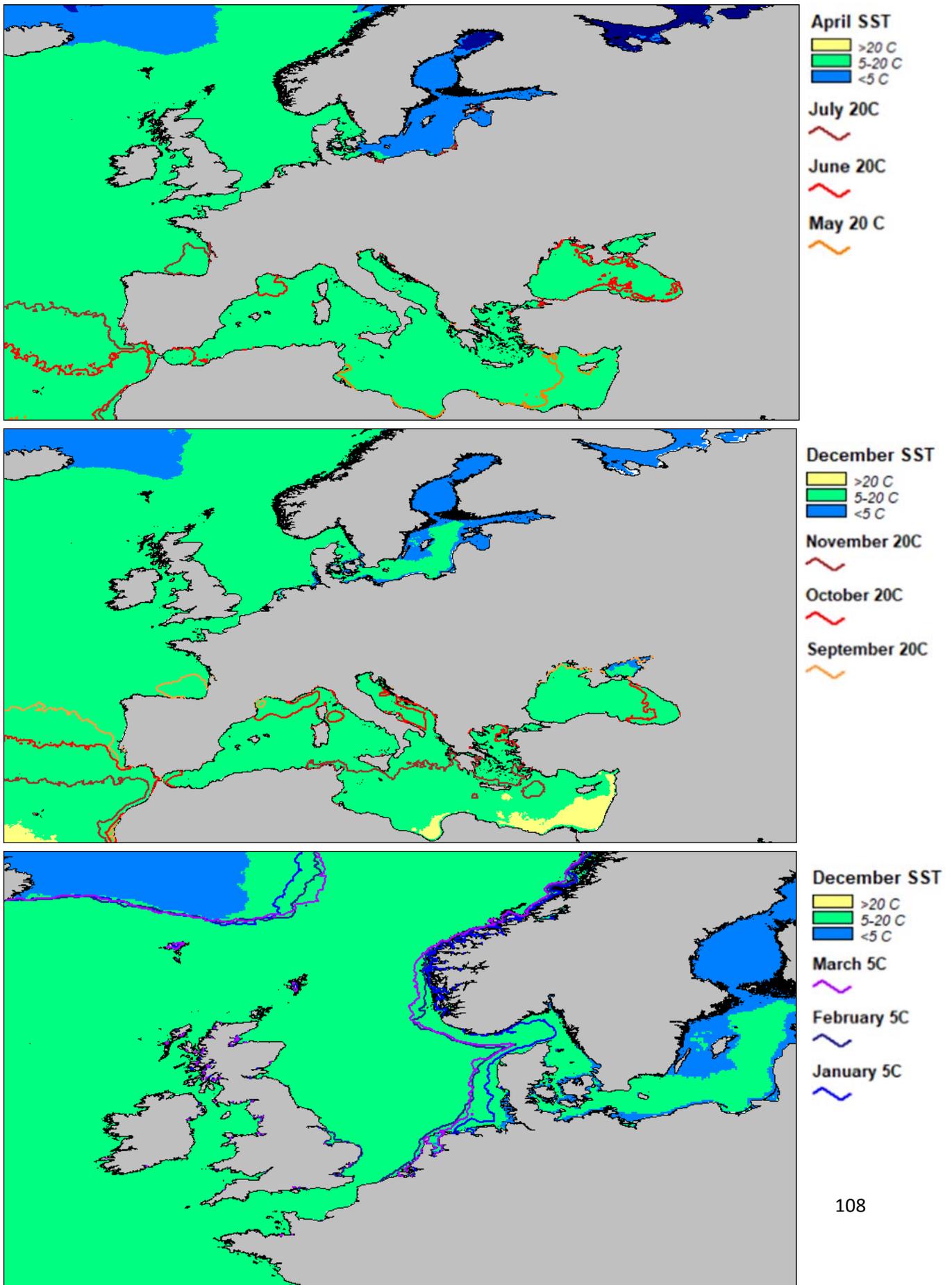
Based on the analysis of Kaluza et al. (2010), Alaska and north-eastern Russia are unlikely to act as donor regions of larvae via ballast water. Ports and coastal waters of Japan, Korea and China are well connected with Mediterranean and Eastern Atlantic ports via the Asia/Europe shipping route and receive a large amount of vessel traffic (Verny et al., 2009; Kaluza et al., 2010). Less intense maritime connections exist between Europe and temperate Australia.

In the Mediterranean Sea, propagules arriving from China in the winter months are likely to find suitable temperatures for development and settlement upon release (Temperature data layers retrieved from MARSPEC, Sbrocco et al., 2013, URL: <http://www.esapubs.org/archive/ecol/E094/086/metadata.php>). However, larvae in ballast arriving from Japan/Korea and from Australia will have a two-month window, in April-May and November-December respectively, to complete development and settle. Between June and October, sea surface temperatures will prevent normal development and survival of *A. amurensis* larvae in the Mediterranean (Figure 1). In Atlantic Europe, sea surface temperatures fall within the 5-20°C range throughout the period that *A. amurensis* larvae may be expected to arrive from Japan/Korea and for the most part from Australia, although larvae arriving from Australia later in the year will encounter increasingly colder temperatures in the Wadden Sea and around Denmark and south-eastern UK. Larvae arriving to Atlantic Europe from China in the winter months will find themselves at the lower limit of their thermal tolerance in the shallow, inshore areas of the western North Sea and along parts of the UK. The Celtic Seas, English Channel and Iberian coast/Bay of Biscay offer favourable establishment conditions throughout the year, with the exception of the Bay of Biscay (BoB), where temperatures are slightly above the 20°C threshold during the summer.

In summary, the likelihood of initial establishment of *A. amurensis* in the RA area will be determined by a combination of donor region, arrival time and abiotic conditions of the recipient region when the first larvae arrive. The survival and settlement of the first introduced larvae is likely to be of high importance for a successful invasion, since, once this happens, the species has demonstrated its adaptability to local conditions by changing the seasonality of its reproductive cycle (see the Australian invasion, also spawning periods throughout its native range). From that point on, natural dispersal and secondary spread can lead to further establishment within the RA area (see following Annex with the results of the species distribution model).

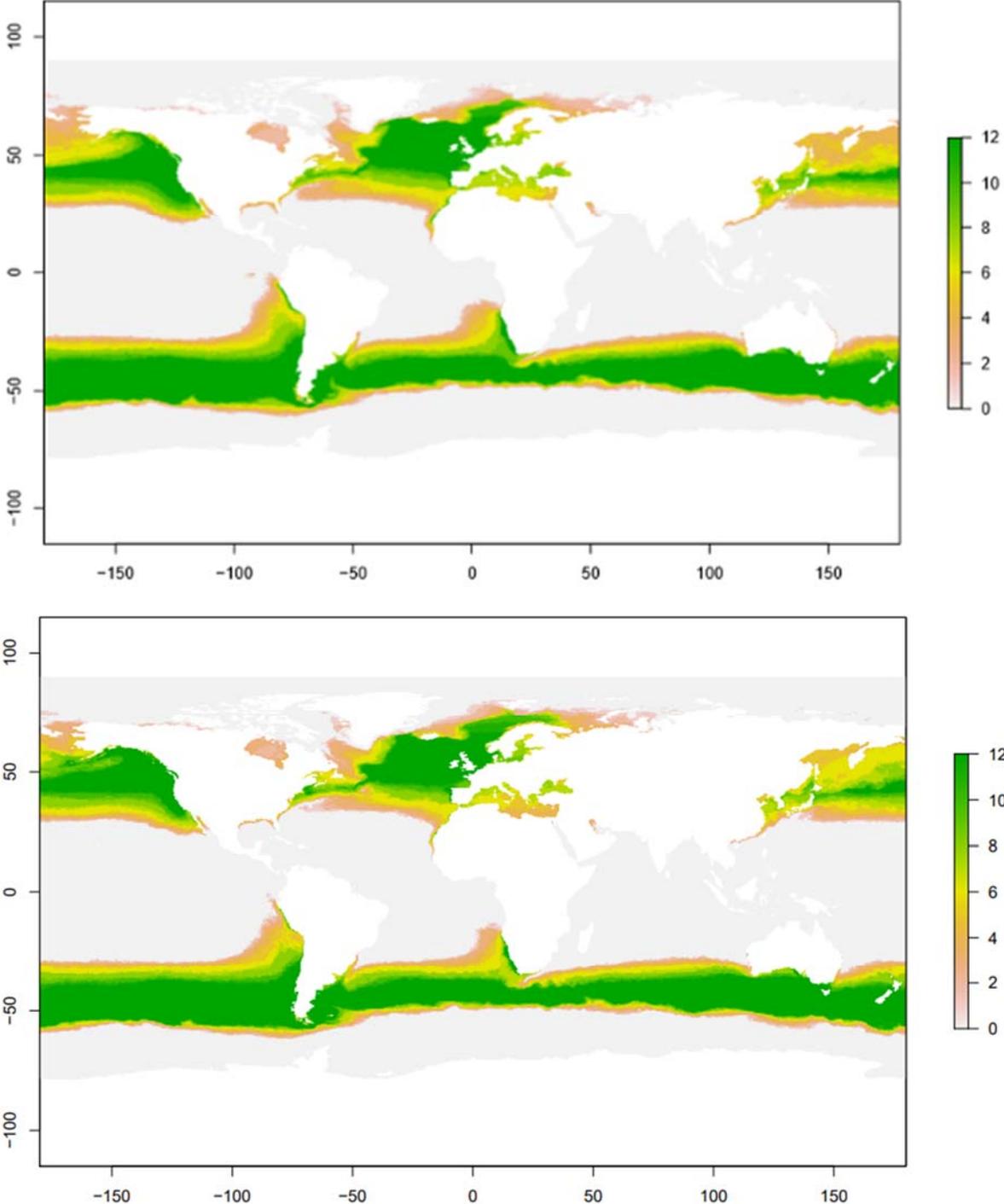
Likelihood of establishment by larvae of <i>A. amurensis</i> (by primary introduction)	
Mediterranean	1 main donor region – suitable in winter – high maritime connectivity only in the south – moderately likely
Iberian Coast	3 donor regions – suitable throughout the year – moderate to low maritime connectivity – moderately likely (not the Bay of Biscay)
Celtic Seas	3 donor regions – suitable throughout the year – moderate maritime connectivity – moderately likely
Greater North Sea	3 donor regions – suitable throughout the year – high maritime connectivity – likely
Black Sea	Restricted by salinity – UNLIKELY (except for the Sea of Marmara)
Baltic Sea	Restricted by salinity – UNLIKELY

Figure 2. Top: April Sea Surface Temperature (SST) and 20°C SST contours for the months of May-July. Middle: December SST and 20°C SST contours for October-November. Suitable conditions (SST<20°C) for *A. amurensis* larval development are encountered westwards and northwards of the contour lines. Bottom: December SST and 5°C SST contours for January-March.



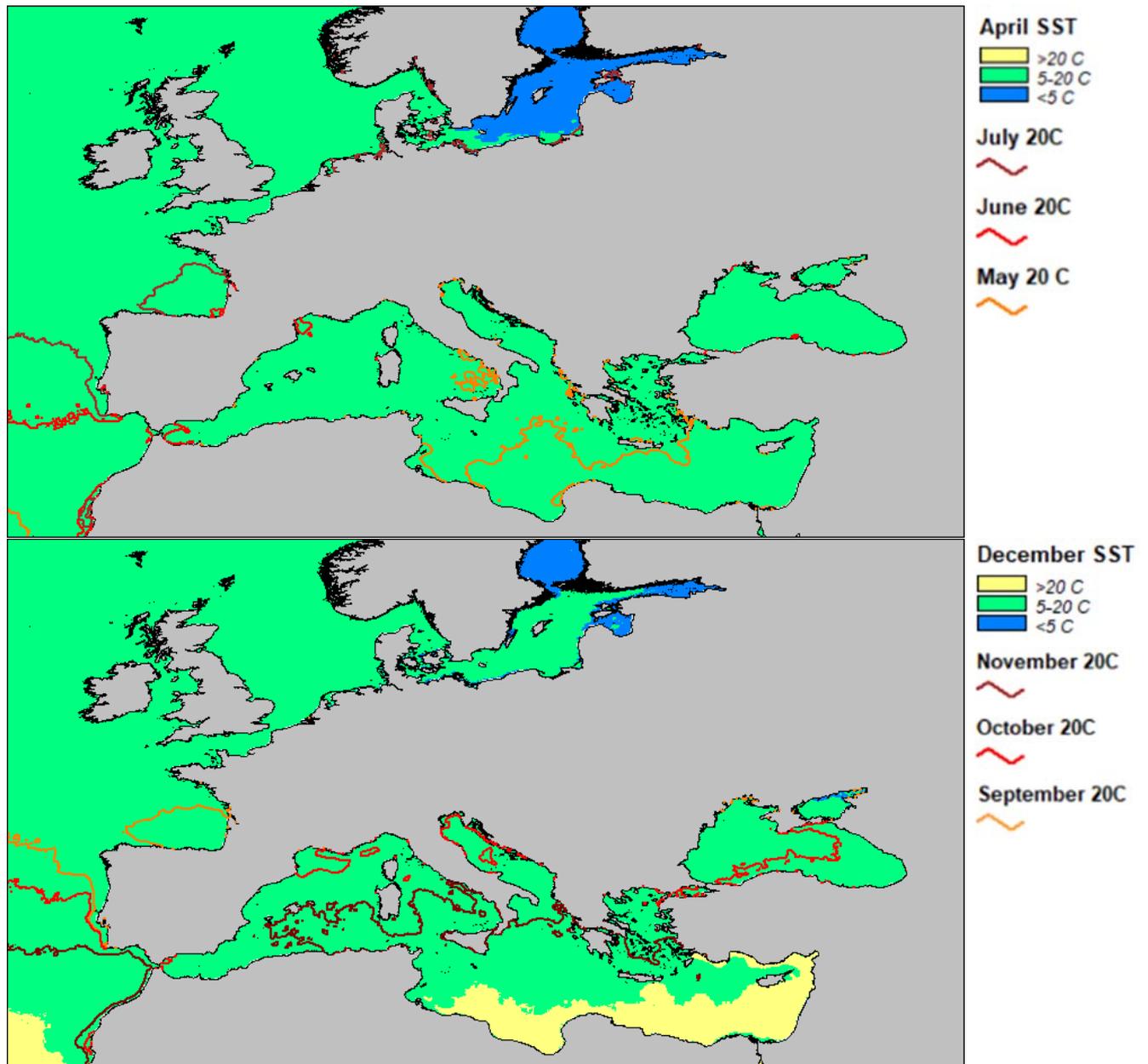
The same principles are expressed in the following map, as number of months with thermal conditions suitable for establishment (i.e. mean monthly temperatures between 5-20 °C).

Figure 3. Number of months with conditions suitable for establishment, i.e. mean monthly temperatures between 5-20 °C. Top: Under current climate conditions. Bottom: Under RCP4.5 in 2070. Maps created by Björn Beckmann.



Similar isotherm maps were produced for a future scenario of climate change (rough estimate based on a maximum increase in seawater temperatures of 0.8 °C by 2070, according to the medium timeframe RCP 4.5 scenario). It appears that, under future climate change, the conditions suitable for establishment in the Mediterranean Sea will shrink even more both in space and in time. Thus, future establishment for the Mediterranean is considered unlikely.

Figure 3: As in Figure 1 but for under RCP4.5 in 2070. Top: April Sea Surface Temperature (SST) and 20°C SST contours for the months of May-July. Bottom: December SST and 20°C SST contours for October-November. Suitable conditions (SST<20°C) for *A. amurensis* larval development are encountered westwards and northwards of the contour lines.



ANNEX IX Species Distribution Model

Projection of environmental suitability for *Asterias amurensis* establishment in Europe

Björn Beckmann, Marika Galanidi and Dan Chapman

10 June 2020

Aim

To project the suitability for potential establishment of *Asterias amurensis* in Europe, under current and predicted future climatic conditions.

Data for modelling

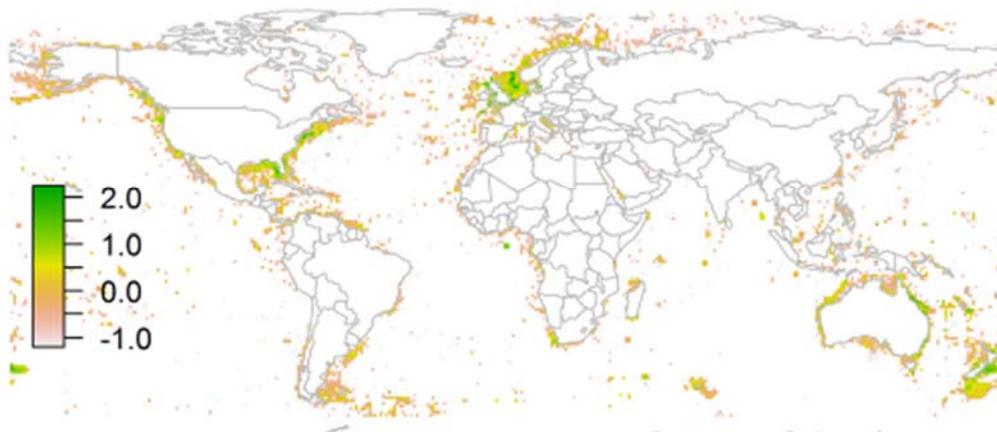
Species occurrence data were obtained from the Global Biodiversity Information Facility (GBIF) (360 records), the Ocean Biogeographic Information System (OBIS) (285 records), the Biodiversity Information Serving Our Nation database (BISON) (219 records), the Integrated Digitized Biocollections (iDigBio) (170 records), the Atlas of Living Australia (99 records), iNaturalist (65 records), and some additional records from the risk assessment team. We scrutinised occurrence records and removed any dubious ones and records where the georeferencing was too imprecise or outside of the coverage of the predictor layers. The records were gridded at a 0.25 x 0.25 degree resolution for modelling, yielding 205 grid cells with occurrences (Figure 1a). As a proxy for recording effort, the density of Asteroidea records held by GBIF was also compiled on the same grid (Figure 1b).

Figure 1. (a) Occurrence records obtained for *Asterias amurensis* and used in the modelling, showing native and invaded distributions. (b) The recording density of Asteroidea on GBIF, which was used as a proxy for recording effort.

(a) Species distribution used in modelling



(b) Estimated recording effort (log10-scaled)



Predictors describing the marine environment were selected from the ‘Bio-ORACLE2’ set of GIS rasters providing geophysical, biotic and environmental data for surface and benthic marine realms (Tyberghein et al., 2012, Assis et al. 2017), supplemented by variables calculated from MARSPEC monthly sea surface temperature data (Sbrocco & Barber 2013). Both were originally at 5 arcminute resolution (0.083 x 0.083 degrees of longitude/latitude) and aggregated to a 0.25 x 0.25 degree grid for use in the model.

Based on the biology of *Asterias amurensis*, the following variables were used in the modelling:

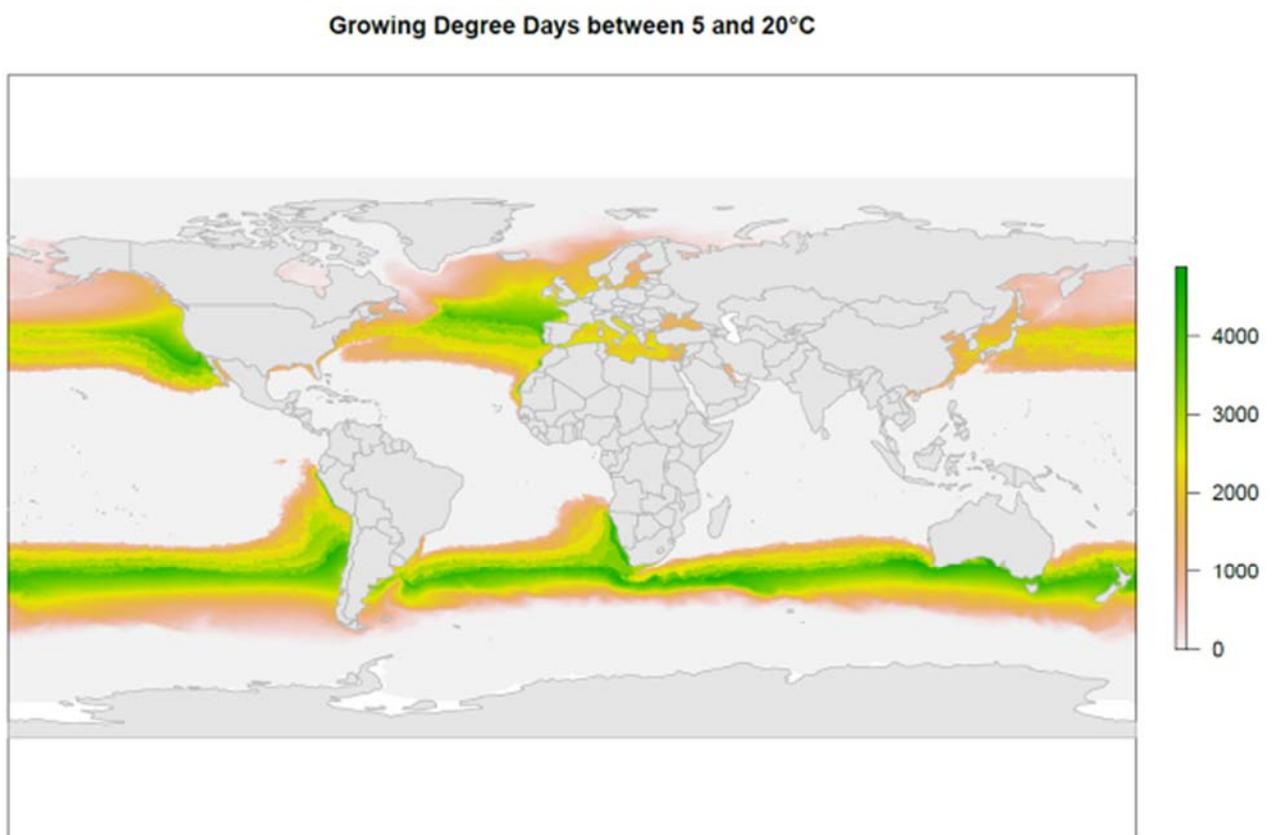
- Mean temperature (tempmean_ss)
- Maximum long-term temperature (templtmax_ss)
- Minimum long-term temperature (templtmin_ss)
- Minimum salinity (salinitymin_ss)

- Mean bathymetry (bathymean)
- Growing Degree Days between 5 and 20°C (gdd_5_20)

All parameters (except depth) are measured at the sea surface.

Growing Degree Days (GDD) have been widely used as a measure of the “biologically useful” sum of warmth between threshold temperatures where processes of interest occur (McMaster and Wilhelm 1997). For *Asterias amurensis*, larval development occurs between approximately 5 and 20°C (see Risk Assessment for details). GDD are usually calculated from daily temperature data, but since these are not available for the marine environment at a global scale, GDD were calculated from MARSPEC monthly mean temperature data: First, all grid cells with temperatures less than 5°C or more than 20°C were excluded for each month. Then, any positive difference from the lower temperature threshold was multiplied by the number of days in the month. For example, a grid cell with a temperature of 7°C in January had a value of 7°C - 5°C = 2°C x 31 days = 62 GDD for January. Finally, GDD values for all months were summed to produce an annual total for each grid cell (Fig. 2).

Figure 2. Annual Growing Degree Days (GDD) between 5 and 20°C for the current climate, calculated from MARSPEC monthly mean sea surface temperatures.



To estimate the effect of climate change on the potential distribution of *Asterias amurensis*, equivalent modelled future conditions for the 2070s under the Representative Concentration Pathways (RCP) 2.6 and 4.5 were also obtained. These represent low and medium emissions scenarios, respectively. Projections for the 2070s were calculated as averages of projections for the 2040s and 2090s (which are the time periods available on Bio-ORACLE2). Future monthly mean temperatures for calculating

Growing Degree Days were approximated by adding the temperature difference between Bio-ORACLE2 current and future scenarios to the MARSPEC monthly temperature layers, because no future layers for MARSPEC are available.

Species distribution model

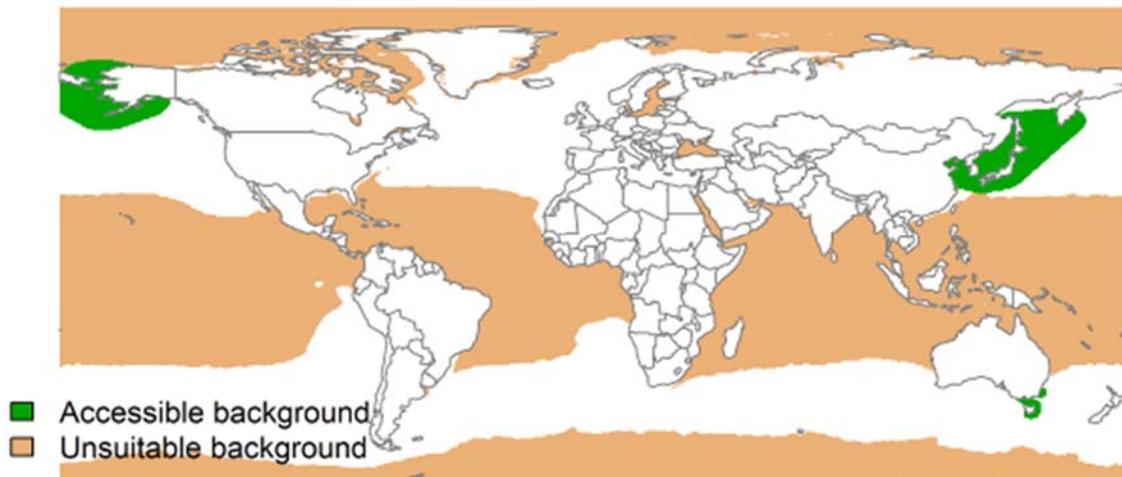
A presence-background (presence-only) ensemble modelling strategy was employed using the BIOMOD2 R package version 3.4.6 (Thuiller et al., 2020, Thuiller et al., 2009). These models contrast the environment at the species' occurrence locations against a random sample of the global background environmental conditions (often termed 'pseudo-absences') in order to characterise and project suitability for occurrence. This approach has been developed for distributions that are in equilibrium with the environment. Because invasive species' distributions are not at equilibrium and subject to dispersal constraints at a global scale, we took care to minimise the inclusion of locations suitable for the species but where it has not been able to disperse to (Chapman et al. 2019). Therefore the background sampling region included:

- The area accessible by native *Asterias amurensis* populations, in which the species is likely to have had sufficient time to disperse to all locations. Based on presumed maximum dispersal distances, the accessible region was defined as a 500km buffer around the native range occurrences; AND
- A 200km buffer around the non-native occurrences, encompassing regions likely to have had high propagule pressure for introduction by humans and/or dispersal of the species; AND
- Regions where we have an *a priori* expectation of high unsuitability for the species so that absence is assumed irrespective of dispersal constraints (see Figure 2). The following rules were applied to define a region expected to be highly unsuitable for *Asterias amurensis* at the spatial scale of the model:
 - Minimum salinity (salinitymin_ss) < 20
 - Maximum long-term temperature (templtmax_ss) < 5
 - Minimum long-term temperature (templtmin_ss) > 20

Altogether, 2.4% of occurrence grid cells were located in the unsuitable background region.

Within the unsuitable background region, 10 samples of 5000 randomly sampled grid cells were obtained. In the accessible background (comprising the accessible areas around native and non-native occurrences as detailed above), the same number of pseudo-absence samples were drawn as there were presence records (205), weighting the sampling by a proxy for recording effort (Figure 3).

Figure 3. The background from which pseudo-absence samples were taken in the modelling of *Asterias amurensis*. Samples were taken from a 500km buffer around the native range and a 200km buffer around non-native occurrences (together forming the accessible background), and from areas expected to be highly unsuitable for the species (the unsuitable background region). Samples from the accessible background were weighted by a proxy for recording effort (Figure 1(b)).



Each dataset (i.e. combination of the presences and the individual background samples) was randomly split into 80% for model training and 20% for model evaluation. With each training dataset, five statistical algorithms were fitted with the default BIOMOD2 settings and rescaled using logistic regression, except where specified below:

- Generalised linear model (GLM)
- Generalised boosting model (GBM)
- Generalised additive model (GAM) with a maximum of four degrees of freedom per smoothing spline
- Random forest (RF)
- Maxent

Since the total background sample was larger than the number of occurrences, prevalence fitting weights were applied to give equal overall importance to the occurrences and the background. Normalised variable importance was assessed and variable response functions were produced using BIOMOD2's default procedure.

Model predictive performance was assessed by the following three measures:

- AUC, the area under the receiver operating characteristic curve (Fielding & Bell 1997). Predictions of presence-absence models can be compared with a subset of records set aside for model evaluation (here 20%) by constructing a confusion matrix with the number of true positive, false positive, false negative and true negative cases. For models generating non-dichotomous scores (as here) a threshold can be applied to transform the scores into a dichotomous set of presence-absence predictions. Two measures that can be derived from the

confusion matrix are sensitivity (the proportion of observed presences that are predicted as such, quantifying omission errors), and specificity (the proportion of observed absences that are predicted as such, quantifying commission errors). A receiver operating characteristic (ROC) curve can be constructed by using all possible thresholds to classify the scores into confusion matrices, obtaining sensitivity and specificity for each matrix, and plotting sensitivity against the corresponding proportion of false positives (equal to $1 - \text{specificity}$). The use of all possible thresholds avoids the need for a selection of a single threshold, which is often arbitrary, and allows appreciation of the trade-off between sensitivity and specificity. The area under the ROC curve (AUC) is often used as a single threshold-independent measure for model performance (Manel, Williams & Ormerod 2001). AUC is the probability that a randomly selected presence has a higher model-predicted suitability than a randomly selected absence (Allouche et al. 2006).

- Cohen's Kappa (Cohen 1960). This measure corrects the overall accuracy of model predictions (ratio of the sum of true presences plus true absences to the total number of records) by the accuracy expected to occur by chance. The kappa statistic ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random. Advantages of kappa are its simplicity, the fact that both commission and omission errors are accounted for in one parameter, and its relative tolerance to zero values in the confusion matrix (Manel, Williams & Ormerod 2001). However, Kappa has been criticised for being sensitive to prevalence (the proportion of sites in which the species was recorded as present) and may therefore be inappropriate for comparisons of model accuracy between species or regions (McPherson, Jetz & Rogers 2004, Allouche et al. 2006).
- TSS, the true skill statistic (Allouche et al. 2006). TSS is defined as sensitivity + specificity - 1, and corrects for Kappa's dependency on prevalence. TSS compares the number of correct forecasts, minus those attributable to random guessing, to that of a hypothetical set of perfect forecasts. Like kappa, TSS takes into account both omission and commission errors, and success as a result of random guessing, and ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random (Allouche et al. 2006).

An ensemble model was created by first rejecting poorly performing algorithms with relatively extreme low AUC values and then averaging the predictions of the remaining algorithms, weighted by their AUC. To identify poorly performing algorithms, AUC values were converted into modified z-scores based on their difference to the median and the median absolute deviation across all algorithms (Iglewicz & Hoaglin, 1993). Algorithms with $z < -2$ were rejected. In this way, ensemble projections were made for each dataset and then averaged to give an overall suitability, as well as its standard deviation. The projections were then classified into suitable and unsuitable regions using the 'minROCDist' method, which minimizes the distance between the ROC plot and the upper left corner of the plot (point (0,1)).

We also produced limiting factor maps for Europe following Elith et al. (2010). For this, projections were made separately with each individual variable fixed at a near-optimal value. These were chosen as the median values at the occurrence grid cells. Then, the most strongly limiting factors were identified as the one resulting in the highest increase in suitability in each grid cell.

Results

The ensemble model suggested that suitability for *Asterias amurensis* was most strongly determined by Mean bathymetry (bathymean), accounting for 42.9% of variation explained, followed by Growing Degree Days between 5 and 20°C (gdd_5_20) (30.8%), Mean temperature (tempmean_ss) (9.9%), Minimum long-term temperature (tempmin_ss) (7.3%), Maximum long-term temperature (tempmax_ss) (6.1%) and Minimum salinity (salinitymin_ss) (3.1%) (Table 1, Figure 4).

Table 1. Summary of the cross-validation predictive performance (ROC, Kappa, TSS) and variable importance of the fitted model algorithms and the ensemble (AUC-weighted average of the best performing algorithms). Results are the average from models fitted to 10 different background samples of the data.

Algorithm	AUC	Kappa	TSS	Used in the ensemble	Variable importance (%)					
					Mean bathymetry (bathymean)	Growing Degree Days between 5 and 20°C (gdd)	Mean temperature (tempmean_ss)	Minimum long-term temperature (tempmin_ss)	Maximum long-term temperature (tempmax_ss)	Minimum salinity (salinitymin_ss)
GLM	0.987	0.759	0.964	no	33	2	20	27	14	4
GAM	0.994	0.812	0.973	yes	32	1	28	18	16	4
GBM	0.992	0.818	0.966	yes	46	51	0	0	0	2
RF	0.994	0.819	0.976	yes	50	40	2	3	2	3
Maxent	0.884	0.692	0.758	no	56	22	4	6	2	10
Ensemble	0.995	0.820	0.978		43	31	10	7	6	3

Figure 4. Partial response plots from the fitted models. Thin coloured lines show responses from the algorithms in the ensemble, while the thick black line is their ensemble. In each plot, other model variables are held at their median value in the training data. Some of the divergence among algorithms is because of their different treatment of interactions among variables.

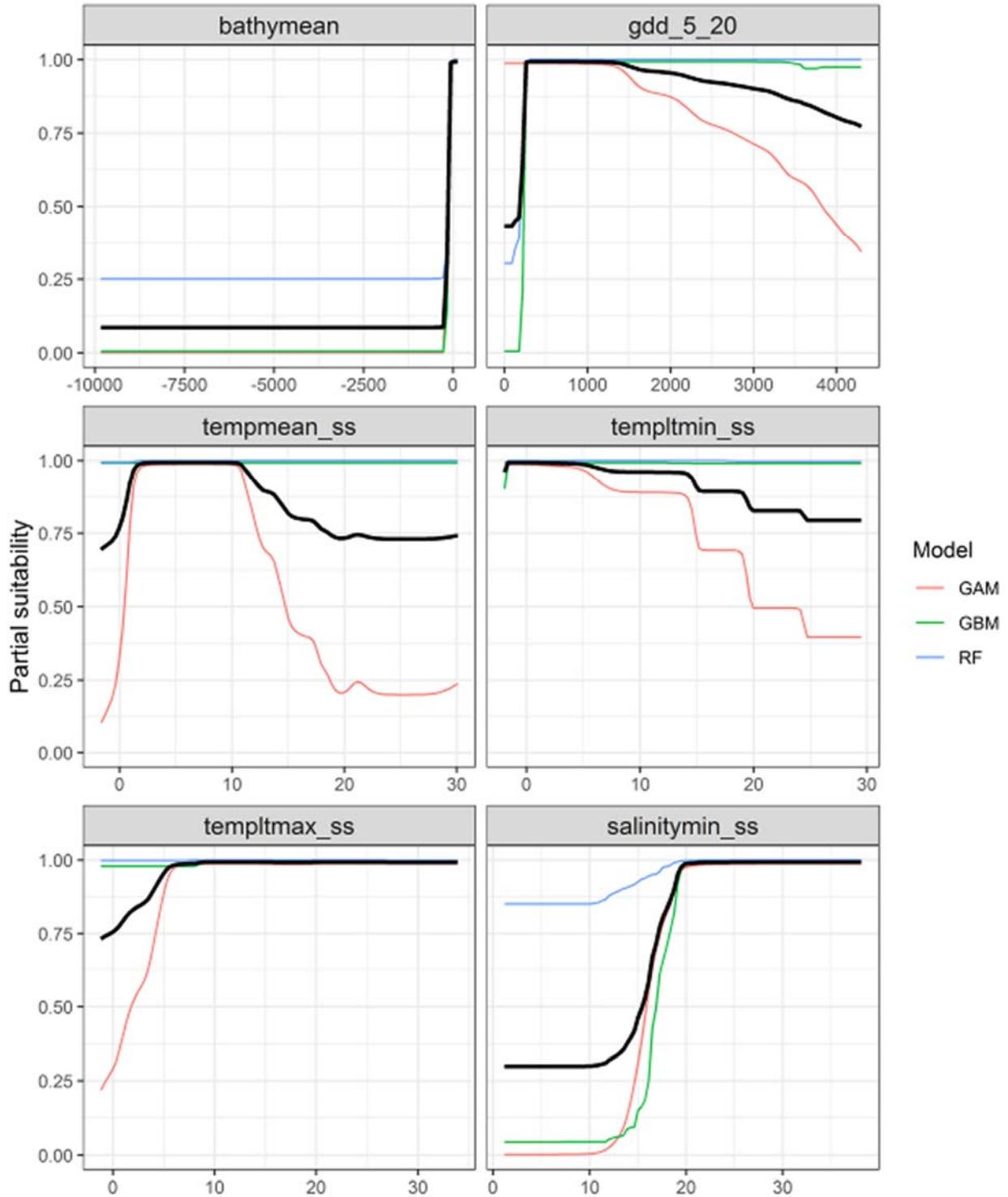
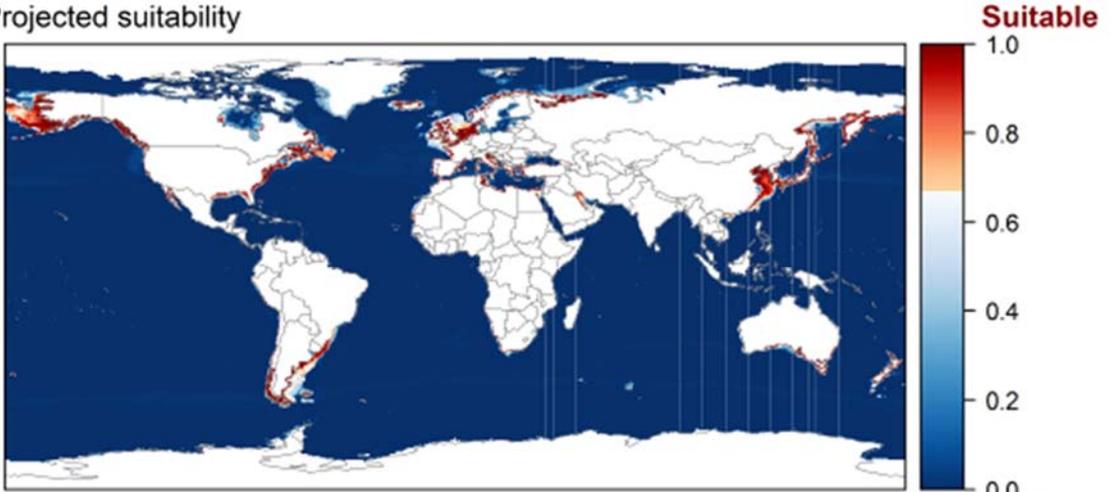


Figure 5. (a) Projected global suitability for *Asterias amurensis* establishment in the current climate. For visualisation, the projection has been aggregated to a 0.5 x 0.5 degree resolution, by taking the maximum suitability of constituent higher resolution grid cells. Values > 0.67 may be suitable for the species. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

(a) Projected suitability



(b) Standard deviation in projected suitability

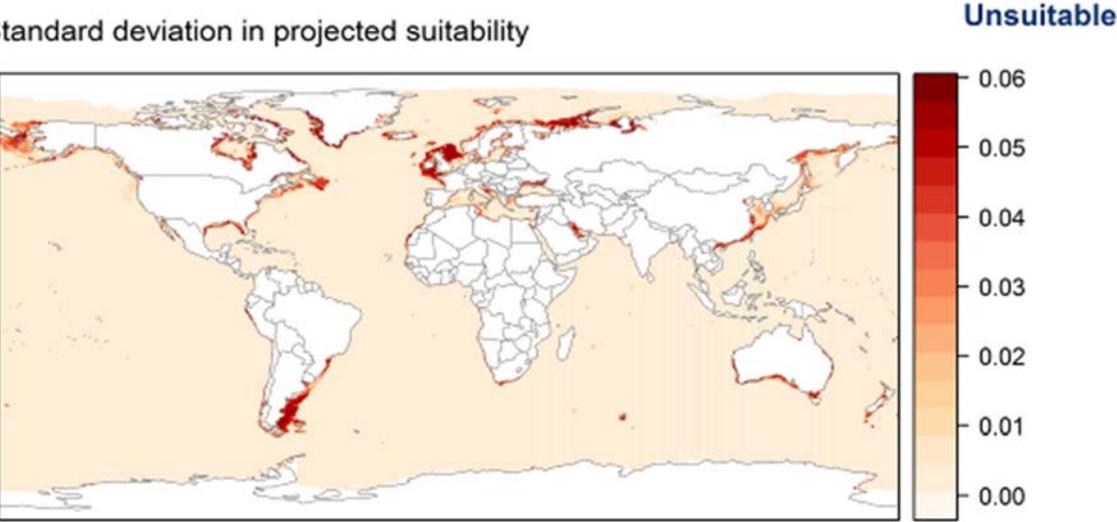


Figure 6. (a) Projected current suitability for *Asterias amurensis* establishment in Europe and the Mediterranean region. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

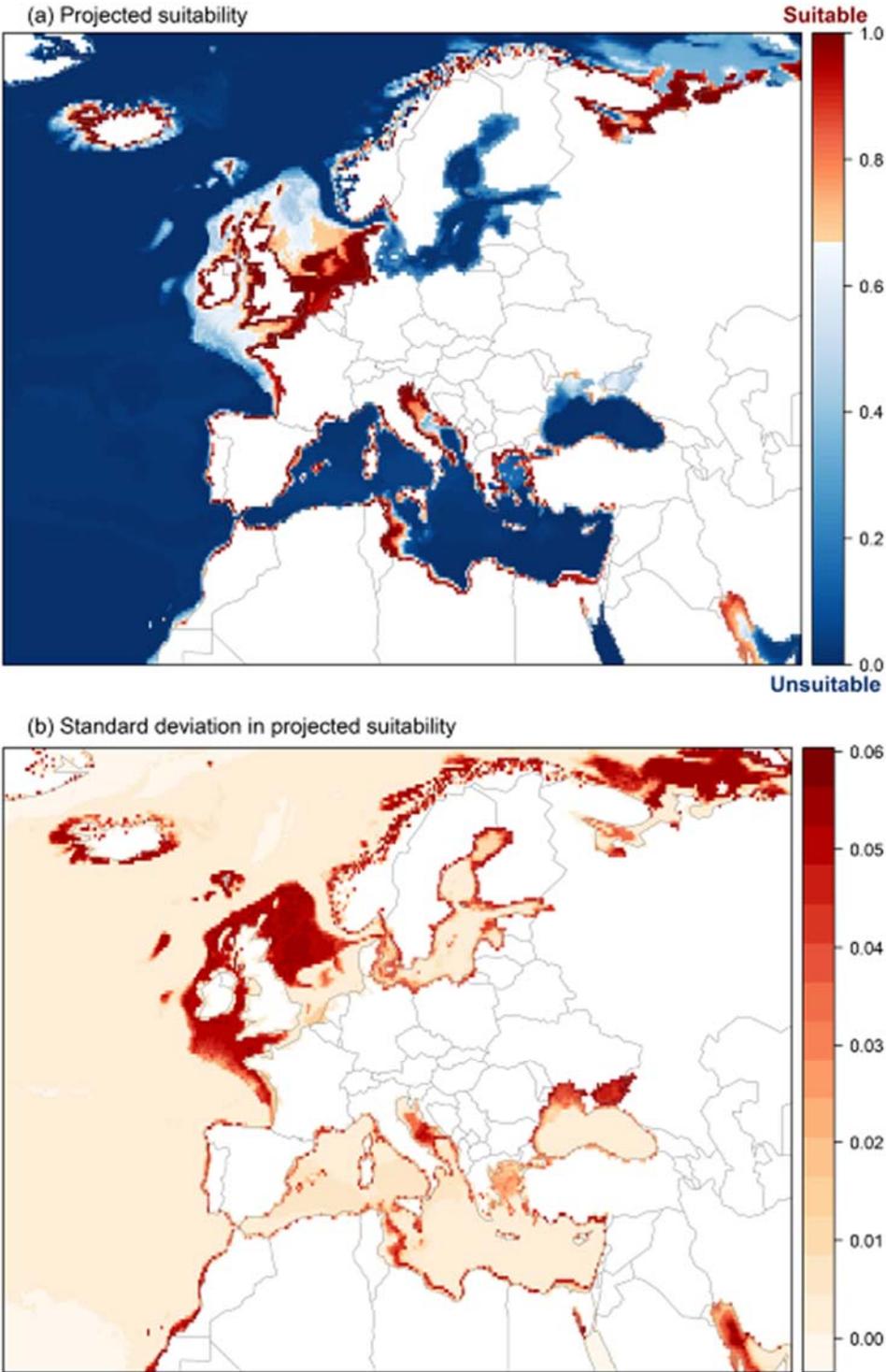


Figure 7. The most strongly limiting factors for *Asterias amurensis* establishment estimated by the model in Europe and the Mediterranean region in current climatic conditions.

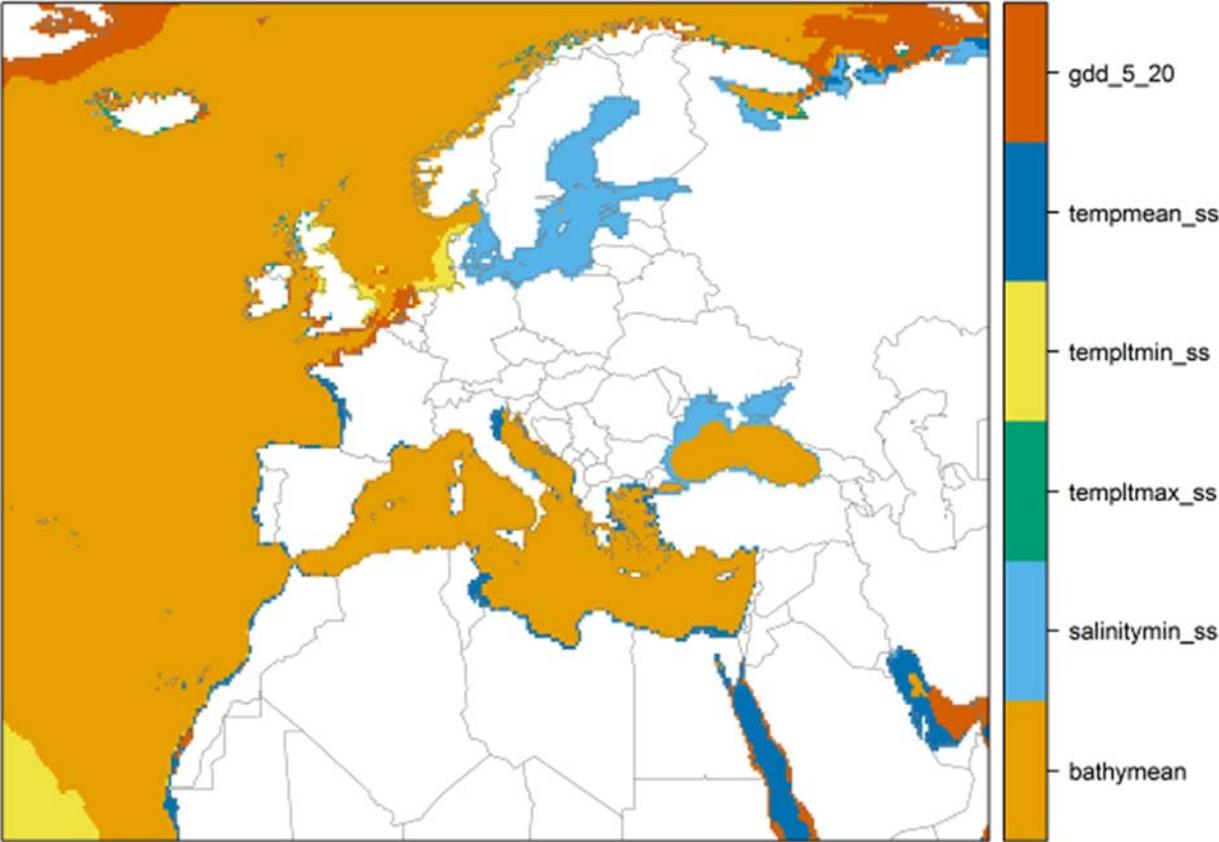


Figure 8. (a) Projected suitability for *Asterias amurensis* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP2.6, equivalent to Figure 6. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

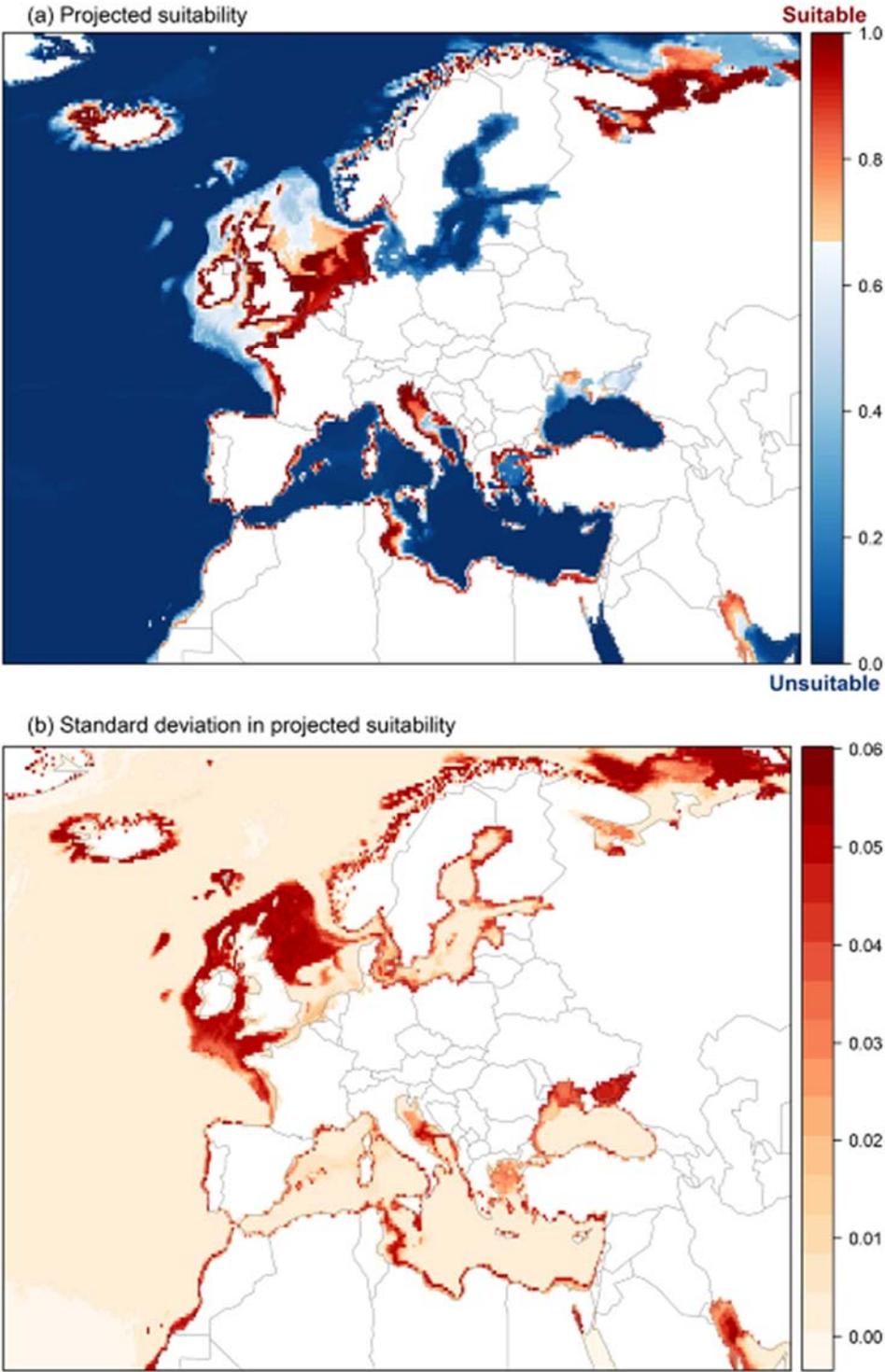


Figure 9. (a) Projected suitability for *Asterias amurensis* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP4.5, equivalent to Figure 6. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

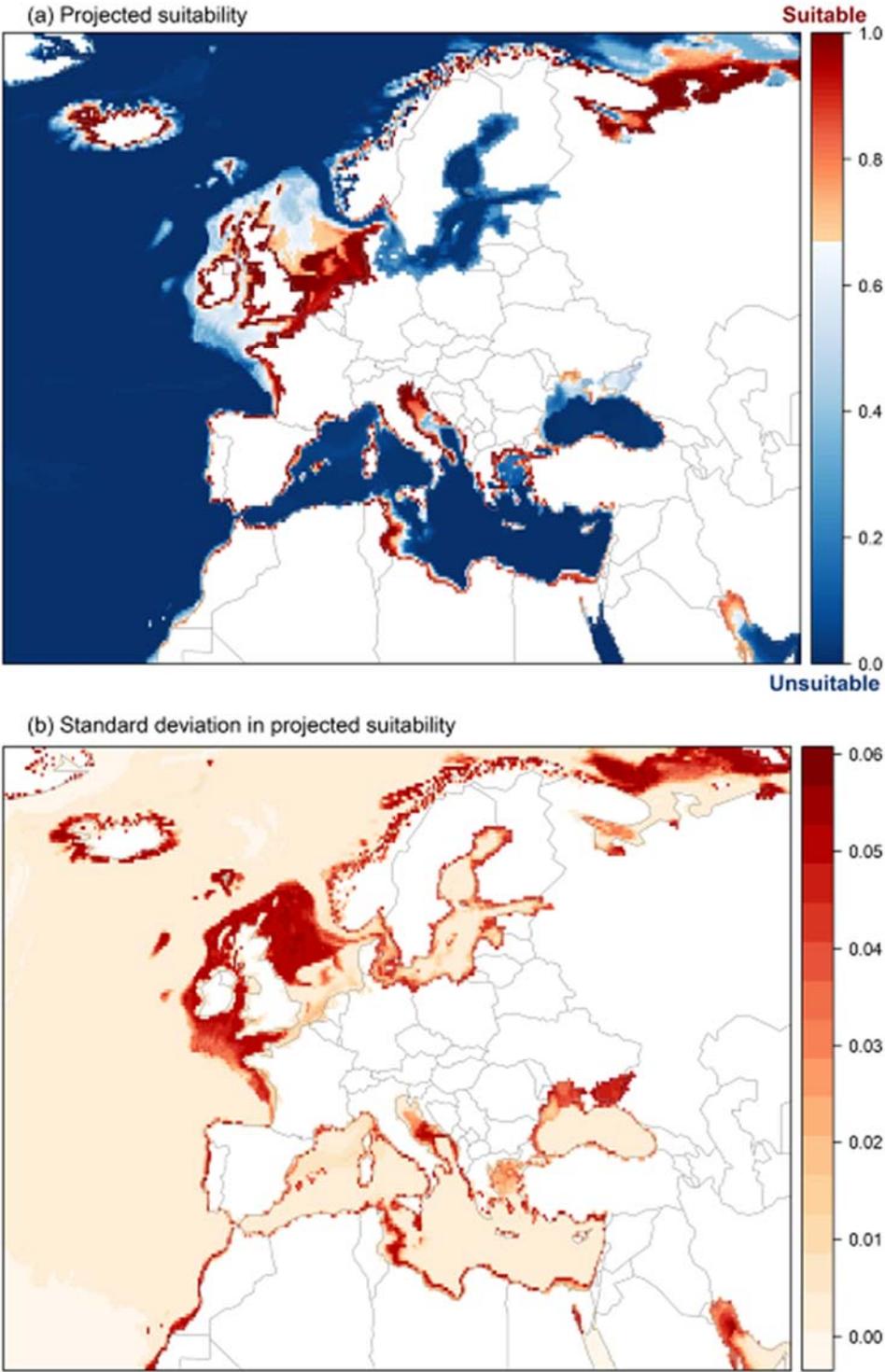


Figure 10. Variation in projected suitability for *Asterias amurensis* establishment among marine subregions of Europe. The bar plots show the proportion of grid cells in each region classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. The location of each region is also shown. Macaronesia is excluded as it is not part of the study area.

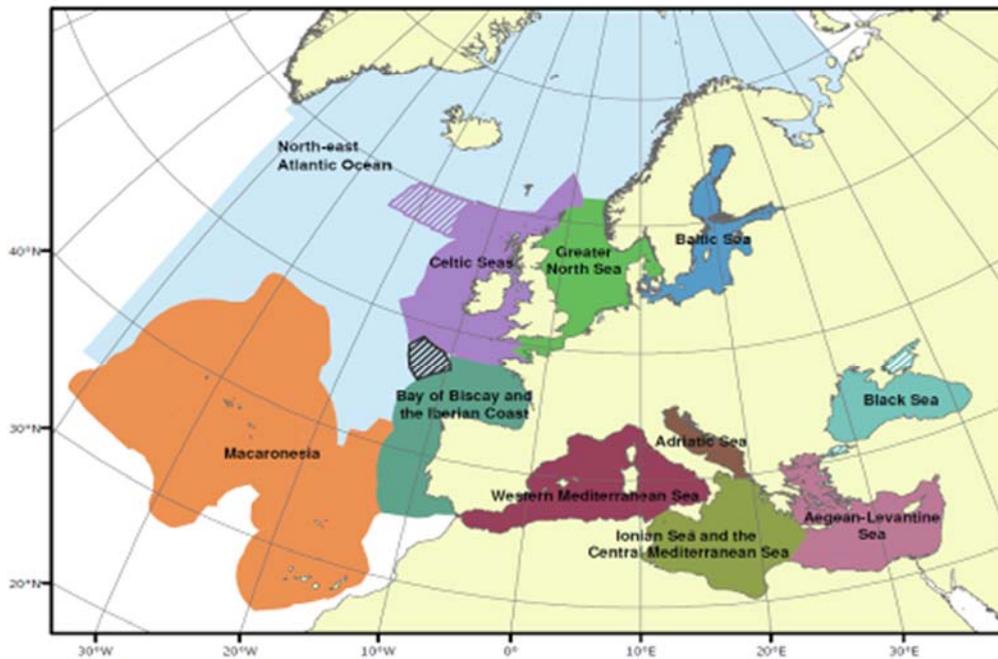
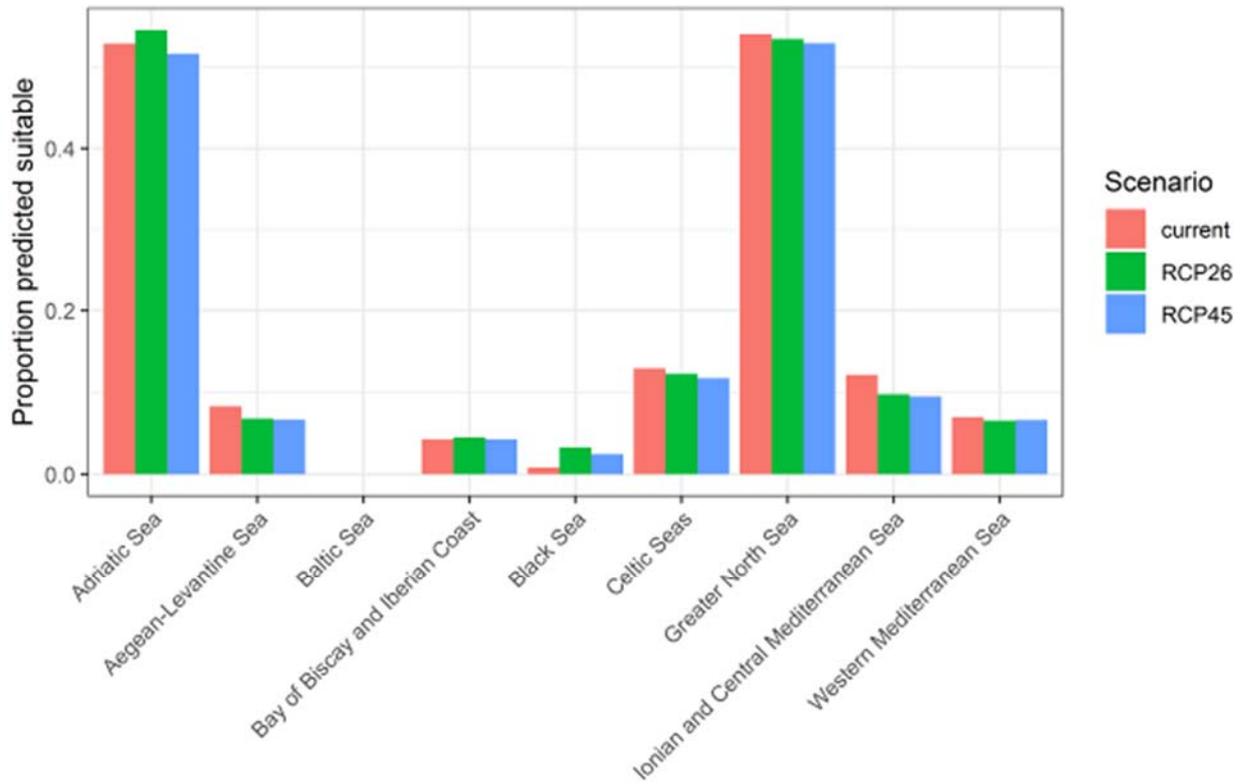


Table 2. Variation in projected suitability for *Asterias amurensis* establishment among marine subregions of Europe (numerical values of Figure 10 above). The numbers are the proportion of grid cells in each region classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios.

Region	Current climate	RCP26	RCP45
Adriatic Sea	0.53	0.55	0.52
Aegean-Levantine Sea	0.08	0.07	0.07
Baltic Sea	0.00	0.00	0.00
Bay of Biscay and Iberian Coast	0.04	0.04	0.04
Black Sea	0.01	0.03	0.02
Celtic Seas	0.13	0.12	0.12
Greater North Sea	0.54	0.53	0.53
Ionian and Central Mediterranean Sea	0.12	0.10	0.10
Western Mediterranean Sea	0.07	0.07	0.07

Figure 11. Variation in projected suitability for *Asterias amurensis* establishment among the 12-nautical-mile national waters of European Union countries and the UK. The bar plots show the proportion of grid cells in each country's waters classified as suitable in the current climate and projected climates for the 2070s under two RCP emissions scenarios. The location of each region is also shown.

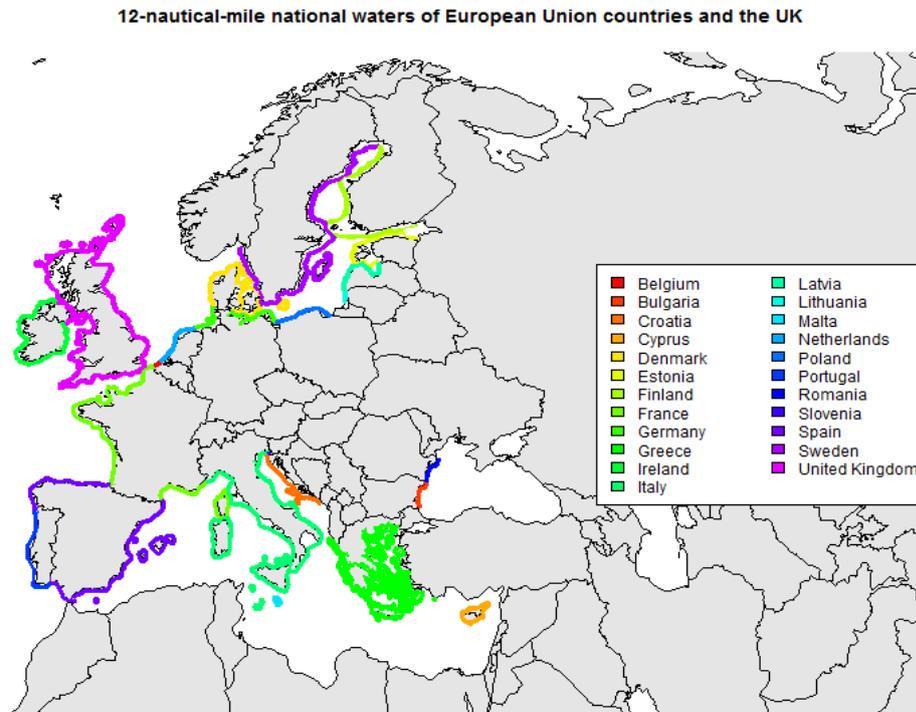
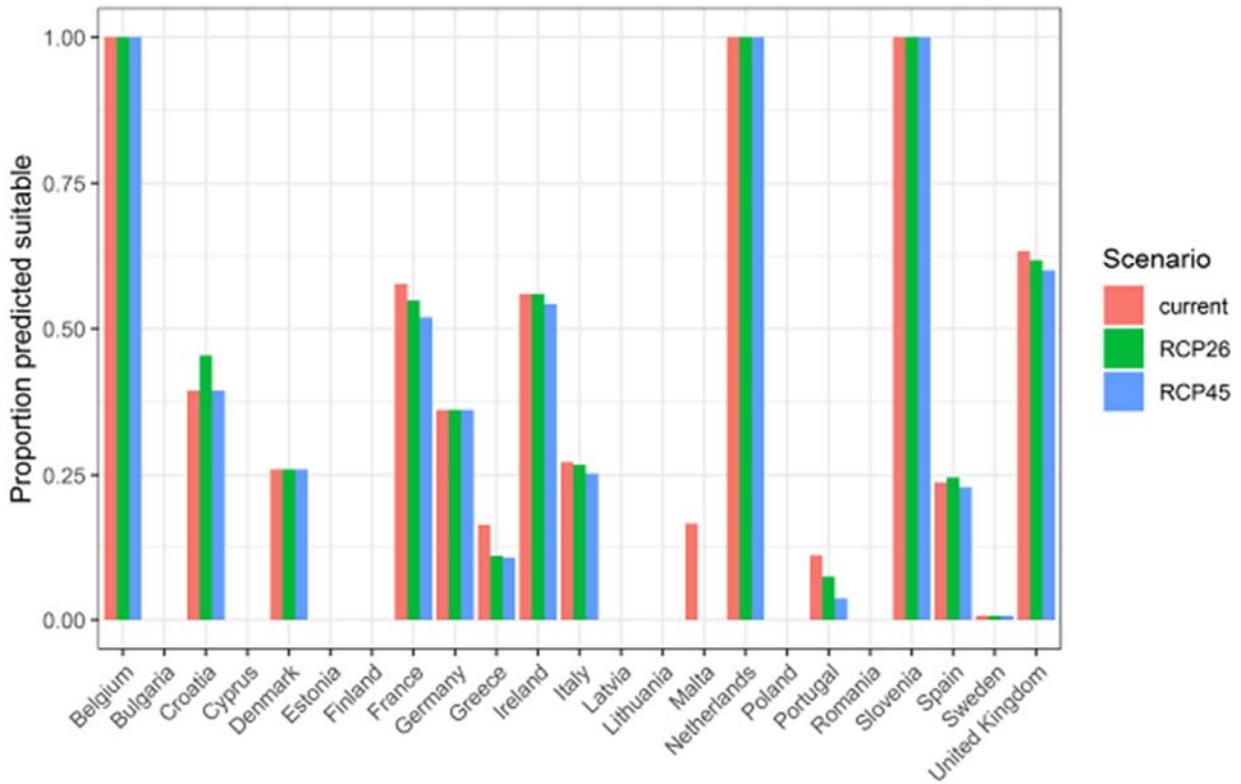


Table 3. Variation in projected suitability for *Asterias amurensis* establishment among 12-nautical-mile national waters of European Union countries and the UK (numerical values of Figure 11 above). The numbers are the proportion of grid cells in each country's waters classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios.

Country	Current climate	RCP26	RCP45
Belgium	1.00	1.00	1.00
Bulgaria	0.00	0.00	0.00
Croatia	0.39	0.45	0.39
Cyprus	0.00	0.00	0.00
Denmark	0.26	0.26	0.26
Estonia	0.00	0.00	0.00
Finland	0.00	0.00	0.00
France	0.58	0.55	0.52
Germany	0.36	0.36	0.36
Greece	0.17	0.11	0.11
Ireland	0.56	0.56	0.54
Italy	0.27	0.27	0.25
Latvia	0.00	0.00	0.00
Lithuania	0.00	0.00	0.00
Malta	0.17	0.00	0.00
Netherlands	1.00	1.00	1.00
Poland	0.00	0.00	0.00
Portugal	0.00	0.00	0.00
Portugal	0.00	0.00	0.00
Portugal	0.11	0.07	0.04
Romania	0.00	0.00	0.00
Slovenia	1.00	1.00	1.00
Spain	0.00	0.00	0.00
Spain	0.24	0.25	0.23
Sweden	0.01	0.01	0.01
United Kingdom	0.63	0.62	0.60

Caveats to the modelling

To remove spatial recording biases, the selection of the background sample from the accessible background was weighted by the density of Asteroidea records on the Global Biodiversity Information Facility (GBIF). While this is preferable to not accounting for recording bias at all, it may not provide the perfect measure of recording bias.

There was substantial variation among modelling algorithms in the partial response plots (Figure 4). In part this will reflect their different treatment of interactions among variables. Since partial plots are made with other variables held at their median, there may be values of a particular variable at which this does not provide a realistic combination of variables to predict from.

Other variables potentially affecting the distribution of the species, such as underwater vegetation were not included in the model.

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**Risk assessment template developed under the "Study on Invasive Alien Species –
Development of risk assessments to tackle priority species and enhance prevention"
Contract No 07.0202/2018/788519/ETU/ENV.D.2¹**

Name of organism: *Axis axis* (Erxleben, 1777)

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Risk Assessment Area: The risk assessment area is the territory of the European Union 27 and the United Kingdom, excluding the EU-outermost regions.

Peer review 1: *Wojciech Solarz, Institute of Nature Conservation, Polish Academy of Sciences, Kraków, Poland*

Peer review 2: *anonymous*

Date of completion: 6 February 2020

Date of revision: 4 March 2021

¹ This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA). A number of amendments have been introduced to ensure compliance with Regulation (EU) 1143/2014 on IAS and relevant legislation, including the Delegated Regulation (EU) 2018/968 of 30 April 2018, supplementing Regulation (EU) No 1143/2014 of the European Parliament and of the Council with regard to risk assessments in relation to invasive alien species (see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>).

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SECTION A – Organism Information and Screening

A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?

including the following elements:

- the taxonomic family, order and class to which the species belongs;
- the scientific name and author of the species, as well as a list of the most common synonym names;
- names used in commerce (if any)
- a list of the most common subspecies, lower taxa, varieties, breeds or hybrids

As a general rule, one risk assessment should be developed for a single species. However, there may be cases where it may be justified to develop one risk assessment covering more than one species (e.g. species belonging to the same genus with comparable or identical features and impact). It shall be clearly stated if the risk assessment covers more than one species, or if it excludes or only includes certain subspecies, lower taxa, hybrids, varieties or breeds (and if so, which subspecies, lower taxa, hybrids, varieties or breeds). Any such choice must be properly justified.

This risk assessment covers one species, axis deer *Axis axis* (Erxleben, 1777), also known as chital, cheetal, spotted deer or Indian spotted deer (Class: Mammalia, Order: Artiodactyla, Family: Cervidae, Subfamily: Cervinae, Genus: *Axis*).

Synonym(s): *Cervus axis* Erxleben, 1777

According to Wilson and Reeder (2005) the genus *Axis* includes three species:

- *A. axis* in India (including Sikkim), Nepal, and Sri Lanka (plus a number of countries where the species is alien, see details in point A5 below);
- the Calamian deer *A. calamaniensis* (Heude, 1888), found in the Calamian Islands in the Philippines;
- the Indian hog deer *A. porcinus* (Zimmermann, 1780), known from Bangladesh, Burma, Cambodia, China, India, Laos, Nepal, Pakistan, Sri Lanka (perhaps introduced), and Vietnam, with introduced populations in Australia and South Africa.

Wilson and Mittermeier (2011) further include the Bawean deer *Axis kuhlii* (Temminck, 1836) in the genus, which other authors include in the genus *Hyelaphus*.

No subspecies of *A. axis* is recognised by Wilson and Reeder (2005) and Wilson and Mittermeier (2011).

There are no hybrids known to occur in the wild, however, as this cannot be completely excluded, as a precaution this risk assessment includes all *A. axis* hybrids. Attempts to cross axis deer with sika deer (*Cervus nippon*) by artificial insemination are reported (Asher et al 1999). One recorded case of hybridization arising from natural mating between sika deer and axis deer is also reported by Asher et al. (1999). In this case, the widest cross yet observed within the subfamily Cervinae, a hind exhibiting physical characteristics intermediate between the two species was born on a Tennessee deer farm sometime in 1995. Electrophoresis

analysis initially verified that hybridization had occurred, but fertility of the hybrid remained to be assessed. The potential for hybridization between axis deer and fallow deer (*Dama dama*) was explored by Willard et al. (2005), also using artificial insemination; in this case, reciprocal hybridization of the two species did not result in the establishment of hybrid pregnancies. Although anecdotal and undocumented accounts for the existence of such hybrids were reported (e.g. between sika deer and axis deer, see Bartos 2009), hybridization between these two species appears unlikely under natural conditions (Willard et al. 2005).

A2. Provide information on the existence of other species that look very similar [that may be detected in the risk assessment area, either in the environment, in confinement or associated with a pathway of introduction]

Include both native and non-native species that could be confused with the species being assessed, including the following elements:

- other alien species with similar invasive characteristics, to be avoided as substitute species (in this case preparing a risk assessment for more than one species together may be considered);
- other alien species without similar invasive characteristics, potential substitute species;
- native species, potential misidentification and mis-targeting

The axis deer is a moderately large deer standing 88-97cm at the shoulders. Hinds are generally smaller than stags, which may weight up to 113 kg (and even over 136 kg in farms, see Centore 2016). Antlers are about 76 cm long and take roughly five months to fully develop (they are present only on stags). The species is characterised by a reddish brown coat covered with typical small white spots (retained at all ages and all seasons), arranged on the lower flanks in longitudinal rows. Under parts are white, as well as inner hind legs and under tail. A dorsal dark stripe is present from the nape to the tip of the tail (for further details see descriptions in Wilson and Mittermeier 2011, GISD 2015, Long 2003, Prater 1965). All the features mentioned above are useful to distinguish this species from other native deer in the risk assessment area. Otherwise only the prominent white throat is absolutely distinctive, because axis deer are in other respects not easy to distinguish (from superficial observation) from fallow deer or some spotted subspecies of sika. For example, in fallow deer younger bucks and does (especially of so-called ‘common’ or ‘menil’ coloration) do not have the distinctive palmated antlers which are typical of mature bucks, therefore they might be confused with axis deer in fleeting observation. Likewise, some colour variants of sika deer and pure Japanese sika deer in summer coat could be confused. Both common-coloured fallow deer, and sika deer, also have a darker dorsal stripe and dark line extending down the tail. Albino animals are occasionally reported (Dinesan et al 2006, Leo Prabu et al. 2013).

A3. Does a relevant earlier risk assessment exist? Give details of any previous risk assessment, including the final scores and its validity in relation to the risk assessment area.

A risk assessment for the axis deer exists for Poland (Okarma et al. 2018a). The result shows that the risk for the country is considered “Medium” and the species is considered moderately invasive on the ground of its impact on the environment. In relation to the risk assessment area, this result may be considered valid particularly for the Continental biogeographic region.

The species was also assessed by Nentwig et al. (2018) according to whom out of 486 alien species established in Europe from a wide range of taxonomic groups, the axis deer ranked 31, among those with the highest environmental and socioeconomic impact (following the generic impact scoring system GISS, as calculated by Nentwig et al. 2010).

In Australia, a risk assessment for the species was made in Western Australia. The risk of establishing populations in the wild and the risk of becoming a pest have been assessed as “extreme” (Massam et al. 2010, Page et al. 2008). The map of Australia included in the risk assessment shows the partial suitability of the Mediterranean climate area for the species (Page et al. 2008). In Western Australia the species (either captive or released animals) was also considered as “moderately dangerous” in relation to public safety, e.g. in relation to the potential for zoonoses, deer-vehicle collisions, injuries following aggressive behaviour (Massam et al. 2010, Page et al. 2008).

A4. Where is the organism native?

including the following elements:

- an indication of the continent or part of a continent, climatic zone and habitat where the species is naturally occurring
- if applicable, indicate whether the species could naturally spread into the risk assessment area

The axis deer is a tropical or sub-tropical species, native to Asia, endemic of the Indian subcontinent, i.e. India (including Sikkim), Nepal, Bhutan, Bangladesh and Sri Lanka (Long 2003, Wilson and Reader 2005, Duckworth et al. 2015).

Axis deer is typical of the grassland-forest ecotone (Wilson and Mittermeier 2011). As summarised by Duckworth et al. (2015) the axis deer thrive in a wide range of habitats throughout its native range (see also Moe and Wegge 1994), but prefers moist and dry deciduous forest near water, interspersed with dry thorn scrublands or grasslands (Eisenberg and Seidensticker 1976). Mangrove forests (Sankar and Acharya 2004), mixed forests or plantations (with Teak *Tectona grandis* and Sal *Shorea robusta*) (Wilson and Mittermeier 2011) and agricultural crops such as coffee areas, are used too (Bali et al. 2007). This species lives mostly in flat areas and at lower elevations, usually below 1000 m, avoiding slopes, hills and mountain areas, but has also been found at high elevations (2,209 m) in India (Wilson and Mittermeier 2011, Duckworth et al. 2015, Schaller 1967, Deepan et al. 2018).

In general, this species avoids extreme habitats such as open semi-desert or desert, dense moist (evergreen) forests but introduced populations show some flexibility in this regard. For example, animals in the Andaman Islands are found in dense evergreen forests (Ali 2004, Sankar and Acharya 2004) and in Hawaii they are found in areas ranging from semi-deserts to rainforest (Moe and Wegge 1994), up to 2150 m (Waring 1996).

The native range is characterized by significant seasonal changes in temperature and, more importantly, extreme swings in precipitation (Anderson 1999), but axis deer have adapted very well to the European eco-climatic zones. For example, the typical habitat occupied in Croatia is represented by scrublands and woodlands of Euro-Mediterranean vegetation (Centore et al. 2018), while in Russia the species was successfully introduced to an area south of Moscow, characterized by deciduous and mixed forests with oak and undergrowth of spindle tree, buckthorn, dogwood, and other shrubs (Bobrov et al. 2008). In Croatia, the species could not adapt to continental climate (Kusak and Krapinec 2010).

Four key factors were identified as delineating the axis deer's distribution: (1) the need for water; (2) the need for shade; (3) an avoidance of high, rugged terrain; and (4) a preference for grass as forage (Schaller 1967, Kushwaha 2018). Habitat use varies seasonally, reflecting food availability (see also Centore 2016). The axis deer easily habituates to human presence, and herds often congregate in open areas near habitation or forest camps to spend the night, possibly due to greater safety from predators and poachers (Duckworth et al. 2015). In fact, the limiting factor seems to be winter conditions, particularly strong frosts and thick snow cover (Okarma et al. 2018b).

A5. What is the global non-native distribution of the organism outside the risk assessment area?

Outside the risk assessment area, the axis deer was successfully introduced in the following countries:

- Europe (Russia, and possibly Moldova and Ukraine, but see below);
- Asia (Armenia, Azerbaijan, Andaman Islands, Pakistan);
- North America (USA: California, Florida, Texas and Hawaiian Islands, México);
- South America (Argentina, Brazil, Uruguay);
- Australia

For details, see Long (2003), Lever (1985), Wilson and Reader (2005), Duckworth et al. (2015), Wilson and Mittermeier (2011), Álvarez-Romero and Medellín (2005). According to Šprem and Zachos (2020) introductions to Ukraine and Moldova (as well as to the British Isles) are sometimes mentioned, but there is no reliable information available about this or any free-living populations resulting from these alleged introductions.

The species is also present in South Africa (<https://www.invasives.org.za/legislation/item/709-axis-deer-axis-axis>, accessed on 26/07/2019).

Axis deer were considered as introduced without success in New Zealand and New Guinea by Long (2003), while Forsyth and Duncan (2001) considered the introduction of this species as “successful” in New Zealand, because the species had a self-sustaining wild population before being eradicated by hunting. This shows that axis deer could have persisted in the climatic and environmental conditions in this country.

A6. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species been recorded and where is it established? The information needs to be given separately for recorded and established occurrences.

A6a. Recorded: List regions

A6b. Established: List regions

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

- Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea.

Comment on the sources of information on which the response is based and discuss any uncertainty in the response.

For delimitation of EU biogeographical regions please refer to <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2> (see also Annex V).

For delimitation of EU marine regions and subregions consider the Marine Strategy Framework Directive areas; please refer to <https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions/technical-document/pdf> (see also Annex V).

(6a): Alpine, Continental, Mediterranean

(6b): Mediterranean

The source of information on which the response is based can be found in Qu. A8.

A7. In which biogeographic region(s) or marine subregion(s) in the risk assessment area could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A7a. Current climate: List regions

A7b. Future climate: List regions

With regard to EU biogeographic and marine (sub)regions, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

(7a):

Boreal, Continental, Mediterranean, Alpine, Atlantic, Black Sea, Pannonian, Steppic (see details in Annex VII).

(7b):

Boreal, Continental, Mediterranean, Alpine, Atlantic, Black Sea, Pannonian, Steppic (see details in Annex VII).

A8. In which EU Member States has the species been recorded and in which EU Member States has it established? List them with an indication of the timeline of

observations. The information needs be given separately for recorded and established occurrences.

A8a. Recorded: List Member States

A8b. Established: List Member States

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden

The description of the invasion history of the species shall include information on countries invaded and an indication of the timeline of the first observations, establishment and spread.

(8a): Czech Republic, France, Ireland, Slovenia; and the United Kingdom

Attempts to introduce axis deer were made as early as 1890 in France, but they did not succeed (Dorst and Giban 1954, Lever 1985).

Axis deer was introduced to west-central Slovenia (from the Brijuni islands) in the late 1940s or in 1950, but this introduction failed (one stag, shot on 12 October 1950 is now in the Natural History Museum of Slovenia, see Duckworth et al. 2015). The species is considered as extinct in Slovenia (Mitchell-Jones et al. 1999).

According to Long (2003) axis deer were reported to be feral in Buckinghamshire, England, in 1944-45, but there is no evidence that they have been present outside a deer park and there is only one record of an escape by a single animal (hence it is dubious whether any population persisted in the wild). According to Fitter (1959) there were a number of reports of individual axis deer in England, but no evidence of breeding (one individual was shot in 1888 in West Sussex, and other animals were seen in 1944-45 in Combe at about the same time in other counties too). According to Šprem and Zachos (2020) introductions to the British Isles are sometimes mentioned, but there is no reliable information available about this or any free-living populations resulting from these alleged introductions.

Occasional records are available also for the following countries:

- Czech Republic: the species was considered as present in game reserves in the Czech Republic as early as 1850 (Mlíkovský and Stýblo 2006) but is now considered extinct (Nobanis 2019).
- Ireland (Fairley 1975).

(8b): Croatia (introduced in 1911)

The only wild populations in the EU are in Croatia, on the islands of Brijuni and Dugi Otok (Šprem and Zachos 2020, Linnell and Zachos 2011, Duckworth et al. 2015).

Axis deer are present on the Veliki Brijun island, the largest island in the Brijuni archipelago. Animals are free on the island, which has a surface of 560 ha. Despite the fact that axis deer are described as capable swimmers (Nowak 1991), Axis deer has never been seen swimming from one island to another, unlike fallow deer (*Dama dama*), so it is considered that the population is restricted in these 560 ha (Public Institution National Park Brijuni and Hunting Directorate of the Ministry of Agriculture, pers. comm. 2020). However, according to Kusak and Krapinec (2010) some animals are observed to swim sometimes from Brijuni to the mainland in the estuary of Mirna river. Also Šprem and Zachos (2020) mention that several cases had been reported of axis deer swimming from Brijuni Islands to the mainland (ca. 3 km), but establishment of new populations was unsuccessful. See also Qu. 4.1.

According to Centore (2016), the first introduction in Croatia dates back to 1911, when several individuals were introduced into Brijuni island from Germany (Šprem et al. 2008). The genetic origin of the introduced animals, however, is unknown (Kusak and Krapinec 2010). According to Long (2003) the population derived from animals which escaped from captivity in 1911 and have increased in numbers substantially. In 2008 the population in the Brijuni National Park reached about 100 individuals, was considered stable and rather numerous (Šprem et al. 2008). According to Šprem and Zachos (2020), some 150 individuals were present in the islands of Brijuni in 2017 (but the same authors also stated that up to 200 animals are removed each year for population control, which may create some confusion about the actual population size in the island). As of April 1, 2019, 76 animals were present according to the Hunting Directorate of the Ministry of Agriculture (Public Institution National Park Brijuni and Hunting Directorate of the Ministry of Agriculture, pers. comm. 2020).

In the area of Dugi Otok island, axis deer is currently present in two hunting grounds: a common open hunting ground number: XIII / 107 " DUGI OTOK – ISTOK" in which the number of axis deer is estimated at 10 individuals, and the state open hunting ground number: XIII / 4 " DUGI OTOK" in which the number is estimated at 12 individuals (as of April 1, 2019). Axis deer in these hunting grounds are not managed, but are removed from the wild in accordance to the relevant legislation (Public Institution National Park Brijuni and Hunting Directorate of the Ministry of Agriculture, pers. comm. 2020). According to Šprem and Zachos (2020) the existence of the axis deer population on the island of Dugi Otok originated in 2012 by 13 individuals escaped from a fenced area from the Brijuni Islands, and increased to about 60 individuals in 2018.

Additional introductions with animals from Brijuni were made in other parts of Croatia but did not succeed. For example, in 1953 the population introduced in the island of Cres declined gradually over the years, the last specimen being recorded in the early 1990s (Frković 2014). The species is considered well established in Brijuni, and according to Centore (2016) survived until the present day due to the favourable climate.

Lever (1985) and Long (2003) report the introduction of two dozen axis deer released in Lithuania in 1954 (which reportedly adapted well and increased to 67 by 1961). However, this information seems not correct, and may well refer to sika deer (*Cervus nippon*) (see for example relevant information on Baleišis et al. 2003), in fact no information was found that axis deer has been ever introduced in Lithuania (Viktorija Maceikaite, pers. comm. 2019). In any case, no mention is made on the species in the review for Baltic countries made by Andersone-Lilley et al. (2010).

A9. In which EU Member States could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A9a. Current climate: List Member States

A9b. Future climate: List Member States

With regard to EU Member States, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)

- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

(9a):

Austria, Belgium, Bulgaria, Croatia, Republic of Cyprus, Czech Republic, Denmark, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden; and the United Kingdom (see details in Annex VII).

(9b):

Austria, Belgium, Bulgaria, Croatia, Republic of Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden; and the United Kingdom (see details in Annex VII).

A10. Is the organism known to be invasive (i.e. to threaten or adversely impact upon biodiversity and related ecosystem services) anywhere outside the risk assessment area?

The species is known to be invasive in several countries outside the risk assessment area (see review in Okarma et al. 2018b). For example, in its alien range it is considered invasive in the Andamane Islands (Banerji 1955, Ali and Pelkey 2013, Mohanty et al 2016) and the US, i.e. in Hawaii (Anderson 1999, GISD 2015) and Texas (Long 2003), as well as in Argentina (Flueck 2009), and Russia (Bobrov et al. 2008).

A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea

Mediterranean: see answer to A12.

A12. In which EU Member States has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden

Croatia: At high population density axis deer was reported to cause significant damage in gardens, orchards and vineyards (Frković 2014). The species is mentioned as invasive in Croatia by CABI database (<https://www.cabi.org/isc/datasheet/89941>) but very little information was found on the impact on biodiversity. As reported by the Public Institution National Park Brijuni and Hunting Directorate of the Ministry of Agriculture (pers. comm. 2020), it is difficult to say how much impact the axis deer itself has on the biodiversity of Brijuni National Park, but it is certain that mouflon and deer species significantly affect the biodiversity of Veliki Brijun islands where axis deer, mouflon and fallow deer are present. In the past, axis deer dominated over the other two species, but due to one harsh winter in the past many died and fallow deer has since prevailed. As the Public Institution National Park Brijuni has been reducing the number of deer specimens in recent years, currently mouflons are predominant. All three species together have a great impact on grasslands and forests of the island Veliki Brijun. Browsing and grazing of large herbivores that live on the island without natural predators affect lower layers of forests causing a problem for the natural reforestation and affect biodiversity of grassland allowing plants that the animals avoid (for example the Spanish oyster thistle *Scolymus hispanicus*) to overly spread.

According to the few data available from literature (see Šprem et al. 2008) the axis deer forages on *Fraxinus ornus*, *Quercus ilex* leaves and acorns, and sometimes browses the areas of *Myrtus communis*, new stems of blackberry (*Rubus spp.*), moss growing on rocks and cedar (*Cedrus spp.*) seeds. However, axis deer on the Brijuni Islands regularly consume supplementary feed such as hay and corn, regardless of the quality of the grassy areas (Šprem and Zachos 2020), therefore is likely that this prevents the species from having a greater (visible) impact on the island ecosystem. The evidence of higher impact may also be hidden by the fact that the populations in Croatia are all controlled through hunting (see Šprem and Zachos 2020). Always according to Šprem and Zachos (2020) axis deer impact on forest regeneration is less than other ungulates (i.e. European mouflon), but both terminal and lateral shoots are damaged.

A13. Describe any known socio-economic benefits of the organism.

including the following elements:

- Description of known uses for the species, including a list and description of known uses in the Union and third countries, if relevant.
- Description of social and economic benefits deriving from those uses, including a description of the environmental, social and economic relevance of each of those uses and an indication of associated beneficiaries, quantitatively and/or qualitatively depending on what information is available.

If the information available is not sufficient to provide a description of those benefits for the entire risk assessment area, qualitative data or different case studies from across the Union or third countries shall be used, if available.

The axis deer is considered by some as amongst the most beautiful of all deer (Prater 1965) and this may explain the popularity in zoological gardens and parks around the world (Schaller 1967, Sankar and Acharya 2004). According to the European Association of Zoos and Aquaria, about 625 specimens were kept by zoos across 12 EU Member States² and the United Kingdom in October 2019 (EAZA, pers. comm. 2019). These numbers concern only zoos that are members of EAZA and can only provide an indication about the situation across the EU.

As summarised by GISD (2015) the meat of axis deer (venison) is highly regarded as it is extremely lean. It consistently ranks in the top ten of all venison in the world (Anderson 1999). As a result, there is an economic value for the meat.

The axis deer is also a prized hunting quarry, owing to its beauty, especially stags with antlers longer than 76 cm (although it was considered as an unattractive trophy animal in Croatia by Frković, 2014). Recreational deer hunting can thus provide both tangible and intangible social benefits (Jesser 2005). Many game ranches receive upwards of US\$1000 for each trophy stag taken (Anderson 1999). In South Africa the costs for a trophy fee is €2,500 (<http://www.fgsafaris.com/PriceList.htm>, accessed on 26/07/2019). Poaching and black-market sales are common wherever the species occurs (Anderson 1999), and some documented evidence of skins and antlers seized from wildlife smugglers is available for India (TRAFFIC 2017).

² Austria, Denmark, Czechia, Estonia, France, Germany, Hungary, Italy, Netherlands, Portugal, Spain, Sweden.

SECTION B – Detailed assessment

Important instructions:

- In the case of lack of information the assessors are requested to use a standardized answer: “No information has been found.”
- With regard to the scoring of the likelihood of events or the magnitude of impacts see Annexes I and II.
- With regard to the confidence levels, see Annex III.
- Highlight the selected response score and confidence level in **bold** but keep the other scores in normal text (so that the selected score is evident in the final document).

1 PROBABILITY OF INTRODUCTION

Important instructions:

- **Introduction** is the movement of the species into the risk assessment area (it may be either in captive conditions and/or in the environment, depending on the relevant pathways).
- **Entry** is the release/escape/arrival in the environment, i.e. occurrence in the wild and is treated in the next section (N.B. introduction and entry may coincide for species entering through pathways such as “corridor” or “unaided”).
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used. For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document³ and the provided key to pathways⁴.
- For organisms which are already present in the risk assessment area, only complete this section for current active pathways and, if relevant, potential future pathways.

Qu. 1.1. List relevant pathways through which the organism could be introduced. Where possible give details about the specific origins and end points of the pathways as well as a description of any associated commodities.

For each pathway answer questions 1.2 to 1.7 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 1.2a, 1.3a, etc. and then 1.2b, 1.3b etc. for the next pathway.

In this context a pathway is the route or mechanism of introduction of the species.

The description of commodities with which the introduction of the species is generally associated shall include a list and description of commodities with an indication of associated risks (e.g. the volume of trade; the likelihood of a commodity being contaminated or acting as vector).

If there are no active pathways or potential future pathways this should be stated explicitly

³ <https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf>

⁴ <https://circabc.europa.eu/sd/a/Oaeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf>

here, and there is no need to answer the questions 1.2-1.9

The following active pathways of introduction have been identified in the risk assessment area:

- a) Hunting (Release in nature)
- b) Farmed animals (including animals left under limited control) (Escape from confinement)
- c) Botanical garden/zoo/aquaria (excluding domestic aquaria) (Escape from confinement)

Another pathway known for the axis deer is “Landscape / flora / fauna “improvement” in the wild (release in nature)”. However, this pathway is only known in regions other than Europe (no evidence was available for the risk assessment area). For example, in Australia, the establishment of wild deer populations began in the mid-1800s, when Acclimatisation Societies released deer for hunting or for aesthetic reasons (Moriarty 2004, Long 2003, Davis et al. 2016). The species was introduced as ornamental also in Argentina (Novillo and Ojeda 2008). As this pathway is considered not active in the risk assessment area, it is not considered further in this document.

The “natural spread” of individuals from neighbouring countries, e.g. Moldova, Russia, Ukraine (Long 2003, Duckworth et al. 2015) is another possibility. The likelihood of the species appearing in the natural environment of Poland as a result of expansion from Ukraine (near Dnipropetrovsk and in the Volga region, i.e. over 1000 km from the Polish border), however, was considered very low within the next 15 years (Okarma et al. 2018b). Moreover, the occurrence of any free-living population in Ukraine and Moldova is considered questionable by Šprem and Zachos (2020). Therefore, also this pathway, is not considered active in the risk assessment area, and not considered further in this document. Similarly, it is likely that some animals are kept as pets by private owners, for example in Croatia (according to the Public Institution National Park Brijuni and Hunting Directorate of the Ministry of Agriculture, pers. comm. 2020) and in France (where according to JF Maillard, OFB/UPADE, as pointed out in a note to the EC, it must be rarely present with private owners due to administrative constraints, hence the risk exists but it must be low). However, as no escapes are reported from this pathway, this is not considered active in the risk assessment area, and not considered further in this document.

a) Hunting (release in nature)

Qu. 1.2a. Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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This pathway refers to animals introduced into the risk assessment area to be hunted for food and/or to provide recreational hunting opportunities (including collection of hunting trophies). This is the typical pathway of introduction also in other regions, for example in Ukraine (Page et al. 2008), USA (GISD 2015), Argentina (Carpinetti and Merino 2000, Novillo and Ojeda 2008), Andamane Islands (Long 2003, Ali and Pelkey 2013, Banerji 1955), Australia (Massam et al. 2010, Moriarty 2004) and South Africa

(<https://www.invasives.org.za/legislation/item/709-axis-deer-axis-axis>, accessed on 26/07/2019).

Qu. 1.3a. How likely is it that large numbers of the organism will be introduced through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in introduction whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Based on evidence relevant to past events, it is possible to expect further introductions and translocations of this species motivated by hunting purposes. Introduction is not expected to take place with large quantities (e.g. hundreds) of animals at one time. It would be expected, however, to be a number large enough to establish viable wild populations (considering that a number just above 7 animals is considered sufficient, see Qu. 2.3a.).

Several introductions occurred in Croatia, despite the unsuccessful result. As summarised by Frković (2014) as part of an extensive programme of introductions to continental hunting grounds, axis deer were brought from the Brijuni islands into several sites in the Croatian Littoral in 1953. A number of factors, such as the inadequately organized capture and transport of the animals, the insufficient preparation of the introduction sites, the poor adaptation of the animals to new habitat conditions, the inability to roam, and the calf mortality in winter season, led to the failure of such introductions. The only site where the number of the introduced axis deer increased was in Punta Križa (island of Cres), where it was hunted as early as in 1955. However, due to the damage it inflicted to vineyards and households, the axis deer was hunted freely without any protection for several years (1965–1970). When the more attractive mouflon (*Ovis musimon*) and fallow deer (*Dama dama*) were introduced to the area in 1962 and 1966, the axis deer population of Punta Križa gradually declined over the years, so that the last specimen was recorded in the early 1990s. Therefore, the only wild populations still present in Croatia are those of the islands of Brijuni and Dugi Otok (Šprem and Zachos 2020).

Qu. 1.4a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely	CONFIDENCE	low medium
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	moderately likely likely very likely		high
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The species is able to survive during passage along the pathway, as demonstrated by the fact that it has been successfully introduced in the past (e.g. in Croatia) and that secondary translocations occurred too. Hence, it is very likely that the animals survive during transport and storage along the pathway (provided appropriate animal welfare standards). The species is unlikely to reproduce or increase during such transport.

Qu. 1.5a. How likely is the organism to survive existing management practices during transport and storage along the pathway?

RESPONSE	N/A very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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There are no management measures applicable during the introduction of animals.

Qu. 1.6a. How likely is the organism to be introduced into the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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The intentional introduction for hunting purposes cannot go undetected (although this is valid for authorised releases only, as any illegal introduction would likely go undetected).

Qu. 1.7a. Estimate the overall likelihood of introduction into the risk assessment area based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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The species was already introduced to the risk assessment area in the past along this pathway, but only in a very limited number of occasions.

b) Farmed animals (including animals left under limited control) (Escape from confinement)

Qu. 1.2b. Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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This pathway refers to animals that have been introduced for farming into confinements, where they were kept with the primary purpose to provide food, resources and/or as working animals (it does not include animals held in zoos, deer parks and the likes, which are treated in the points below 1.2c to 1.7c). However, the number of axis deer farms present in the EU is unknown, and no information is available about the numbers of axis deer kept in such facilities.

The only documented evidence is a small population occurring in Croatia in a fenced area in the island of Rab (Centore 2016). In Germany, the species is kept in enclosures since 1707, although no occurrences are documented in the wild (Geiter et al. 2002, Nehring et al. 2015).

In Australia, the axis deer is the most popular farmed species among deer, and the most commonly released (Moriarty 2004). According to Massam et al. (2010), the species is used as livestock, e.g. for venison production, since the early 1800s in New South Wales (Moriarty 2004). In Texas the species occurs as a confined animal on ranches in 67 counties (Davis and Schmidly 1997, Long 2003).

Qu. 1.3b. How likely is it that large numbers of the organism will be introduced through this pathway from the point(s) of origin over the course of one year? including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in introduction whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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It is possible to expect further introductions and translocations of this species motivated by farming purposes, although data on this regard are not available. It is moderately likely that large numbers of animals are introduced for farming within one year.

Qu. 1.4b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the

organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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As demonstrated by the fact that it has been frequently kept in captive facilities, the likelihood of the animals to survive during transport and storage along the pathway is high, provided that appropriate animal welfare standards are ensured. Also, the likelihood of the axis deer to survive, reproduce, or increase in a fenced area is high, provided that the species requirements are duly considered and ensured (see for example Centore 2016, Centore et al. 2018).

Qu. 1.5b. How likely is the organism to survive existing management practices during transport and storage along the pathway?

RESPONSE	N/A very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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The likelihood of the axis deer to survive existing management practices in a fenced area will vary depending on the type of deer management and extent of disturbance in the area. In principle it might be high, provided that the species requirements are duly considered and ensured (see for example Centore 2016). For example, as reported by Centore et al. (2018), the population in the fenced area in the island of Rab is actively managed through hunting. The hunting technique is stalking, distributed year round, depending on hunting season, and is characterised by an annual hunting bag of 6 animals (4 adults and 2 yearling) with a sex ratios of 0.86:1 in favour of stags. However, this is not deemed to affect the population, which in fact was specifically created and maintained for hunting purposes.

Qu. 1.6b. How likely is the organism to be introduced into the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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The intentional introduction for farming purposes cannot go undetected.

Qu. 1.7b. Estimate the overall likelihood of introduction into the risk assessment area based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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The species was already introduced into the risk assessment area in the past along this pathway, but only in a very limited number of occasions.

c) Botanical garden/zoo/aquaria (excluding domestic aquaria) (Escape from confinement)

Qu. 1.2c. Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Axis deer are known to be kept in zoos and wildlife parks for ornamental reasons. In fact this species was considered for centuries a favourite with zoological gardens and parks around the world (Schaller 1967, Sankar and Acharya 2004), and managed herds still occur in parks throughout the native and introduced range (Duckworth et al. 2015).

In Europe, the species is currently known to be present in captive facilities for ornamental reasons in many countries, like in the UK (Long 2003), Italy (Boitani et al. 2003), in Poland (Okarma et al. 2018b), as well as in Denmark, Estonia, France, Croatia, Netherlands, Austria, Portugal, Sweden, Spain, Czech Republic, Cyprus and Germany (see <https://www.zootierliste.de/?klasse=1&ordnung=121&familie=12110&art=1160403>).

According to the data from the European Association of Zoos and Aquaria (EAZA, pers. comm. 2019) taken from Species360 ZIMS the axis deer population in EAZA associated facilities is represented by 96 males, 236 females and 294 animals of unknown sex across 12 EU Member States⁵ and the United Kingdom (information correct as of 03/10/2019).

Qu. 1.3c. How likely is it that large numbers of the organism will be introduced through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in introduction whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely	CONFIDENCE	low
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⁵ Austria, Denmark, Czechia, Estonia, France, Germany, Hungary, Italy, Netherlands, Portugal, Spain, Sweden

	unlikely moderately likely likely very likely		medium high
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It is moderately likely that large numbers of animals are introduced for keeping in zoos and deer parks within one year. So far, according to the data from the European Association of Zoos and Aquaria (EAZA, pers. comm. 2019) taken from Species360 ZIMS the axis deer population in EAZA associated facilities, there are 29 zoos hosting axis deer, with a number of animals ranging from 3 to 76 in each single facility, and about 2/3 of the facilities have at least 10 animals.

Qu. 1.4c. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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The likelihood of the animals to survive during transport and storage along the pathway is high, as demonstrated by the fact that it has been frequently kept in captive facilities (hence provided that appropriate animal welfare standards are ensured). Also, the likelihood of the axis deer to survive, reproduce, or increase in a fenced area is high, provided that the species requirements are duly considered and ensured (see for example Centore 2016, Centore et al. 2018).

Qu. 1.5c. How likely is the organism to survive existing management practices during transport and storage along the pathway?

RESPONSE	N/A very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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There are no management measures applicable during the introduction of animals.

Qu. 1.6c. How likely is the organism to be introduced into the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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The intentional introduction into a zoological facility cannot go undetected.

Qu. 1.7c. Estimate the overall likelihood of introduction into the risk assessment area based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Axis deer is abundant in zoos and deer parks and the likelihood of further introductions or transport of animals between existing facilities (from outside the EU into the risk assessment area) is moderately likely.

End of pathway assessment, repeat Qu. 1.3 to 1.7 as necessary using separate identifier.

Qu. 1.8. Estimate the overall likelihood of introduction into the risk assessment area based on all pathways and specify if different in relevant biogeographical regions in current conditions.

Provide a thorough assessment of the risk of introduction in relevant biogeographical regions in current conditions: providing insight in to the risk of introduction into the Union.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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The species is already present in the risk assessment area through the described pathways, possibly leading to a risk of introduction in all biogeographical regions (but paucity of information on animals held in farms and parks does not allow to assess which regions exactly). It is to be noted, however, that apart from the one wild population in Croatia, itself restricted to an island, all current populations (by whatever route to date) are relative to animals held in confinement.

Qu. 1.9. Estimate the overall likelihood of introduction into the risk assessment area based on all pathways in foreseeable climate change conditions?

Thorough assessment of the risk of introduction in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of introduction (e.g. change in trade or user preferences)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely introduction within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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There is no evidence that climate change will have any effect on the likelihood of introduction via hunting, farming or keeping animals in zoological facilities.

2 PROBABILITY OF ENTRY

Important instructions:

- Entry is the release/escape/arrival in the environment, i.e. occurrence in the wild. Entry is not to be, the movement of an organism within the risk assessment area.
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used. For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document⁶ and the provided key to pathways⁷.
- For organisms which are already present in the risk assessment area, only complete this section for current active or if relevant potential future pathways. This section need not be completed for organisms which have entered in the past and have no current pathway of entry.

Qu. 2.1. List relevant pathways through which the organism could enter into the environment.

For each pathway answer questions 2.2 to 2.7 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 2.2a, 2.3a, etc. and then 2.2b, 2.3b etc. for the next pathway. In this context a pathway is the route or mechanism of entry of the species into the environment.

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 2.2-2.8

Pathway name:

The following active pathways of entry have been identified in the risk assessment area:

- a) Hunting (Release in nature)
- b) Farmed animals (including animals left under limited control) (Escape from confinement)
- c) Botanical garden/zoo/aquaria (excluding domestic aquaria) (Escape from confinement)

The “natural spread” of individuals from neighbouring countries, e.g. Moldova, Russia, Ukraine (Long 2003, Duckworth et al. 2015) is another possibility (but the occurrence of any free-living population in Ukraine and Moldova is considered questionable by Šprem and Zachos 2020). However, as no detailed information is available on the exact location and relevant population size, or the population and expansion trends, this is not considered an active pathway for the time being and the relevant risk cannot be quantified.

a) Hunting (Release in nature)

Qu. 2.2a. Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from a confinement)?

⁶ <https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf>

⁷ <https://circabc.europa.eu/sd/a/Oaeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf>

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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This pathway refers to animals released intentionally into the natural environment to be hunted for food and/or to provide recreational hunting opportunities (including collection of hunting trophies). The release for hunting purpose used to be the main pathway for the species in Europe, as documented in Croatia (Frković 2014; Centore et al. 2018), where several entries into the wild occurred, some of which with successful result (although ultimately only one population has been kept viable until present).

This has been a typical pathway of entry also in other regions, for example in Ukraine (Page et al. 2008), USA (GISD 2015), Argentina (Carpinetti and Merino 2000, Novillo and Ojeda 2008), Andamane Islands (Long 2003, Ali and Pelkey 2013, Banerji 1955), Australia (Massam et al. 2010, Moriarty 2004) and South Africa (<https://www.invasives.org.za/legislation/item/709-axis-deer-axis-axis>, accessed on 26/07/2019).

Qu. 2.3a. How likely is it that large numbers of the organism will enter into the environment along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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It seems that a very small number of hinds and a few stags is sufficient to found a new population. Despite some uncertainty regarding the outcome of the introduction of axis deer, the propagule size in deer introductions is considered a highly significant predictor of establishment success, as introduction involving four or fewer individuals failed, whereas involving seven or more individuals succeeded (Forsyth et al. 2004).

In Croatia the axis deer population is managed only in the fenced part of the state open hunting ground (number: VIII / 6 - "KALIFRONT") on the island of Rab, where a parental stock of 63 heads and an increase of 15 heads per year is defined by the game management plan (Public Institution National Park Brijuni and Hunting Directorate of the Ministry of

Agriculture, pers. comm. 2020). This population originated from seven axis deer released in 1974 and resulting in a total of 78 animals during the 2015/2016 season, according to Centore et al. (2018). Axis deer in the island of Rab are all kept in a fenced area, but there are also animals reported out of the fence (Nikica Šprem pers. comm. 2020). However, any axis deer out of the enclosure needs to be removed from the wild in accordance to the relevant regulations (Public Institution National Park Brijuni and Hunting Directorate of the Ministry of Agriculture, pers. comm. 2020).

In other introductions occurred in countries outside Europe, the animals were subject to active management (i.e. hunting) therefore the data cannot be considered representative of any specific trend. However, several other introductions occurred in Croatia, although these were unsuccessful (see Qu 1.3a).

Outside the risk assessment area, in the Hawaiian Islands, deer populations flourished on Oahu, Molokai, and Lanai following releases. For example, as reported by Waring (1996) 8 axis deer (3 stags, 4 hinds, and one male fawn) were released in 1868 on Molokai Island where the population increased to 1,000 within 20 years and reached perhaps 7,500 before specific control measures were taken (see also Anderson, 1999). Similar trends were reported in other islands (Anderson 1999). In Queensland (Australia), one herd reported as still present by Bentley (1957) was established about 1866 by the introduction of a stag and two hinds. Similarly, in Rita Island (in Queensland) a population starting in the late 1970s from 20 individuals reached 2,000 or more in 2004 (Jesser 2005). In Ukraine, the number of axis deer increased from 25 individuals to 448 in 15 years (Anderson 1999), but no specific pathways are described. Also in Russia a population of axis deer grew rapidly, from 50 head in 1973 to 109 head in 1989 (Bobrov et al. 2008) but also in this case no specific pathways are described.

Qu. 2.4a. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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The intentional release of the species in the wild for hunting purposes cannot go undetected (although this is valid for authorised releases only, as any illegal introduction would likely go undetected).

Qu. 2.5a. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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There is no documented evidence about which particular time of the year would be more appropriate for establishment. The diverse diet and habitats requirements along with the

aseasonal reproduction patterns may open the window of opportunity for the entry of the species into the environment during most (if not all) months of the year. The likelihood of the animals to enter into the environment during the period most appropriate for establishment along this pathway therefore is high. Moreover, it is likely that hunters will release the animals in the most appropriate time and place, although there is no documented evidence that this has been systematically done (hence the low confidence).

Qu. 2.6a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Same as in 2.5a.

Qu. 2.7a. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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The species has already entered the risk assessment area through this pathway, although there is no evidence that this is going to happen regularly.

b) Farmed animals (including animals left under limited control) (Escape from confinement)

Qu. 2.2b. Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from a confinement)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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This pathway refers to the unintentional escape of animals from confinements where they were kept with the primary purpose to provide food, resources and/or as working animals. However, the number of deer farms present in the EU is unknown, and no information is available about the number of axis deer kept in such facilities.

Escapes from farms is a well known risk also in regions other than the EU, e.g. Ukraine (Page et al. 2008). In Australia, axis deer is the most popular farmed species among deer and the most commonly released (Moriarty 2004). According to Massam et al. (2010) escapes occurred since the early 1800s in New South Wales. Also, escapes from private captive facilities are reported in the US, particularly in Texas (Long 2003). In the USA, the origin a

population introduced in the 1930s in Volusia County in Florida was caused by the escape from a private collection (Long 2003, Page et al. 2008).

Qu. 2.3b. How likely is it that large numbers of the organism will enter into the environment along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Although there are no specific data for axis deer (with the exception of some generic reference of animals escaped from fenced areas, see Šprem and Zachos 2020), escapes of other species of deer from farms are well known in Europe, as in the case of the sika deer (Bartos 2009). For example, in France an increasing number of small free-living sika deer populations have been reported to enter the wild (and establish) during the last decades, mostly as a result of escapes from deer parks (Baiwy et al. 2013) which share many analogies with deer farms. Also in Germany, according to Bartos (2009), frequent escapes of sika deer from an enclosure near Neuhaus, Möhnensee, occurred (here axis deer were present too, thus showing the inherent risk of entry associated to this pathway). Escapes of sika deer occurred also in Lithuania (Baleišis et al. 2003) and in Poland (Solarz et al. 2018).

Escapes of axis deer from farms are documented in other countries beyond Europe, e.g. in Australia (Jesser 2005). There, axis deer is known to be farmed since 1803, and already 6 years later the escape of 400 animals was recorded (Moriarty 2004).

Qu. 2.4b. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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This is a medium sized deer heavily spotted in all seasons, and although mostly active around dawn and dusk (Wilson and Mittermeier 2011) it may be easily detected by hunters,

naturalists, farmers, etc., hence it is unlikely to be introduced in the risk assessment area undetected. Nevertheless, the occurrence of other deer species throughout much of the risk assessment area may allow the entry of axis deer into the wild to go undetected by landowners and the general public not fully familiar with deer species differences.

Qu. 2.5b. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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There is no documented evidence about which particular time of the year would be more appropriate for establishment, but it can be assumed that it is not during winter months (see for example limiting factors in Qu. 1.3a and 2.3a). The diverse diet and habitats requirements along with the aseasonal reproduction patterns may open the window of opportunity for the entry of escaped animals into the environment during most (if not all) months of the year.

Qu. 2.6b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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The likelihood of the animals to be able to transfer from the pathway to a suitable habitat in the environment through this pathway depends on the actual location of the deer farm. It is considered unlikely because of the lack of documented evidence on this regard, but on the basis of the experience with other deer species, it is not possible to exclude that this may happen.

Qu. 2.7b. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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The species has not yet entered the wild through this pathway, however, there is some risk for such events to happen as long as animals are kept in such facilities. For example in relation to the population on the island of Rab, as the species is known to be a good swimmer and move across islands by covering also distances of 10 km (see Qu. 4.1). However, the sound assessment of this point is affected by the lack of information about the distribution of deer farms in Europe where the species is held and their biosecurity.

c) **Botanical garden/zoo/aquaria (excluding domestic aquaria) (Escape from confinement)**

Qu. 2.2c. Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from a confinement)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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This pathway refers to the unintentional escape of animals from facilities such as zoological and deer parks where they are confined within enclosures, displayed to the public, and in which they may also breed. Nevertheless, a part from the number of zoos associated to EAZA, the total number of zoos and deer parks present in the EU is unknown, and no information is available about the number of axis deer kept in such facilities.

In Europe, there is no documented evidence on escapes of the species from captive facilities, except for some general references for the UK (Long 2003), but this possibility cannot be completely ruled out. In fact, escapes from private captive facilities is a well known risk in regions other than Europe, e.g. a release from a zoo in Armenia is reported (Long 2003), and escapes from captive facilities are reported too, e.g. for Ukraine and the US, particularly in Texas (Long 2003). Also the origin of the population introduced during early 1940s to Point Reyes Peninsula (Marin County, California) was the San Francisco Zoo (Long 2003, Page et al. 2008).

Qu. 2.3c. How likely is it that large numbers of the organism will enter into the environment along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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No specific information has been found for axis deer, but it is considered unlikely that large numbers of animals escape from zoos or deer parks within one year.

Escapes of animals from deer farms and deer parks are however well known in Europe, as in the case of the sika deer (Bartos 2009). For example, in France an increasing number of small free-living sika deer populations have been reported during the last decades, mostly as a result of escapes from deer parks (Baiwy et al. 2013). According to Bartos (2009) frequent escapes of sika deer occurred in Germany, from an enclosure near Neuhaus, Möhnensee, where axis deer were present too, thus showing the inherent risk of entry associated to this pathway. Additionally, it is known that some populations of free-ranging fallow deer in Europe derive from escapes from deer parks.

It is however unknown how many axis deer are kept in deer farms and parks in Europe (with the notable exception of the animals kept in EAZA associated facilities (see Qu. 1.2c and 1.3c), and there is no documented evidence on escapes of this species from such facilities. Also in Croatia, the Ministry of Agriculture (responsible for hunting) and the Ministry of Environment and Energy (responsible for nature protection) confirmed that they do not have any data or information on the existence of axis deer populations held in captivity (Public Institution National Park Brijuni and Hunting Directorate of the Ministry of Agriculture, pers. comm. 2020).

Qu. 2.4c. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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This is a medium sized deer heavily spotted in all seasons, and although mostly active around dawn and dusk (Wilson and Mittermeier 2011) it may be easily detected by hunters, naturalists, farmers, etc. hence it is unlikely to enter the wild in the risk assessment area as an escape from a zoo or deer park undetected. Nevertheless, the occurrence of other deer species throughout much of the risk assessment area may allow the entry of axis deer to go undetected by landowners and the general public not fully familiar with deer species differences.

Qu. 2.5c. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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There is no documented evidence about which particular time of the year would be more appropriate for establishment, but it can be assumed that it is not during winter months (see for example limiting factors in Qu. 1.3a and 2.3a). The diverse diet and habitats requirements along with the aseasonal reproduction patterns may open the window of opportunity for the entry of escaped animals into the environment during most (if not all) months of the year

Qu. 2.6c. How likely is the organism to be able to transfer from the pathway to a

suitable habitat or host in the environment?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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The likelihood of the animals to be able to transfer from the pathway to a suitable habitat in the environment through this pathway depends on the actual location of the zoological garden or deer park. It is considered unlikely because of the lack of documented evidence on this regard, but on the basis of the experience with other deer species, it is not possible to exclude that this may happen.

Qu. 2.7c. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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The species has not yet entered the wild through this pathway (as escapee from a zoo or deer park), but some risk for such events to happen exists as long as animals are kept in such facilities. However, the sound assessment of this point is affected by the lack of information about the distribution of zoos and deer parks in Europe where the species is held and their biosecurity.

End of pathway assessment, repeat Qu. 2.2 to 2.7. as necessary using separate identifier.

Qu. 2.8. Estimate the overall likelihood of entry into the environment within the risk assessment area based on all pathways in current conditions and specify if different in relevant biogeographical regions.
Provide a thorough assessment of the risk of entry into the environment in relevant biogeographical regions in current conditions.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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The most likely pathway of axis deer entry into the wild within the EU is the deliberate release for hunting (as it has happened in the Mediterranean biogeographical region in the past) and, less likely, the accidental/deliberate releases of individuals from deer farms and zoological gardens or deer parks.

Qu. 2.9. Estimate the overall likelihood of entry into the environment within the risk assessment area based on all pathways in foreseeable climate change conditions and

specify if different in relevant biogeographical regions.

Thorough assessment of the risk of entry in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if likelihood of entry is likely to increase or decrease for specific pathways.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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There is no evidence that climate change will have any effect on the likelihood of entry via the active pathways.

3 PROBABILITY OF ESTABLISHMENT

Important instructions:

- For organisms which are already established in parts of the risk assessment area, answer the questions with regard to those areas, where the species is not yet established.

Qu. 3.1. How likely is it that the organism will be able to establish in the risk assessment area based on the history of invasion by this organism elsewhere in the world (including similarity between other abiotic conditions within it and the organism’s current distribution)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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The only population established in the wild in the EU is in Croatia, on the islands of Brijuni, off Istria (Duckworth et al. 2015). However, environmental conditions similar to those present in the native and alien range of the species are present in other areas of the EU, particularly in the Mediterranean region, therefore it is likely that suitable sites exist elsewhere in all biogeographic regions in the risk assessment area (see Annex VII).

This may be partly confirmed by the fact that before being eradicated the axis deer was considered as successfully introduced also in New Zealand (Forsyth and Duncan 2001), a country partly sharing bio-climatic conditions similar to those found in Europe, as demonstrated by the many successful introductions of alien species of European origin.

Although native to tropical and subtropical areas of the Indian subcontinent, axis deer have adapted well to other ecoclimatic zones, including those present in the EU, such as the Mediterranean, and more continental climates in Russia (and the Ukraine, although the occurrence of any free-living population in this country is considered questionable by Šprem and Zachos 2020).

Qu. 3.2. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area?

RESPONSE	very isolated isolated moderately widespread widespread ubiquitous	CONFIDENCE	low medium high
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Axis deer seem characterised by an extreme degree of flexibility and are well adapted to a wide variety of natural and semi-natural habitats and food, according to availability. Therefore, habitats or species (food sources) necessary for the survival, development and

multiplication of axis deer are moderately widespread in the risk assessment area, particularly in the Mediterranean region.

Axis deer occupy a wide range of habitats throughout their native range, and are most commonly associated with a mixture of forest and more open grass-shrub, often avoiding rugged terrain, almost exclusively at lower elevations, below 1000 m a.s.l. (GISD 2015). Axis deer are typically associated with forest and grasslands interfaces but are highly adaptable to a wide range of habitats and changing conditions, including suburban settings (Duckworth et al. 2015). In particular, axis deer are found throughout dry and mixed deciduous forest habitat and secondary forest lands broken by glades, with a presence of understorey of grasses, forbs and tender shoots which supply adequate drinking water and shade. Axis deer consume an extremely wide variety of plants throughout their native and introduced range: about 160–190 of plant species (Duckworth et al. 2015, Sankar and Acharya 2004). Axis deer are predominantly generalist grazers that also browse leaves, flowers, fruits, and seeds, as well as bark when the preferred food items are scarce or during droughts (Anderson, 1999, Long 2003, Wilson and Mittermeier 2011 Duckworth et al. 2015, Schaller 1967), and possibly also during winter. Moreover, when natural forage is insufficient, axis deer forage in cultivated crops and cause economic damage (Anderson 1999). As summarised by Duckworth et al. (2015), axis deer is known to feed on mushrooms, crabs, rubbish and occasionally even human faeces in areas close to human habitation. Moreover, like in other deer species, antler and bone chewing is also common. The need to drink water once a day, more frequently in summer, in general restricts them to forest areas with assured presence of water, even if widely scattered.

However, the species is characterised by flexibility as shown by the significant seasonal changes in temperature and, more significantly, extreme swings in precipitation in their native range. These conditions force the species to deal regularly with long periods of drought and poor forage availability, as well as widespread flooding and lush seasonal growth during the rainy season (GISD 2015, Anderson, 1999). Outside its native range, in Hawaii, for example, axis deer is present from semi-deserts to rainforests (Moe and Wegge 1994).

Qu. 3.3. If the organism requires another species for critical stages in its life cycle then how likely is the organism to become associated with such species in the risk assessment area?

RESPONSE	N/A very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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No specific organism is required to be associated to axis deer for critical stages in its life cycle.

In their native range, axis deer are known to be associated with other animals, particularly monkeys, which produce alarm sounds on the presence of predators like leopard (*Panthera pardus*) or tiger (*Panthera tigris*) (Dinesan et al. 2006). However, this facilitative/mutualistic relationship is opportunistic and not obligate, and there is no evidence that this is required for critical stages in the life cycle of the species.

Qu. 3.4. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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There is potential for competition with the native red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*), as well as other ungulates in the risk assessment area, but as noted for other introduced deer species such competition is unlikely to prevent establishment. However, no specific studies on axis deer exist in the EU on this regard. Studies carried out in regions outside the EU, e.g. in USA (Texas), showed aggressive and dominant behaviour in axis deer toward white-tailed deer (*Odocoileus virginianus*), demonstrating that species coexistence is unlikely, at least at the spatial scale of the study and depending on factors such as population density of the two species and habitat quality (Faas and Weckerly 2010).

Axis deer seem unable to tolerate the presence of feral pigs (Lever 1994), however explicit research on this possible relation is not available.

Qu. 3.5. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?

RESPONSE	N/A very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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The enemies of axis deer in its native range vary from tiger to leopard, wild dog, jackal and python (Dinesan et al. 2006). In particular, jackals may kill fawns (Moe and Wegge 1994). As summarised by Sankar and Acharya (2004) in its native range in India the main cause of death is predation, mostly from tiger (*Panthera tigris*) and leopard (*Panthera pardus*). Outside the native range, predation was thought to limit the spread of axis deer, like in Australia, as a consequence of high density of dingo populations in some areas (Moriarty 2004). Wild boar (*Sus scrofa*) may also predate on axis deer fawns or juveniles, as reported in Argentina (Gürtler et al. 2017). However, the community of predators differ in the risk assessment area and their strategies are presumably different as well (see discussion below).

Other mortality factors in its native range are diseases (e.g. foot and mouth disease). The potential impact of an exotic epidemic like foot and mouth is demonstrated by the 1924 outbreak in California (Clements 2007). Also in Azerbaijan an introduced population was reduced by foot and mouth disease (Long 2003).

The risk assessment area is certainly characterised by the presence of potential predators, parasites or pathogens of axis deer, however there are several species of native and alien deer already occurring, and this does not seem to represent a limiting factor for their populations.

Predation from large carnivores may be less effective in the risk assessment area, given the lack of tigers and leopards, and the potential impact of the large carnivores occurring in Europe is unknown. Therefore, natural enemies and diseases are unlikely to affect the likelihood of species establishment. Moreover, the role of predators in controlling ungulate populations remains uncertain, and is considered not effective, at least in some systems (Côté et al. 2004). The situation may be different in island ecosystems, where ungulates, as a consequence of their co-evolutionary history with large predators, may have very high reproductive rates, causing rapid population growth. For example in Hawaii, in the absence of predators, introduced populations of axis deer exhibit annual population growth rates of 20–30% (Hess 2008). A detailed discussion is beyond the scope of this assessment, and in any case due to the lack of specific data may be too speculative. Moreover, predicting the impact of native predators on axis deer would be a challenging task also because the impact of predators may be sensitive to the composition of the multi-prey species community (e.g. for the wolf see Sand et al. 2016)

Qu. 3.6. How likely is the organism to establish despite existing management practices in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Deer in Europe are usually subject to hunting and culling, which are regulated by law (see Apollonio et al. 2010). Poaching and overhunting has been a factor which led to the extinction of introduced populations of axis deer, e.g. in Croatia (Frković 2014). However, controlling axis deer may be problematic because it is a charismatic species, and there may be a conflict of interest between sectors obtaining recreational or economic gains from the exploitation of exotic wildlife and sectors promoting the conservation of biodiversity, as reported for Argentina (Gürtler et al. 2017).

Qu. 3.7. How likely are existing management practices in the risk assessment area to facilitate establishment?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Usually axis deer populations respond positively to higher levels of protection, water availability, forage quality, flat terrain and low predation, factors that are relatively widespread in the risk assessment area, although poaching and livestock grazing may be limiting factors (Duckworth et al. 2015). The availability of food and cover, which is usually provided to deer in game management reserves or in protected areas (where hunting may be forbidden, depending on the national legislation) may certainly favour the species establishment. Axis deer may benefit from water troughs established for cattle plus water sources on golf courses and homesites, as reported in Texas (Waring 1996). Also habitat restoration measures (i.e., prescribed burns and opening of fire breakers offering permanent

pastures) may benefit axis deer (Gürtler et al. 2017). In addition, reducing competition (and perhaps predation) from wild boar due to its heavy hunting in the risk assessment area, may lead to an increase of axis deer abundance, as shown by a study assessing the result of a control program targeting both species in Argentina (Gürtler et al. 2017), which would increase the chance of successful establishment.

Qu. 3.8. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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There is no information for the risk assessment area, but overhunting has been a clear factor which led to the extinction of introduced populations (see for example Frković 2014). Nevertheless it is interesting to consider a long-term study of hunted axis deer in the introduced range in Argentina (for detail see Gürtler et al. 2017), which showed that contrary to park managers’ expectations, the control program failed to reduce the axis deer population over a 10-year period despite increasing shooting effort and increasing deer harvest. Failure to reduce deer abundance may be explained by the combined effects of several putative processes: (1) population growth of axis deer over nearly two decades; (2) deer range expansion in the region leading to increasing immigration to the park; (3) sex- and stage-biased hunting mortality which kept per capita deer recruitment rates at sub-maximal levels, and (4) release from the pressure of wild boar (which was also a target of the control program) as a competitor (and perhaps as a predator).

Overall, the success of an eradication programme may depend on several factors, including the population size and the availability of resources. For example, in Russia the axis deer population of the Prioksko-Terrasny Nature Reserve (5,000 hectares) was reduced from 109 heads in 1989 to 5 in 2006 due to a control program (Bobrov et al. 2008).

Qu. 3.9. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?

including the following elements:

- a list and description of the reproduction mechanisms of the species in relation to the environmental conditions in the Union
- an indication of the propagule pressure of the species (e.g. number of gametes, seeds, eggs or propagules, number of reproductive cycles per year) of each of those reproduction mechanisms in relation to the environmental conditions in the Union.

If relevant, comment on the likelihood of establishment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely	CONFIDENCE	low medium high
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	likely very likely		
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Key biological characteristic which may facilitate the establishment of axis deer in the risk assessment area are the behavioural variability, opportunism and the species' extreme adaptability to changing circumstances (Anderson, 1999). As summarised by Duckworth et al. (2015) and references therein, the axis deer is a prolific breeder, which is documented by several empirical studies of the speed of increase by newly introduced subpopulations or in those where a factor restraining subpopulations was removed. For example, the population explosion in the Andaman Islands is considered a consequence of a series of factors (beside the presence of good vegetation) such as fast maturity, high annual pregnancy rate, low fawn mortality (Sivakumar 2003). In Bhadra, India, following the departure from the park of human settlements and consequent removal of anthropogenic pressures on axis deer and habitats, axis deer populations bounced back by nearly seven times in less than four years (Duckworth et al. 2015).

In the wild, axis deer are characterised by an aseasonal reproduction pattern (Centore 2016, Graf and Nichols 1966). The reproductive cycle of individual stags is not synchronised with that of other stags in the herd, hence they are found in rutting conditions throughout the year, do not retain harems and mate with hinds in more herds as they become receptive (GISD 2015). Hinds also experience non-synchronised oestrous cycles, with each cycle lasting about 3 weeks, and typically produce one fawn per pregnancy after a 210-238 days gestation period (Davis and Schmidly 1997).

Qu. 3.10. How likely is the adaptability of the organism to facilitate its establishment?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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The diverse diet requirements and the ecological flexibility which characterise the axis deer, along with the aseasonal reproduction patterns may facilitate the establishment of the species. Several other features of the species biology may explain the invasion success of the axis deer within the many introductions which occurred worldwide. For example, it is known to be a gregarious species, found in herds ranging from a few animals to 100 or more. In its native range, population densities fall within three to 50 animals per km² in India, up to around 200 axis deer per km² in Nepal (Duckworth et al. 2015). In Hawaii a herd as large as 300 was reported (Hess 2008). Natural lifespan of the species is 9-13 years, although zoo animals may reach 18-22 years (Davis and Schmidly 1997, Page et al. 2008).

Qu. 3.11. How likely is it that the organism could establish despite low genetic diversity in the founder population?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Apparently, a very small number of hinds and a few stags seems sufficient to fund a new population (which may show the negligible impact of genetic diversity), although no data on the impact of low genetic diversity in the founder population are available. See also **Qu. 2.3a**.

Qu. 3.12. If the organism does not establish, then how likely is it that casual populations will continue to occur?

Consider, for example, a species which cannot reproduce in the risk assessment area, because of unsuitable climatic conditions or host plants, but is present because of recurring introduction, entry and release events. This may also apply for long-living organisms.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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It is likely that high number of individuals are still kept and bred in captivity in the risk assessment area, which leads to a certain risk of some being intentionally or accidentally released in the wild, building up casual occurrences. The overall likelihood of casual population to occur seems low, but no sufficient data are available to support any statement on this regard.

Qu. 3.13. Estimate the overall likelihood of establishment in the risk assessment area based on the similarity between climatic conditions within it and the organism’s current distribution under current climatic conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under current climatic conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in current conditions: providing insight in the risk of establishment in (new areas in) the Union.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Providing that sufficient founder individuals are encountered (see point 2.3.a), the axis deer is likely to establish self-sustaining populations in almost all EU Member States (with the exception of Estonia and Finland, see Annex VII) because appropriate climatic conditions, habitats and food are present and local natural enemies and diseases are unlikely to affect establishment.

Qu. 3.14 Estimate the overall likelihood of establishment in the risk assessment area under foreseeable climate change conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under foreseeable climate change conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in

foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of establishment (e.g. increase in average winter temperature, increase in drought periods)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely establishment within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Providing that sufficient founder individuals are encountered (see point 2.3.a), the axis deer is likely to establish self-sustaining populations in all EU Member States (see Annex VII) because appropriate climatic conditions, habitats and food are likely to be widespread (even more than in current conditions) and local natural enemies and diseases are unlikely to affect establishment.

4 PROBABILITY OF SPREAD

Important instructions:

- Spread is defined as the expansion of the geographical distribution of an alien species within the risk assessment area.
- Repeated releases at separate locations do not represent continuous spread and should be considered in the probability of entry section. In other words, intentional anthropogenic “spread” via release or escape (“jump-dispersal”), should be dealt within the entry section. However, as repeated releases contribute to the spread of the target organism in the risk assessment area, the relevant pathway(s) should be briefly discussed here too, with an explicit reference to the entry section for additional details.

Qu. 4.1. How important is the expected spread of this organism within the risk assessment area by natural means? (List and comment on each of the mechanisms for natural spread.)

including the following elements:

- a list and description of the natural spread mechanisms of the species in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of each of those spread mechanisms in relation to the environmental conditions in the Union.

The description of spread patterns should include elements of the species life history and behavioural traits able to explain its ability to spread, including: reproduction or growth strategy, dispersal capacity, longevity, dietary requirements, environmental and climatic requirements, specialist or generalist characteristics.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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The potential of axis deer to spread within the risk assessment area by natural means is likely to be minimal, but there are no data about the rate of spread of individuals in Europe (which may vary depending on the extent of deer management and disturbance, as well as habitat availability and connectivity, appropriate food resources, presence of other species acting as competitor/predators etc.). For example, Okarma et al. (2018b) pointed out that current information based on the lack of success of previous introductions in Europe and on biological characteristics of the species (size, life history, fertility, behaviour) allow to consider the spread rate of the population rather small.

Studies on spacing behaviour and habitat use in other parts of their native and alien range, show that animals are mostly sedentary and with small home ranges, usually between 180-890 ha (Long 2003, Moe and Wegge 1994), depending on resource availability (Waring 1996). Herds travel slowly at some 0.5 km/hour (Schaller 1967), but occasionally axis deer may make long trips to reach feeding grounds and water sources, for example during the dry season, and daily movements of up to 8 km for water have been reported (Graf and Nichols

1966). In Russia, the species was introduced approx. 100 km south of Moscow, in the Serpukhovskoe Hunting Reserve, and dispersed in about 10 years spontaneously to the Prioksko-Terrasnyi Biosphere Reserve through the Oka valley, just a few kilometres from the release site (Bobrov et al. 2008). In Queensland, although much of the area appears climatically suited to the species, axis deer were mostly concentrated surrounding their original release point, although drought may lead to wider dispersals of the animals (Jesser 2005).

Isolation of the axis deer in a small island, may not prevent the species from spreading. Axis deer are capable swimmers (Nowak 1991), and have been observed to swim fairly long distances between islands, i.e. about 3 km in Croatia (Šprem and Zachos 2020) and about 10 km in the Andaman Islands (Ali 2004, Ali and Pelkey 2003). In Brazil, the species is supposed to have reached the country from Uruguay by crossing the Uruguay River at the border between the two countries (Sponchiado et al. 2011).

Qu. 4.2. How important is the expected spread of this organism within the risk assessment area by human assistance? (List and comment on each of the mechanisms for human-assisted spread and provide a description of the associated commodities.)

including the following elements:

- a list and description of the anthropogenic spread mechanisms of the species in relation to the environmental conditions in the Union.
- an indication of the rate of each of those spread mechanisms in relation to the environmental conditions in the Union.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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The main (potential) pathway of spread is the natural dispersal across borders.

Otherwise axis deer were reportedly translocated and released intentionally in the risk assessment area for hunting purposes, e.g. in Croatia (Frković 2014). Moreover, the potential for spread after escapes from deer farms and deer parks should not be underestimated. The relevant introduction and entry pathways are already discussed in the corresponding sections above.

Qu. 4.2a. List and describe relevant pathways of spread. Where possible give detail about the specific origins and end points of the pathways. For each pathway answer questions 4.3 to 4.9 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 4.3a, 4.4a, etc. and then 4.3b, 4.4b etc. for the next pathway.

including the following elements:

- a list and description of pathways with an indication of their importance and associated risks (e.g. the likelihood of spread in the Union, based on these pathways; likelihood of survival, or reproduction, or increase during transport and storage; ability and likelihood of transfer from the pathway to a suitable habitat or host).

Where possible details about the specific origins and end points of the pathways shall be included.

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication.
- All relevant pathways should be considered. The classification of pathways developed by the Convention of Biological Diversity shall be used.

Natural dispersal across borders of invasive alien species that have been introduced through pathways 1 to 5.

Qu. 4.3a. Is spread along this pathway intentional or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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This pathway is unintentional, as it depends on the dispersal capacities of the species. It is facilitated by the habitat conditions which characterise the area (including, for instance, the forest management regime and the recreational hunting practices, the extent of suitable ecological corridors etc.).

Qu. 4.4a. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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No specific information is available on this regard. However as discussed in the sections above (see for example point 1.3a), it seems that in general a very small number of hinds and a few stags are sufficient to found a new population.

Qu. 4.5a. How likely is the organism to survive, reproduce, or increase during transport

and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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The likelihood of the animals to survive, reproduce, or increase during spread (there is no transport and storage as such along this pathway) will vary depending on the extent of deer management and disturbance in the area (for examples in relation to land use practices, hunting, and other pressures).

Qu. 4.6a. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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The likelihood of the animals to survive existing management practices during spread will vary depending on the extent of deer management and disturbance in the area (for examples in relation to the hunting regime for ungulates).

Qu. 4.7a. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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This is a medium sized deer heavily spotted in all seasons, and although mostly active around dawn and dusk (Wilson and Mittermeier 2011) it may be easily detected by hunters, naturalists, and farmers; hence it is unlikely to be spreading in the risk assessment area undetected. Nevertheless, the occurrence of other deer species throughout much of the risk assessment area may allow the spread of axis deer to go undetected by landowners and the general public not fully familiar with deer species differences.

Qu. 4.8a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread? (including, where possible, details about the specific origins and end points of the pathway)

RESPONSE	very unlikely unlikely moderately likely	CONFIDENCE	low medium high
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	likely very likely		
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No information available on this regard. Based on information from similar species, animals dispersing through natural spread are highly likely to find suitable habitats for survival throughout the risk assessment area, except in areas devoid of any woodland (see GB Non-Native Species Secretariat 2011). The species would not spread by natural means along unsuitable habitats.

Qu. 4.9a. Estimate the overall potential rate of spread within the Union based on this pathway? (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Overall, natural spread from localised population is likely to be slow, but there are no data about the rate of spread of individuals in Europe (which may vary depending on e.g. the extent of deer management and disturbance).

End of pathway assessment, repeat Qu. 4.3 to 4.9. as necessary using separate identifiers.

Qu. 4.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?

RESPONSE	very easy easy with some difficulty difficult very difficult	CONFIDENCE	low medium high
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Effective containment measures to prevent the spread of axis deer through the pathway above are the same as those to control/eradicate the species (see for example discussion on **Qu. 3.8.**), hence their applicability is context dependent, and depends on the size of the population and the invasion stage.

Qu. 4.11. Estimate the overall potential rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (indicate any key issues and provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in current conditions, providing insight in the risk of spread into (new areas in) the Union.

RESPONSE	very slowly slowly moderately rapidly	CONFIDENCE	low medium high
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	very rapidly		
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See Qu. 4.9a.

Qu. 4.12. Estimate the overall potential rate of spread in relevant biogeographical regions in foreseeable climate change conditions (provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if rates of spread are likely slowed down or accelerated.

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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No information has been found.

5 MAGNITUDE OF IMPACT

Important instructions:

- Questions 5.1-5.5 relate to biodiversity and ecosystem impacts, 5.6-5.8 to impacts on ecosystem services, 5.9-5.13 to economic impact, 5.14-5.15 to social and human health impact, and 5.16-5.18 to other impacts. These impacts can be interlinked, for example a disease may cause impacts on biodiversity and/or ecosystem functioning that leads to impacts on ecosystem services and finally economic impacts. In such cases the assessor should try to note the different impacts where most appropriate, cross-referencing between questions when needed.
- Each set of questions starts with the impact elsewhere in the world, then considers impacts in the risk assessment area (=EU excluding outermost regions) separating known impacts to date (i.e. past and current impacts) from potential future impacts (including foreseeable climate change).
- Only negative impacts are considered in this section (socio-economic benefits are considered in Qu. A.7)

Biodiversity and ecosystem impacts

Qu. 5.1. How important is the impact of the organism on biodiversity at all levels of organisation caused by the organism in its non-native range excluding the risk assessment area?

including the following elements:

- Biodiversity means the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems
- impacted chemical, physical or structural characteristics and functioning of ecosystems

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Axis deer may cause significant direct impacts on native vegetation, e.g. through browsing and bark stripping, and may have a number of indirect effects on fauna and ecosystem processes. In general their impact (as an invasive alien species) is similar to that of other native deer, however it could amplify the pressure caused by ungulates on the natural environment. The impact may be even more severe where also other alien ungulates occur.

As summarised by Page et al. (2008) axis deer can feed on many species of native plants, as documented in the Hawaiian Islands (Hess 2008). Negative impact on natural regeneration of the native forests is also reported (Novillo and Ojeda 2008). In the Andaman Islands, where axis deer feed on over 70 different plant species (Sivakumar 2003), a negative impact on

seedling and sapling survival, as well as on forest regeneration and forest structure is documented (Ali 2004; Ali and Pelkey 2013). In northern Patagonia, Argentina, introduced deer (among which axis deer) cause significant modification of the forest understory and impair the regeneration of canopy tree species (Veblen et al. 1989, Veblen et al. 1992), which seemed to negatively affect also the endemic conifer *Austrocedrus chilensis* (Relva and Veblen 1998).

Significant impact to individual trees which may limit the forests renewal is known to occur during the rut (reproductive season) when stags rub and wipe the antlers against the bark, frequently causing secondary infections, which may lead to the death of the trees, for example in Hawaii (Anderson 1999). Additionally, in extreme drought conditions (and possibly in winter) axis deer may feed on the bark of trees (Anderson 1999).

Another threat to the habitat and native vegetation may be caused by the deer trampling behaviour, which may lead to the creation of trails and increasing erosion and runoff (Hess et al. 2015, Page et al. 2008), for example in the Hawaiian Islands (Anderson 1999, Hess 2008). As summarised by GISD (2015) this results in a loss of the stability that vegetation provides, with resulting destabilisation of stream banks, subsequent changes in stream flow and increasing erosion and sedimentation of streams, ponds and rivers. When deer populations become very large, their trailing behaviour creates dirt paths even through the thickest of vegetation. These trails can lead to significant erosion and, in wet forest areas, increase runoff by decreasing the moss layer from soil that would normally retain water (Centore 2016, Anderson 1999). Soil erosion induced by the species leading to consequent siltation of offshore coral reefs is reported in Hawaii (Lever 1994).

Additionally, by opening up of habitat or by selective browsing of understory vegetation, axis deer could help in the spread and establishment of alien, and probably invasive, plants (Mohanty et al. 2016). Anecdotal observations exist that high axis deer densities lead to exposing bare ground, e.g. by removing the vegetation, which in turn may increase light levels and disrupt moisture dynamics, hence facilitating the invasion of exotic weeds (Jesser 2005, Davis et al. 2016). An example is the parthenium (*Parthenium hysterophorus*), a native to the New World accidentally introduced into several countries, including Australia, where it is flourishing in areas where axis deer are not adequately controlled (Jesser 2005).

Axis deer may also have a potential for endozoochoric dispersal of native and exotic plants, as documented in the case of the exotic hog deer (*Axis porcinus*) in south-eastern Australia (Davis et al. 2010).

Competitive displacement of native deer is another (potential) impact, as reported in Argentina (Novillo and Ojeda 2008). Axis deer is a generalist species, and scarcity of forage in the dry (or cold) season may lead to niche overlap with other cervids (Bhattarai 2019). For example, axis deer outcompeted white-tailed deer (*Odocoileus virginianus*) in experimental enclosures over an eight-year follow-up in Texas (Anonymous 2016). This study was within enclosures, where by definition competition may be enhanced because there is no opportunity to avoid competition through niche differentiation or use of species-specific refugia, therefore the results are only indicative (but may reflect situations in closed environment, e.g. small islands). Another research conducted in Texas showed aggressive and dominant behaviour in axis deer toward white-tailed deer, which subsequently modified the habitat selection and feeding patterns (Faas and Weckerly 2010). Axis deer may have a competitive advantage over white-tailed deer for being less specialized in food requirements, while the role played by the different susceptibilities to parasitic disease (Richardson and Demarais 1992). Another study

carried out in an enclosure (although about the size of a small island or a small protected area) demonstrates that coexistence of these two species is unlikely, at least at the spatial scale of the studies and in any case depending on factors such as population density of each species and habitat quality (Faas and Weckerly 2010). Ferretti and Lovari (2014) stressed the difficulty to use an experimental approach in field conditions, but pointed out that evidence on overlap in the use of resources, opposing trends in population size, and behavioural interactions support the hypothesis of competition between alien ungulates and native ones. This however needs to be evaluated on a case by case.

Indirect effects on native biodiversity by altering ecosystem processes may be more subtle and affect also animals other than ungulates. For example, a study showed that in the Andaman archipelago axis deer depressed the abundance of forest floor and semi arboreal lizards approximately five-fold, by reducing vegetative cover in the understory (Mohanty et al. 2016).

Detrimental effects of axis deer are reported from outside the risk assessment area in relation to the conservation status of threatened species at the global level. This is mostly as a consequence of the habitat degradation, as documented by the IUCN Red List, in this case with examples limited to the situation in the Hawaii (BirdLife International 2016a, 2016b, 2016c, 2016d, 2018a, 2018b, 2018c, Bruegmann and Caraway 2003, Heddle 2004). For instance, this is deemed to affect four species that are considered Critically endangered (CR): the Pacific Lacefern (*Ctenitis squamigera*), the Olomao (*Myadestes lanaiensis*), the Maui Parrotbill (*Pseudonestor xanthophrys*) and the Ou (*Psittirostra psittacea*). Two additional species, the Maui Alauahio (*Paroreomyza montana*) and the Fabulous Green Sphinx Moth (*Tinostoma smaragditi*), are considered Endangered (EN). Because of its burrows trampled by axis deer and other ungulates, the Hawaiian Petrel (*Pterodroma sandwichensis*) is considered Vulnerable (VU). Axis deer also contributed to the destruction of the habitats of two extinct Hawaiian species (EX), the Black Mamo (*Drepanis funereal*) and the Bishop's Oo (*Moho bishop*).

Overabundant deer may apparently exert cascading effects on other animals by competing directly for resources with other herbivores and omnivores and by indirectly modifying the composition and physical structure of habitats of both invertebrates and vertebrates (Côté 2005). High deer abundance resulting from the introduction of alien deer species, may have strong indirect effect on forest birds through their impact on vegetation and associated insects. For example, as documented by Allombert et al. (2005) overabundance of white-tailed deer (*Odocoileus virginianus*) populations in North America, resulted in a decrease in songbird habitat quality through decreased food resources and nest site quality and may partly explain continental-scale decreases in songbird populations. An introduced herbivore may even lead to the indirect extirpation of an abundant large carnivore, as documented in a large island in Canada, where the near eradication of shrubs producing berries by introduced white-tailed deer (*O. virginianus*) was considered as the main cause of the extirpation of black bears (*Ursus americanus*) within approximately 50–70 years (Côté 2005). As a remark, the examples above pertain to other deer species in countries other than the EU, and as such do not necessarily apply to axis deer in particular, especially if those deer are not at high densities. However, the information was deemed indicative for the purpose of this assessment, to show the diversity of impacts potentially emerging from the introduction of a new deer species in the EU.

Qu. 5.2. How important is the current known impact of the organism on biodiversity at all levels of organisation (e.g. decline in native species, changes in native species)

communities, hybridisation) in the risk assessment area (include any past impact in your response)?

Discuss impacts that are currently occurring or are likely occurring or have occurred in the past in the risk assessment area. Where there is no direct evidence of impact in the risk assessment area (for example no studies have been conducted), evidence from outside of the risk assessment area can be used to infer impacts within the risk assessment area.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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No direct evidence of impact in the risk assessment area exists other than what is reported for the presence of the species in Croatia.

As reported by the Public Institution National Park Brijuni and Hunting Directorate of the Ministry of Agriculture (pers. comm. 2020) it is difficult to say how much impact the axis deer itself has on the biodiversity of Brijuni National Park, but it is certain that mouflon and deer species significantly affect the biodiversity of Veliki Brijun islands (where axis deer, mouflon and fallow deer are present). In the past, axis deer dominated over the other two species, but due to one harsh winter in the past many died and fallow deer has since prevailed. As the Public Institution National Park Brijuni has been reducing the number of deer specimens in recent years, currently mouflons are predominant. All three species together have a great impact on grasslands and forests of the island Veliki Brijun. Browsing and grazing of large herbivores that live on the island without natural predators affect lower layers of forests causing a problem for the natural reforestation and affect biodiversity of grassland allowing plants that the animals avoid (for example the Spanish oyster thistle *Scolymus hispanicus*) to overly spread. According to the few available data from literature, in the island of Brijuni, axis deer are known to feed on grasses and ash (*Fraxinus ornus*) leaves and holm oak (*Quercus ilex*) leaves and acorns, and sometimes browse the leaves of myrtle (*Myrtus communis*), new stems of blackberry (*Rubus* spp.), mosses growing on rocks, and cedar (*Cedrus* spp.) seeds (Šprem et al. 2008). No information on the type and scale of impact is available. It must be noted that the island of Brijuni is characterised by a very intense human use, limiting the possibility of observing impacts on natural ecosystems of the axis deer.

A couple of studies were carried out in the hunting reserve in the Island of Rab (Krapinec 2002a, 2002b), but their results may be of limited applicability for the assessment of impacts in the wild, because the location was inside an actively managed forest in a fenced area.

Based on evidence from outside the risk assessment area it can be expected that overabundant deer may have a substantial impact on woodland vegetation (modifying patterns of relative abundance and vegetation dynamics), and play a significant role in woodland ecosystem function. In case axis deer would get established on islands, the impacts on the local ecosystems as well as on some bird species (e.g. petrels) could be severe. In the absence of control (either by predators or humans), deer populations can rise to very high densities. This may be further facilitated by human management of forests providing ideal habitats. Vegetation changes brought about by browsing and trampling axis deer are detrimental to other deer species as well as other vertebrate and invertebrate species (see note by Gill 2000). Cascading effects on other species may extend to insects, birds, and other vertebrates. Hence,

axis deer may tip forest ecosystems toward alternative states by acting as “ecosystem engineers” or “keystone herbivores”, as generally noted for deer (Côté et al. 2004).

According to Okarma et al. (2018b) in the worst case axis deer may locally cause hardy reversible changes in ecosystem functioning. According to Okarma et al. (2018b) it can therefore be assumed that in the event of spreading in Poland, the impact could lead to serious decreases in the population size of some native protected species. In Poland, it can be expected that axis deer may exert a certain negative impact on native deer species, as also remarked by the Council of Europe (2002) for Croatia. The potential for competition with native deer may be particularly strong because such species have not shared a common evolutionary history. Additionally, some possible competition with European bison (*Bison bonasus*) may be expected in Poland, should axis deer become established and widespread in this country (Okarma et al. 2018b).

Qu. 5.3. How important is the potential future impact of the organism on biodiversity at all levels of organisation likely to be in the risk assessment area?

See comment above. The potential future impact shall be assessed only for the risk assessment area.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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In the event of substantial spread and increase in numbers of axis deer to new parts of the risk assessment area, the impact may be expected to increase accordingly, even despite the possible presence of predators. However, there are no elements to foresee that the impact would lead to any irreversible change, therefore the risk is considered “moderate”. Because there is no documented evidence the confidence is low.

Qu. 5.4. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism currently in the risk assessment area?

including the following elements:

- native species impacted, including red list species, endemic species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	minimal minor moderate major	CONFIDENCE	low medium high
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	massive		
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The axis deer may represent a potential threat for a series of species and habitats protected by the Birds and Habitats directives, as well as a number of IUCN red-listed species, as shown in countries outside the risk assessment area. The effect of axis deer on protected species of plants and relevant habitats would reflect its browsing habits and diet, as well as the ability of the plants to withstand damage (including from trampling, etc.). Therefore, several plants may be susceptible to axis deer impact, not to consider the cascading effects that overabundant axis deer populations may apparently exert on other ungulates (see for the possible competition with *Bison bonasus*, which could occur if the species were to establish in Poland, Qu. 5.2) and other groups of animals as well, including birds on islands.

Qu. 5.5. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism likely to be in the future in the risk assessment area?

including the following elements:

- native species impacted, including red list species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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In the event of substantial spread and increase in numbers of axis deer to new parts of the risk assessment area, the impact may be expected to increase accordingly. In case of a future expansion of the species range, other native species may be affected. While there is no documented evidence of the species being able to cause the extinction of any native species, the level of risk is assessed as being “moderate” also in the future.

Ecosystem Services impacts

Qu. 5.6. How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?

- For a list of relevant services use the CICES classification V5.1 provided as an annex.
- Impacts on ecosystem services build on the observed impacts on biodiversity (habitat, species, genetic, functional) but focus exclusively on reflecting these changes in relation to their links with socio-economic well-being.
- Quantitative data should be provided whenever available and references duly reported.

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Axis deer may affect several ecosystem services, not only through the discussed impacts on biodiversity, but also due to the impacts documented on ornamental plants and agricultural crops through browsing and bark stripping, for example in the Hawaiian Islands (Hess 2008). The erosion caused by the trampling behaviour associated with the death of trees caused by the habit to wipe their antlers on the barks, may results in destabilisation of stream banks, changes in stream flow and increased erosion and sedimentation of waterways (Anderson 1999, GISD 2015). Additionally, it is known that the trailing behaviour has caused erosion and damage to a variety of culturally or archaeologically significant sites in Hawaii (Anderson 1999). The role of axis deer in the regulation of zoonosis, because of its pathogens and parasites, is another possible threat to both wildlife and livestock, and to humans (Okarma et al. 2018b).

Here follows a list of potential impacts on ecosystem services (based on the CICES classification V5.1):

Provisioning (Biomass)

- Cultivated terrestrial plants
- Reared animals
- Wild plants (terrestrial and aquatic)
- Wild animals (terrestrial and aquatic)

Regulation & Maintenance (Regulation of physical, chemical, biological conditions)

- Baseline flows and extreme event regulation
- Lifecycle maintenance, habitat and gene pool protection
- Pest and disease control
- Soil quality regulation
- Water conditions

Cultural (Direct, in-situ and outdoor interactions with living systems that depend on presence in the environmental setting)

- Physical and experiential interactions with natural environment
- Intellectual and representative interactions with natural environment

Qu. 5.7. How important is the impact of the organism on provisioning, regulating, and cultural services currently in the different biogeographic regions or marine sub-regions where the species has established in the risk assessment area (include any past impact in your response)?

- See guidance to Qu. 5.6.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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No information has been found. It is worth mentioning that the value of hunting provided by game species can be an argument for introducing, translocating and preserving populations of these species, e.g. the fallow deer (*Dama dama*) in the risk assessment area. However, only little information is available for the target species (see point A13).

Qu. 5.8. How important is the impact of the organism on provisioning, regulating, and cultural services likely to be in the different biogeographic regions or marine sub-regions where the species can establish in the risk assessment area in the future?

- See guidance to Qu. 5.6.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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In the event of substantial spread and increase in numbers of axis deer to new parts of the risk assessment area, the impact may be expected to increase accordingly (at the moment there is no evidence of impact, but should the population grow and spread, the impact may become evident). As there is no documented evidence of the species being able to cause other types of impact, the level of risk can be expected to be “moderate” in the future. However, because of paucity of information, confidence of this assessment is low.

Economic impacts

Qu. 5.9. How great is the overall economic cost caused by the organism within its current area of distribution (excluding the risk assessment area), including both costs of / loss due to damage and the cost of current management.

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the world these should be reported here. The assessment of the potential costs of / loss due to damage shall describe those costs quantitatively and/or qualitatively depending on what information is available. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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As pointed out by Page et al. (2008), the species is capable of having an impact on sheep, cattle, cereal grain, grain legumes, and other fruit (pineapple) commodities. Crop damages by

axis deer, particularly when other available forage is scarce, have been described in both the native and the introduced range (Anderson 1999, Hess et al. 2015, GISD 2015, Page et al. 2008). For example, in Hawaii severe and extensive damage to the pineapple industry on Lānai was reported (Lever 1994, Hess et al. 2015). In Maui, more specifically, deer were blamed to be responsible of an estimated \$35,000 to \$55,000 in crop losses to Maui Pineapple Co., and one farmer claimed about 40 deer caused US \$20,000 in fence and corn crop damage in one night (Kubota 2001).

When overgrazing occurs, axis deer are known to compete with livestock and native wildlife (Long 2003). Being primarily grazers, axis deer compete for food mainly with domestic cattle and sheep (Lever 1994). In particular, direct competition for forage with cattle is reported in both California and in Texas (Anderson 1999). In California, in Point Reyes National Seashore, the cost to the park for staff, equipment, vehicles and supplies to monitor and manage non-native deer (both axis deer and fallow deer) was approximately \$140,000, or 2.5% of the park annual budget (GISD 2015, National Park Service 2004). In Argentina, although regularly hunted, axis deer populations have increased in some provinces, interfering with livestock production (Flueck 2009).

Deer may transmit infectious diseases directly to livestock (as well as to other deer and to humans), especially if deer density is high (Côté et al. 2004). In particular, axis deer have been shown to carry and transmit bovine tuberculosis (*Mycobacterium bovis*) and several other diseases in both the native range, i.e. in India (Schaller 1967) and the introduced range. For example, in Hawaii, bovine tuberculosis was found in five percent of deer from Molokai, posing an ongoing threat to cattle trade throughout the islands (Hess et al. 2015). In California, in addition to carrying several livestock and wildlife diseases, a small percentage of axis deer also harboured John's disease (*Mycobacterium paratuberculosis*), a contagious bacterial disease of the small intestines of ruminants (Hess et al. 2015). In Russia, the species was considered responsible for the introduction of the deer louse fly (*Lipoptena cervi*) (Bobrov et al. 2008), although this parasite is considered native to the region. However, some studies suggest that the indigenous parasite fauna of small founder populations of introduced exotic ungulates, such as the axis deer in Hawaii, frequently does not persist in their free-ranging progeny and that subsequent parasite communities acquired from sympatric ungulates are of limited diversity and comprised primarily of species exhibiting a broad host range (McKenzie and Davidson 1989).

Besides carrying parasites and pathogens, axis deer are responsible for a number of deer-vehicle collisions, as regularly documented on Molokai, Hawaii (Anderson 1999, Page et al. 2008), however the economic impact is not quantified.

Qu. 5.10. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism currently in the risk assessment area (include any past costs in your response)?

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the EU these should be reported here. Assessment of the potential costs of damage on human health, safety, and the economy, including the cost of non-action. A full economic assessment at EU scale might not be possible, but qualitative data or different case studies from across the EU (or third countries if relevant) may provide useful information to inform decision making. In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. Cost of / loss due to damage within

different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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No direct evidence of impact in the risk assessment area exists other than what is reported in Croatia. For example, in the island of Cres, the axis deer inflicted damage to vineyards and households (Frković 2014). No economic damage was recorded in the Island of Rab in a study aimed at the analysis of the feeding activities of axis deer and mouflon (*Ovis ammon*) in an actively managed (fenced) forest community of holm oak and manna ash (*Fraxino orn-Quercetum ilicis*) (Krapinec 2002a).

Okarma et al. (2018b) considered that in case the species established in Poland, the impact of the species on crops would be “medium”.

Qu. 5.11. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 5.10.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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In the event of substantial spread and increase in numbers of axis deer to new parts of the risk assessment area, the impact may be expected to increase accordingly.

Overabundant deer are known to inflict major economic losses in forestry, agriculture, and transportation and contribute to the transmission of several animal and human diseases (Côté et al. 2004). See also comments in **Qu. 5.19** and **Qu. 5.10**.

Qu. 5.12. How great are the economic costs / losses associated with managing this organism currently in the risk assessment area (include any past costs in your response)?

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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No information has been found.

Qu. 5.13. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 5.12.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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In the event of substantial spread and increase in numbers of axis deer to new parts of the risk assessment area, the costs may be expected to increase accordingly. If the species spreads and is to be managed, some costs are bound to be incurred, even if there is no info on what these costs are currently. But it is not possible to estimate the monetary value, as it depends on deer management systems and policies involved, which vary considerably across the different countries of Europe depending on species present, legislation, cultural tradition and the status of deer as *res nullius* or *res communis*.

Social and human health impacts

Qu. 5.14. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism for the risk assessment area and for third countries, if relevant (e.g. with similar eco-climatic conditions).

The description of the known impact and the assessment of potential future impact on human health, safety and the economy, shall, if relevant, include information on

- illnesses, allergies or other affections to humans that may derive directly or indirectly from a species;
- damages provoked directly or indirectly by a species with consequences for the safety of people, property or infrastructure;
- direct or indirect disruption of, or other consequences for, an economic or social activity due to the presence of a species.

Social and human health impacts can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Deer may transmit infectious diseases directly to humans (as well as to other deer and to livestock), especially if deer density is high (Côté et al. 2004). The axis deer, as other ungulates (hence similar to native species), can be a carrier of a number of diseases and parasites that may be harmful to humans. For example, they carry common parasites that may

directly affect humans, i.e. if droppings enter freshwater systems (GISD 2015). Parasitic zoonoses harbored by the species include: leptospirosis, cryptosporidiosis, and strains of *Escherichia coli* (Anderson 1999). A potential role of axis deer and their associated ticks (e.g. *Ixodes pacificus*) in the ecology of the Lyme disease spirochete, *Borrelia burgdorferi*, was evidenced through a study in California, USA (Lane and Burgdorfer 1986, Page et al. 2008). However the relationship between density of deer (and other large herbivores) in the environment and environmental tick burden is controversial, with different studies coming to different conclusions, hence the information above should be considered only indicative.

Overall, as pointed out for alien mammals in general (Capizzi et al. 2018), axis deer can act as vectors of both alien and native pathogens, and as host of either native or alien parasites (which in turn can be acting as vectors of either native or alien pathogens). In this way axis deer may either introduce new pathogens, alter the epidemiology of local pathogens, become reservoir hosts, and increase disease risk for humans, along with other species (e.g. by introducing changes in the vector-host-parasite relationship). However, we could not find any evidence of the species hosting new alien species, or increasing the rate or intensity of infections of pathogens.

In addition to carrying diseases that can infect humans, axis deer may cause road collisions, e.g. as reported in the Hawaiian Islands (Hess 2008). On Maui roads, for example, at least 36 motor vehicle collisions with axis deer occurred during an 18-month period between 1999 and 2000, see <http://archives.starbulletin.com/2001/08/28/news/story8.html>).

An indirect human health issue that deer axis pose in Hawaii is the potential for stray bullets to hit people as poaching increases (Anderson 1999). In any case shooting for managing the species is considered potentially dangerous and has led to complaints as it may represent a safety risk for residents, e.g. mostly because is conducted at night, as reported in Australia (Mitchell 2015).

Axis deer is an animal that is unlikely to make an unprovoked attack but such attacks can cause serious injury (requiring hospitalisation) or fatality if animals are cornered or handled (Page et al. 2008).

Qu. 5.15. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism in the future for the risk assessment area.

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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In the event of substantial spread and increase in numbers of axis deer to new parts of the risk assessment area, the impact may be expected to increase accordingly.

Other impacts

Qu. 5.16. How important is the impact of the organism as food, a host, a symbiont or a vector for other damaging organisms (e.g. diseases)?

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Deer, in general, may transmit infectious diseases directly to other species of deer (as well as to livestock, and to humans), especially if their density is high (Côté et al. 2004). The axis deer, as other ungulates, can be a carrier of a number of diseases and parasites that may be harmful to native species. For example, this species is involved in the transmission of bovine tuberculosis (Anderson 1999, Schaller 1967), which is a deadly disease for native ungulates, including the European bison (*Bison bonasus*), as pointed out by Okarma et al. (2018b). Other diseases transmitted by axis deer in their native range are leptospirosis and cryptosporidiosis (Anderson 1999, Schaller 1967). The species may also act as a new host for native parasites, as in the case of the tick *Amblyomma dubitatum* found on axis deer in northern Argentina, and this interrelationship may have potential deleterious effects on the native fauna, due to acquisition and amplification of the native parasite by an introduced host (Debárbora et al. 2012).

Qu. 5.17. How important might other impacts not already covered by previous questions be resulting from introduction of the organism?

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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No information has been found.

Qu. 5.18. How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area?

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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As described in Qu.3.5 the risk assessment area is certainly characterised by the presence of potential predators, parasites or pathogens of axis deer. However, there are several species of native and alien deer already occurring here, and this does not seem to represent a limiting factor for the relevant populations (predation from the large carnivores may be less effective than in the native range, given the lack of tigers and leopards). In fact, the role of predators in

controlling ungulate populations remains uncertain as pointed out by Côté et al. (2004), and is considered not effective, at least in some ecosystems.

The situation may be different in island ecosystems, where ungulates, as a consequence of their co-evolutionary history with large predators, may have very high reproductive rates, causing rapid population growth in the absence of predators. For example, in Hawaii, introduced axis deer exhibit annual population growth rates of 20–30% (Hess 2008).

Qu. 5.19. Estimate the overall impact in the risk assessment area under current climate conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, in current conditions.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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The species is known to exert a multifaceted impact on both biodiversity and ecosystem services, by feeding on native vegetation and contributing to the loss of habitat structure and function (hence indirectly affecting other species, including birds, reptiles, invertebrates, etc.). Competition with other ungulates is documented. The species is known to contribute to the spread of diseases and pathogens affecting both livestock and humans. It can also damage crops and compete with livestock. It can be a threat in relation to possible deer/vehicle collisions. No documented exists to provide discuss in details the overall impact in the biogeographical regions.

Qu. 5.20. Estimate the overall impact in the risk assessment area in foreseeable climate change conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, under future conditions.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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In foreseeable climate change conditions, the area suitable for the species in the risk assessment area may increase (see Annex VII), and the impact may be expected to increase accordingly. For example, in case of a future expansion of the species range, other native species may be affected. No documented evidence exists to discuss in details the overall impact in the biogeographical regions.

RISK SUMMARIES			
	RESPONSE	CONFIDENCE	COMMENT
Summarise Introduction*	very unlikely unlikely moderately likely likely very likely	low medium high	The species is already present in the risk assessment area (in the wild and in confinements). Further introductions for hunting, farming or exhibitions are considered possible.
Summarise Entry*	very unlikely unlikely moderately likely likely very likely	low medium high	Releases or escapes from captive facilities have been documented in the past in the risk assessment area and may take place again.
Summarise Establishment*	very unlikely unlikely moderately likely likely very likely	low medium high	Axis deer, although native to tropical and subtropical areas of the Indian subcontinent, has the ability to establish in other ecoclimatic zones, including those present in the EU, such as the Mediterranean. In Croatia it is currently established. The species life-history, available habitat conditions and management practices in the EU offer the potential to support self-sustaining populations of axis deer also in other countries and biogeographical regions.
Summarise Spread*	very slowly slowly moderately rapidly very rapidly	low medium high	The species has a sedentary habit, but is also known to spread over some distance in specific circumstances (e.g. suitability of habitat, lack of predators), including across islands given the good swimming skills.
Summarise Impact*	minimal minor moderate major massive	low medium high	The species is known to exert a multifaceted impact on both biodiversity and ecosystem services, by feeding on native vegetation and contributing to the loss of habitat structure and function (hence indirectly affecting other species, including birds, reptiles, invertebrates, etc.). Competition with other

			<p>ungulates is documented. The species is known to contribute to the spread of diseases and pathogens affecting both livestock and humans. It can also damage crops and compete with livestock. It can be a threat in relation to possible deer/vehicle collisions.</p>
<p>Conclusion of the risk assessment (overall risk)</p>	<p>low moderate high</p>	<p>low medium high</p>	<p>The axis deer represents a potential threat in the risk assessment area, given the ability to establish in the wild, the potential for spread, and the documented impact in other parts of the introduced range.</p> <p>Further warming of the climate due to climate change may increase impacts by increasing the amount of suitable habitat.</p>

*in current climate conditions and in foreseeable future climate conditions

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Distribution Summary

Please answer as follows:

- Yes if recorded, established or invasive
- if not recorded, established or invasive
- ? Unknown; data deficient

The columns refer to the answers to Questions A5 to A12 under Section A.

For data on marine species at the Member State level, delete Member States that have no marine borders. In all other cases, provide answers for all columns.

Member States and the United Kingdom

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Austria			Yes	Yes	
Belgium			Yes	Yes	
Bulgaria			Yes	Yes	
Croatia	Yes	Yes	Yes	Yes	Yes
Cyprus			Yes	Yes	
Czech Republic	Yes		Yes	Yes	
Denmark			Yes	Yes	
Estonia				Yes	
Finland				Yes	
France	Yes		Yes	Yes	
Germany			Yes	Yes	
Greece			Yes	Yes	
Hungary			Yes	Yes	
Ireland	Yes		Yes	Yes	
Italy			Yes	Yes	
Latvia			Yes	Yes	
Lithuania			Yes	Yes	
Luxembourg			Yes	Yes	
Malta			Yes	Yes	
Netherlands			Yes	Yes	
Poland			Yes	Yes	
Portugal			Yes	Yes	
Romania			Yes	Yes	
Slovakia			Yes	Yes	
Slovenia	Yes		Yes	Yes	
Spain			Yes	Yes	
Sweden			Yes	Yes	
United Kingdom	Yes		Yes	Yes	

Biogeographical regions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Alpine	Yes		Yes	Yes	
Atlantic			Yes	Yes	
Black Sea			Yes	Yes	
Boreal			Yes	Yes	
Continental	Yes		Yes	Yes	
Mediterranean	Yes	Yes	Yes	Yes	Yes
Pannonian			Yes	Yes	
Steppic			Yes	Yes	

ANNEX I Scoring of Likelihoods of Events

(adapted from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Description	Frequency
Very unlikely	This sort of event is theoretically possible, but is never known to have occurred and is not expected to occur	1 in 10,000 years
Unlikely	This sort of event has not occurred anywhere in living memory	1 in 1,000 years
Moderately likely	This sort of event has occurred somewhere at least once in recent years, but not locally	1 in 100 years
Likely	This sort of event has happened on several occasions elsewhere, or on at least one occasion locally in recent years	1 in 10 years
Very likely	This sort of event happens continually and would be expected to occur	Once a year

ANNEX II Scoring of Magnitude of Impacts

(modified from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Biodiversity and ecosystem impact	Ecosystem Services impact	Economic impact (Monetary loss and response costs per year)	Social and human health impact, and other impacts
	<i>Question 5.1-5</i>	<i>Question 5.6-8</i>	<i>Question 5.9-13</i>	<i>Question 5.14-18</i>
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected ⁸	Up to 10,000 Euro	No social disruption. Local, mild, short-term reversible effects to individuals.
Minor	Some ecosystem impact, reversible changes, localised	Local and temporary, reversible effects to one or few services	10,000-100,000 Euro	Significant concern expressed at local level. Mild short-term reversible effects to identifiable groups, localised.
Moderate	Measureable long-term damage to populations and ecosystem, but reversible; little spread, no extinction	Measureable, temporary, local and reversible effects on one or several services	100,000-1,000,000 Euro	Temporary changes to normal activities at local level. Minor irreversible effects and/or larger numbers covered by reversible effects, localised.
Major	Long-term irreversible ecosystem change, spreading beyond local area	Local and irreversible or widespread and reversible effects on one / several services	1,000,000-10,000,000 Euro	Some permanent change of activity locally, concern expressed over wider area. Significant irreversible effects locally or reversible effects over large area.
Massive	Widespread, long-term population loss or extinction, affecting several species with serious ecosystem effects	Widespread and irreversible effects on one / several services	Above 10,000,000 Euro	Long-term social change, significant loss of employment, migration from affected area. Widespread, severe, long-term, irreversible health effects.

⁸ Not to be confused with “no impact”.

ANNEX III Scoring of Confidence Levels

(modified from Bacher et al. 2017)

Each answer provided in the risk assessment must include an assessment of the level of confidence attached to that answer, reflecting the possibility that information needed for the answer is not available or is insufficient or available but conflicting. The responses in the risk assessment should clearly support the choice of the confidence level.

Confidence level	Description
Low	There is no direct observational evidence to support the assessment, e.g. only inferred data have been used as supporting evidence <i>and/or</i> Impacts are recorded at a spatial scale which is unlikely to be relevant to the assessment area <i>and/or</i> Evidence is poor and difficult to interpret, e.g. because it is strongly ambiguous <i>and/or</i> The information sources are considered to be of low quality or contain information that is unreliable.
Medium	There is some direct observational evidence to support the assessment, but some information is inferred <i>and/or</i> Impacts are recorded at a small spatial scale, but rescaling of the data to relevant scales of the assessment area is considered reliable, or to embrace little uncertainty <i>and/or</i> The interpretation of the data is to some extent ambiguous or contradictory.
High	There is direct relevant observational evidence to support the assessment (including causality) <i>and</i> Impacts are recorded at a comparable scale <i>and/or</i> There are reliable/good quality data sources on impacts of the taxa <i>and</i> The interpretation of data/information is straightforward <i>and/or</i> Data/information are not controversial or contradictory.

ANNEX IV Ecosystem services classification (CICES V5.1, simplified) and examples

For the purposes of this risk assessment, please feel free to use what seems as the most appropriate category / level / combination of impact (Section – Division – Group), reflecting information available.

Section	Division	Group	Examples (i.e. relevant CICES “classes”)
Provisioning	Biomass	Cultivated <i>terrestrial</i> plants	Cultivated terrestrial plants (including fungi, algae) grown for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from cultivated plants, fungi, algae and bacteria for direct use or processing (excluding genetic materials); Cultivated plants (including fungi, algae) grown as a <u>source of energy</u> <i>Example: negative impacts of non-native organisms to crops, orchards, timber etc.</i>
		Cultivated <i>aquatic</i> plants	Plants cultivated by in- situ aquaculture grown for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from in-situ aquaculture for direct use or processing (excluding genetic materials); Plants cultivated by in- situ aquaculture grown as an <u>energy source</u> . <i>Example: negative impacts of non-native organisms to aquatic plants cultivated for nutrition, gardening etc. purposes.</i>
		Reared animals	Animals reared for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from reared animals for direct use or processing (excluding genetic materials); Animals reared to provide <u>energy</u> (including mechanical) <i>Example: negative impacts of non-native organisms to livestock</i>
		Reared <i>aquatic</i> animals	Animals reared by in-situ aquaculture for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from animals grown by in-situ aquaculture for direct use or processing (excluding genetic materials); Animals reared by in-situ aquaculture as an <u>energy source</u> <i>Example: negative impacts of non-native organisms to fish farming</i>
		Wild plants (terrestrial and aquatic)	Wild plants (terrestrial and aquatic, including fungi, algae) used for <u>nutrition</u> ; <u>Fibres and other materials</u> from wild plants for direct use or processing (excluding genetic materials); Wild plants (terrestrial and aquatic, including fungi, algae) used as a <u>source of energy</u> <i>Example: reduction in the availability of wild plants (e.g. wild berries, ornamentals) due to non-native organisms (competition, spread of disease etc.)</i>
		Wild animals (terrestrial and aquatic)	Wild animals (terrestrial and aquatic) used for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from wild animals for direct use or processing (excluding genetic materials); Wild animals (terrestrial and aquatic) used as a <u>source of energy</u> <i>Example: reduction in the availability of wild animals (e.g. fish stocks, game) due to non-native organisms (competition, predations, spread of disease etc.)</i>
		Genetic material from all biota	Genetic material from plants, algae or fungi

			<p>strains or varieties; Individual genes extracted from higher and lower plants for the <u>design and construction of new biological entities</u></p> <p><i>Example: negative impacts of non-native organisms due to interbreeding</i></p>	
		Genetic material from animals	<p>Animal material collected for the purposes of maintaining or establishing a population; Wild animals (whole organisms) used to breed new strains or varieties; Individual genes extracted from organisms for the design and construction of new biological entities</p> <p><i>Example: negative impacts of non-native organisms due to interbreeding</i></p>	
		Water⁹	Surface water used for nutrition, materials or energy	<p>Surface water for <u>drinking</u>; Surface water used as a material (<u>non-drinking purposes</u>); Freshwater surface water, coastal and marine water used as an <u>energy source</u></p> <p><i>Example: loss of access to surface water due to spread of non-native organisms</i></p>
			Ground water for used for nutrition, materials or energy	<p>Ground (and subsurface) water for <u>drinking</u>; Ground water (and subsurface) used as a material (<u>non-drinking purposes</u>); Ground water (and subsurface) used as an <u>energy source</u></p> <p><i>Example: reduced availability of ground water due to spread of non-native organisms and associated increase of ground water consumption by vegetation.</i></p>
Regulation & Maintenance	Transformation of biochemical or physical inputs to ecosystems	Mediation of wastes or toxic substances of anthropogenic origin by living processes	<p><u>Bio-remediation</u> by micro-organisms, algae, plants, and animals; <u>Filtration/sequestration/storage/accumulation</u> by micro-organisms, algae, plants, and animals</p> <p><i>Example: changes caused by non-native organisms to ecosystem functioning and ability to filtrate etc. waste or toxics</i></p>	
		Mediation of nuisances of anthropogenic origin	<p><u>Smell reduction</u>; <u>noise attenuation</u>; <u>visual screening</u> (e.g. by means of green infrastructure)</p> <p><i>Example: changes caused by non-native organisms to ecosystem structure, leading to reduced ability to mediate nuisances.</i></p>	
	Regulation of physical, chemical, biological conditions	Baseline flows and extreme event regulation	<p>Control of <u>erosion</u> rates; Buffering and attenuation of <u>mass movement</u>; <u>Hydrological cycle and water flow regulation</u> (Including flood control, and coastal protection); <u>Wind</u> protection; <u>Fire</u> protection</p> <p><i>Example: changes caused by non-native organisms to ecosystem functioning or structure leading to, for example, destabilisation of soil, increased risk or intensity of wild fires etc.</i></p>	
		Lifecycle maintenance , habitat and gene pool protection	<p><u>Pollination</u> (or 'gamete' dispersal in a marine context); <u>Seed dispersal</u>; Maintaining <u>nursery populations and habitats</u> (Including gene pool protection)</p> <p><i>Example: changes caused by non-native organisms to the abundance and/or distribution of wild pollinators; changes to the availability / quality of nursery habitats for fisheries</i></p>	
		Pest and disease control	<p>Pest control; Disease control</p>	

⁹ Note: in the CICES classification provisioning of water is considered as an abiotic service whereas the rest of ecosystem services listed here are considered biotic.

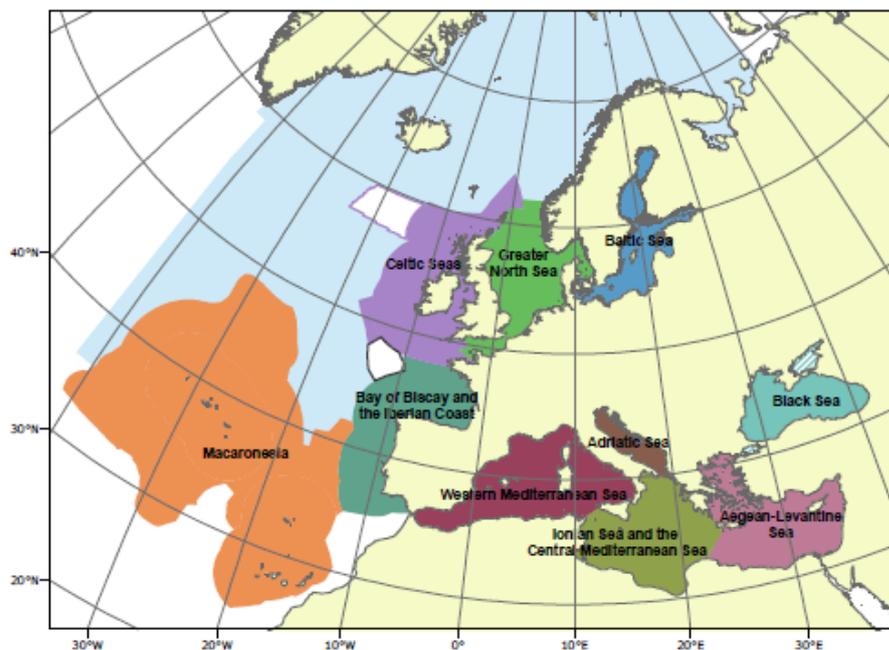
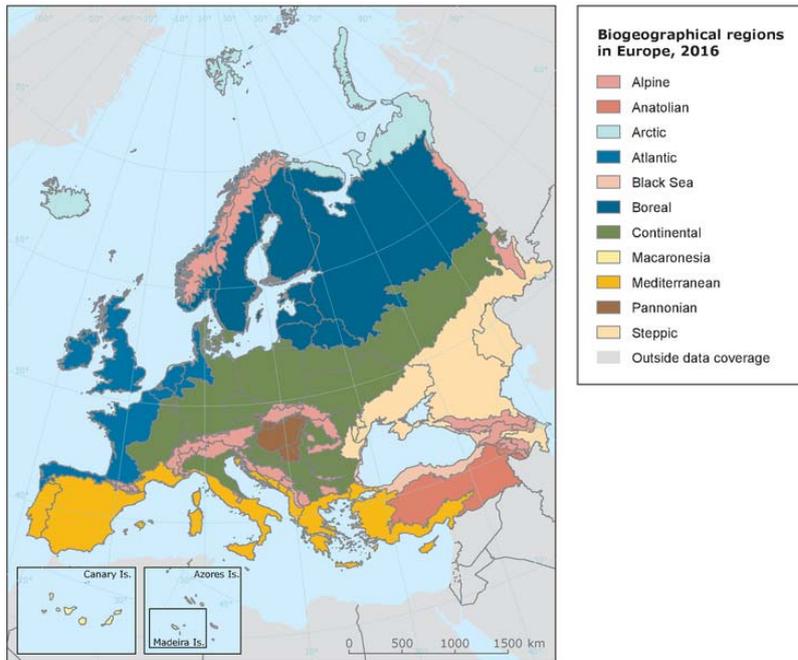
			<i>Example: changes caused by non-native organisms to the abundance and/or distribution of pests</i>
		Soil quality regulation	Weathering processes and their effect on soil quality; <u>Decomposition and fixing processes</u> and their effect on soil quality <i>Example: changes caused by non-native organisms to vegetation structure and/or soil fauna leading to reduced soil quality</i>
		Water conditions	Regulation of the <u>chemical condition</u> of freshwaters by living processes; Regulation of the chemical condition of salt waters by living processes <i>Example: changes caused by non-native organisms to buffer strips along water courses that remove nutrients in runoff and/or fish communities that regulate the resilience and resistance of water bodies to eutrophication</i>
		Atmospheric composition and conditions	Regulation of <u>chemical composition</u> of atmosphere and oceans; Regulation of <u>temperature and humidity</u> , including ventilation and transpiration <i>Example: changes caused by non-native organisms to ecosystems' ability to sequester carbon and/or evaporative cooling (e.g. by urban trees)</i>
Cultural	Direct, in-situ and outdoor interactions with living systems that depend on presence in the environmental setting	Physical and experiential interactions with natural environment	Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through <u>active or immersive interactions</u> ; Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through <u>passive or observational interactions</u> <i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that make it attractive for recreation, wild life watching etc.</i>
		Intellectual and representative interactions with natural environment	Characteristics of living systems that enable <u>scientific investigation</u> or the creation of traditional ecological knowledge; Characteristics of living systems that enable <u>education and training</u> ; Characteristics of living systems that are resonant in terms of <u>culture or heritage</u> ; Characteristics of living systems that enable <u>aesthetic experiences</u> <i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have cultural importance</i>
	Indirect, remote, often indoor interactions with living systems that do not require presence in the environmental setting	Spiritual, symbolic and other interactions with natural environment	Elements of living systems that have <u>symbolic meaning</u> ; Elements of living systems that have <u>sacred or religious meaning</u> ; Elements of living systems used for <u>entertainment or representation</u> <i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have sacred or religious meaning</i>
		Other biotic characteristics that have a non-use value	Characteristics or features of living systems that have an <u>existence value</u> ; Characteristics or features of living systems that have an <u>option or bequest value</u> <i>Example: changes caused by non-native organisms to ecosystems designated as wilderness areas, habitats of endangered species etc.</i>

ANNEX V EU Biogeographic Regions and MSFD Subregions

See <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2> ,
http://ec.europa.eu/environment/nature/natura2000/biogeog_regions/

and

<https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions-1/technical-document/pdf>



ANNEX VI Delegated Regulation (EU) 2018/968 of 30 April 2018

see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>

ANNEX VII Projection of climatic suitability for *Axis axis* establishment

Björn Beckmann, Riccardo Scalera, Beth Purse and Dan Chapman

30 October 2019

Aim

To project the suitability for potential establishment of *Axis axis* in Europe, under current and predicted future climatic conditions.

Data for modelling

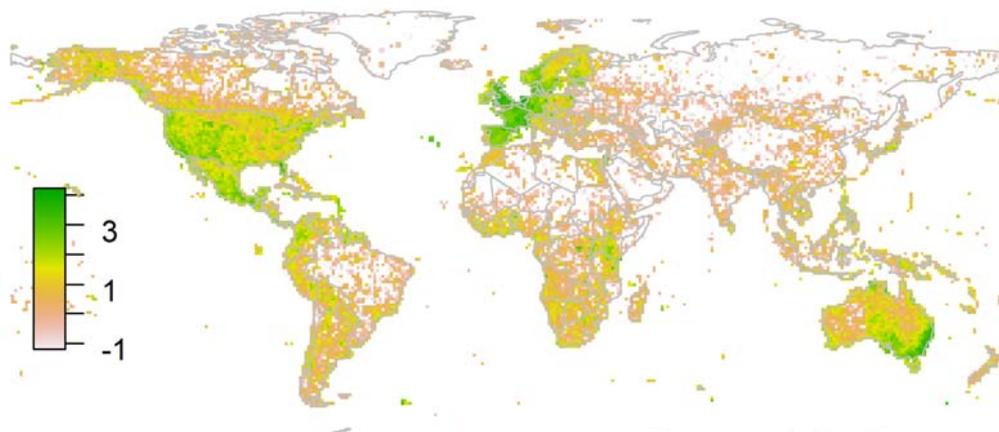
Species occurrence data were obtained from the Global Biodiversity Information Facility (GBIF) (394 records), the Biodiversity Information Serving Our Nation database (BISON) (85 records), the Atlas of Living Australia (19 records), the Integrated Digitized Biocollections (iDigBio) (8 records), and a small number of additional records from the risk assessment team. We scrutinised occurrence records from regions where the species is not known to be established and removed any dubious records (e.g. fossils, captive records) or where the georeferencing was too imprecise (e.g. records referenced to a country or island centroid) or outside of the coverage of the predictor layers (e.g. small island or coastal occurrences). The remaining records were gridded at a 0.25 x 0.25 degree resolution for modelling, yielding 156 grid cells with occurrences (Figure 1a). As a proxy for recording effort, the density of Mammalia records held by GBIF was also compiled on the same grid (Figure 1b).

Figure 1. (a) Occurrence records obtained for *Axis axis* and used in the modelling, showing native and invaded distributions. (b) The recording density of Mammalia on GBIF, which was used as a proxy for recording effort.

(a) Species distribution used in modelling



(b) Estimated recording effort (log10-scaled)



Climate data were selected from the ‘Bioclim’ variables contained within the WorldClim database (Hijmans et al., 2005), originally at 5 arcminute resolution (0.083 x 0.083 degrees of longitude/latitude) and aggregated to a 0.25 x 0.25 degree grid for use in the model.

Based on the biology of *Axis axis*, the following climate variables were used in the modelling:

- Minimum temperature of the coldest month (Bio6)
- Mean temperature of the warmest quarter (Bio10)
- Annual precipitation (Bio12)

To estimate the effect of climate change on the potential distribution, equivalent modelled future climate conditions for the 2070s under the Representative Concentration Pathways (RCP) 2.6 and 4.5 were also obtained. There represent low and medium emissions scenarios, respectively. The above variables were obtained as averages of outputs of eight Global Climate Models (BCC-CSM1-1, CCSM4, GISS-E2-R, HadGEM2-AO, IPSL-CM5A-LR, MIROC-ESM, MRI-CGCM3, NorESM1-M), downscaled and calibrated against the WorldClim baseline (see http://www.worldclim.org/cmip5_5m).

Species distribution model

A presence-background (presence-only) ensemble modelling strategy was employed using the BIOMOD2 R package v3.3-7.1 (Thuiller et al., 2019, Thuiller et al., 2009). These models contrast the environment at the species' occurrence locations against a random sample of the global background environmental conditions (often termed 'pseudo-absences') in order to characterise and project suitability for occurrence. This approach has been developed for distributions that are in equilibrium with the environment. Because invasive species' distributions are not at equilibrium and subject to dispersal constraints at a global scale, we took care to minimise the inclusion of locations suitable for the species but where it has not been able to disperse to (Chapman et al. 2019). Therefore the background sampling region included:

- The area accessible by native *Axis axis* populations, in which the species is likely to have had sufficient time to disperse to all locations. Based on presumed maximum dispersal distances, the accessible region was defined as a 300km buffer around the native range occurrences; AND
- A 30km buffer around the non-native occurrences, encompassing regions likely to have had high propagule pressure for introduction by humans and/or dispersal of the species; AND
- Regions where we have an *a priori* expectation of high unsuitability for the species so that absence is assumed irrespective of dispersal constraints (see Figure 2). The following rules were applied to define a region expected to be highly unsuitable for *Axis axis* at the spatial scale of the model:
 - Minimum temperature of the coldest month (Bio6) < -12°C
 - Mean temperature of the warmest quarter (Bio10) < 11°C
 - Annual precipitation (Bio12) < 6 (i.e. < 403mm, as the Bioclim variable is on a natural log scale)

Altogether, only 0.6% of occurrence grid cells were located in the unsuitable background region. Within the background region, 10 samples of 5000 randomly sampled grid cells were obtained, weighting the sampling by recording effort (Figure 2).

Figure 2. The background from which pseudo-absence samples were taken in the modelling of *Axis axis*. Samples were taken from a 300km buffer around the native range and a 30km buffer around non-native occurrences (together forming the accessible background), and from areas expected to be highly unsuitable for the species (the unsuitable background region). Samples were weighted by a proxy for recording effort (Figure 1(b)).



Each dataset (i.e. combination of the presences and the individual background samples) was randomly split into 80% for model training and 20% for model evaluation. With each training dataset, seven statistical algorithms were fitted with the default BIOMOD2 settings and rescaled using logistic regression, except where specified below:

- Generalised linear model (GLM)
- Generalised boosting model (GBM)
- Generalised additive model (GAM) with a maximum of four degrees of freedom per smoothing spline
- Artificial neural network (ANN)
- Multivariate adaptive regression splines (MARS)
- Random forest (RF)
- Maxent

Since the background sample was much larger than the number of occurrences, prevalence fitting weights were applied to give equal overall importance to the occurrences and the background. Normalised variable importance was assessed and variable response functions were produced using BIOMOD2's default procedure.

Model predictive performance was assessed by the following three measures:

- AUC, the area under the receiver operating characteristic curve (Fielding & Bell 1997). Predictions of presence-absence models can be compared with a subset of records set aside for model evaluation (here 20%) by constructing a confusion matrix with the number of true positive, false positive, false negative and true negative cases. For models generating non-dichotomous scores (as here) a threshold can be applied to transform the scores into a dichotomous set of presence-absence predictions. Two measures that can be derived from the confusion matrix are sensitivity (the proportion of observed presences that are predicted as such, quantifying omission errors), and specificity (the proportion of

observed absences that are predicted as such, quantifying commission errors). A receiver operating characteristic (ROC) curve can be constructed by using all possible thresholds to classify the scores into confusion matrices, obtaining sensitivity and specificity for each matrix, and plotting sensitivity against the corresponding proportion of false positives (equal to $1 - \text{specificity}$). The use of all possible thresholds avoids the need for a selection of a single threshold, which is often arbitrary, and allows appreciation of the trade-off between sensitivity and specificity. The area under the ROC curve (AUC) is often used as a single threshold-independent measure for model performance (Manel, Williams & Ormerod 2001). AUC is the probability that a randomly selected presence has a higher model-predicted suitability than a randomly selected absence (Allouche et al. 2006).

- Cohen's Kappa (Cohen 1960). This measure corrects the overall accuracy of model predictions (ratio of the sum of true presences plus true absences to the total number of records) by the accuracy expected to occur by chance. The kappa statistic ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random. Advantages of kappa are its simplicity, the fact that both commission and omission errors are accounted for in one parameter, and its relative tolerance to zero values in the confusion matrix (Manel, Williams & Ormerod 2001). However, Kappa has been criticised for being sensitive to prevalence (the proportion of sites in which the species was recorded as present) and may therefore be inappropriate for comparisons of model accuracy between species or regions (McPherson, Jetz & Rogers 2004, Allouche et al. 2006).
- TSS, the true skill statistic (Allouche et al. 2006). TSS is defined as sensitivity + specificity - 1, and corrects for Kappa's dependency on prevalence. TSS compares the number of correct forecasts, minus those attributable to random guessing, to that of a hypothetical set of perfect forecasts. Like kappa, TSS takes into account both omission and commission errors, and success as a result of random guessing, and ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random (Allouche et al. 2006).

An ensemble model was created by first rejecting poorly performing algorithms with relatively extreme low AUC values and then averaging the predictions of the remaining algorithms, weighted by their AUC. To identify poorly performing algorithms, AUC values were converted into modified z-scores based on their difference to the median and the median absolute deviation across all algorithms (Iglewicz & Hoaglin, 1993). Algorithms with $z < -2$ were rejected. In this way, ensemble projections were made for each dataset and then averaged to give an overall suitability, as well as its standard deviation. The projections were then classified into suitable and unsuitable regions using the 'minimum ROC distance' method, which minimizes the distance between the ROC plot and the upper left corner of the plot (point (0,1)), i.e. which maximises both sensitivity (correctly classified presences) and specificity (correctly classified absences).

We also produced limiting factor maps for Europe following Elith et al. (2010). For this, projections were made separately with each individual variable fixed at a near-optimal value. These were chosen as the median values at the occurrence grid cells. Then, the most strongly limiting factors were identified as the one resulting in the highest increase in suitability in each grid cell.

Results

The ensemble model suggested that suitability for *Axis axis* was most strongly determined by Annual precipitation (Bio12), accounting for 45.9% of variation explained, followed by Minimum temperature

of the coldest month (Bio6) (35.1%) and Mean temperature of the warmest quarter (Bio10) (19%) (Table 1, Figure 3).

Table 1. Summary of the cross-validation predictive performance (AUC, Kappa, TSS) and variable importance of the fitted model algorithms and the ensemble (AUC-weighted average of the best performing algorithms). Results are the average from models fitted to 10 different background samples of the data.

Algorithm	AUC	Kappa	TSS	Used in the ensemble	variable importance (%)		
					Annual precipitation (Bio12)	Minimum temperature of the coldest month (Bio6)	Mean temperature of the warmest quarter (Bio10)
GLM	0.984	0.653	0.948	yes	47	35	18
GAM	0.982	0.668	0.949	yes	43	40	18
ANN	0.982	0.648	0.949	yes	44	37	19
GBM	0.977	0.671	0.946	no	45	33	22
MARS	0.981	0.660	0.950	yes	42	40	18
RF	0.978	0.639	0.938	yes	54	23	23
Maxent	0.976	0.675	0.943	no	46	32	22
Ensemble	0.983	0.655	0.952		46	35	19

Figure 3. Partial response plots from the fitted models. Thin coloured lines show responses from the algorithms in the ensemble, while the thick black line is their ensemble. In each plot, other model variables are held at their median value in the training data. Some of the divergence among algorithms is because of their different treatment of interactions among variables.

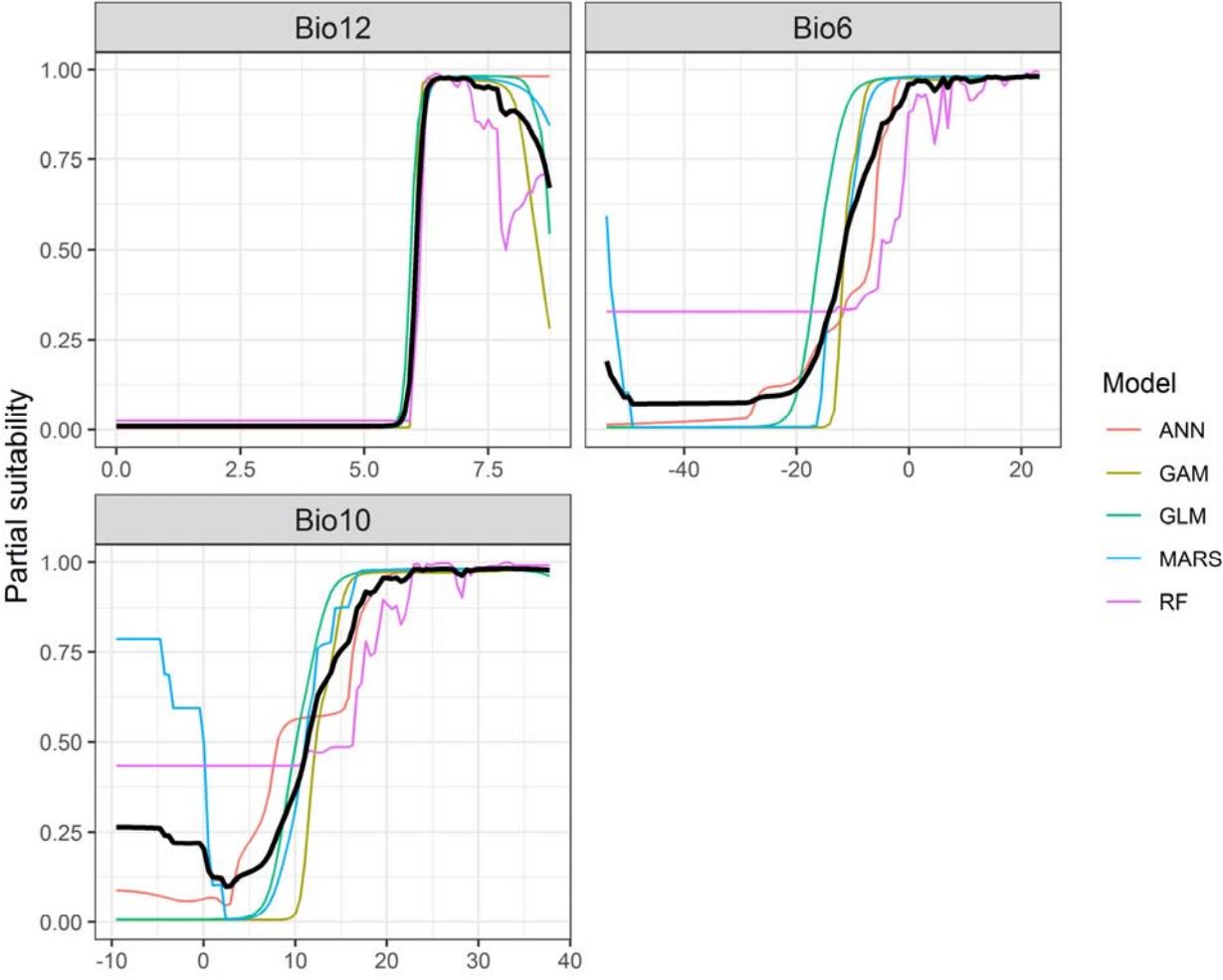


Figure 4. (a) Projected global suitability for *Axis axis* establishment in the current climate. For visualisation, the projection has been aggregated to a 0.5 x 0.5 degree resolution, by taking the maximum suitability of constituent higher resolution grid cells. Values > 0.45 may be suitable for the species ('minimum ROC distance' threshold at which both correctly classified presences and correctly classified absences are maximised). Grey areas have climatic conditions outside the range of the training data and were excluded from the projection. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

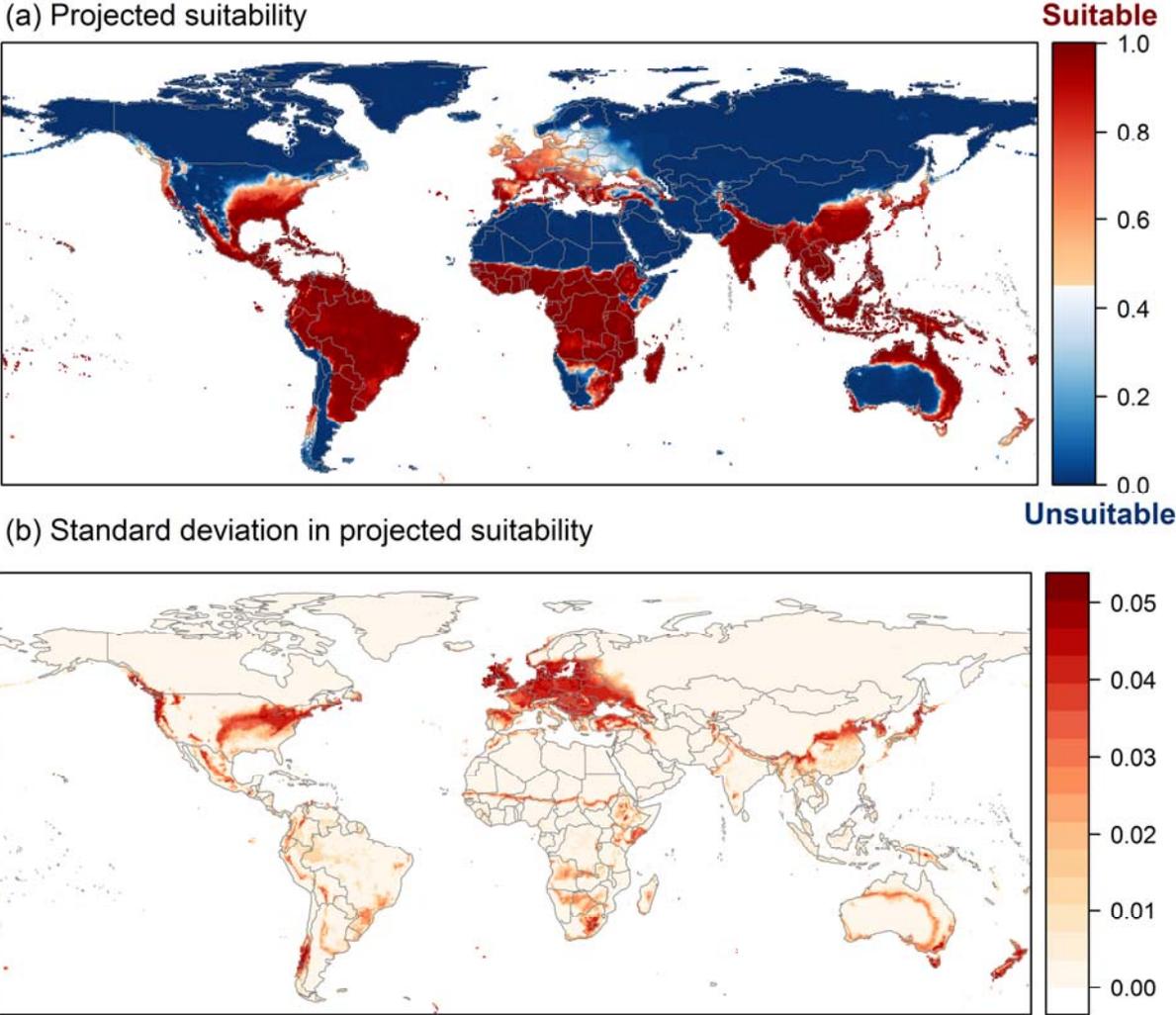


Figure 5. (a) Projected current suitability for *Axis axis* establishment in Europe and the Mediterranean region. Values > 0.45 may be suitable for the species ('minimum ROC distance' threshold at which globally both correctly classified presences and correctly classified absences are maximised). Grey areas have climatic conditions outside the range of the training data and were excluded from the projection. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

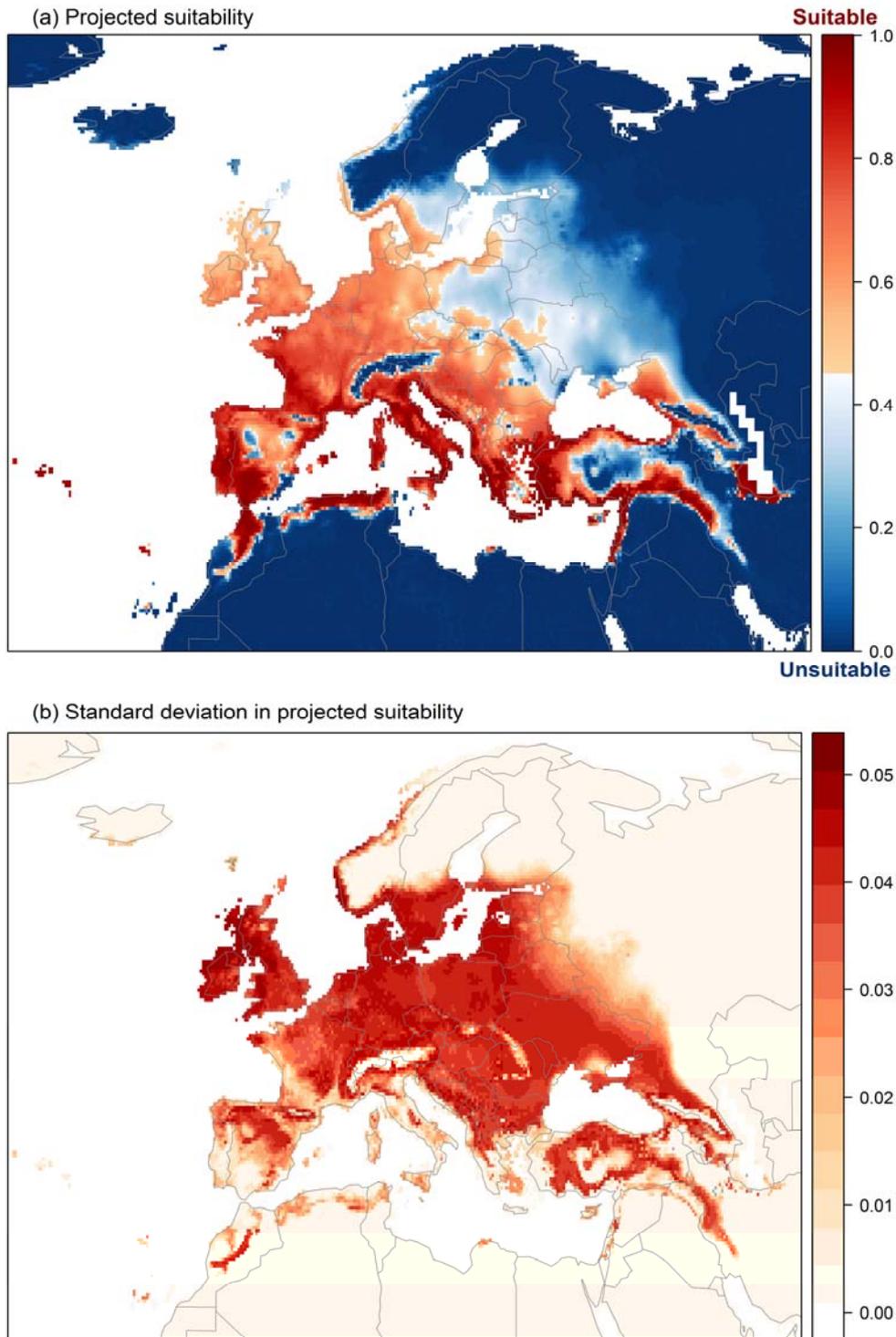


Figure 6. The most strongly limiting factors for *Axis axis* establishment estimated by the model in Europe and the Mediterranean region in current climatic conditions.



Figure 7. (a) Projected suitability for *Axis axis* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP2.6, equivalent to Figure 5. Values > 0.45 may be suitable for the species ('minimum ROC distance' threshold at which globally both correctly classified presences and correctly classified absences are maximised). Grey areas have climatic conditions outside the range of the training data and were excluded from the projection. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

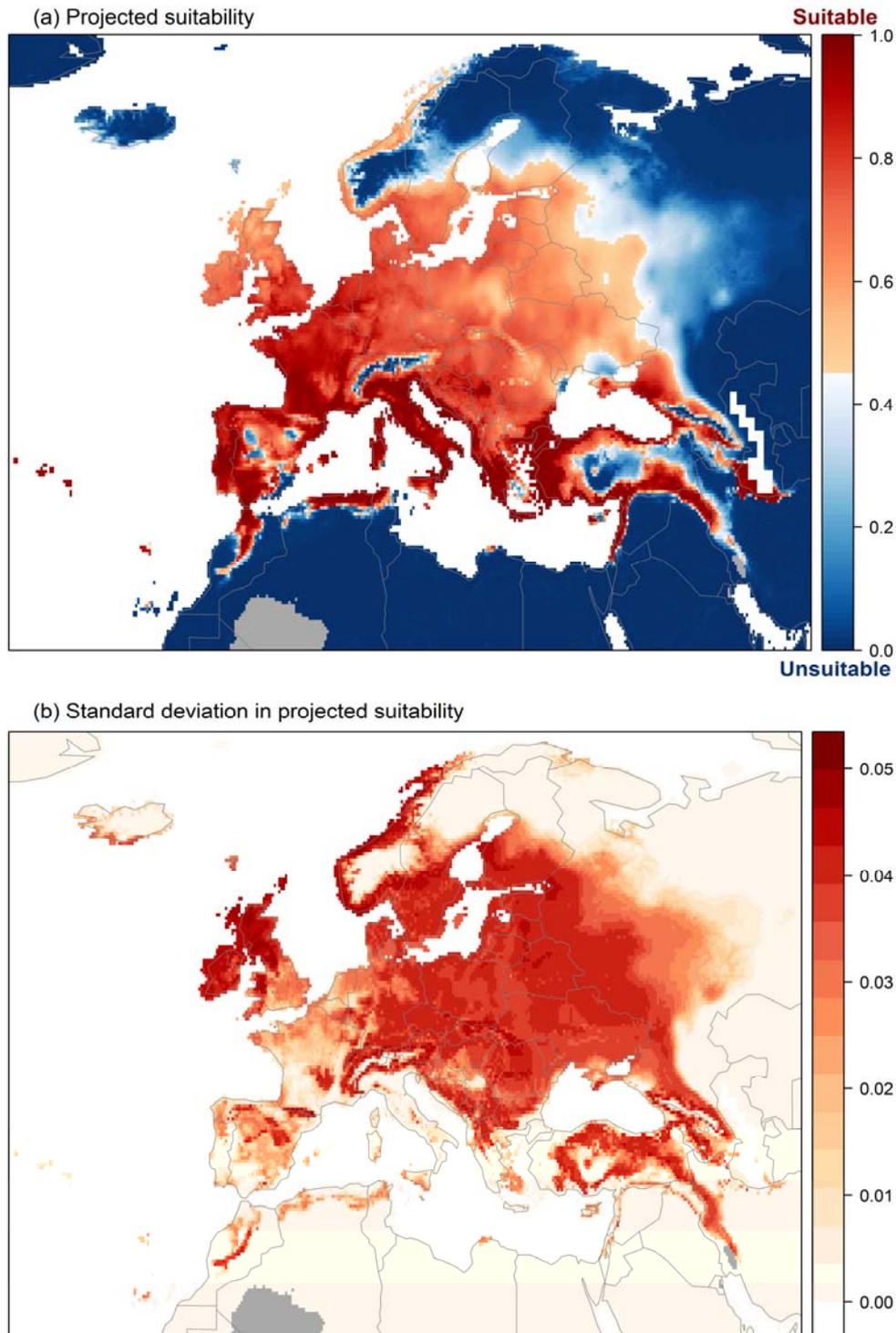


Figure 8. (a) Projected suitability for *Axis axis* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP4.5, equivalent to Figure 5. Values > 0.45 may be suitable for the species ('minimum ROC distance' threshold at which globally both correctly classified presences and correctly classified absences are maximised). Grey areas have climatic conditions outside the range of the training data and were excluded from the projection. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

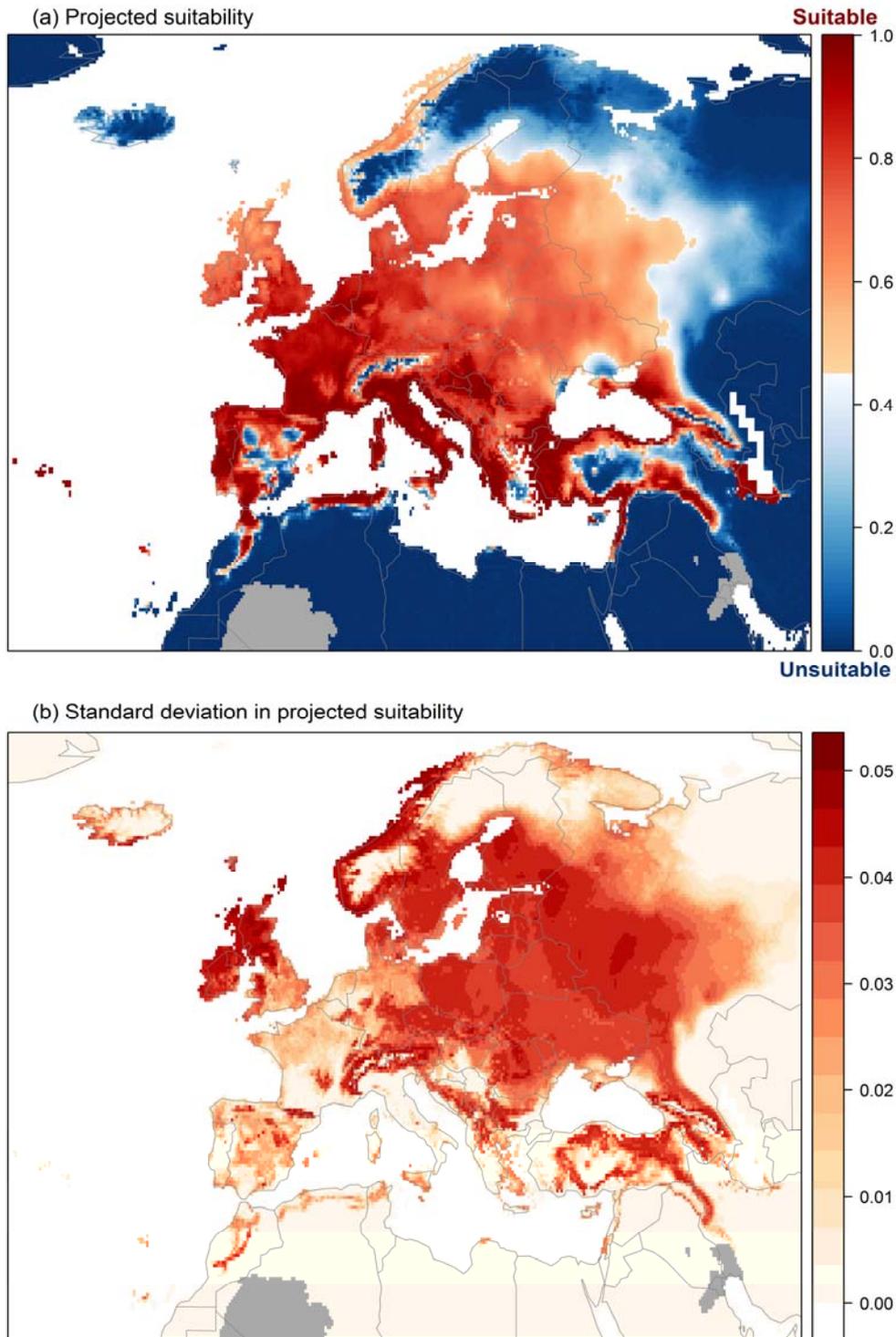


Figure 9. Variation in projected suitability for *Axis axis* establishment among Biogeographical regions of Europe (<https://www.eea.europa.eu/data-and-maps/data/biogeographical-regions-europe-3>). The bar plots show the proportion of grid cells in each region classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. The classification threshold is the same as in Figures 4, 5, 7 and 8 above (minimum ROC distance), i.e. the bars in the below diagram show the proportion of each Biogeographical region that is coloured in a shade of red in the above figures. The location of each region is also shown. The Arctic and Macaronesian biogeographical regions are not part of the study area, but are included for completeness.

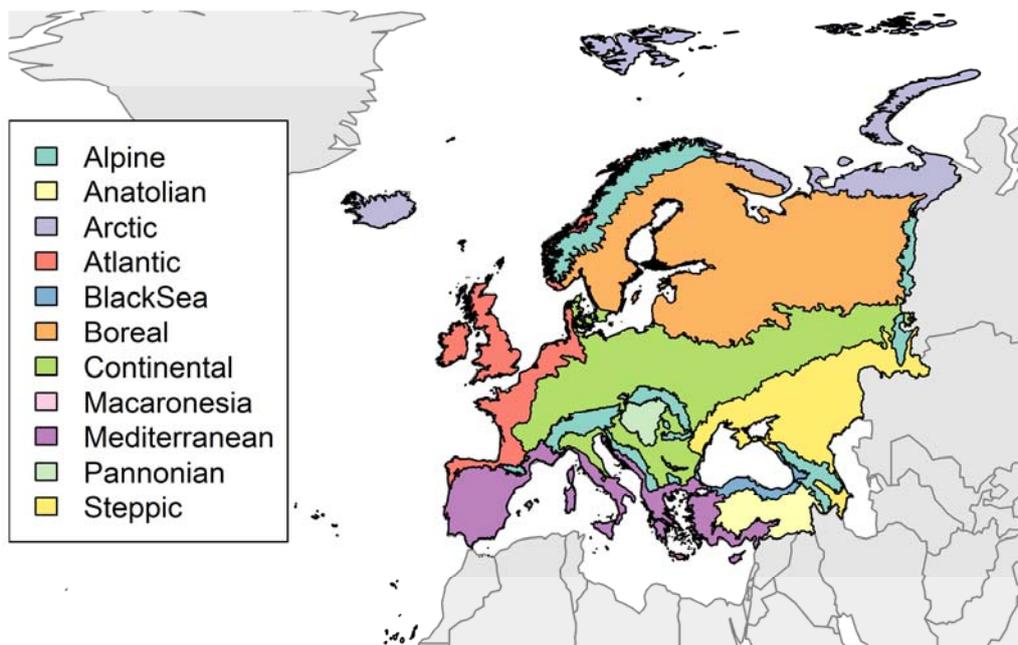
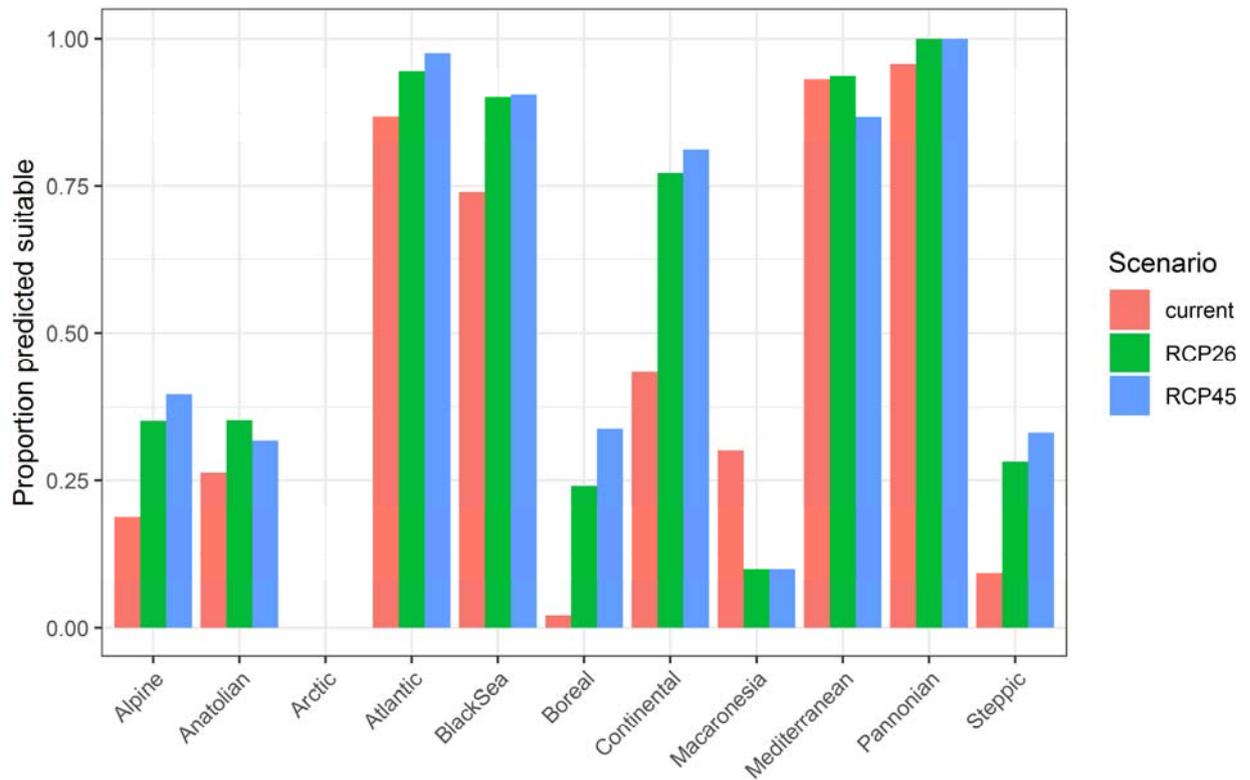
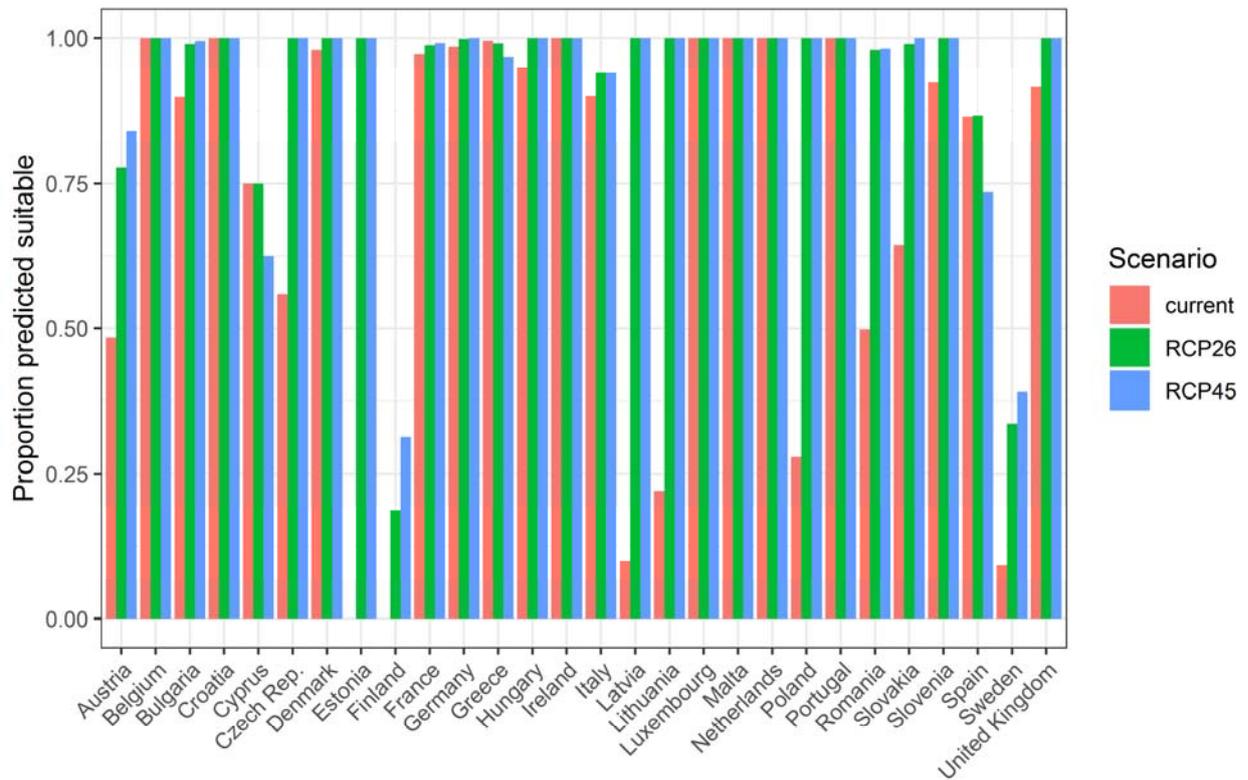


Figure 10. Variation in projected suitability for *Axis axis* establishment among European Union countries. The bar plots show the proportion of grid cells in each country classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. The classification threshold is the same as in Figures 4, 5, 7 and 8 above (minimum ROC distance), i.e. the bars in the below diagram show the proportion of each country that is coloured in a shade of red in the above figures.



Caveats to the modelling

To remove spatial recording biases, the selection of the background sample was weighted by the density of Mammalia records on the Global Biodiversity Information Facility (GBIF). While this is preferable to not accounting for recording bias at all, it may not provide the perfect measure of recording bias.

There was substantial variation among modelling algorithms in the partial response plots (Figure 3). In part this will reflect their different treatment of interactions among variables. Since partial plots are made with other variables held at their median, there may be values of a particular variable at which this does not provide a realistic combination of variables to predict from. Other variables potentially affecting the distribution of the species, such as land cover were not included in the model.

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**Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention"
Contract No 07.0202/2019/812602/ETU/ENV.D.2¹**

Name of organism: *Broussonetia papyrifera* (L.) L'Hér ex Vent.

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Risk Assessment Area: The risk assessment area is the territory of the European Union 27 and UK, excluding the outermost regions.

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Date of completion: 15/10/2020

Date of revision: 15/10/2021

¹ This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA). A number of amendments have been introduced to ensure compliance with Regulation (EU) 1143/2014 on IAS and relevant legislation, including the Delegated Regulation (EU) 2018/968 of 30 April 2018, supplementing Regulation (EU) No 1143/2014 of the European Parliament and of the Council with regard to risk assessments in relation to invasive alien species (see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>).

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SECTION A – Organism Information and Screening

A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?

including the following elements:

- the taxonomic family, order and class to which the species belongs;
- the scientific name and author of the species, as well as a list of the most common synonym names;
- names used in commerce (if any)
- a list of the most common subspecies, lower taxa, varieties, breeds or hybrids

As a general rule, one risk assessment should be developed for a single species. However, there may be cases where it may be justified to develop one risk assessment covering more than one species (e.g. species belonging to the same genus with comparable or identical features and impact). It shall be clearly stated if the risk assessment covers more than one species, or if it excludes or only includes certain subspecies, lower taxa, hybrids, varieties or breeds (and if so, which subspecies, lower taxa, hybrids, varieties or breeds). Any such choice must be properly justified.

Response: This risk assessment covers one species, the paper mulberry tree *Broussonetia papyrifera* (L.) L'Hér ex Vent.

Taxonomy

Regnum	<i>Plantae</i>
Infraregnum	<i>Streptophyta</i>
Divisio	<i>Tracheophyta</i> (Vascular plants)
Subdivisio	<i>Spermatophytina</i> (Seed plants)
Class	<i>Magnoliopsida</i>
Superordo	<i>Rosanae</i>
Ordo	<i>Rosales</i> Bercht. & J. Presl
Familia	<i>Moraceae</i> (mulberries)
Genus	<i>Broussonetia</i>

Broussonetia papyrifera (L.) L'Hér ex Vent. is an accepted name of a species with a temperate to subtropical East Asian origin (Chang et al. 2015) in the genus *Broussonetia* (Family *Moraceae*) (Euro+Med 2006-; The Plant List 2020). The accepted name is based on *Morus papyrifera* L. (basionym, Linnaeus 1753) (Euro+Med 2006-; IPNI 2020a). The lectotype for the name was described by Etienne Pierre Ventenat in the Tableau du Règne Végétal, Selon de Méthode de Jussieu (IPNI 2020b).

The genus name honors Pierre Auguste Marie Broussonet (1761-1807), French physician, naturalist and one-time professor of botany at Le Jardin des Plantes de Montpellier. Specific epithet comes from the Egyptian word papyrus meaning paper and the Latin word “ferre” meaning to bear in reference to the use of tree bark to make paper.

Synonyms

According to The Plant List (2013) and Plants of the World Online (2020) synonyms for the species are:

- *Broussonetia billardii* Carruth.
- *Broussonetia cordata* Blume
- *Broussonetia dissecta* Bureau
- *Broussonetia elegans* K. Koch
- *Broussonetia kasii* Dippel
- *Broussonetia kazi* Siebold ex Blume
- *Broussonetia maculata* Steud.
- *Broussonetia nana* Bureau
- *Broussonetia navicularis* Lodd. ex Bureau
- *Broussonetia navicularis* Lodd. ex K. Koch
- *Broussonetia papyrifera* var. *integrifolia* Miq.
- *Broussonetia spathulata* Steud.
- *Broussonetia tricolor* K. Koch
- *Morus papyrifera* L.
- *Papyrius papyrifera* (L.) Kuntze
- *Smithiodendron artocarpoideum* Hu
- *Stenochasma ancolanum* Miq.
- *Streblus cordatus* Lour.
- *Trophis cordata* (Lour.) Poir.

Common names

According to CABI (2020) and Euro+Med (2006-) common names in the European region are:

- Dutch (Belgian): papiermoerbe
- Catalan/ Valencian: morera de paper; morera de Xina; morera femella; morera paperera
- Croatian: brusonecija, dudovac
- Czech: papírovník čínský, brousonetie papíronosná
- English: paper mulberry, paper mulberry tree; tapa cloth tree
- French: mûrier à papier; mûrier de Chine; arbre à tapa
- German: Papiermaulbeerbaum; japanischer Papierbaum
- Italian: gelso papirifero del Giappone; moro della China, Brussonezia; gelso da carta; gelso della China;
- Spanish: mora de papel; moral de la China; morera del Japón; morera del papel; papelero

- Portuguese: amoreira do papel; amoreira-do-papel
- Slovak: brusonécia papierová

Most common subspecies, lower taxa, varieties, breeds or hybrids

About 16 or 17 varieties (including five wild) are recognized within this species (CABI 2020; HEAR 2020; Watt 1972). Watt 1972 refers to eleven or twelve varieties in cultivated plants besides which there are five varieties of the wild species, though there are no variety names available in this reference.

These cultural varieties have their origin in the very long use of the plant by humans. Varieties can be distinguished by different leaf shapes and margins and colors of the petiole (Seelenfreund et al. 2017).

Seelenfreund et al. (2017) report three main varieties of paper mulberry (masi) for Fiji: *masi vula* (white masi); *masi damu* (red masi); *masi vutu*. *Masi vula* can be recognized by the green petiole of the leaves, while *masi damu* is recognized by the reddish or brown petiole. *Masi vutu* is recognized not by the color of the petiole, but by the round shape of leaves. On the Vatulele Island, two varieties (*bele damu*, *bele vula*) with white and red stemmed leaves independently if the leaves are rounded or lobed. In Tonga, varieties are recognized according to the shape of the leaves: *lau ma'opo'opo* (more heart-shaped leaves with occasional incuts on one side), *lau mahaehae* (deeply lobed variety), *hiapo lai mangamanga* (deeply lobed leaves) and *hiapo lau a'opo'opo* (crenate leaves). Tapa makers in Hawaii basically recognize two varieties based on leaf shape: *mana mana lima* (regularly lobed leaves) and *poa'a'aha/poa aha* (non-lobed leaves).

Hybrids are identified on a regional level, e.g. *Broussonetia* × *kazinoki* Siebold has long been utilized as a major component in the manufacturing of Korean traditional paper (hanji) and Won (2019) confirmed by a genetic analysis that *B. × kazinoki* is a hybrid between *B. monoica* and *B. papyrifera*. The hybrid is most likely formed naturally in Korea.

Payacan et al. (2017) show a genetic diversity among paper mulberry accessions from Remote Oceania. A clear separation between West and East Polynesia was found that may be indicative of pulses during its dispersal history.

Hybrids and varieties are included in this risk assessment.

A2. Provide information on the existence of other species that look very similar [that may be detected in the risk assessment area, either in the environment, in confinement or associated with a pathway of introduction]

Include both native and non-native species that could be confused with the species being assessed, including the following elements:

- other alien species with similar invasive characteristics, to be avoided as substitute species (in this case preparing a risk assessment for more than one species together may be considered);
- other alien species without similar invasive characteristics, potential substitute species;
- native species, potential misidentification and mis-targeting

Response: Paper mulberry is a medium to large deciduous tree with milky sap. The crown is round and spreading. It is a hardy, fast-growing tree (CABI 2020; Saito et al. 2009, Tanasombat et al. 2005; Whistler & Elevitch 2006). Its stout, grey-brown, spreading branches are brittle and susceptible to wind damage. The branches are marked with stipular scars. Young branchlets are subtomentose and shoots are pubescent when young. The bark is light-grey, smooth, with shallow fissures or ridges. The stem, branches and petioles contain a milky latex. The twigs of paper mulberry are hairy reddish brown, the bark is tan and smooth to moderately furrowed, the wood is soft and brittle, and it has conical buds.

Broussonetia papyrifera has variable mulberry-like papery leaves. The leaves are densely gray-pubescent, often lobed or mitten-shaped, and are alternate, opposite or whorled along the stem. Some leaves are distinctly deep lobed, while others are unlobed. Several different shapes of leaves may appear on the same shoot. The leaves are alternate/subopposite, ovate, acuminate, dentate-crenate, their bases often oblique, scabrous above, with a woolly surface on the lower side. The leaf margin is sharply toothed, the leaf base is heart-shaped to rounded with pointed tips, and the upper leaf surface is rough feeling (CABI 2020).

Broussonetia papyrifera is a dioecious species, with unisexual male and female flowers in inflorescences on separate plants. Trees with staminate inflorescences (hereafter male trees) produce catkins that are long clusters of flowers. Trees with pistillate inflorescences (hereafter female trees) produce ball-shaped flower clusters, which mature into red, globose aggregate fruits. The male flower is 3.5-7.5 cm long, yellowish-white, with pendulous catkin-like spikes. The perianth is campanulate, hairy, 4-fid, and its segments are valvate (CABI 2020). The female flowers are in rounded clusters in globose pedunculate heads about 1.3 cm in diameter. Persistent, hairy, clavate bracts subtend flowers. The fruit is shiny-reddish, fleshy, globose and compound with the achenes hanging on long fleshy stalks. The achenes are 1-2 cm long and wide. Globose infructescence (syncarp), 2-3 cm in diameter, orange-red when ripe, composed of drupes thinly pulpy, invested by the perianth, hanging on long fleshy stalks. Seeds ovoid, slightly compressed, papillate-asperous, crustaceous, with a keel double at the base, 1.8-2.4 mm long (Barker 2002).

Existence of other native species that look very similar

Broussonetia papyrifera has no known close relatives in the risk assessment area. In the Mediterranean biogeographic region there is some risk of confusion with *Ficus carica* L., in particular with the wild type.

Existence of other non-native species that look very similar

The combination of hairy twigs and leaves, variable leaves, male and female flowers appearing on separate trees, and the fleshy globular fruits helps differentiate paper mulberry from potential look-alikes. The white mulberry (*Morus alba*) has leaves with larger teeth and its fruits resemble raspberries or blackberries rather than spheres. The fruits of the red mulberry (*Morus rubra*) resemble cylindrical raspberries (Rawlins et al. 2018; Sarver et al. 2008). Some leaf forms can be confused with white mulberry; however, paper mulberry leaves have a rough, sand paper-like surface, whereas mulberry leaves are smooth (Whistler & Elevitch 2006). There is also a potential risk of confusion with *Morus kagayamae* Koidz.

Basswood (*Tilia americana*) has leaves that are very similar to leaves of species of the mulberry family, but are never lobed. Basswood also has clear sap, whereas the sap of the mulberries is milky (Sarver et al. 2008).

A3. Does a relevant earlier risk assessment exist? Give details of any previous risk assessment, including the final scores and its validity in relation to the risk assessment area.

Response: The paper mulberry was added to the EPPO Alert List in 2016 and was prioritized in 2019 as a species with moderate risk and added to the EPPO Observation List in 2019.

Inside of the risk assessment area

Italy

Crosti et al. (2010) used a modified version of the Australian Weed Risk Assessment (A-WRA) adapted for the Mediterranean region of Central Italy, to assess the risk for a number of invasive alien plants in Lazio (Italy, Mediterranean biogeographical region). *Broussonetia papyrifera* scored 7, resulting in a “reject” decision (potentially invasive) according to the A-WRA.

Outside of the risk assessment area

Hawaii

The Hawaiian Ecosystems at Risk project conducted a risk assessment of *B. papyrifera* for the Hawaiian Islands (HEAR 2020). The method followed the Australian/New Zealand Weed Risk Assessment adapted for Hawaii. The species is assessed as “low risk” with a score of three. A rating of “low risk” indicates that the plant has a low probability of becoming a serious pest. It is one of the principle plants introduced by early voyaging Polynesians who settled in Hawaiian region. It has become naturalized throughout Asia, from India and Pakistan to the Pacific Islands, and also in North America. *Broussonetia papyrifera* is an extremely fast growing tree highly suitable for rehabilitating eroded areas and controlling further erosion. However, a few years after introduction it often becomes invasive and is difficult to eradicate. The tree shows the characteristics of a vigorous pioneer, spreading rapidly following extreme canopy disturbance (e.g. cyclones) or farming. Following invasion of an area, it excludes other species and it is very difficult to eradicate. Its growth is extremely fast and it quickly out-competes other species. Mature fruits have not been observed in Hawaii.

As the Hawaii Islands belong to a different climatic and biogeographical region than the area of the risk assessment, basic information of e.g. biology or habitat preferences can be taken into account, though the assessment of e.g. invasiveness has to be reviewed accordingly to different climate conditions.

Australia/Queensland

The Department of Agriculture and Fisheries (Csurhes 2016) conducted an invasive species risk assessment of paper mulberry for the federal state Queensland of Australia. In summary, the species is currently in its early stages of population development in Queensland. It appears to be a ‘high-risk’ species and a worthy candidate for preventative control. However, an assessment of the feasibility of eradication and/or control needs to be made. Currently, *B. papyrifera* is sparingly naturalized in Queensland. Wild populations have been detected in Brisbane and coastal northern Queensland. Substantial areas of eastern Queensland appear climatically suitable. Elsewhere in Australia, *B. papyrifera* has been recorded in South Australia and New South Wales. It seems reasonable to predict that *B. papyrifera* could become widespread and problematic in suitable habitat types in coastal and subcoastal Queensland (where climate, soil and land use are favourable). Habitats most at risk are predicted to include riparian areas; semi-deciduous (closed) forests and vine thickets (especially margins and gaps); and disturbed, open sites generally, where there is relatively well-drained, moist, fertile soil. On some islands in the Pacific, only male plants are cultivated and regeneration is purely vegetative. If

male and female plants are present, long-range dispersal is via seeds. Seeds rarely germinate under dense forest canopies, but germination can be prolific in large canopy gaps, roadsides and abandoned farmland. Root suckers are produced when the main stem is cut. Over time, this can lead to the formation of dense thickets. Root suckers can be cut, dug out and replanted (the main form of reproduction when ‘male clones’ are used for agroforestry).

As Australia belongs to a different climatic and biogeographical region than the area of this risk assessment, basic information of e.g. biology or habitat preferences can be taken into account, though the assessment of e.g. invasiveness has to be reviewed accordingly to different climate conditions.

Georgia – United States of America

In 2017, the paper mulberry has been evaluated for the region of the US federal state Georgia (Bucalo 2017). The outcome is an indication of the invasive potential of Paper mulberry in Georgia, and therefore should not be considered for sale or propagation. According to Georgia Exotic Pest Plant Council (GA-EPPC), the species is ranked in the category 3: Exotic plant that is a minor problem in Georgia natural areas, or is not yet known to be a problem in Georgia but is known to be a problem in adjacent states. PRE is a 20-question plant risk assessment tool. It is also an online platform and database enabling those involved in non-native, terrestrial plant production to know before they grow if a plant poses a potential regional invasive risk. In summary, *B. papyrifera* can spread via seed and vegetative suckers, coppicing and re-sprouting, and it is a very vigorous grower. It is reported as invasive in many states that are a climate match for Georgia. Paper mulberry exhibits aggressive growth and quickly invades disturbed lands, competing with more desirable plant species. The fruits of *B. papyrifera* are consumed by many types of birds and other small animals which are thought to disseminate the seeds. Detailed species information about such animals dispersing seeds are not available. Water may also play a role of dispersal alongside water courses or irrigation channels. The seeds are spread far and wide by wildlife who feed on the fruits.

As Georgia belongs to a different climatic and biogeographical region than the area of this risk assessment, basic information of e.g. biology or habitat preferences can be taken into account, though the assessment of e.g. invasiveness has to be reviewed accordingly to different climate conditions.

A4. Where is the organism native?

including the following elements:

- an indication of the continent or part of a continent, climatic zone and habitat where the species is naturally occurring
- if applicable, indicate whether the species could naturally spread into the risk assessment area

Response: *Broussonetia papyrifera* is native to East Asia and mainland Southeast Asia (González-Lorca et al. 2015; Huston 2004; Peñailillo et al. 2016). An accurate native range distribution cannot be given as the species was introduced already prehistorically in the surroundings as a species of cultural importance (González-Lorca et al. 2015), e.g. to Polynesia as a source of bark fiber by Austronesian-speaking voyagers (Peñailillo et al. 2016).

While CABI (2020) lists China, Japan and Taiwan as countries within the native distribution (according to Ghafoor 1985), Csurhes (2016) includes parts of China (Anhui, Fujian, Gansu, Guangdong, Guangxi, Guizhou, Hainan, Hebei, Henan, Hubei, Hunan, Jiangsu, Jiangxi, Shaanxi, Shandong, Shanxi, Sichuan,

Xizang, Yunnan and Zhejiang), Japan, Korea, Taiwan, Cambodia, Laos, Myanmar, Thailand, Vietnam and Malaysia into a larger native range (compare POWO 2020). On the other hand, EPPO (2019) considers China, Japan, Korea, Taiwan and Thailand within the native range. Chung et al. (2017) give a comprehensive overview about considerable discrepancies existing in literature regarding distribution ranges of the species.

Broussonetia papyrifera can tolerate a wide range of climates, including humid tropical (monsoon), humid and sub-humid subtropical as well as warm temperate areas (EPPO 2019; Whistler & Elevitch 2006). It can grow in areas with an annual rainfall of 700–2 500 mm (EPPO 2019; CABI 2020), though the species can survive a 3–4 month dry season (Whistler & Elevitch 2006). The paper mulberry is considered being a pioneer species adapted to colonize disturbed sites (Csurhes 2016). The species does not grow well under full shade and is suited to disturbed habitats, in particular to riparian habitats and gaps within subtropical forest (EPPO 2019; Saito et al. 2009; Luna 1996). It exhibits a high reproductive potential, by means of both sexual and vegetative propagation (Maan et al. 2020; Morgan et al. 2019). *Broussonetia papyrifera* is insect- and wind-pollinated, and can be grown from seed, stem cuttings, coppice and root suckers (CABI 2020). The fruit, which is a compound (syncarp) of juicy, orange-red drupelets are dispersed by birds and small mammals (Seelenfreund et al. 2017). Its fecundity is high by virtue of its ability to fruit twice in a year and also achieving reproductive maturity at relatively small size. Thus sexual reproduction maybe the most important mode of reproduction that drives its invasiveness. Though trees start fruiting early, fruiting percentages increase with tree size (Kyereh et al. 2014).

Since the native distribution center in Asia and the European continent are separated by a large distance, the natural spread into the risk assessment area is very unlikely.

A5. What is the global non-native distribution of the organism outside the risk assessment area?

Response: Table 1 and Figure 1 give an overview about the global non-native distribution outside the risk assessment area. Considering the broader native range, available spatial and non-spatial data describe the non-native distribution of *B. papyrifera* covering parts of North, Middle and South America, Europe, Africa, Asia and Oceanica.

In the neighborhood of the risk assessment area, the species' status is “introduced” in Switzerland (Aeschimann & Burdet 1994; Hess 1998). In some countries of the Balkan peninsula (Bosnia and Herzegovina and Montenegro; Euro+Med 2006-, Stešević & Petrović 2010) the paper mulberry is assessed as “introduced and naturalized”. Furthermore the occurrence of *B. papyrifera* is documented in Albania, Georgia, Macedonia, Moldova, Russia and Ukraine (Table 1).

Broussonetia papyrifera

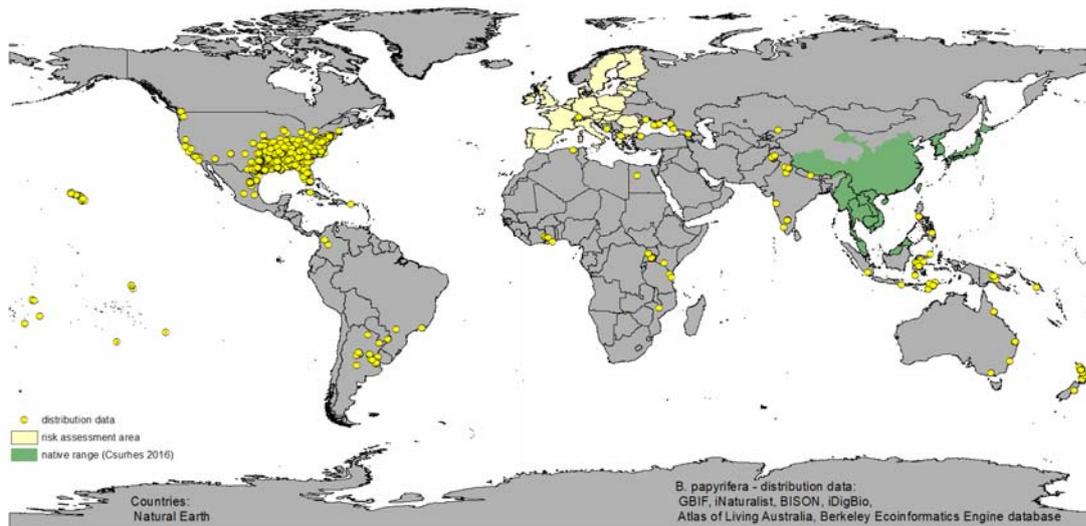


Figure 1 Global non-native occurrences records of *Broussonetia papyrifera* outside the risk assessment area (map designed by Umweltbundesamt/Environment Agency Austria – 2020/07/01)

Table 1 countries with *Broussonetia papyrifera* occurrences mentioned in references and databases

continent	country	general remarks	source
Africa	Algeria	no data available	GBIF 2020; iNaturalist 2020
	Egypt	no data available	GBIF 2020
	Ghana	invasive	Adigbli et al. 2019; Apetorgbor & Bosu 2011; GBIF 2020; Kyereh et al. (2014); Witt et al. 2018; Haysom & Murphy 2003; Witt & Luke 2017
	Kenia	no data available	iNaturalist 2020; BioNET-EAFRINET 2020
	Malawi	no data available	GBIF 2020
	Tanzania	naturalized	GBIF 2020; iNaturalist 2020; Dawson et al. 2008; Witt & Luke 2017
	Uganda	introduced; invasive	CABI 2020; POWO 2020; GBIF 2020; Witt et al. 2018; Haysom & Murphy 2003; Witt & Luke 2017
Zimbabwe	planted	CABI 2020	
Asia	India	planted; introduced; invasive	CABI 2020; GBIF 2020; iNaturalist 2020; ALA 2020; Haysom & Murphy 2003
	Indonesia	Planted	CABI 2020; GBIF 2020; iNaturalist 2020
	Kyrgyzstan	no data available	iDigBio 2020
	Nepal	no data available	iNaturalist 2020
	Pakistan	planted; introduced; widespread; invasive	CABI 2020; POWO 2020; GBIF 2020; iNaturalist 2020; Witt et al. 2018; Haysom & Murphy 2003; Ahmed et al. 2019; Khan et al. 2020
	Papua New Guinea	no data available	GBIF 2020
	Philippines	no data available	Chang et al. 2015; GBIF 2020; iNaturalist 2020
	Tadzhikistan	no data available	POWO 2020
Uzbekistan	no data available	POWO 2020	
Europe	Albania	no data available	Euro+Med 2006-; Vangjeli 2017
	Bosnia and Herzegovina	introduced; naturalized	Euro+Med 2006-; iNaturalist 2020
	Georgia	no data available	POWO 2020; iNaturalist 2020

	Macedonia	no data available	iNaturalist 2020
	Moldova	Cultivated	Euro+Med 2006-; Tzvelev 2004
	Montenegro	introduced: naturalized	Euro+Med 2006-; Stešević & Petrović 2010; Bulatović et al. 2016
	Russia	no data available	CABI 2020; GBIF 2020; iNaturalist 2020
	Serbia	no data available	Rat et al. 2016
	Switzerland	Introduced: casual	GBIF 2020; iNaturalist 2020; Aeschmann & Burdet 1994; Hess 1998; Mangili et al. 2018; Schoenenberger et al. 2014
	Ukraine	cultivated; planted	Euro+Med 2006-; CABI 2020; iNaturalist 2020
North and Middle America (incl. Caribbean)	Canada	no data available	BISON 2020
	Cuba	no data available	GBIF 2020
	Mexico	no data available	GBIF 2020; iNaturalist 2020
	United States of America: Alabama, Arkansas, Connecticut, Florida, Georgia, Hawaii, Illinois, Indiana, Kansas, Kentucky, Louisiana, Missouri, New York, North Carolina, Ohio, Oklahoma, Pennsylvania, South Carolina, Tennessee, Texas, Virginia, West Virginia	Introduced: planted	CABI 2020; GBIF 2020; iNaturalist 2020; iDigBio 2020; ALA 2020; BISON 2020; Haysom & Murphy 2003; USDA 2020
Oceania	Australia	no data available	ALA 2020; GBIF 2020
	East Timor	no data available	GBIF 2020; Haysom & Murphy 2003
	Easter Island	no data available	POWO 2020; Haysom & Murphy 2003
	New Zealand	no data available	GBIF 2020; iNaturalist 2020; ALA 2020; Haysom & Murphy 2003
	Samoa	no data available	CABI 2020; GBIF 2020; Haysom & Murphy 2003
	Society Island	no data available	POWO 2020; Haysom et al. 2003
	Solomon Island	planted	CABI 2020; GBIF 2020; ALA 2020; Haysom & Murphy 2003
	Tonga	planted	CABI 2020; POWO 2020; Haysom & Murphy 2003
	Vanuatu	no data available	POWO 2020; Haysom & Murphy 2003
	Wallis-Futuna Island (Pacific Islands)	no data available	POWO 2020; Haysom & Murphy 2003
		Naturalized	Haysom & Murphy 2003
South America	Argentina	no data available	POWO 2020; GBIF 2020; iNaturalist 2020; iDigBio 2020
	Brazil	no data available	GBIF 2020; iNaturalist 2020; iDigBio 2020
	Colombia	no data available	iNaturalist 2020
	Peru	no data available	Haysom et al. 2003
	Uruguay	no data available	GBIF 2020; iNaturalist 2020

A6. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species been recorded and where is it established? The information needs be given separately for recorded (including casual or transient occurrences) and established occurrences. “Established” means the process of an alien species successfully producing viable offspring with the likelihood of continued survival².

A6a. Recorded: List regions

² Convention on Biological Diversity, Decision VI/23

A6b. Established: List regions

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

- Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea.

Comment on the sources of information on which the response is based and discuss any uncertainty in the response.

For delimitation of EU biogeographical regions please refer to <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2> (see also Annex VI).

For delimitation of EU marine regions and subregions consider the Marine Strategy Framework Directive areas; please refer to <https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions/technical-document/pdf> (see also Annex VI).

Response: Figure 2 and Table 2 give an overview about occurrences of the species in the biogeographical regions. The Arctic, Anatolian and Macaronesian biogeographical regions are not part of the risk assessment area, but included for completeness. In the Steppic region, no occurrence information is available.

Sources of information are observation data (GBIF 2020, iDigBio 2020, iNaturalist 2020, ALA 2020), European wide overviews like Euro+Med PlantBase (providing an on-line database and information system for the vascular plants of Europe and the Mediterranean region, against an up-to-date and critically evaluated consensus taxonomic core of the species concerned) or Flora Europaea (Tutin et al. 1964-1980) as well as national floristic or invasive species references (e.g. Greuter et al. 1989, Conti et al. 2005, Nikolić 1994; Boršić et al. 2008; Strid & Tan 1997; Mifsud 2020).

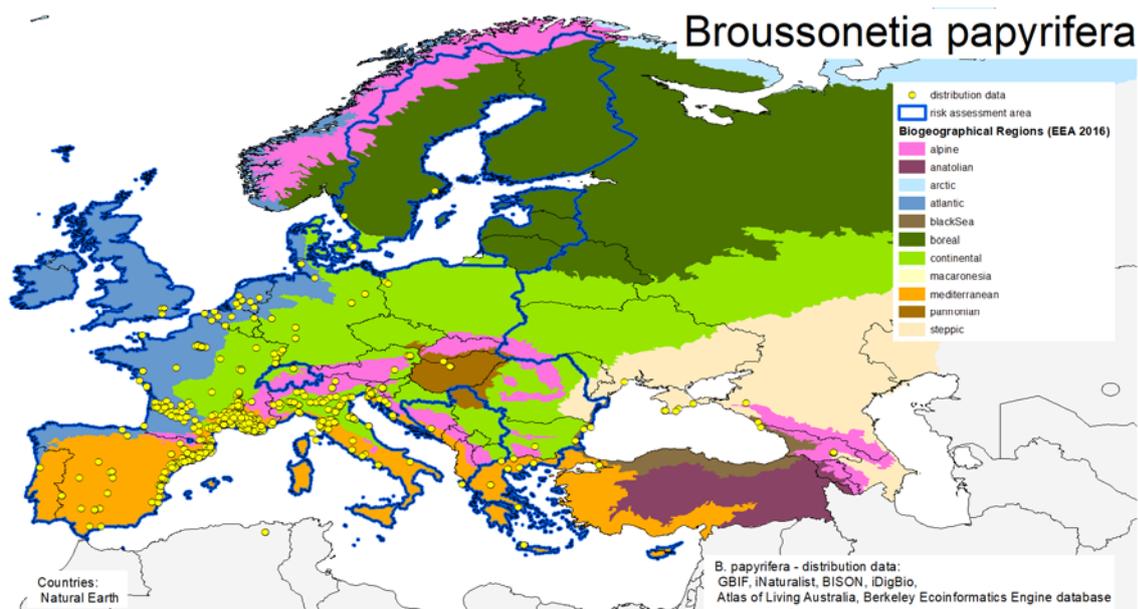


Figure 2 Occurrences records of *Broussonetia papyrifera* in the biogeographical regions (map designed by Umweltbundesamt/Environment Agency Austria – 2020/07/01)

Table 2 biogeographical regions with recorded/established *Broussonetia papyrifera* occurrences (green cell – recorded/established yes; white cell – recorded/established no; grey cell – no examined or no data available)

biogeographical region	recorded	established	source of recorded occurrences	source of established occurrences
Alpine			GBIF 2020; iDigBio 2020; Aeschimann et al. 2004	
Anatolian				
Arctic				
Atlantic			ALA 2020; GBIF 2020; iNaturalist 2020	Euro+Med 2006-; Tutin et al. 1964-1980; Greuter et al. 1989;
Black Sea			GBIF 2020; iNaturalist 2020	
Boreal				
Continental			ALA 2020; GBIF 2020; iNaturalist 2020	Euro+Med 2006-; Tutin et al. 1964-1980; Greuter et al. 1989; Conti et al. 2005
Macaronesian				
Mediterranean			GBIF 2020; iNaturalist 2020	Euro+Med 2006-; Tutin et al. 1964-1980; Greuter et al. 1989; Conti et al. 2005; Tutin

				et al. 1964-1980; Greuter et al. 1989; Nikolić 1994; Boršić et al. 2008; Strid & Tan 1997; Mifsud 2020
Pannonian			GBIF 2020; iNaturalist 2020	
Steppic				

Response (6a):

Paper mulberry is recorded in the Alpine, Atlantic, Black Sea, Continental, Mediterranean and Pannonian biogeographical regions. No occurrence data is available for the Steppic region. In the Boreal biogeographic region, occurrence data is available only in botanical gardens.

Response (6b):

According to the available information, *B. papyrifera* can be considered as “established” in the Atlantic, Continental and Mediterranean biogeographical regions.

“Established” means the process of an alien species successfully producing viable offspring with the likelihood of continued survival. In the available references (mainly Euro+Med PlantBase and Flora Europaea), the term “naturalized” is used. In order to answer question 6b, “naturalized” is used as a synonym for “established”. The assessment of established occurrences in biogeographical regions is mainly deduced from the data available on national level (compare Table 5).

A7. In which biogeographic region(s) or marine subregion(s) in the risk assessment area could the species establish in the future under current climate and under foreseeable climate change? The information needs to be given separately for current climate and under foreseeable climate change conditions.

A7a. Current climate: List regions

A7b. Future climate: List regions

With regard to EU biogeographic and marine (sub)regions, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065)

and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

According to SDM results (Figure 3 and Figure 4, Table 3 and Table 4, see also Annex IX) the biogeographical regions paper mulberry could establish in are as follows:

Response (7a):

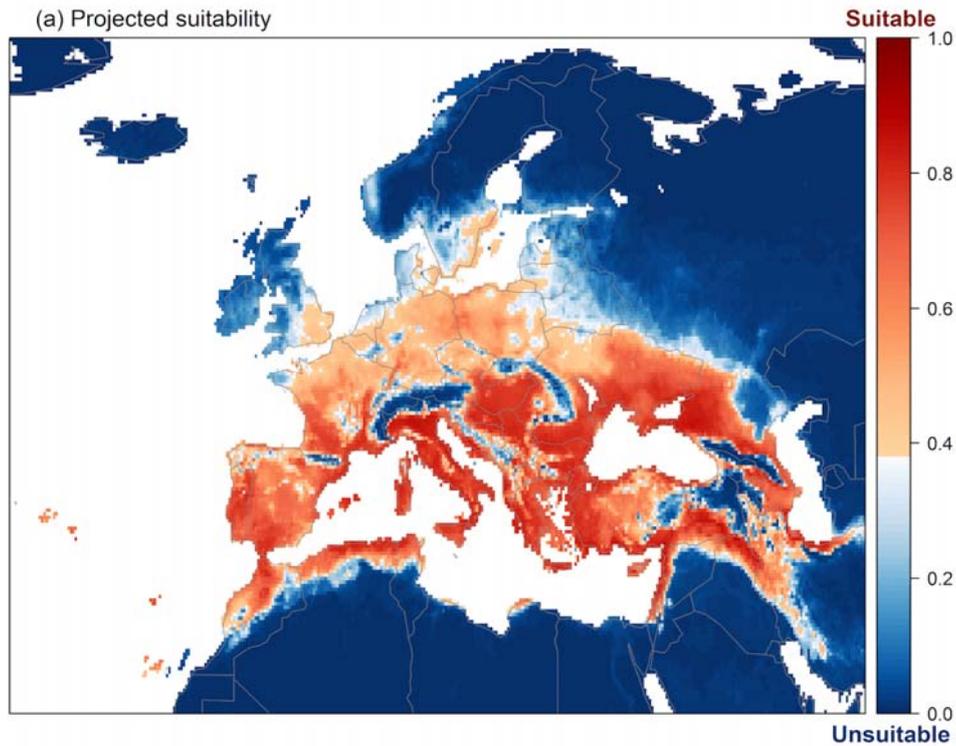


Figure 3 Projected current suitability for *Broussonetia papyrifera* establishment in Europe

Table 3 Biogeographic regions and suitability under current conditions

Biogeographic region	likeliness	confidence
Alpine	unlikely	medium
Anatolian	likely	high
Arctic	unlikely	high
Atlantic	likely	high
Black Sea	likely	high
Boreal	unlikely	high
Continental	likely	high
Macaronesian	likely	high
Mediterranean	likely	high
Pannonian	likely	high
Steppic	likely	high

Response (7b):

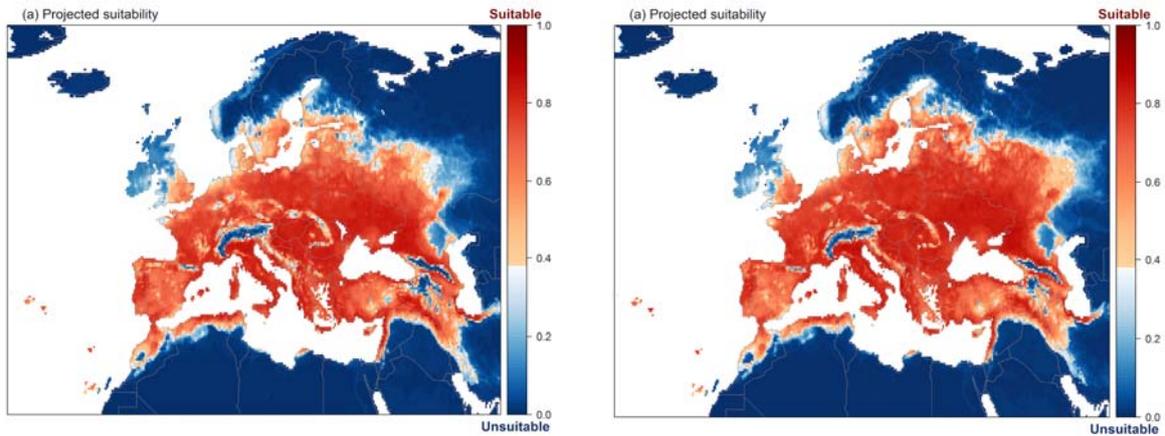


Figure 4 Projected suitability for *Broussonetia papyrifera* establishment in European region in the 2070s under climate change scenarios; Left: RCP 2.6 scenario, right: RCP 4.5 scenario

Table 4 Biogeographic regions and suitability under projected scenario conditions

biogeographic region	Scenario RCP 2.6		Scenario RCP 4.5	
	likeliness	confidence	likeliness	confidence
Alpine	likely	medium	likely	medium
Anatolian	likely	high	likely	high
Arctic	unlikely	high	unlikely	high
Atlantic	likely	high	likely	high
Black Sea	likely	high	likely	high
Boreal	likely	medium	likely	high
Continental	likely	high	likely	high
Macaronesian	likely	high	likely	high
Mediterranean	likely	high	likely	high
Pannonian	likely	high	likely	high
Steppic	likely	high	likely	high

A8. In which EU Member States has the species been recorded and in which EU Member States has it established? List them with an indication of the timeline of observations. The information needs be given separately for recorded and established occurrences.

A8a. Recorded: List Member States

A8b. Established: List Member States

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden

The description of the invasion history of the species shall include information on countries invaded and an indication of the timeline of the first observations, establishment and spread.

Response: Figure 5 and Table 5 give an overview about occurrences of the species in the EU Member States. Sources of information are observation data (GBIF 2020, iDigBio 2020, iNaturalist 2020, ALA 2020), European wide overviews like Euro+Med PlantBase or Flora Europaea as well as national floristic or invasive species references (see Table 5). An indication of the first observation is not possible for every member state but given if possible. Mainly based upon Euro+Med PlantBase and CABI (2020) additional information like cultivated/planted respectively naturalized status is available in some cases. The available European wide (e.g. Euro+Med 2006-; Tutin et al. 1964-1980), national or regional (e.g. Aeschimann et al. 2004) floristic compilations are a reliable source of information.

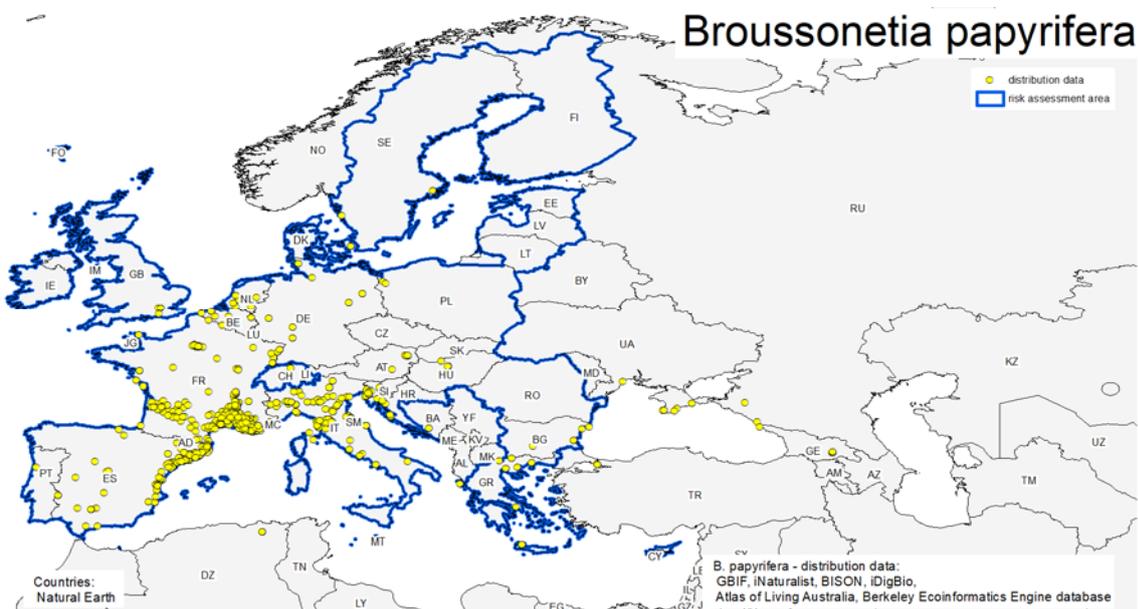


Figure 5 distribution data records of *Broussonetia papyrifera* in the EU member states (including United Kingdom) (map designed by Umweltbundesamt/Environment Agency Austria – 2020/07/01)

Table 5 EU member states and United Kingdom with recorded/established *Broussonetia papyrifera* occurrences; * information given by Euro+Med 2006-; ** information by CABI 2020; N/A not available (green cell – recorded/established yes; white cell – recorded/established no; grey cell – no examined or no data available)

country	recorded	established	source recorded	source established	general remarks	timeline of observations
Austria			Euro+Med 2006-; GBIF 2020; iNaturalist 2020; Fischer et al. 2008; Stöhr et al. 2012; Essl 2006; Essl 2008		Cultivated*	1863-2019

Belgium			GBIF 2020; iNaturalist 2020		casual	2005-2019
Bulgaria			GBIF 2020; iNaturalist 2020	Petrova et al. 2013		1885-2019
Croatia			Euro+Med 2006-; GBIF 2020; iNaturalist 2020	Euro+Med 2006-; Nikolić 1994; Boršić et al. 2008; Mitić et al. 2006; EPPO 2020a, Duplić & Vrdoljak 2016	cultivated; introduced: naturalized*	1996-2019
Cyprus						
Czechia						
Denmark			ALA 2020; GBIF 2020		cultivated	1869-N/A
Estonia						
Finland						
France			Euro+Med 2006-; GBIF 2020; iNaturalist 2020; Tutin et al. 1964-1980	Euro+Med 2006-; Tutin et al. 1964-1980; Greuter et al. 1989; Aeschimann et al. 2004; Tela Botanica 2020	introduced: naturalized*	1890-2019
Germany			ALA 2020; GBIF 2020			1828-2019
Greece			Euro+Med 2006-; GBIF 2020; iNaturalist 2020; iDigBio 2020	Euro+Med 2006-; Strid & Tan 1997; Zenetos et al. 2016	introduced: naturalized*	1985-2019
Hungary			Euro+Med 2006-; GBIF 2020; iNaturalist 2020; Király 2009; CABI 2020; Varga 2016		introduced*; planted**	N/A-2019
Ireland						
Italy			Euro+Med 2006-; ALA 2020; GBIF 2020; iNaturalist 2020	Euro+Med 2006-; Aeschimann et al. 2004; Conti et al. 2005; Tutin et al. 1964-1980; Greuter et al. 1989; Celesti- Grapow et al. 2009; Banfi & Galasso 2010; Galasso et al. 2018; EPPO 2020b	introduced: naturalized*; planted, introduced, widespread**	1923-2020
Latvia						
Lithuania						

Luxembourg						
Malta			Euro+Med 2006-	Euro+Med 2006-; Greuter et al. 1989; Mifsud 2020	introduced: naturalized*	N/A-N/A
Netherlands			GBIF 2020; iNaturalist 2020; Waarneming.nl (2021)			1993-2020
Poland			iNaturalist 2020			2005-2011
Portugal			iNaturalist 2020			N/A-2018
Romania			Euro+Med 2006-	Euro+Med 2006-; Tutin et al. 1964-1980; Sîrbu & Oprea 2011; Anastasiu et al. 2016	introduced: naturalized*	N/A- N/A
Slovakia			Euro+Med 2006-; iNaturalist 2020; Goliašová & Michalková 2006		cultivated*; planted**	N/A-2017
Slovenia			GBIF 2020; iNaturalist 2020; EPPO 2020c; Follak et al. 2018; Tavzes 2016		planted**	1982-2019
Spain			Euro+Med 2006-; GBIF 2020; iNaturalist 2020; Tutin et al. 1964-1980	Euro+Med 2006-; Tutin et al. 1964-1980; Greuter et al. 1989; Dana et al. 2004; Bayón & Vilá 2019; EPPO 2020d	introduced: naturalized*; planted**	1892-2019
Sweden			GBIF 2020		cultivated	N/A-2008
United Kingdom			GBIF 2020; iNaturalist 2020			N/A-2019

Response (8a): Paper mulberry is recorded in 19 (Austria, Belgium, Bulgaria, Croatia, Denmark, France, Germany, Greece, Hungary, Italy, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden) out of 27 Member States and in the United Kingdom.

Response (8b): Based upon the available data, *B. papyrifera* can be assessed as established in 8 (Bulgaria, Croatia, France, Greece, Italy, Malta, Romania, Spain) out of 27 Member States. Some of the member states with first observations about 100 or more years ago and Atlantic / Mediterranean climatic conditions (France 1890, Italy 1923, Spain 1892) exhibit a wide distribution of the species (see Figure 5).

By using a subset of GBIF records with a spatial accuracy $\leq 100\text{m}$, the EUNIS habitat classes at Level 2 (EEA 2019) can be spatially retrieved from the Ecosystem types of Europe 2012 raster data set (compare Table 6).

Table 6 Selected 283 *Broussonetia papyrifera* occurrences by GBIF with coordinate accuracy $\leq 100\text{m}$ and spatially related EUNIS habitat classes at Level 2 (EEA 2019)

EUNIS Label	AT	BE	BG	CH	CY	ES	FR	GR	HR	HU	IT	NL
C1: Surface standing waters											1	
C2: Surface running waters							1					
E1: Dry grasslands							2					
E2: Mesic grasslands					1		16				6	
E3: Seasonally wet and wet grasslands			1									
F3: Temperate and mediterranean-montane scrub		1										
FB: Shrub plantations							8					
G1: Broadleaved deciduous woodland						1	9				5	
G2: Broadleaved evergreen woodland											2	
G4: Mixed deciduous and coniferous woodland							4					
G5: Lines of trees, small anthropogenic woodlands, recently felled woodland, early-stage woodland and coppice							3				2	
I1: Arable land and market gardens						1	12	1			17	
I2: Cultivated areas of gardens and parks		2				2	5	1		1	9	
J1: Buildings of cities, towns and villages						10	66	1	2	1	22	1
J2: Low density buildings	1			2		2	42	1			7	
J4: Transport networks and other constructed hard-surfaced areas						1	4				5	

Most of the selected GBIF records are related to urban ecosystem types like *Buildings of cities, towns and villages* or *Low density buildings*.

Though for countries like e.g. Italy and France, the records are additionally related to *Mesic grasslands*, *Broadleaved deciduous woodland*, *Lines of trees, small anthropogenic woodlands, recently felled woodland, early-stage woodland and coppice*, *Arable land and market gardens*, *Transport networks and other constructed hard-surfaced areas*.

In summary, there is enough evidence that the used occurrence data cover several ecosystem categories from urban to more natural types.

A9. In which EU Member States could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A9a. Current climate: List Member States

A9b. Future climate: List Member States

With regard to EU Member States, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)

- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

The ensemble SDM model (see Annex IX) suggested that suitability for *B. papyrifera* was most strongly determined by minimum temperature of the coldest month (Bio6), accounting for 31,6 % of variation explained, followed by mean temperature of the warmest quarter (Bio10) (26.6 %), Climatic Moisture Index (CMI) (22.6 %) and Human Influence Index (HII) (19.2 %).

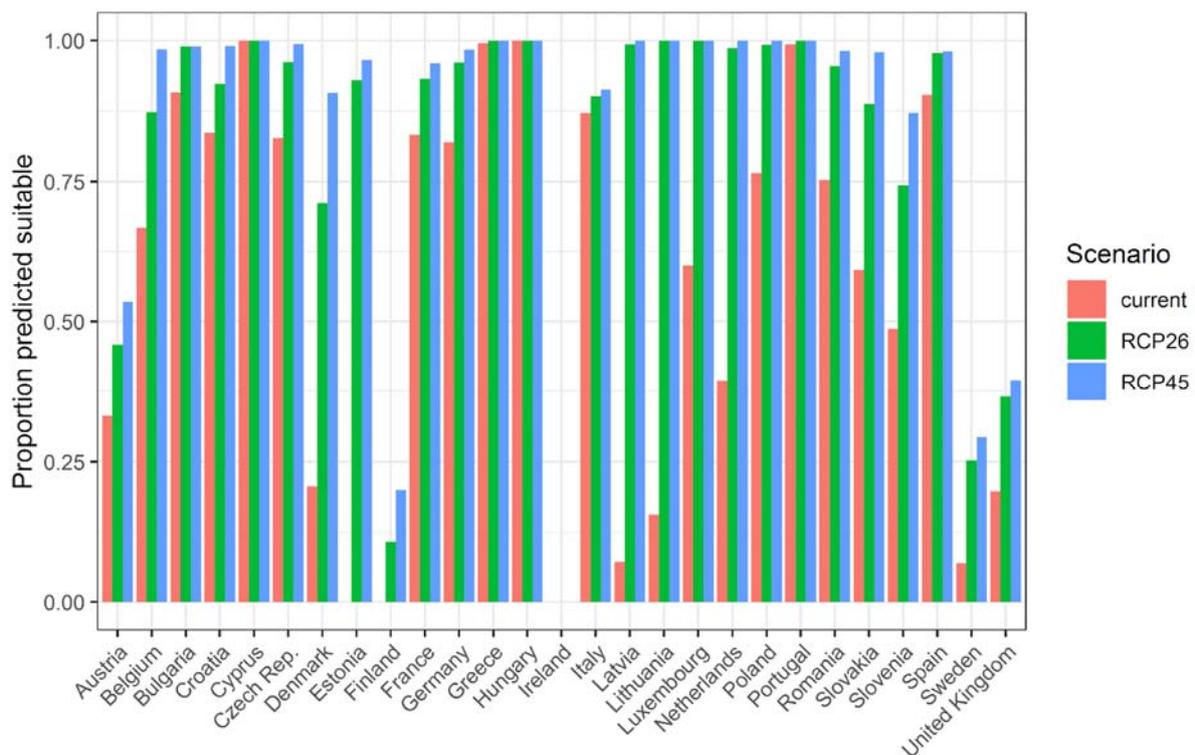


Figure 6 Variation in projected suitability for *Broussonetia papyrifera* establishment among European Union countries and the United Kingdom. The bar plots show the proportion of grid cells in each country classified as suitable in the current climate and projected climate for the 2070 using two different RCP pathways

Response (9a):

Table 6 EU Member States and the United Kingdom with suitability under current conditions

country	likeliness	confidence
Austria	unlikely	medium

Belgium	likely	high
Bulgaria	likely	high
Croatia	likely	high
Cyprus	likely	high
Czechia	likely	medium
Denmark	unlikely	medium
Estonia	unlikely	medium
Finland	unlikely	medium
France	likely	high
Germany	likely	medium
Greece	likely	high
Hungary	likely	high
Ireland	unlikely	high
Italy	likely	high
Latvia	unlikely	medium
Lithuania	unlikely	medium
Luxembourg	likely	medium
Malta	likely	high
Netherlands	likely	medium
Poland	likely	medium
Portugal	likely	high
Romania	likely	high
Slovakia	likely	medium
Slovenia	likely	high
Spain	likely	high
Sweden	unlikely	high
United Kingdom	unlikely	high

Response (9b):

Table 7 EU Member States and the United Kingdom with suitability under projected conditions

country	Scenario RCP 2.6		Scenario RCP 4.5	
	likeliness	confidence	likeliness	confidence
Austria	likely	medium	likely	high
Belgium	likely	high	likely	high
Bulgaria	likely	high	likely	high
Croatia	likely	high	likely	high
Cyprus	likely	high	likely	high
Czechia	likely	medium	likely	high
Denmark	likely	medium	likely	high
Estonia	likely	medium	likely	high
Finland	unlikely	medium	unlikely	high
France	likely	high	likely	high
Germany	likely	high	likely	high
Greece	likely	high	likely	high

Hungary	likely	high	likely	high
Ireland	unlikely	high	unlikely	high
Italy	likely	high	likely	high
Latvia	likely	medium	likely	high
Lithuania	likely	medium	likely	high
Luxembourg	likely	high	likely	high
Malta	likely	high	likely	high
Netherlands	likely	high	likely	high
Poland	likely	high	likely	high
Portugal	likely	high	likely	high
Romania	likely	high	likely	high
Slovakia	likely	high	likely	high
Slovenia	likely	high	likely	high
Spain	likely	high	likely	high
Sweden	likely	medium	likely	medium
United Kingdom	likely	medium	likely	medium

A10. Is the organism known to be invasive (i.e. to threaten or adversely impact upon biodiversity and related ecosystem services) anywhere outside the risk assessment area?

Response: In Pakistan, paper mulberry was intentionally introduced to make the Islamabad (Capital) and Rawalpindi area green. In less than 30 years it not only became highly invasive in the natural vegetation but also caused health problems in the human population (Qazi et al. 2019). It is now commonly found in India and Pakistan from sea level to 1000 m altitude and has become highly invasive and a troublesome weed in many localities. In Pakistan, direct competition of *B. papyrifera* limits the growth of the native *Dalbergia sissoo*, *Morus alba* and *Ziziphus sp.*, an important source of nectar for honey bees, especially near Islamabad and Rawalpindi and Murree Hills (Ahmed et al. 2019; Khan et al. 2020). The thick monocultures at different sites have rapidly replaced the native flora and fauna, although these thickets have also become refuges for wild boar and other mammals, and enhanced the buildup of the crow population (CABI 2020; Qureshi et al. 2020a).

In Uganda, *B. papyrifera* forms dense stands which displace native plant species and prevent forest regeneration as well as reduce water availability. The plant produces vast quantities of allergenic pollen (Witt et al. 2018).

Paper mulberry was introduced into Ghana in 1969 by the Forestry Research Institute of Ghana (FORIG). The reason for its deliberate introduction was to evaluate its potential for pulp and paper production. However, the plant has now become an invasive of alarming proportion. It is perhaps the most serious non-indigenous woody invasive plant in the closed forest zone of Ghana and the second most important plant invasive after *Chromolaena odorata*. In Ghana, paper mulberry is highly concentrated in the two forest reserves, namely Pra-Anum and Afram Headwaters Forest Reserves. It is in these forest reserves that the initial experimental trial were carried out. The two reserves are located within the Moist Semi-Deciduous (MSDF) and Dry Semi-Deciduous Forest (DSDF) zones, respectively. These two forest types are types among the most floristically diverse and economically important of all the forest types in Ghana. The high concentration of paper mulberry in these two reserves and nearby reserves or forests were facilitated by extensive deforestation and bushfires. From these two centers, the

plant is spreading extensively. Dense stands of *Broussonetia* can be seen conspicuously in farms and along roads in and around Pra-Anum and Afram Headwaters forest reserves. The species is spreading to other areas up to perhaps 100 km from these point of introduction. In the Pacific Islands paper mulberry is not invasive because only male clones were introduced. Thus, no seeds are produced and propagation is by vegetative means, using root shoot suckers. In Ghana and other places where both fertile male and female plants were introduced the invasive potential of the plant increases significantly (Bosu & Apetorgor 2020).

A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea

Response: Paper mulberry exhibits a widespread distribution in parts of the Atlantic and Mediterranean biogeographical regions (compare Figure 2). There are documented signs of invasiveness in the Continental biogeographical region (Northern Italy - Portale della Flora d'Italia 2020: Friuli Venezia Giulia, Lombardia (Montagnani et al. 2018), Piemonte (Gruppo di Lavoro Specie Esotiche della Regione Piemonte 2015), Veneto) and in the triangle of the Continental, Alpine and Mediterranean regions around Auvergne-Rhône-Alpes (Pôle d'information flore-habitats-fonge d'Auvergne-Rhône-Alpes 2020) and Provence Alpes-Côte d'Azur (Terrin et al. 2014). Regarding Croatia (Vuković et al. 2014, Borsic et al. 2008), no biogeographical region information is available. The endangered area includes natural and ruderal/disturbed habitats.

A12. In which EU Member States has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden

Response: The species exhibits a widespread distribution in Spain, France and Italy (compare Figure 5). There are documented signs of invasiveness in Italy (Northern Italy - Portale della Flora d'Italia 2020: Friuli Venezia Giulia, Lombardia (Montagnani et al. 2018), Piemonte (Gruppo di Lavoro Specie Esotiche della Regione Piemonte 2015), Veneto). Paper mulberry is listed as invasive in Croatia

(Vuković et al. 2014, Borsic et al. 2008). France has some reports of local invasiveness including in the Auvergne-Rhône-Alpes (Pôle d'information flore-habitats-fonge d'Auvergne-Rhône-Alpes 2020) where it is also recorded as an emerging invasive (Debay et al. 2020) and it is listed as “one-to-watch” in the Pays de la Loire (Dortel & Le Bail 2019) and Provence Alpes-Côte d’Azur (Terrin et al. 2014). The endangered area includes natural and ruderal/disturbed habitats.

A13. Describe any known socio-economic benefits of the organism.

including the following elements:

- Description of known uses for the species, including a list and description of known uses in the risk assessment area and third countries, if relevant.
- Description of social and economic benefits deriving from those uses, including a description of the environmental, social and economic relevance of each of those uses and an indication of associated beneficiaries, quantitatively and/or qualitatively depending on what information is available.

If the information available is not sufficient to provide a description of those benefits for the entire risk assessment area, qualitative data or different case studies from across the risk assessment area or third countries shall be used, if available.

Response: Inside of the risk assessment area: Paper mulberry is grown as an ornamental in parks and gardens. No other kind of use is known for the risk assessment area.

Outside of the risk assessment area: Paper mulberry has been cultivated in Asia and some Pacific Islands for many centuries for food, fiber (CABI 2020; POWO 2020), medicine (Hussain et al. 2008) and other uses (Matthews 1996).

The species played a significant role in the development of paper-making (Chang et al. 2015; González-Lorca et al. 2015; POWO 2020). For centuries, the fiber from the inner bark of paper mulberry has been used to make paper in Japan and textiles throughout the Pacific. In both cases the raw material is the soft, inner bark. For paper-making, the inner bark is pounded and mixed with water, and the resulting paste then spread evenly on a mesh to make 'washi' (Japanese handmade paper). Paper-making with paper mulberry fiber was established in China by around 100 AD, and reached Japan by about 600 AD (POWO 2020).

In contrast, in the Pacific region textiles are made by beating together strips of inner bark. This tapa cloth is used for various items of clothing, such as sarongs, scarves and hats, as well as for making bags and other items such as bedding. Until relatively recently, tapa cloth was the main source of clothing worn on Pacific islands such as Fiji, Tonga and Tahiti. Tapa cloth is still worn on ceremonial occasions, during festivals and for traditional dances. The bark fiber (and indeed the roots) can also be made into rope and cord (POWO 2020).

The wood is light and easily worked, and is used for making cups, bowls and furniture (ALA 2020; POWO 2020). The timber, being soft and brittle, is used mainly in the manufacture of cheap furniture, match sticks, packing cases, boxes, plywood, building-boards, sports equipment and pencils (FAO 1980; Sheikh 1993).

The fruits of *B. papyrifera* are edible, as are the young leaves, when steamed. The leaves, fruit and bark have a variety of traditional medicinal uses. In China the leaves are fed to silk-worms (POWO 2020).

Paper mulberry is a vigorous pioneer species, which can rapidly colonise forest clearings and abandoned farmland. Its ability to colonise degraded lands may make it suitable for reforestation programs in some situations (Luna 1996), although it can become invasive when both male and female trees are present, followed by pollination and seed set. Paper mulberry is frequently planted as a shade tree. It tolerates air pollution, making it suitable for planting along roadsides and in urban settings. It is also grown as an ornamental in parks and gardens. The tree is widely coppiced for tapa and paper production, with the young trees cut every 12-18 months (POWO 2020).

Environmental pollution is an increasing global problem. In this context, *B. papyrifera* is tested for a possible use of tree planting for short-rotation coppicing and phytoremediation (Capuana 2020; Huang et al. 2019; Huimin et al. 2019; Kang et al. 2018). Soils are often polluted by different metals or organic compounds, so phytoremediation may require multiple plant species and ecotypes since most of the plants suited to this purpose show an aptitude to accumulate only one or a few pollutants. A pot experiment was carried out with the aim of determining the phytoextraction potential of the hyperaccumulator *Pteris vittata* when co-planted with a woody tree (*Morus alba* or *B. papyrifera*) in soil contaminated with Cd, Pb, Zn, or As. The uptake of As was significantly increased when co-planted with *Morus* or *Broussonetia* (by 80.0% and 64.2% respectively). However, co-plantation did not have a promoting effect on the metal accumulation of both *M. alba*. and *B. papyrifera* (Capuana 2020).

Energy derived from biomass has been considered for many years, but was not pursued as a viable energy alternative to fossil fuels until recently as a result of declining fossil fuel sources and increasing oil prices. *Broussonetia papyrifera* may be considered as a 'second-generation' biofuel plant in short rotation woody crops providing lingo-cellulose, which is the woody structural material of plants that is abundant and diverse and has increasing appeal because it does not divert food away from the animal or the human food chain (Witt 2010; Dimitrou & Rutz 2015).

SECTION B – Detailed assessment

Important instructions:

- In the case of lack of information the assessors are requested to use a standardized answer: “No information has been found.”
- With regard to the scoring of the likelihood of events or the magnitude of impacts see Annexes I and II.
- With regard to the confidence levels, see Annex III.
- Highlight the selected response score and confidence level in **bold** but keep the other scores in normal text (so that the selected score is evident in the final document).

1 PROBABILITY OF INTRODUCTION AND ENTRY

Important instructions:

- **Introduction** is the movement of the species into the risk assessment area (it may be either in captive conditions and/or in the environment, depending on the relevant pathways).
- **Entry** is the release/escape/arrival in the environment, i.e. occurrence in the wild
- Introduction and entry may coincide for species entering through pathways such as “corridor” or “unaided”, but it also may differ. If different, please consider all relevant pathways, both for the introduction into the risk assessment area and the entry in the environment.
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used (see Annex IV). For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document³ and the provided key to pathways⁴.
- For organisms which are already present (recorded or established) in the risk assessment area, the likelihood of introduction and entry should be scored as “very likely” by default.
- Repeated (independent) introductions and entries at separate locations in the risk assessment area should be considered here (see Qu. 1.7).

Qu. 1.1. List relevant pathways through which the organism could be introduced into the risk assessment area and/or enter into the environment. Where possible give details about the specific origins and end points of the pathways as well as a description of any associated commodities.

For each pathway answer questions 1.2 to 1.7 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 1.2a, 1.3a, etc. and then 1.2b, 1.3b etc. for the next pathway.

In this context a pathway is the route or mechanism of introduction and/or entry of the species.

³ <https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf>

⁴ <https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf>

The description of commodities with which the introduction of the species is generally associated shall include a list and description of commodities with an indication of associated risks (e.g. the volume of trade; the likelihood of a commodity being contaminated or acting as vector).

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 1.2-1.9.

Table 8 list of pathways with relevance assessment

Category pathways	Subcategory	relevance
Release in Nature	Biological control	No information has been found.
	Erosion control / dune stabilization	No information has been found.
	Landscape / flora / fauna "improvement" in the wild	No information has been found.
	Introduction for conservation purposes	No information has been found.
	Release in nature for use	No information has been found.
	Other intentional release	No information has been found.
Escape from confinement	Agriculture	No information has been found.
	Botanical garden	relevant
	Forestry	No information has been found.
	Horticulture	relevant
	Ornamental purpose other than horticulture	relevant
	Research and ex-situ breeding	No information has been found.
	Other escape from confinement	No information has been found.
Transport Containment	Seed contaminant	No information has been found.
	Timber trade	No information has been found.
	Transportation of habitat material	No information has been found.
Transport - Stowaway	Machinery / equipment	No information has been found.
Unaided	Natural dispersal	No information has been found.

Pathways considered but excluded from the risk assessment.

Forestry: *Broussonetia papyrifera* is mentioned in the context of biofuel plant in short rotation woody crops (Witt 2010; Dimitrou & Rutz 2015). This pathway was not considered for the risk assessment as there is no further evidence that this is an (active) pathway in the EU.

Other escape from confinement: *Broussonetia papyrifera* is mentioned in the context of phytoremediation (Capuana 2020; Huimin et al. 2019; Kang et al. 2018). This pathway was not considered for the risk assessment as there is no further evidence that this is an (active) pathway in the EU.

Pathway name: Botanical garden/zoo/aquaria (excluding domestic aquaria)

Qu. 1.2a. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional	CONFIDENCE	low
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	unintentional		medium high
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Response: Long before Linnaeus' time, paper mulberry had been cultivated widely in European gardens (Barker 2002). Currently, the species is regularly displayed in Botanical Gardens across the risk assessment area.

Selection of occurrences in botanical gardens inside the risk assessment area:

- Bochum (Germany)
- Córdoba (Spain)
- Frankenburg (Austria)
- Freiburg (Germany)
- Graz (Austria)
- Ljubljana (Slovenia)
- Meise (Belgium)
- Tübingen (Germany)
- Wrocław (Poland)
- Vienna (Austria)

Qu. 1.3a. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Broussonetia papyrifera* is unisexual and dioecious. Thus, a male and female tree are needed if the ornamental and attractive fruits are to be shown to visitors. The fruits of *B. papyrifera* are consumed by birds and other small animals which are able to disseminate the seeds outside of botanical gardens. There is evidence in literature about spreading and seed dispersal by birds and small mammals (e.g. fruit bats), though no particular bird or mammal species is mentioned, neither for regions outside

of the risk assessment area nor for the risk assessment area. The seed density may be locally high. Root sprouts are produced when the main stem is cut. It can be assumed that these clonal sprouts are local and managed in botanical gardens according to the European Code of Conduct for Botanic Gardens on Invasive Alien Species (Heywood & Sharrock 2013). Nagodă et al. (2014) examined the “Dimitrie Brandza” Botanic Garden (Bucharest) as a potential centre for the dispersal of invasive plants. Paper mulberry has been assessed as: a species surviving in the climatic conditions of the Botanic Garden (Romania), it flowers, bears fruit and produces seeds and seedlings around the parent plant, without any human intervention.

Qu. 1.4a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely	CONFIDENCE	low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response: As the pathway is intentional, it is likely that the organism will survive. As the intention is showing the species itself as well as the ornamental and attractive fruits to visitors of the botanical garden, it is likely that male and female trees are planted in proximity. Then it is likely that the trees will reproduce during their life. Vegetative growth is controlled by the botanical garden management.

Qu. 1.5a. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely	CONFIDENCE	low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response: As the pathway is intentional to display paper mulberry in botanical gardens, it is likely that the organism will survive.

Qu. 1.6a. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely	CONFIDENCE	low
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	unlikely moderately likely likely very likely		medium high
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Response: As the pathway is intended, it is unlikely that the organism introduction into the risk assessment area is undetected. On the other hand, an undetected dispersal of seeds by animals out of the botanical garden into the environment may be possible. A requirement for the latter is that male and female plants are present in the garden. In very rare cases, visitors may collect some fruits/seeds for planting in their garden or the wild.

Qu. 1.7a. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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Response: Botanical gardens are neither ubiquitous nor widespread. Thus, the points of introduction into the environment in the risk assessment area are isolated.

Qu. 1.8a. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: As a tree life cycle in botanical gardens normally span over several decades, planting *B. papyrifera* in such localities happens rarely. Though, if there are male and female trees in proximity, seeds may be produced over the whole life cycle. Vegetative growth is controlled by the botanical garden management. The overall likelihood of introduction is assessed as unlikely based upon the European Code of Conduct for Botanic Gardens on Invasive Alien Species (Heywood & Sharrock 2013).

Pathway name: Horticulture

Qu. 1.2b. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: The horticulture pathway covers large scale/commercial cultivation of plants in a controlled or confined environment and is the intentional introduction of the species into the risk assessment area for commercial culturing. Paper mulberry had been cultivated widely for ornamental uses in European gardens for hundreds of years (Barker 2002).

The Horticulture pathway focuses on plants kept in commercial culturing facilities (nurseries, greenhouses) from where they may accidentally escape due to mismanagement, or during transport to/from locations as part of the nursery trade. Therefore, this pathway includes the bulk shipment of live plants for nurseries and gardens centres, and the intentional introduction of seeds (and potentially rhizomes) for planting.

An internet search reveals that the plant can be ordered (online) at local or regional tree nurseries in several EU member states (e.g. Spain, France, Ireland, Netherlands, Belgium, Germany, and Austria) as well as at online retailers like eBay or Amazon. Seeds, cuttings or trees are offered.

Qu. 1.3b. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Seeds, cuttings or trees are offered at tree nurseries or at online retailers. *Broussonetia papyrifera* is unisexual and dioecious. Thus, it needs at least one male and well one female, tree for fruits to be produced. The fruits of paper mulberry are consumed by birds and other small animals which are able to disseminate the seeds outside of tree nurseries. There is evidence in the global literature on

spreading and seed dispersal by birds and small mammals (e.g. fruit bats), though no particular bird or mammal species is mentioned. The seed density may be locally very high, though no figures are available. Root sprouts are produced when the main stem is cut; though no information about management of root sprouts is available in the context of horticulture.

Qu. 1.4b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The pathway ‘Horticulture’ is the deliberate movement of plant material into the risk assessment area and as such plant material would be maintained and moved to ensure survival. Three types of material can potentially enter the risk assessment area via this pathway (1) seed, (2) live plants and (2) rhizomes. Although seed import cannot be ruled out, it is unlikely to be part of the horticulture pathway for the commercial production of the species.

As the pathway is intentional, it is likely that the organism will survive. As the intention is to produce cuttings and, to a lesser extent, seeds for sale, it is likely that male and female trees are planted in proximity. Therefore, it is very likely that the trees will reproduce during their life.

Qu. 1.5b. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: As the pathway is intentional, it is likely that the organism will survive existing management practices. Plant material is the commodity itself and it is deliberately moved for sale within the risk assessment area.

Qu. 1.6b. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: As the pathway is intended, it is unlikely that the organism would be introduced into the risk assessment area undetected. On the other hand, the undetected dispersal of seeds by animals out of the tree nursery or root sprouts into the environment may be possible.

Qu. 1.7b. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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Response: For live plant imports in the risk assessment area (introduction), each EU Member country has official entry points where plant material can be imported into. Tree nurseries or plant retailers are common in all EU member states; a selection of them may sell seeds, cuttings or trees of paper mulberry. Furthermore, these points are not evenly distributed over the member states area. In summary, possible points of introduction respectively the entry into the environment are assessed as isolated.

Qu. 1.8b. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The horticulture pathway involves the movement of plant material for planting from outside of the risk assessment area via nurseries and garden centers. As in tree nurseries or plant retailers, seeds, cuttings or trees of the paper mulberry are processed more frequently than e.g. in botanical gardens, an introduction into the risk assessment area or into the environment is assessed as moderately likely.

Pathway name: Ornamental purpose other than horticulture

Qu. 1.2c. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: *Broussonetia papyrifera* had been cultivated widely for ornamental uses in European gardens and parks for hundreds of years (Barker 2002). The introduction into the risk assessment area is intentional for ornamental purposes in gardens and parks. A check based on a small random subset of the available spatial data (GBIF 2020) indicates that private and public gardens are the main locations recorded.

Qu. 1.3c. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Seeds, cuttings or trees are sold at tree nurseries or at online retailers. *B. papyrifera* is unisexual and dioecious. Thus, it needs a male, as well as a female tree at least if the desired fruits are to be produced. Though, no information about sex ratio among the plant material sold in the risk assessment area is available to be able giving figures about reproduction. The fruits of *B. papyrifera* are consumed by birds and other small animals which are able to disseminate the seeds outside of gardens or parks. The seed density may be locally very high. Root sprouts are produced when the main stem is cut; though no information about management of root sprouts is available. In gardens, containment management is less common than in botanical gardens and parks.

Qu. 1.4c. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: As the pathway is intentional (ornamental use in garden and parks), it is likely that the organism will survive. *Broussonetia papyrifera* is dioecious. Thus, it needs a male as well as a female tree to be planted in gardens or parks if fruits are to be produced. However, no information about sex ratio among the plant material sold in the risk assessment area is available to support the estimation of reproduction and the likelihood of increasing populations at such sites.

Qu. 1.5c. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: As the pathway is intentional for ornamental purpose in gardens and parks, it is likely that the organism will survive existing management practices.

Qu. 1.6c How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: As the pathway is intended for ornamental use in gardens and parks, it is very unlikely that the organism could be introduced into the risk assessment area undetected. On the other hand, an

undetected dispersal of seeds by animals out of the gardens or parks or root sprouts into the environment may be possible. However, no information about sex ratio among the plant material in the risk assessment area is available to be able to estimate undetected seed dispersal.

Qu. 1.7c. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated	CONFIDENCE	low
	widespread		medium
	ubiquitous		high

Response: Occurrences in gardens and parks are neither ubiquitous nor widespread in the risk assessment area; there may be spatial clusters like in the Mediterranean or Atlantic regions. Additionally, occurrences in gardens may be more common than in botanical gardens, parks and nurseries. However in summary, possible points of introduction are assessed as isolated.

Qu. 1.8c. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely	CONFIDENCE	low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response: There are no available sales figures regarding paper mulberry use in gardens. Thus, an introduction into the risk assessment area or into the environment is assessed as moderately likely with a low confidence; because the species is offered by tree nurseries, plant retailers and online in many EU member states.

Qu. 1.9. Estimate the overall likelihood of introduction into the risk assessment area or entry into the environment based on all pathways and specify if different in relevant biogeographical regions in current conditions.

Provide a thorough assessment of the risk of introduction in relevant biogeographical regions in current conditions: providing insight in to the risk of introduction into the risk assessment area.

RESPONSE	very unlikely	CONFIDENCE	low
	unlikely		medium

	moderately likely likely very likely		high
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Response: Paper mulberry is recorded in 19 EU member states (plus the United Kingdom) and already established in 8 out of 27 EU member states (see Table 5). According to the guidelines: For organisms which are already present (recorded or established) in the risk assessment area, the likelihood of introduction and entry should be scored as “very likely” by default. This has been scored with a high confidence.

However, there are differences at the biogeographical region level (see Figure 2). The species is recorded in the Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean and Pannonian biogeographical regions and can be already assessed as “established” in the Atlantic, Continental and Mediterranean biogeographical regions. The core areas of occurrences are the Atlantic, Mediterranean and Continental region parts in Western Europe.

Qu. 1.10. Estimate the overall likelihood of introduction into the risk assessment area or entry into the environment based on all pathways in foreseeable climate change conditions?

Thorough assessment of the risk of introduction in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of introduction (e.g. change in trade or user preferences)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely introduction within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Paper mulberry is recorded in 19 EU member states (plus the United Kingdom) and already established in 8 out of 27 (see Table 5). However, there are differences on the biogeographical region level (see Figure 3). The species is recorded in the Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean and Pannonian biogeographical regions and can be already assessed as “established” in

the Atlantic, Continental and Mediterranean biogeographical regions. The core areas are the Atlantic, Mediterranean and Continental region parts in Western Europe.

The ensemble SDM model (see Annex IX) suggested that suitability for *B. papyrifera* was most strongly determined by Minimum temperature of the coldest month (Bio6), accounting for 35% of variation explained, followed by Mean temperature of the warmest quarter (Bio10) (28%), Climatic moisture index (CMI) (25%) and Human influence index (HII) (11%).

The climate change scenarios RCP 2.6 and RCP 4.5 indicate a higher suitability in the Northern parts of Europe (compare Annex IX). Future climate change conditions may change the offer or availability of seeds, cuttings or trees by local or regional tree nurseries in the northern part of Europe as the demand from gardeners may increase with more suitable climate conditions.

2 PROBABILITY OF ESTABLISHMENT

Important instructions:

- For organisms which are already established in parts of the risk assessment area or have previously been eradicated, the likelihood of establishment should be scored as “very likely” by default.
- Discuss the risk also for those parts of the risk assessment area, where the species is not yet established.

Qu. 2.1. How likely is it that the organism will be able to establish in the risk assessment area based on similarity of climatic and abiotic conditions in its distribution elsewhere in the world?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Paper mulberry is already established in 8 EU Member States (see Table 5) encompassing the Atlantic, Continental and Mediterranean biogeographical regions. Therefore establishment in the risk assessment area is considered very likely with a high confidence.

Paper mulberry can tolerate a wide range of climates, including humid tropical (monsoon), humid and sub-humid subtropical as well as warm temperate areas (EPPO 2019; Whistler & Elevitch 2006). The explicit Alpine, Boreal, Continental and Steppic parts of the risk assessment area are out of these climatic preferences to some extent (compare SDM in Annex IX).

Broussonetia papyrifera is unisexual and dioecious. Thus, it needs male as well as female trees to produce seeds. As information about sex distribution within the European occurrences is not available, it is not feasible to estimate the likelihood of species establishing from reproductive populations into new areas with suitable climatic and abiotic conditions. Root suckers are produced when the main stem is cut. Over time, this can lead to the formation of dense thickets and can be considered a means of establishment to some extent.

Qu. 2.2. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area? Consider if the organism specifically requires another species to complete its life cycle.

RESPONSE	very isolated isolated moderately widespread widespread	CONFIDENCE	low medium high
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	ubiquitous		
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Response: paper mulberry is considered a pioneer species adapted to colonize disturbed sites (Csurhes 2016). The species does not grow well under full shade and is suited to disturbed habitats, in particular to riparian habitats and gaps within subtropical forest (EPPO 2019). The fruits are dispersed by birds and small mammals (Seelenfreund et al. 2017; Wilcox 2004). The habitats are disturbed, open sites generally, where there is relatively well-drained, moist, fertile soil (e.g. along roadsides, Follak et al. 2018). There is some evidence that *B. papyrifera* also colonizes natural and semi-natural habitats such as thermophilic forests and dry grasslands (Montagnani et al. 2018). Such habitats are unevenly distributed but widespread within the European landscape.

Qu. 2.3. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: There is an indication of competition with other tree species as the species does not grow well under full shade. For the risk assessment area, no explicit information is available on competitive interactions with native plant species.

Qu. 2.4. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?

RESPONSE	N/A very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: No explicit information is available on predators, parasites or pathogens already present in the risk assessment area. Ellis (2020) gives some indication about whiteflies (*Aleyrodidae*) and scale insects (*Diaspididae*) as pests (Table 9).

Table 9 parasites on *Broussonetia papyrifera* (Ellis 2020)

organ	taxonomic group	parasite
leaf	Aleyrodidae	<i>Parabemisia myricae</i>
stem	Diaspididae	<i>Diaspidiotus ostreaeformis</i>

Broussonetia papyrifera can be lopped for fodder. In a study in Taiwan, the leaves were studied for digestibility. Of the various components, 67.7% of dry matter was digestible, crude protein 84.8%, crude fiber 65.5%, crude fat 35.0% and ash 50.3% (Lin et al. 1996). Paper mulberry seedlings and saplings are browsed by cattle (CABI 2020).

Pollack & Stevenson (1973) reported *Corynespora cassicola*, a fungal pathogen, causing “frog-eye” lesions on leaves of paper mulberry in Alabama (United States of America). A new disease associated with paper mulberry causing leaf yellowing and curling symptoms was observed in Nanjing, Jiangsu Province, China, in 2014 and 2015. Mei et al. (2016) identified a *Candidatus* Phytoplasma as the cause of the disease.

Qu. 2.5. How likely is the organism to establish despite existing management practices in the risk assessment area? Explain if existing management practices could facilitate establishment.

RESPONSE	CONFIDENCE
very unlikely	low
unlikely	medium
moderately likely	high
likely	
very likely	

Response: Paper mulberry is already established in 8 EU Member States as well as in the Atlantic, Continental and Mediterranean biogeographical regions. Thus, existing management practices related to introduction pathways/vectors could not prevent the entry into the environment of sufficient propagules for establishment. Considering the already established populations in Europe and the large EU area to be monitored, further establishment is considered very likely. The planting of paper mulberry trees in parks and gardens would serve as a new possible source of propagules (seeds). Furthermore, intensified land use facilitates establishment by disturbing semi-natural and natural habitats.

Qu. 2.6. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?

RESPONSE	CONFIDENCE
very unlikely	low
unlikely	medium
moderately likely	high
likely	
very likely	

Response: Root shoot suckers are produced when the main stem is cut. Over time, this can lead to the formation of dense thickets (e.g. Bosu & Apetorgor 2020). The organism may survive eradication campaigns as the removal of all plant parts is difficult (CABI 2020, Csurhes 2016), though an eradication on a local level may be feasible.

Qu. 2.7. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?

including the following elements:

- a list and description of the reproduction mechanisms of the species in relation to the environmental conditions in the risk assessment area
- an indication of the propagule pressure of the species (e.g. number of gametes, seeds, eggs or propagules, number of reproductive cycles per year) of each of those reproduction mechanisms in relation to the environmental conditions in the risk assessment area.
- If relevant, comment on the likelihood of establishment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in establishment whereas for others high propagule pressure (many thousands of individuals) may not.
- If relevant, comment on the adaptability of the organism to facilitate its establishment and if low genetic diversity in the founder population would have an influence on establishment.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Paper mulberry exhibits a high reproductive potential, by means of both sexual and vegetative propagation (Maan et al. 2020; Morgan et al. 2019). *Broussonetia papyrifera* is an extremely fast growing tree. Whistler & Elevitch (2006) indicate a fast growth rate with just 12-18 months required to reach a size of 3-4m. The tree shows the characteristics of a vigorous pioneer on disturbed sites. Lu et al. (1984) carried out a study on *B. papyrifera* in Taiwan to determine the variation in seed size and weight, average number of days for germination, and growth of seedlings under different nursery conditions. Seeds have a germination rate of 50 % or less in moist soils (EPPO 2019). Studies about the genetic diversity are available for the origin and their surroundings in East Asia (e.g. Liao et al. (2014), Payacan et al. (2017), Peng et al. (2019), Seelenfreund et al. (2011)), though no relevant information can be found for the risk assessment area. Furthermore, no relevant information about propagule pressure within the risk assessment can be found.

The species is dioecious. Thus, it needs male as well as female trees to produce seeds. If male and female plants are present, mid- to long-range dispersal is possible via seeds. The fruits are dispersed by birds and small mammals. Seeds rarely germinate under dense forest canopies, but germination can be prolific in large canopy gaps, roadsides and abandoned farmland.

Qu. 2.8. If the organism does not establish, then how likely is it that casual populations will continue to occur?

Consider, for example, a species which cannot reproduce in the risk assessment area, because of unsuitable climatic conditions or host plants, but is present because of recurring introduction, entry and release events. This may also apply for long-living organisms.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The paper mulberry is already established in 8 out of 27 EU member states as well as in the Atlantic, Continental and Mediterranean biogeographical regions. According to the above specified pathways for introduction/entry (e.g. horticulture, ornamental purpose other than horticulture), recurring introductions may occur.

Broussonetia papyrifera can tolerate a wide range of climates, including humid tropical (monsoon), humid and sub-humid subtropical as well as warm temperate areas (EPPO 2019; Whistler & Elevitch 2006). It can grow in areas with an annual rainfall of 700–2 500 mm (EPPO 2019; CABI 2020), though the species can survive a 3–4 month dry season (Whistler & Elevitch 2006).

It is very likely that casual populations will continue to occur, as long as habitats with disturbed site conditions are available in the neighborhood. The tree shows the characteristics of a vigorous pioneer on disturbed sites. Paper mulberry exhibits a high reproductive potential, by means of both sexual and vegetative propagation (Maan et al. 2020; Morgan et al. 2019).

Qu. 2.9. Estimate the overall likelihood of establishment in the risk assessment area under current climatic conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under current climatic conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in current conditions: providing insight in the risk of establishment in (new areas in) the risk assessment area.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The paper mulberry is currently established in 7 EU Member States as well as in the Atlantic, Continental and Mediterranean biogeographical regions.

Broussonetia papyrifera can tolerate a wide range of climates, including humid tropical (monsoon), humid and sub-humid subtropical as well as warm temperate areas (EPPO 2019; Whistler & Elevitch

2006). It can grow in areas with an annual rainfall of 700–2 500 mm (EPPO 2019; CABI 2020), though the species can survive a 3–4 month dry season (Whistler & Elevitch 2006).

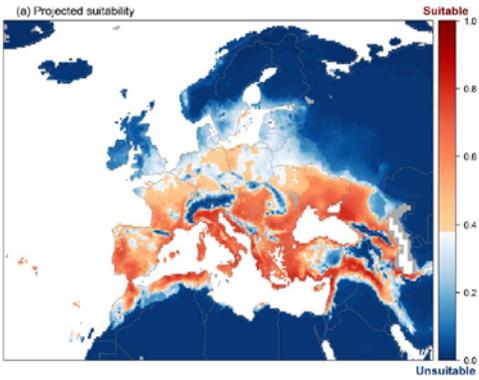


Figure 8 Projected current suitability for *Broussonetia papyrifera* establishment in Europe

According to the species distribution model (see Annex IV), large parts of the risk assessment area (compare Figure 8) are already suitable under current climatic conditions. The suitability declines in the North (Boreal biogeographical region) and Northwest regions of Europe.

Qu. 2.10. Estimate the overall likelihood of establishment in the risk assessment area under foreseeable climate change conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under foreseeable climate change conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of establishment (e.g. increase in average winter temperature, increase in drought periods)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely establishment within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Paper mulberry is already established in 8 EU Member States as well as in the Atlantic, Continental and Mediterranean biogeographical regions. *Broussonetia papyrifera* can tolerate a wide range of climates, including humid tropical (monsoon), humid and sub-humid subtropical as well as warm temperate areas (EPPO 2019; Whistler & Elevitch 2006). It can grow in areas with an annual rainfall of 700–2 500 mm (EPPO 2019; CABI 2020), though the species can survive a 3–4 month dry season (Whistler & Elevitch 2006). The climate change scenarios RCP 2.6 and RCP 4.5 indicate a higher suitability in the northern parts of Europe (compare Annex IX) by an increase in average winter temperature.

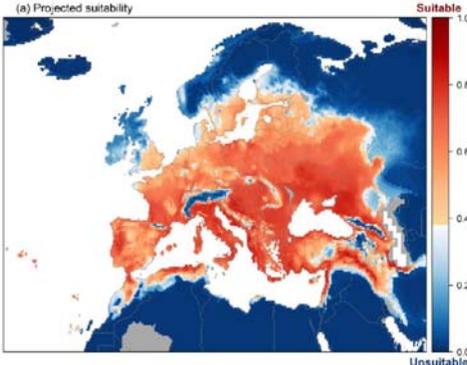


Figure 9 Projected suitability for *Broussonetia papyrifera* establishment in Europe in the 2070s under climate change scenario RCP2.6

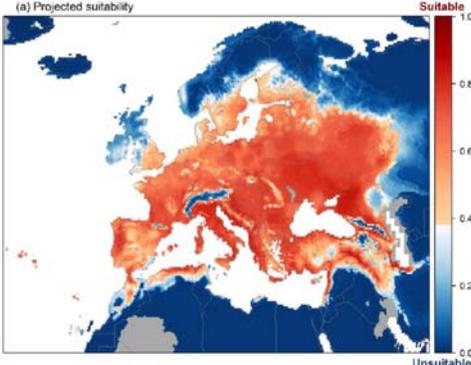


Figure 10 Projected suitability for *Broussonetia papyrifera* establishment in Europe in the 2070s under climate change scenario RCP4.5

Both climate change scenarios RCP 2.6 and RCP 4.5 indicate a higher suitability in the northern and eastern parts of Europe (compare Annex IX) by an increase in average summer temperature as well low temperature in winter (Mean temperature of the warmest quarter - Bio10, Minimum temperature of the coldest month - Bio6). Boreal and Continental regions are affected by an increased climatic suitability for *B. papyrifera*.

3 PROBABILITY OF SPREAD

Important instructions:

- Spread is defined as the expansion of the geographical distribution of an alien species within the risk assessment area.
- Repeated releases at separate locations do not represent continuous spread and should be considered in the probability of introduction and entry section (Qu. 1.7).

Qu. 3.1. How important is the expected spread of this organism within the risk assessment area by natural means? (List and comment on each of the mechanisms for natural spread.)

including the following elements:

- a list and description of the natural spread mechanisms of the species in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of spread discussed in relation to the species biology and the environmental conditions in the risk assessment area.

The description of spread patterns here refers to the CBD pathway category “Unaided (Natural Spread)”. It should include elements of the species life history and behavioural traits able to explain its ability to spread, including: reproduction or growth strategy, dispersal capacity, longevity, dietary requirements, environmental and climatic requirements, specialist or generalist characteristics.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: The potential for natural dispersal is assessed as moderate with a medium confidence. References or figures of the spread rate of populations already established in the risk assessment area are not available.

Broussonetia papyrifera is dioecious. Thus, it needs male as well as female trees to produce seeds. Peñailillo et al. (2016) found that contemporary paper mulberry plants are all female in near and remote Oceania, with the exception of Hawaii, where plants of both sexes are found. Information about the sex distribution/ratio within the European occurrences is not available. If male and female plants are present, (long) to mid-range dispersal may occur via seeds as fruits are dispersed by birds and small mammals. Root suckers are produced when the main stem is cut. Over time, this can lead to the formation of dense thickets so clonal growth would allow slow spreading to some extent. Natural spread may occur as long as suitably disturbed habitats are available.

Qu. 3.2a. List and describe relevant pathways of spread other than "unaided". For each pathway answer questions 3.3 to 3.9 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 3.3a, 3.4a, etc. and then 3.3b, 3.4b etc. for the next pathway.

including the following elements:

- a list and description of pathways of spread with an indication of their importance and associated risks (e.g. the likelihood of spread in the risk assessment area, based on these pathways; likelihood of survival, or reproduction, or increase during transport and storage; ability and likelihood of transfer from the pathway to a suitable habitat or host) in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of spread for each pathway discussed in relation to the species biology and the environmental conditions in the risk assessment area.
- All relevant pathways of spread (except “Unaided (Natural Spread)”, which is assessed in Qu. 4.1) should be considered. The classification of pathways developed by the Convention of Biological Diversity shall be used (see Annex IV).

Table 10 list of spread pathways including relevant assessment and estimated rate of spread

Category pathways	Subcategory	relevance	rate of spread
Release in Nature	Biological control	No information has been found.	
	Erosion control / dune stabilization	No information has been found.	
	Landscape / flora / fauna "improvement" in the wild	No information has been found.	
	Introduction for conservation purposes	No information has been found.	
	Release in nature for use	No information has been found.	
	Other intentional release	No information has been found.	
Escape from confinement	Agriculture	No information has been found.	
	Botanical garden	not relevant	
	Forestry	No information has been found.	
	Horticulture	relevant	slow
	Ornamental purpose other than horticulture	relevant	slow
	Research and ex-situ breeding	No information has been found.	
	Other escape from confinement	No information has been found.	
Transport Containment	Seed contaminant	No information has been found.	
	Timber trade	No information has been found.	
	Transportation of habitat material	No information has been found.	
Transport - Stowaway	Machinery / equipment	No information has been found.	

Horticulture and ornamental purpose other than horticulture are considered as relevant spread pathways. *Broussonetia papyrifera* is unisexual and dioecious. Thus, it needs male as well as female trees to produce seeds. Along both pathways, male and female individuals are not always available. There is evidence in the literature of spreading and seed dispersal by birds and small mammals (e.g. fruit bats), though no particular bird or mammal species are mentioned, neither for regions outside of the risk assessment area nor for within the risk assessment area. The rate of reproduction and rate of spread are considered to be slow.

Pathway name: Horticulture

Qu. 3.3a. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: This spread pathway is unintentional. Nurseries and retailers are moving plants or seeds from site a to site b within the risk assessment area. Seeds may be lost while the nursery is transporting trees or seeds. Birds or mammals may disseminate seeds outside of the nurseries.

Qu. 3.4a. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Although the plant can be ordered online at local or regional tree nurseries in several EU member states as well as at online retailers, information on the number male as well as female trees are kept and sold are not available. The seed density may be locally very high, though in many cases low as they are young plants. The fruits of paper mulberry are consumed by birds and other small animals which are able to disseminate the seeds outside of horticulture. Root spread is very unlikely as they will be potted beforehand. In summary it is considered as very unlikely that a number of individuals sufficient to originate a viable population over the course of a year would be spread via this pathway.

Qu. 3.5a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Reproduction during transport is very unlikely, though moderate likely during storage; while surviving is likely. The higher score is given.

Qu. 3.6a. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Existing management practices related to this spread pathway could not prevent the entry into the environment of sufficient propagules for establishment. The confidence level is low, as no references or figures are available.

Qu. 3.7a. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Undetected dispersal of seeds by animals from a horticultural setting or of plant material during transport by nurseries into the environment may be possible.

Qu. 3.8a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Paper mulberry is considered to be a pioneer species adapted to colonize disturbed sites. The suitable habitats are disturbed, open sites generally, where there is relatively well-drained, moist,

fertile soil. Such suitable habitats are unevenly spatially distributed. Furthermore, the plants are grown in protected conditions and correct disposal of plant waste may be applied. The transfer from the pathway to a suitable habitat is assessed as unlikely as all conditions above have to be fulfilled.

Qu. 3.9a. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area. (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: The overall potential rate of spread is assessed as very slow as all conditions mentioned in Qu. 3.8a have to be fulfilled.

Pathway name: Ornamental purpose other than horticulture

Qu. 3.3b. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: This spread pathway is unintentional. While maintaining garden stock (bushes and trees), plant materials like seeds and cuttings may be carelessly disposed of. Birds or mammals may disseminate seeds outside of the gardens. There is evidence in literature about spreading and seed dispersal by birds and small mammals (e.g. fruit bats), though no particular bird or mammal species is mentioned, neither for regions outside of the risk assessment area nor for the risk assessment area.

Qu. 3.4b. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway

- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Broussonetia papyrifera* is unisexual and dioecious. It is unlikely that both, male and female, trees are planted in e.g. gardens in most cases. The fruits of paper mulberry are consumed by birds and other small animals which are able to disseminate the seeds outside of gardens and parks. Plant material may be disposed of to a small extent. In summary it is considered as very unlikely that a number of individuals sufficient to originate a viable population over the course of a year is spread via this pathway.

Qu. 3.5b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: It is very unlikely that the organism will survive, reproduce or increase during transport and storage along this pathway.

Qu. 3.6b. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Existing management practices related to this spread pathways could not prevent the entry into the environment of sufficient propagules for establishment.

Qu. 3.7b. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Undetected dispersal of seeds by animals out of gardens or of plant material by gardeners' disposal into the environment may be possible.

Qu. 3.8b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Paper mulberry is considered to be a pioneer species adapted to colonize disturbed sites. The habitats are disturbed, open sites generally, where there is relatively well-drained, moist, fertile soil. Such suitable habitats are unevenly spatially distributed. The transfer from the pathway to a suitable habitat is assessed as unlikely as all conditions above have to be fulfilled.

Qu. 3.9b. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area. (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: The overall potential rate of spread is assessed as very slow as all conditions mentioned in Qu. 3.8b have to be fulfilled.

Qu. 3.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?

RESPONSE	very easy easy with some difficulty difficult very difficult	CONFIDENCE	low medium high
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Response: Naturally dispersing organisms are difficult to contain, especially plants with pioneer character. Though, the removal of flowering trees could prevent further spread.

Qu. 3.11. Estimate the overall potential rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (indicate any key issues and provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in current conditions, providing insight in the risk of spread into (new areas in) the risk assessment area.

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: *Broussonetia papyrifera* is dioecious. If male and female plants are present, mid-range dispersal may occur via seeds as birds and small mammals disperse fruits. Information on sex distribution within the European occurrences is not available. Some of the member states with early observations from about 100 or more years ago and Atlantic / Mediterranean climatic conditions (e.g. France 1890, Italy 1923, Spain 1892) exhibit a wide distribution (see Figure 3). Paper mulberry is considered to be a pioneer species adapted to colonize disturbed sites. The habitats are disturbed, open sites generally, where there is relatively well-drained, moist, fertile soil. Such suitable habitats are unevenly spatially distributed.

The overall potential rate of spread under current conditions is assessed as “slowly” because all constraint conditions (i.e. male and female plant availability, animals disseminating fruits and seeds, suitable habitats in a sufficient quantity) have to be fulfilled. Information on the rate of spread within the risk assessment area could not be found.

Qu. 3.12. Estimate the overall potential rate of spread in relevant biogeographical regions in foreseeable climate change conditions (provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if rates of spread are likely slowed down or accelerated.

RESPONSE		CONFIDENCE	
	very slowly		low
	slowly		medium
	moderately		high
	rapidly		
	very rapidly		

Response: The overall potential rate of spread is expected to remain “slowly” as all constraint conditions (i.e. male and female plant availability, animals disseminating fruits and seeds, suitable habitats in a sufficient quantity) have to be fulfilled, but this will likely apply to an even more extensive part of the risk assessment area due to favorable climatic conditions.

4 MAGNITUDE OF IMPACT

Important instructions:

- Questions 4.1-4.5 relate to biodiversity and ecosystem impacts, 4.6-4.8 to impacts on ecosystem services, 4.9-4.13 to economic impact, 4.14-4.15 to social and human health impact, and 4.16-4.18 to other impacts. These impacts can be interlinked, for example, a disease may cause impacts on biodiversity and/or ecosystem functioning that leads to impacts on ecosystem services and finally economic impacts. In such cases the assessor should try to note the different impacts where most appropriate, cross-referencing between questions when needed.
- Each set of questions starts with the impact elsewhere in the world, then considers impacts in the risk assessment area (=EU+UK, excluding outermost regions) separating known impacts to date (i.e. past and current impacts) from potential future impacts (including foreseeable climate change).
- Only negative impacts are considered in this section (socio-economic benefits are considered in Qu. A.7)
- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

Biodiversity and ecosystem impacts

Qu. 4.1. How important is the impact of the organism on biodiversity at all levels of organisation caused by the organism in its non-native range excluding the risk assessment area?

including the following elements:

- Biodiversity means the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems
- impacted chemical, physical or structural characteristics and functioning of ecosystems

RESPONSE		CONFIDENCE	
	minimal		low
	minor		medium
	moderate		high
	major		
	massive		

Response: *Broussonetia papyrifera* is a recognized invader in many countries around the world. Much research has been done in sensitive habitats in Pakistan where it is considered to be “one of the most unwieldy woody exotics in the Himalayas foothills due to its adverse effect on the native vegetation” (Malik & Husain 2006) and was further reported as the most critical issue confronting the managers of the Margalla Hills National Park in that country. Malik & Husein (2006) also reported that the weed “has shown vigorous growth extending over large areas excluding many other plant species, so invaded areas have considerably lower richness and biodiversity of herbaceous as well as woody species”. In a thorough biodiversity study of 77 plots in and around Islamabad, Malik & Husein (2007) described the

plant as a real threat to scrub forest floristic diversity because instead of small herbaceous vegetation, nothing else grows under the canopy of *B. papyrifera*. They observed at some plots a high density of *B. papyrifera* where vegetation is more or less undisturbed so it would seem capable of invading intact vegetation. In Pakistan, direct competition of *B. papyrifera* limits the growth of the native *Dalbergia sissoo*, *Morus alba* and *Ziziphus sp.*, an important source of nectar for honey bees, especially near Islamabad and Rawalpindi. The thick monocultures at different sites have rapidly replaced the native flora and fauna, although these thickets have also become refuges for wild boar and other mammals, and enhanced the build-up of the crow population (CABI 2020). Qureshi et al. (2020a) demonstrated that *B. papyrifera* reduced the values of diversity indices in invaded plots compared to the control plots in 5 districts in Pakistan. The authors go on to state that this trend of decreased values of ecological indices is “similar to invasion studies on *B. papyrifera* from Australia, Argentina, Carolina, Columbia, Florida, Georgia, Louisiana, Maryland, North Carolina, Oklahoma, Pennsylvania, South Tennessee, Uganda and Virginia” where it is considered invasive in natural areas (see e.g Csurhes, 2016 and Ghersa et al, 2002).

In Ghana, *B papyrifera* has the ability to reduce the abundance of indigenous broadleaf forest species (Bosu et al. 2013). According to Agyeman & Kyereh (1997) and Agyeman (2000), paper mulberry is a major threat to native species regeneration and succession in Ghana’s forest ecosystems and is gradually establishing itself as one of the dominant species in the forest–savannah transition ecosystem. It is even able to displace *Chromolaena odorata*, a highly invasive species in Ghana (Bosu et al. 2013). In Uganda a recent study confirmed that areas that had been invaded by *B. papyrifera* had much lower diversity indices than the forest reserve which it is actively invading (Yamungu, 2020)

Ghersa et al. (2002) consider *B. papyrifera* to be one of the worst invasive species in Pampa grasslands in Argentina.

Given the fact that many of the examples listed are at significant scale and of high impact despite being technically reversible a score of major is given with medium confidence.

Qu. 4.2. How important is the current known impact of the organism on biodiversity at all levels of organisation (e.g. decline in native species, changes in native species communities, hybridisation) in the risk assessment area (include any past impact in your response)?

Discuss impacts that are currently occurring or are likely occurring or have occurred in the past in the risk assessment area. Where there is no direct evidence of impact in the risk assessment area (for example no studies have been conducted), evidence from outside of the risk assessment area can be used to infer impacts within the risk assessment area.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: *Broussonetia papyrifera* is reported as invasive in the Lombardy and Piedmont and Ticino (Mangili et al. 2018) areas of Italy. The greatest environmental damage is reported to occur when it colonizes natural and semi-natural formations such as thermophilic forests and dry grasslands

(Montagnani et al. 2018). In the framework of the Italian LIFE project LIFE03 NAT/IT/000119 dense stands of *B. papyrifera* have been highlighted as a clear menace to the conservation of Habitat 6210* (Semi-natural dry grasslands and scrubland facies on calcareous substrates - *Festuco-Brometalia* - *important orchid sites) so that are tackled in the management plan (Lasen et al. 2008).

Further, the species is listed as invasive in Croatia (Vuković et al. 2014, Borsic et al. 2008), Montenegro (Bulatovic et al. 2016). France has several reports of invasiveness including in the Auvergne-Rhône-Alpes (Pôle d'information flore-habitats-fonge d'Auvergne-Rhône-Alpes 2020) where it is also recorded as an emerging invasive (Debay et al. 2020) and it is listed as “one-to-watch” in the Pays de la Loire (Dortel & Le Bail 2019) and Provence Alpes-Côte d’Azur (Terrin et al. 2014).

Dana et al. (2001), in their assessment of plant invaders in Spain, recorded *B. papyrifera* on a checklist of species that are considered as highly invasive in other European countries and which may represent a potential threat for Spanish ecosystems.

According to Montagnani et al. (2018) the species creates monotypic populations that exclude other species, inhibiting their renewal and changing the light conditions on the ground. It is able to absorb large quantities of water, with negative effects on water availability for other plants

The score is moderate because of the limited evidence of any impact on biodiversity in the risk assessment area.

Qu. 4.3. How important is the potential future impact of the organism on biodiversity at all levels of organisation likely to be in the risk assessment area?
 See comment above. The potential future impact shall be assessed only for the risk assessment area.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: The tree is likely to continue to spread slowly and is more likely to displace native species as it has in other parts of the world, even though there is only limited evidence of impact in the risk assessment area as yet, so the score is assessed as major but confidence level is .

Qu. 4.4. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism currently in the risk assessment area?
 including the following elements:

- native species impacted, including red list species, endemic species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats

- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Only limited information has been found, and the species predominantly is found in areas that are impacted by humans. However, the threats to the goals of the Habitats Directive have been highlighted by the Italian LIFE project LIFE03 NAT/IT/000119.

Qu. 4.5. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.4.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: As there is no evidence of decline in conservation value in the risk assessment area, there is no baseline on which to extrapolate likely future impacts. It is likely that the tree will spread out from ruderal habitats especially along transport corridors and this is likely to increase the propagule pressure in natural and sensitive environments, much as it has in non-European invasions. Therefore these impacts are likely to become more significant in the future so a score of moderate with low confidence is given.

Ecosystem Services impacts

Qu. 4.6. How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?

- For a list of services use the CICES classification V5.1 provided in Annex V.
- Impacts on ecosystem services build on the observed impacts on biodiversity (habitat, species, genetic, functional) but focus exclusively on reflecting these changes in relation to their links with socio-economic well-being.
- Quantitative data should be provided whenever available and references duly reported.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Qureshi et al. (2020a) refer to damaged ecosystem services due to *Broussonetia* invasion in Pakistan. It is believed to be allelopathic in the Philippines (Combalicer et al. 2019) which will have an impact on local biodiversity and succession, by affecting soil quality regulation provisioning biomass of wild plants. Further impacts on regulation services, specifically soil quality are through soil nutrient manipulation to its own benefit in Ghana (Kofi Anning et al. 2018). Additional evidence of soil quality regulation and local biodiversity comes from a recent study in India summarised that “the soil in highly invaded areas was more acidic with greater conductivity, phenolic content, and organic carbon content (Maan et al, 2021), but the authors go on to query whether *B. papyrifera* actually alters soil physico-chemical properties or prefers to inhabit soil with such characteristics.

Broussonetia papyrifera can affect provisioning for water as it is recorded as having the capability of massive water consumption, but also slowing the flow of water in channels and suppressing the growth of other plants. (CABI 2019). Additionally, the flow of most of the drains of Nallah Lai near Rawalpindi and Islamabad, Pakistan, are affected resulting in increased flood risks and impacting on Regulation of baseline flows and extreme events. Its shallow root system makes it susceptible to blowing over during high winds (Malik 2007), posing a hazard to people and causing slope erosion and further degradation of an area. It has a moderate impact score because the impacts are technically reversible and confidence is medium because the impact is more than minor with multiple aspects of provisioning affected but the supporting information could be better.

Qu. 4.7. How important is the impact of the organism on provisioning, regulating, and cultural services currently in the different biogeographic regions or marine sub-regions where the species has established in the risk assessment area (include any past impact in your response)?

- See guidance to Qu. 4.6.

RESPONSE	N/A minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: According to Montagnani et al. (2018) the species creates monotypic populations that exclude other species, inhibiting their renewal and changing the light conditions on the ground. It is able to absorb large quantities of water, with negative effects on water availability for other plants. Therefore, the ecosystem services impacts of *B. papyrifera* include provisioning of water with local impacts on provisioning of biomass of wild plants with associated impacts on cultural ecosystem services in the category of cultural and experiential. The score is assessed as minor given that it is reported as forming

monotypic populations that affect light and water thereby having significant ecosystem impact but since there is little direct assessment published and therefore confidence levels are low.

Qu. 4.8. How important is the impact of the organism on provisioning, regulating, and cultural services likely to be in the different biogeographic regions or marine sub-regions where the species can establish in the risk assessment area in the future?

- See guidance to Qu. 4.6.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: There is limited evidence of impact thus far in the risk assessment area but in future conditions, impact on ecosystem services is likely to increase along with other impacts but perhaps not enough to be scored as moderate. Confidence is low because of the lack of published evidence.

Economic impacts

Qu. 4.9. How great is the overall economic cost caused by the organism within its current area of distribution (excluding the risk assessment area), including both costs of / loss due to damage and the cost of current management.

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the world these should be reported here. The assessment of the potential costs of / loss due to damage shall describe those costs quantitatively and/or qualitatively depending on what information is available. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage. As far as possible, it would be useful to separate costs of / loss due to the organism from costs of current management.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Quereshi et al. (2020) report choking of sewerage lines in urban set-up and increased crow population acting as seed dispersal vector. The mechanical removal of paper mulberry in Islamabad alone is several million rupees per year (Rashid et al. 2014) which equates to tens of thousands of euros per year. This economic impact appears low in a European setting as it was calculated from Pakistan data, but those costs would be much higher in a European country and management costs are also being incurred in the USA, but no figures were found.

Broussonetia papyrifera, as other invasive tree species, are reported as “not only reducing the land value and causing economic loss to agriculture communities, but are also a source of allergy and related health problems in Islamabad and Peshawar” (Marwat et al. 2010), though no detail is given on the nature and extent of the economic impact.

Qu. 4.10. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism currently in the risk assessment area (include any past costs in your response)?

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the EU these should be reported here. Assessment of the potential costs of damage on human health, safety, and the economy, including the cost of non-action. A full economic assessment at EU scale might not be possible, but qualitative data or different case studies from across the EU (or third countries if relevant) may provide useful information to inform decision making. In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	N/A minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No information has been found on the issue but the question is applicable so a minimal score is given with low confidence.

Qu. 4.11. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.10.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: It is assumed that increased spread and associated impact will have a greater than 10,000 euro per year cost across the many countries that would be host to the plant, especially given its allergenic impacts, but confidence is low.

Qu. 4.12. How great are the economic costs / losses associated with managing this organism currently in the risk assessment area (include any past costs in your response)?

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: There is no evidence that any management is being undertaken in the risk assessment area.

Qu. 4.13. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.12.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Due to the vigorous regrowth from cut stumps, vegetative root regeneration and seed dispersal, control using traditional methods is both labour intensive and costly with repeated applications needed to achieve a restored habitat. Manual removal (uprooting) and cutting coupled with an application of systemic herbicides have been shown to be effective in controlling shrub forms of the plant. Spread may be limited in the short term so a minor score is given with low confidence given the lack of current management figures in the area on which to build.

Social and human health impacts

Qu. 4.14. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism for the risk assessment area and for third countries, if relevant (e.g. with similar eco-climatic conditions).

The description of the known impact and the assessment of potential future impact on human health, safety and the economy, shall, if relevant, include information on

- illnesses, allergies or other affections to humans that may derive directly or indirectly from a species;

- damages provoked directly or indirectly by a species with consequences for the safety of people, property or infrastructure;
- direct or indirect disruption of, or other consequences for, an economic or social activity due to the presence of a species.

Social and human health impacts can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: As far back as early 20th Century the allergenic pollen of *B. papyrifera* was recognized as a causal agent for respiratory problems like hay fever and asthma in sensitized patients (Bernton 1928; Balyeat & Rinkel 1931) particularly due to its vast pollen production (Velasco-Jiménez et al. 2014). Asthma leads to difficulty breathing whilst allergic rhinitis (hay fever) is a type of inflammation in the nose that occurs when the immune system overreacts to allergens in the air. Signs and symptoms include a runny or stuffy nose, sneezing, red, itchy, and watery eyes, and swelling around the eyes.

In Islamabad, Pakistan, *B. papyrifera* is the main source of airborne allergens (Malik & Husein 2007). Its pollen cause rhinitis and asthma in Pakistan (Qureshi et al. 2020a, 2020b) and it is a major cause of urticaria and severe respiratory symptoms (Aslam et al. 2015). Also in Taiwan (Hsu et al. 2008) it is an emerging aeroallergen associated with allergic illness (Wu et al. 2019). During 1995, the Pakistan Medical Research Council found more than 45% of the allergic patients in Islamabad and Rawalpindi showed positive sensitivity to pollen of *B. papyrifera*, and it was considered extremely important for pollen allergy tests not only because of allergenicity of its pollen, but also for the high quantity of pollen produced and pollen dispersibility (CABI 2019). A study in the same region showed a clear correlation between rate of patient admission for allergies with the flowering period of *B. papyrifera* (Ghufran et al. 2013), later supported also by a study by Qazi et al. (2019). There is a suggestion that the human reaction is triggered by two proteins of 40kDa which may lead to the development of immunological treatments (Aslam et al, 2015)

In addition, the growth and excessive root systems of *B. papyrifera* has impeded the flow of most of the drains of Nallah Lai near Rawalpindi and Islamabad, Pakistan, resulting in increased flood risks, and is considered to have assisted in the worst flood of the history in Rawalpindi where many people died or lost their homes (CABI 2019). Its shallow root system makes it susceptible to blowing over during high winds, posing a hazard to people and causing slope erosion and further degradation of an area (National Park Service 2010).

There is limited evidence of human impacts in the risk assessment areas apart from a report of pollenosis relating to *Broussonetia* in the hospital of Este (Zanforlin & Incorvaia, 2004). Despite this, the evidence from other countries shows that the human health impact is major.

Qu. 4.15. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism in the future for the risk assessment area.

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	N/A minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

There is limited evidence of current allergies caused by the pollen of *B. papyrifera* in the risk assessment area bar one case in Italy (Zanforlin & Incorvaia, 2004). *Broussonetia papyrifera* pollen is under monitoring in **Italy** at the national level since 2019 with a recent study showing the highest levels in excess of 100 parts per m³ in Veneto in April 2019 (Arpa FVG report online) . The regional authority for environmental protection of the Tuscany region (ARPAT Toscana) states that so far pollen allergenicity in Italy is low, but the process needs to be monitored in consideration of what is known for other parts the world (ARPAT 2014). The few *B papyrifera* individuals recorded in a recent assessment in Brussels were not thought by the authors to play a role in pollinosis in the region (yet) (Aerts et al. 2021).

Broussonetia pollen could be confused with the one from *Urticaceae* species, so that there is some risk of underestimating its presence (http://www.pollnet.it/default_it.asp).

Other impacts

Qu. 4.16. How important is the organism in facilitating other damaging organisms (e.g. diseases) as food source, a host, a symbiont or a vector etc.?

RESPONSE	N/A minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Whilst there is no evidence of any facilitation of damaging organisms actually occurring in the risk assessment area, there are numerous species of plant damaging organisms present in the native range of the plant that could impact other species in the risk assessment area. Among the most serious pathogenic organisms of which *B. papyrifera* can being a carrier is the mulberry cerambicide, *Apriona germani* which can cause serious damage to several deciduous species (EPPO, 2014). In Japan the bacterial blight *Pseudomonas syringe* pv. *broussonetiae* affects paper mulberry (Whistler & Elevitch,

2006) as well as the spider mite *Tetranychus piercei* (Ohno et al. 2010). The fungi *Phytophthora boehmeriae* and *Dendryphiella broussonetiae* are also known to attack the plant (Berg 2003) but the host range of these pathogens are not fully known and may be limited.

In China the plant is known to host crown gall (*Agrobacterium tumefaciens*), which causes tumor-like growths on the plant (Whistler & Elevitch 2006; Li et al. 2015) as well as *Candidatus phytoplasma asteris* (Mei et al. 2016).

Paper Mulberry is identified as a known host of the citrus long-horned beetle *Anoplophora chinensis* (OEPP/EPPO (2013)) as well as the spotted lanternfly, *Lycorma delicatula* (EPPO, 2016). It is also a host of the cassava white peach scale *Pseudaulacaspis pentagona* in the Solomon Islands (Tsatsia & Jackson 2020). It is also on the host plant list for *Xylella fastidiosa* (EFSA 2020).

The score of minor is given with low confidence as the impacts are hypothetical assuming the arrival of the other pests.

Qu. 4.17. How important might other impacts not already covered by previous questions be resulting from introduction of the organism?

RESPONSE	N/A minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No information has been found on the issue.

Qu. 4.18. How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area?

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: There are no known natural enemies that would significantly restrict its performance in the risk assessment area.

Qu. 4.19. Estimate the overall impact in the risk assessment area under current climate conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, in current conditions.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: At this stage of the invasion process the impact of *B. papyrifera* could be considered relatively minor as there are very few published studies on it. It is considered established in 8 Member States, and can be locally widespread but with only limited records of impacts on biodiversity, ecosystem services and social and human health so far. However, in other relevant biogeographical regions, it has a significant impact on biodiversity by invading not only disturbed ecosystems but also intact forest. Its effects on ecosystem services range from provisioning of wild plants through competition, shading and allelopathy to regulation services through changes in soil nutrients. It can have further provisioning effects on water through its massive water consumption which can reduce availability overall and the rate of flow in specific circumstances, but it can also have a regulating impact on water causing flooding by blocking invaded drainage channels. Given that it has serious impacts outside the risk assessment area and is relatively well-established within the overall impact can be considered moderate but confidence remains low given the lack of published studies in the risk assessment area.

Qu. 4.20. Estimate the overall impact in the risk assessment area in foreseeable climate change conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, under future conditions.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Although the impact on biodiversity and ecosystem services could be assumed to be likely to increase significantly based on the experience in Pakistan, where only 30 years after a planting regime impacts were felt some *B. papyrifera* populations in Europe have been in place for a very long time without major reported problem. If populations do grow as more habitats become suitable for invasion, then direct competition for resources by this dominant tree will decrease biodiversity in invaded areas. The main impact is likely to be as an allergen impacting on human health in a similar way to *Ambrosia*

artemisiifola. This could become a very serious issue, as it has in Pakistan and more recently emerging in Taiwan (Wu et al. 2019).

RISK SUMMARIES			
	RESPONSE	CONFIDENCE	COMMENT
Summarise Introduction and Entry*	very unlikely unlikely moderately likely likely very likely	low medium high	The main introduction and entry pathways are “escapes from confinement” (botanical garden, horticulture and ornamental purpose other than horticulture). Future climate change conditions may change the demand and availability of seeds, cuttings or trees by regional tree nurseries, especially in northern Europe.
Summarise Establishment*	very unlikely unlikely moderately likely likely very likely	low medium high	The paper mulberry is recorded in 19 EU Member states (plus UK) and established in 8. The species is recorded in the Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean and Pannonian biogeographical regions and can be assessed as “established” (naturalized) in the Atlantic, Continental and Mediterranean biogeographical regions. The core areas are the Atlantic, Mediterranean and Continental region parts in Western Europe. The climate change scenarios RCP 2.6 and RCP 4.5 indicate a higher suitability to establish in the Northern and Eastern parts of Europe.
Summarise Spread*	very slowly slowly moderately rapidly very rapidly	low medium high	The overall potential rate of spread is expected to remain slowly as all constraint conditions (i.e. male and female plant availability, animals disseminating fruits and seeds, suitable habitats in a sufficient quantity) have to be fulfilled, but this will likely apply to an even more extensive part of the risk assessment area due to favourable climatic conditions.
Summarise Impact*	minimal minor moderate major massive	low medium high	Paper mulberry is considered a pioneer species adapted to colonize disturbed habitats. It exhibits a high reproductive potential, by means of both sexual and vegetative propagation. Root suckers are produced when the main stem is cut. Over time, this

			<p>can lead to the formation of dense thickets. It is believed to be allelopathic (Combalicer et al. 2019) which will have an impact on local biodiversity and succession. <i>Broussonetia papyrifera</i>, as other invasive tree species is reported as not only reducing the land value and causing economic loss to agriculture communities, but is also a source of allergens which have a direct effect on people and exacerbate other pre-existing respiratory health problems. At this stage of the invasion process the impact of <i>B. papyrifera</i> appears to be minor. Regarding future climate scenarios: although the impact on biodiversity and ecosystem services is likely to increase as more regions become suitably for invasion, the main impact is likely to be as an allergen impacting on human health.</p> <p>On the topic of biodiversity and ecosystems, the impact on biodiversity on all levels in the risk assessment area is assessed a moderate as the species is reported to invade natural and semi-natural formations such as thermophilic forests and dry grasslands (e.g. in Italy). It is likely that the tree will spread out from ruderal habitats especially along transport corridors and this is likely to increase the propagule pressure in natural and sensitive environments, much as it has in non-European invasions. If populations do grow as more habitats become suitable for invasion, then direct competition for resources by this dominant tree will decrease biodiversity in invaded areas. There is some evidence of current allergies caused by the pollen of <i>B. papyrifera</i> in the risk assessment area as one case in Italy.</p>
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<p>Conclusion of the risk assessment (overall risk)</p>	<p>low moderate high</p>	<p>low medium high</p>	<p><i>Broussonetia papyrifera</i> is recorded in 19 (plus UK) and already established in 8 out of 27 EU member states. The species is recorded in the Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean and Pannonian biogeographical regions and can be assessed as established in the Atlantic, Continental and Mediterranean biogeographical regions. The core areas are the Atlantic, Mediterranean and Continental region parts in Western Europe. Under future climate conditions, further establishment may be expected, especially in the Northern parts of Europe and to the Alpine biogeographical region to some extent. The main introduction and entry pathways are manmade (botanical garden, horticulture, ornamental purpose other than horticulture). Spread may occur from the introduction and entry pathways as well as from already established occurrences. Spread rate is considered as slow because all constraint conditions (i.e. male and female plant availability, animals disseminating fruits and seeds, suitable habitats in a sufficient quantity) have to be fulfilled. Impacts on biodiversity, ecosystem services and human health are known. Considering all aspects, <i>Broussonetia papyrifera</i> is assessed as a moderately risk species for the European Union.</p>
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*in current climate conditions and in foreseeable future climate conditions

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Distribution Summary

Please answer as follows:

- Yes if recorded, established or invasive
- if not recorded, established or invasive
- ? Unknown; data deficient

The columns refer to the answers to Questions A5 to A12 under Section A.

For data on marine species at the Member State level, delete Member States that have no marine borders.

In all other cases, provide answers for all columns.

Member States and the United Kingdom

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Austria	Yes	-	-	Yes	-
Belgium	Yes	-	-	Yes	-
Bulgaria	Yes	-	Yes	Yes	-
Croatia	Yes	Yes	Yes	Yes	-
Cyprus	?	-	Yes	Yes	-
Czech Republic	-	-	-	Yes	-
Denmark	Yes	-	-	Yes	-
Estonia	-	-	-	Yes	-
Finland	-	-	-	-	-
France	Yes	Yes	Yes	Yes	-
Germany	Yes	-	-	Yes	-
Greece	Yes	Yes	Yes	Yes	-
Hungary	Yes	-	Yes	Yes	-
Ireland	-	-	-	-	-
Italy	Yes	Yes	Yes	Yes	-
Latvia	-	-	-	Yes	-
Lithuania	-	-	-	Yes	-
Luxembourg	-	-	-	Yes	-
Malta	Yes	Yes	Yes	Yes	-
Netherlands	Yes	-	-	Yes	-
Poland	Yes	-	-	Yes	-
Portugal	Yes	-	Yes	Yes	-
Romania	Yes	Yes	Yes	Yes	-
Slovakia	Yes	-	-	Yes	-
Slovenia	Yes	-	Yes	Yes	-
Spain	Yes	Yes	Yes	Yes	-
Sweden	-	-	-	Yes	-
United Kingdom	Yes	-	-	Yes	-

Biogeographical regions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Alpine	Yes	-	-	Yes	-
Atlantic	Yes	Yes	Yes	Yes	-
Black Sea	Yes	-	Yes	Yes	-
Boreal	Yes	-	-	Yes	-
Continental	Yes	Yes	Yes	Yes	-
Mediterranean	Yes	Yes	Yes	Yes	-
Pannonian	Yes	-	Yes	Yes	-
Steppic	-	-	Yes	Yes	-

ANNEX I Scoring of Likelihoods of Events

(taken from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Description	Frequency
Very unlikely	This sort of event is theoretically possible, but is never known to have occurred and is not expected to occur	1 in 10,000 years
Unlikely	This sort of event has occurred somewhere at least once in the last millenium	1 in 1,000 years
Moderately Likely	This sort of event has occurred somewhere at least once in the last century	1 in 100 years
Likely	This sort of event has happened on several occasions elsewhere, or on at least once in the last decade	1 in 10 years
Very likely	This sort of event happens continually and would be expected to occur	Once a year

ANNEX II Scoring of Magnitude of Impacts

(modified from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Biodiversity and ecosystem impact	Ecosystem Services impact	Economic impact (Monetary loss and response costs per year)	Social and human health impact, and other impacts
	<i>Question 5.1-5</i>	<i>Question 5.6-8</i>	<i>Question 5.9-13</i>	<i>Question 5.14-18</i>
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected ⁵	Up to 10,000 Euro	No social disruption. Local, mild, short-term reversible effects to individuals.
Minor	Some ecosystem impact, reversible changes, localised	Local and temporary, reversible effects to one or few services	10,000-100,000 Euro	Significant concern expressed at local level. Mild short-term reversible effects to identifiable groups, localised.
Moderate	Measureable long-term damage to populations and ecosystem, but reversible; little spread, no extinction	Measureable, temporary, local and reversible effects on one or several services	100,000-1,000,000 Euro	Temporary changes to normal activities at local level. Minor irreversible effects and/or larger numbers covered by reversible effects, localised.
Major	Long-term irreversible ecosystem change, spreading beyond local area	Local and irreversible or widespread and reversible effects on one / several services	1,000,000-10,000,000 Euro	Some permanent change of activity locally, concern expressed over wider area. Significant irreversible effects locally or reversible effects over large area.
Massive	Widespread, long-term population loss or extinction, affecting several species with serious ecosystem effects	Widespread and irreversible effects on one / several services	Above 10,000,000 Euro	Long-term social change, significant loss of employment, migration from affected area. Widespread, severe, long-term, irreversible health effects.

⁵ Not to be confused with “no impact”.

ANNEX III Scoring of Confidence Levels

(modified from Bacher et al. 2017)

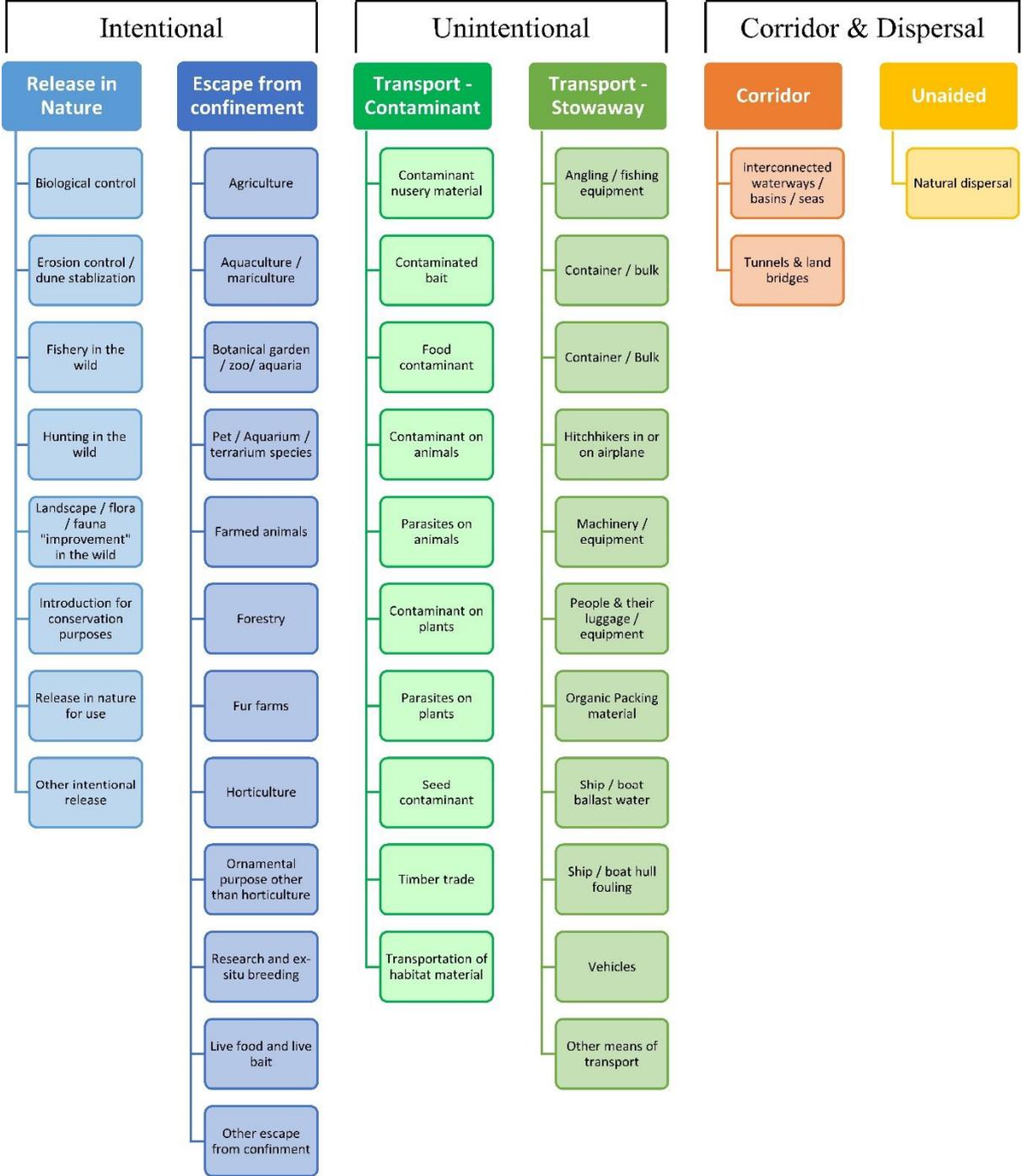
Each answer provided in the risk assessment must include an assessment of the level of confidence attached to that answer, reflecting the possibility that information needed for the answer is not available or is insufficient or available but conflicting.

The responses in the risk assessment should clearly support the choice of the confidence level.

Confidence level	Description
Low	There is no direct observational evidence to support the assessment, e.g. only inferred data have been used as supporting evidence <i>and/or</i> Impacts are recorded at a spatial scale which is unlikely to be relevant to the assessment area <i>and/or</i> Evidence is poor and difficult to interpret, e.g. because it is strongly ambiguous <i>and/or</i> The information sources are considered to be of low quality or contain information that is unreliable.
Medium	There is some direct observational evidence to support the assessment, but some information is inferred <i>and/or</i> Impacts are recorded at a small spatial scale, but rescaling of the data to relevant scales of the assessment area is considered reliable, or to embrace little uncertainty <i>and/or</i> The interpretation of the data is to some extent ambiguous or contradictory.
High	There is direct relevant observational evidence to support the assessment (including causality) <i>and</i> Impacts are recorded at a comparable scale <i>and/or</i> There are reliable/good quality data sources on impacts of the taxa <i>and</i> The interpretation of data/information is straightforward <i>and/or</i> Data/information are not controversial or contradictory.

ANNEX IV CBD pathway categorisation scheme

Overview of CBD pathway categorisation scheme showing how the 44 pathways relate to the six main pathway categories. All of the pathways can be broadly classified into 1) those that involve intentional transport (blue), 2) those in which the taxa are unintentionally transported (green) and 3) those where taxa moved between regions without direct transportation by humans and/or via artificial corridors (orange and yellow). **Note that the pathways in the category “Escape from confinement” can be considered intentional for the introduction into the risk assessment area and unintentional for the entry into the environment.**



ANNEX V Ecosystem services classification (CICES V5.1, simplified) and examples

For the purposes of this risk assessment, please feel free to use what seems as the most appropriate category / level / combination of impact (Section – Division – Group), reflecting information available.

Section	Division	Group	Examples (i.e. relevant CICES “classes”)
Provisioning	Biomass	Cultivated <i>terrestrial</i> plants	Cultivated terrestrial plants (including fungi, algae) grown for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from cultivated plants, fungi, algae and bacteria for direct use or processing (excluding genetic materials); Cultivated plants (including fungi, algae) grown as a <u>source of energy</u> <i>Example: negative impacts of non-native organisms to crops, orchards, timber etc.</i>
		Cultivated <i>aquatic</i> plants	Plants cultivated by in- situ aquaculture grown for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from in-situ aquaculture for direct use or processing (excluding genetic materials); Plants cultivated by in- situ aquaculture grown as an <u>energy source</u> . <i>Example: negative impacts of non-native organisms to aquatic plants cultivated for nutrition, gardening etc. purposes.</i>
		Reared animals	Animals reared for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from reared animals for direct use or processing (excluding genetic materials); Animals reared to provide <u>energy</u> (including mechanical) <i>Example: negative impacts of non-native organisms to livestock</i>
		Reared <i>aquatic</i> animals	Animals reared by in-situ aquaculture for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from animals grown by in-situ aquaculture for direct use or processing (excluding genetic materials); Animals reared by in-situ aquaculture as an <u>energy source</u> <i>Example: negative impacts of non-native organisms to fish farming</i>
		Wild plants (terrestrial and aquatic)	Wild plants (terrestrial and aquatic, including fungi, algae) used for <u>nutrition</u> ; <u>Fibres and other materials</u> from wild plants for direct use or processing (excluding genetic materials); Wild plants (terrestrial and aquatic, including fungi, algae) used as a <u>source of energy</u> <i>Example: reduction in the availability of wild plants (e.g. wild berries, ornamentals) due to non-native organisms (competition, spread of disease etc.)</i>
		Wild animals (terrestrial and aquatic)	Wild animals (terrestrial and aquatic) used for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from wild animals for direct use or processing (excluding genetic materials); Wild animals (terrestrial and aquatic) used as a <u>source of energy</u> <i>Example: reduction in the availability of wild animals (e.g. fish stocks, game) due to non-native organisms (competition, predations, spread of disease etc.)</i>

	Genetic material from all biota	Genetic material from plants, algae or fungi	<p><u>Seeds, spores and other plant materials</u> collected for maintaining or establishing a population; Higher and lower plants (whole organisms) used to <u>breed new strains or varieties</u>; Individual genes extracted from higher and lower plants for the <u>design and construction of new biological entities</u></p> <p><i>Example: negative impacts of non-native organisms due to interbreeding</i></p>
		Genetic material from animals	<p>Animal material collected for the purposes of maintaining or establishing a population; Wild animals (whole organisms) used to breed new strains or varieties; Individual genes extracted from organisms for the design and construction of new biological entities</p> <p><i>Example: negative impacts of non-native organisms due to interbreeding</i></p>
	Water ⁶	Surface water used for nutrition, materials or energy	<p>Surface water for <u>drinking</u>; Surface water used as a material (<u>non-drinking purposes</u>); Freshwater surface water, coastal and marine water used as an <u>energy source</u></p> <p><i>Example: loss of access to surface water due to spread of non-native organisms</i></p>
		Ground water for used for nutrition, materials or energy	<p>Ground (and subsurface) water for <u>drinking</u>; Ground water (and subsurface) used as a material (<u>non-drinking purposes</u>); Ground water (and subsurface) used as an <u>energy source</u></p> <p><i>Example: reduced availability of ground water due to spread of non-native organisms and associated increase of ground water consumption by vegetation.</i></p>
Regulation & Maintenance	Transformation of biochemical or physical inputs to ecosystems	Mediation of wastes or toxic substances of anthropogenic origin by living processes	<p><u>Bio-remediation</u> by micro-organisms, algae, plants, and animals; <u>Filtration/sequestration/storage/accumulation</u> by micro-organisms, algae, plants, and animals</p> <p><i>Example: changes caused by non-native organisms to ecosystem functioning and ability to filtrate etc. waste or toxics</i></p>
		Mediation of nuisances of anthropogenic origin	<p><u>Smell reduction; noise attenuation; visual screening</u> (e.g. by means of green infrastructure)</p> <p><i>Example: changes caused by non-native organisms to ecosystem structure, leading to reduced ability to mediate nuisances.</i></p>
	Regulation of physical, chemical, biological conditions	Baseline flows and extreme event regulation	<p>Control of <u>erosion</u> rates; Buffering and attenuation of <u>mass movement</u>; <u>Hydrological cycle and water flow regulation</u> (Including flood control, and coastal protection); <u>Wind</u> protection; <u>Fire</u> protection</p> <p><i>Example: changes caused by non-native organisms to ecosystem functioning or structure leading to, for example, destabilisation of soil, increased risk or intensity of wild fires etc.</i></p>
Lifecycle maintenance , habitat and gene pool protection		<p><u>Pollination</u> (or 'gamete' dispersal in a marine context); <u>Seed dispersal</u>; Maintaining <u>nursery populations and habitats</u> (Including gene pool protection)</p>	

⁶ Note: in the CICES classification provisioning of water is considered as an abiotic service whereas the rest of ecosystem services listed here are considered biotic.

			<p><i>Example: changes caused by non-native organisms to the abundance and/or distribution of wild pollinators; changes to the availability / quality of nursery habitats for fisheries</i></p>
		Pest and disease control	<p>Pest control; Disease control</p> <p><i>Example: changes caused by non-native organisms to the abundance and/or distribution of pests</i></p>
		Soil quality regulation	<p><u>Weathering processes</u> and their effect on soil quality; <u>Decomposition and fixing processes</u> and their effect on soil quality</p> <p><i>Example: changes caused by non-native organisms to vegetation structure and/or soil fauna leading to reduced soil quality</i></p>
		Water conditions	<p>Regulation of the <u>chemical condition</u> of freshwaters by living processes; Regulation of the chemical condition of salt waters by living processes</p> <p><i>Example: changes caused by non-native organisms to buffer strips along water courses that remove nutrients in runoff and/or fish communities that regulate the resilience and resistance of water bodies to eutrophication</i></p>
		Atmospheric composition and conditions	<p>Regulation of <u>chemical composition</u> of atmosphere and oceans; Regulation of <u>temperature and humidity</u>, including ventilation and transpiration</p> <p><i>Example: changes caused by non-native organisms to ecosystems' ability to sequester carbon and/or evaporative cooling (e.g. by urban trees)</i></p>
Cultural	Direct, in-situ and outdoor interactions with living systems that depend on presence in the environmental setting	Physical and experiential interactions with natural environment	<p>Characteristics of living systems that that enable activities promoting health, recuperation or enjoyment through <u>active or immersive interactions</u>;</p> <p>Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through <u>passive or observational interactions</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that make it attractive for recreation, wild life watching etc.</i></p>
		Intellectual and representative interactions with natural environment	<p>Characteristics of living systems that enable <u>scientific investigation</u> or the creation of traditional ecological knowledge;</p> <p>Characteristics of living systems that enable <u>education and training</u>;</p> <p>Characteristics of living systems that are resonant in terms of <u>culture or heritage</u>;</p> <p>Characteristics of living systems that enable <u>aesthetic experiences</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have cultural importance</i></p>
	Indirect, remote, often indoor interactions with living systems that do not require presence in the environmental setting	Spiritual, symbolic and other interactions with natural environment	<p>Elements of living systems that have <u>symbolic meaning</u>;</p> <p>Elements of living systems that have <u>sacred or religious meaning</u>;</p> <p>Elements of living systems used for <u>entertainment or representation</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have sacred or religious meaning</i></p>

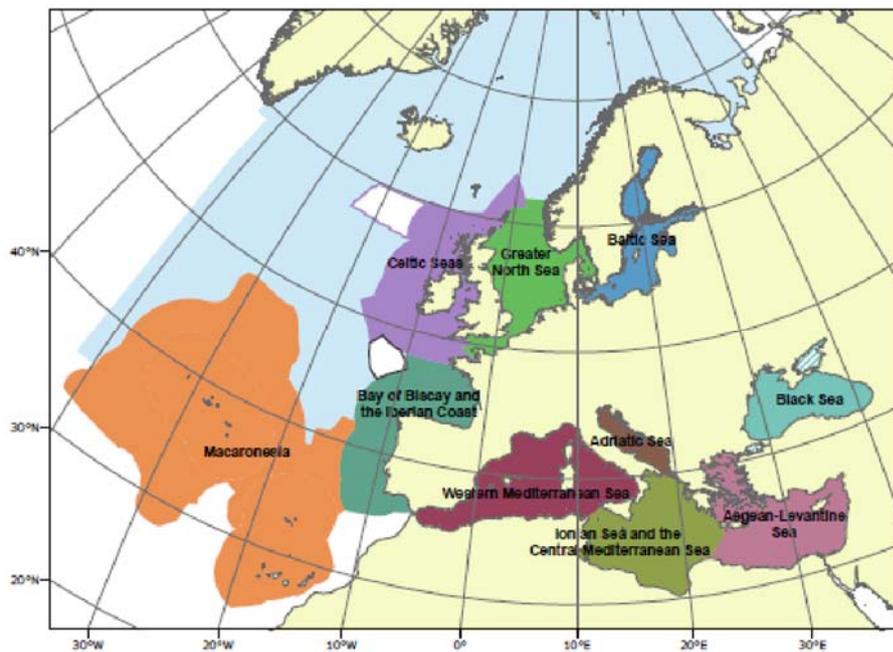
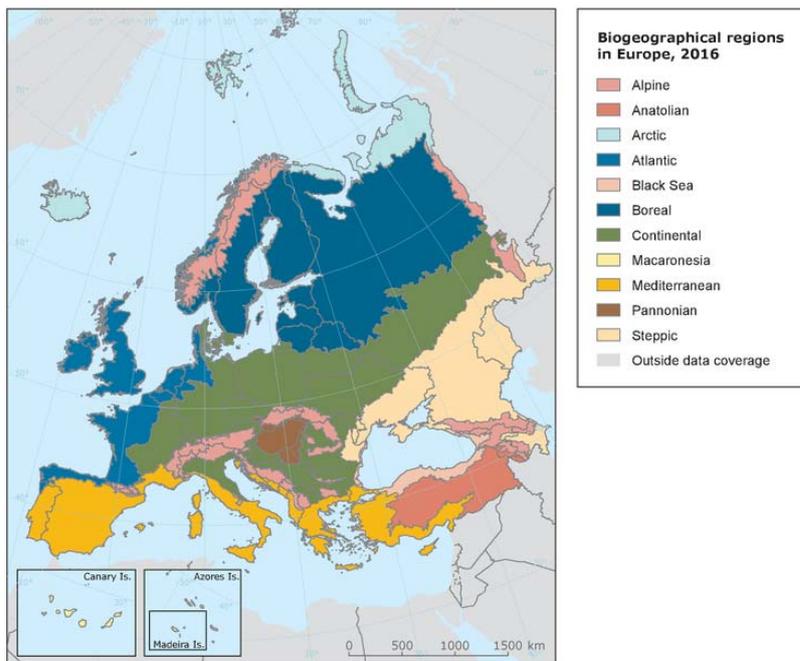
		<p>Other biotic characteristics that have a non-use value</p>	<p>Characteristics or features of living systems that have an <u>existence value</u>; Characteristics or features of living systems that have an <u>option or bequest value</u></p> <p><i>Example: changes caused by non-native organisms to ecosystems designated as wilderness areas, habitats of endangered species etc.</i></p>
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ANNEX VI EU Biogeographic Regions and MSFD Subregions

See <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2> ,
http://ec.europa.eu/environment/nature/natura2000/biogeog_regions/

and

<https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions-1/technical-document/pdf>



ANNEX VII Delegated Regulation (EU) 2018/968 of 30 April 2018

see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>

ANNEX VIII Species distribution model

Projection of climatic suitability for *Broussonetia papyrifera* establishment in Europe

Björn Beckmann, Helmut Kudrnovsky, Richard Shaw, David Paternoster and Dan Chapman

10 October 2020

Aim

To project the suitability for potential establishment of *Broussonetia papyrifera* in Europe, under current and predicted future climatic conditions.

Data for modelling

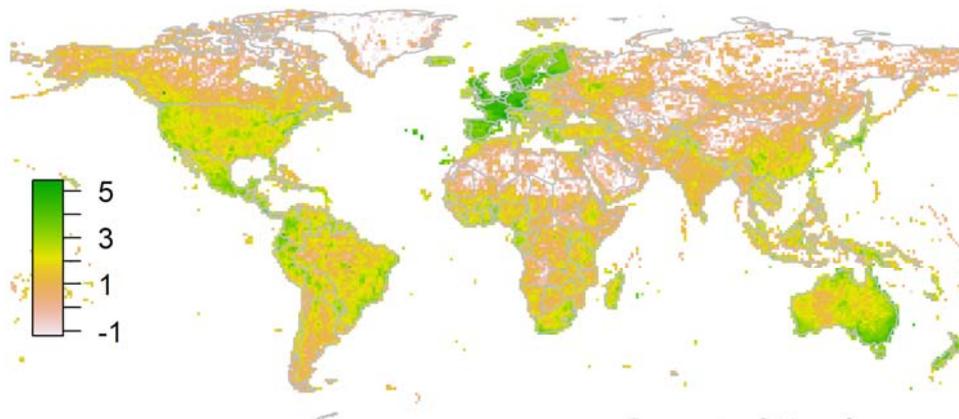
Species occurrence data were obtained from the Global Biodiversity Information Facility (GBIF) (8684 records), iNaturalist (5474 records), the Biodiversity Information Serving Our Nation database (BISON) (259 records), and additional records from the risk assessment team. We classified records as native- and invaded-range according to the CABI Invasive Species Compendium. We scrutinised occurrence records from regions where the species is not known to be established and removed any which may not refer to populations currently established in the wild (e.g. museum specimens, records from botanical gardens) or where the georeferencing was too imprecise (e.g. records referenced to a country or island centroid) or outside of the coverage of the predictor layers (e.g. small island or coastal occurrences). The remaining records were gridded at a 0.25 x 0.25 degree resolution for modelling, yielding 1773 grid cells with occurrences (Figure 1a). As a proxy for recording effort, the density of Tracheophyta records held by GBIF was also compiled on the same grid (Figure 1b).

Figure 1. (a) Occurrence records obtained for *Broussonetia papyrifera* and used in the modelling, showing native and invaded distributions. (b) The recording density of Tracheophyta on GBIF, which was used as a proxy for recording effort.

(a) Species distribution used in modelling



(b) Estimated recording effort (log10-scaled)



Climate data were selected from the ‘Bioclim’ variables contained within the WorldClim database (Hijmans et al. 2005), originally at 5 arcminute resolution (0.083 x 0.083 degrees of longitude/latitude) and aggregated to a 0.25 x 0.25 degree grid for use in the model.

Based on the biology of *Broussonetia papyrifera*, the following climate variables were used in the modelling:

- Minimum temperature of the coldest month (Bio6)
- Mean temperature of the warmest quarter (Bio10)
- Climatic moisture index (CMI): ratio of mean annual precipitation to potential evapotranspiration, log+1 transformed. For its calculation, monthly potential evapotranspirations were estimated from the WorldClim monthly temperature data and solar radiation using the simple method of Zomer et al. (2008) which is based on the Hargreaves evapotranspiration equation (Hargreaves, 1994).

To estimate the effect of climate change on the potential distribution, equivalent modelled future climate conditions for the 2070s under the Representative Concentration Pathways (RCP) 2.6 and 4.5 were also obtained. These represent low and medium emissions scenarios, respectively. The above variables were obtained as averages of outputs of eight Global Climate Models (BCC-CSM1-1, CCSM4, GISS-E2-R, HadGEM2-AO, IPSL-CM5A-LR, MIROC-ESM, MRI-CGCM3, NorESM1-M), downscaled and calibrated against the WorldClim baseline (see http://www.worldclim.org/cmip5_5m).

The following habitat layers were also used:

- Human influence index (HII): As many non-native invasive species associate with anthropogenically disturbed habitats. We used the Global Human Influence Index Dataset of the Last of the Wild Project (Wildlife Conservation Society - WCS & Center for International Earth Science Information Network - CIESIN - Columbia University, 2005), which is developed from nine global data layers covering human population pressure (population density), human land use and infrastructure (built-up areas, nighttime lights, land use/land cover) and human access (coastlines, roads, railroads, navigable rivers). The index ranges between 0 and 1 and was $\ln+1$ transformed for the modelling to improve normality.

Species distribution model

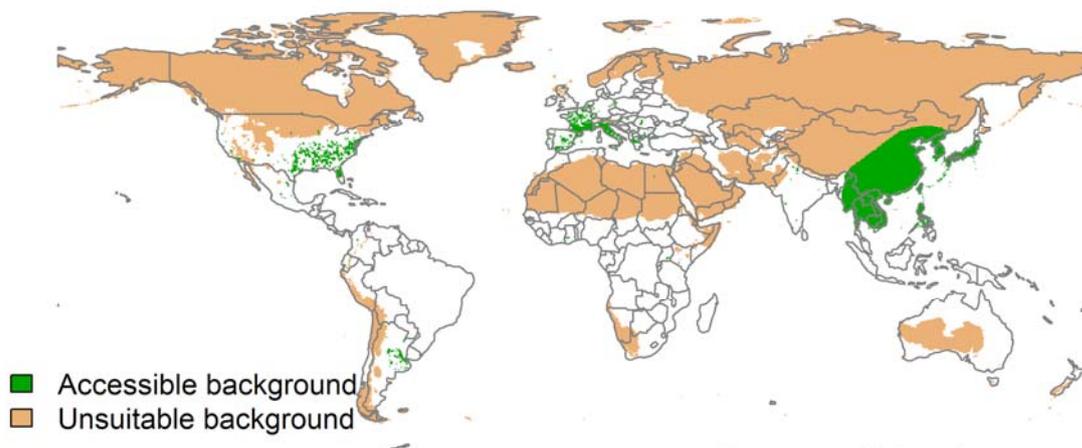
A presence-background (presence-only) ensemble modelling strategy was employed using the BIOMOD2 R package version 3.4.6 (Thuiller et al., 2020, Thuiller et al., 2009). These models contrast the environment at the species' occurrence locations against a random sample of the global background environmental conditions (often termed 'pseudo-absences') in order to characterise and project suitability for occurrence. This approach has been developed for distributions that are in equilibrium with the environment. Because invasive species' distributions are not at equilibrium and subject to dispersal constraints at a global scale, we took care to minimise the inclusion of locations suitable for the species but where it has not been able to disperse to (Chapman et al. 2019). Therefore the background sampling region included:

- The area accessible by native *Broussonetia papyrifera* populations, in which the species is likely to have had sufficient time to disperse to all locations. Based on presumed maximum dispersal distances, the accessible region was defined as a 400km buffer around the native range occurrences; AND
- A 30km buffer around the non-native occurrences, encompassing regions likely to have had high propagule pressure for introduction by humans and/or dispersal of the species; AND
- Regions where we have an *a priori* expectation of high unsuitability for the species so that absence is assumed irrespective of dispersal constraints (see Figure 2). The following rules were applied to define a region expected to be highly unsuitable for *Broussonetia papyrifera* at the spatial scale of the model:
 - Minimum temperature of the coldest month (Bio6) < -12
 - Mean temperature of the warmest quarter (Bio10) < 13
 - Climatic moisture index (CMI) < 0.15

Altogether, only 0.5% of occurrence grid cells were located in the unsuitable background region.

Within the unsuitable background region, 10 samples of 5000 randomly sampled grid cells were obtained. In the accessible background (comprising the accessible areas around native and non-native occurrences as detailed above), the same number of pseudo-absence samples were drawn as there were presence records (1773), weighting the sampling by a proxy for recording effort (Figure 2).

Figure 2. The background from which pseudo-absence samples were taken in the modelling of *Broussonetia papyrifera*. Samples were taken from a 400km buffer around the native range and a 30km buffer around non-native occurrences (together forming the accessible background), and from areas expected to be highly unsuitable for the species (the unsuitable background region). Samples from the accessible background were weighted by a proxy for recording effort (Figure 1(b)).



Each dataset (i.e. combination of the presences and the individual background samples) was randomly split into 80% for model training and 20% for model evaluation. With each training dataset, five statistical algorithms were fitted with the default BIOMOD2 settings and rescaled using logistic regression, except where specified below:

- Generalised linear model (GLM)
- Generalised boosting model (GBM)
- Generalised additive model (GAM) with a maximum of four degrees of freedom per smoothing spline
- Random forest (RF)
- Maxent

Since the total background sample was larger than the number of occurrences, prevalence fitting weights were applied to give equal overall importance to the occurrences and the background. Normalised

variable importance was assessed and variable response functions were produced using BIOMOD2's default procedure.

Model predictive performance was assessed by the following three measures:

- AUC, the area under the receiver operating characteristic curve (Fielding & Bell 1997). Predictions of presence-absence models can be compared with a subset of records set aside for model evaluation (here 20%) by constructing a confusion matrix with the number of true positive, false positive, false negative and true negative cases. For models generating non-dichotomous scores (as here) a threshold can be applied to transform the scores into a dichotomous set of presence-absence predictions. Two measures that can be derived from the confusion matrix are sensitivity (the proportion of observed presences that are predicted as such, quantifying omission errors), and specificity (the proportion of observed absences that are predicted as such, quantifying commission errors). A receiver operating characteristic (ROC) curve can be constructed by using all possible thresholds to classify the scores into confusion matrices, obtaining sensitivity and specificity for each matrix, and plotting sensitivity against the corresponding proportion of false positives (equal to $1 - \text{specificity}$). The use of all possible thresholds avoids the need for a selection of a single threshold, which is often arbitrary, and allows appreciation of the trade-off between sensitivity and specificity. The area under the ROC curve (AUC) is often used as a single threshold-independent measure for model performance (Manel, Williams & Ormerod 2001). AUC is the probability that a randomly selected presence has a higher model-predicted suitability than a randomly selected absence (Allouche et al. 2006).
- Cohen's Kappa (Cohen 1960). This measure corrects the overall accuracy of model predictions (ratio of the sum of true presences plus true absences to the total number of records) by the accuracy expected to occur by chance. The kappa statistic ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random. Advantages of kappa are its simplicity, the fact that both commission and omission errors are accounted for in one parameter, and its relative tolerance to zero values in the confusion matrix (Manel, Williams & Ormerod 2001). However, Kappa has been criticised for being sensitive to prevalence (the proportion of sites in which the species was recorded as present) and may therefore be inappropriate for comparisons of model accuracy between species or regions (McPherson, Jetz & Rogers 2004, Allouche et al. 2006).
- TSS, the true skill statistic (Allouche et al. 2006). TSS is defined as sensitivity + specificity - 1, and corrects for Kappa's dependency on prevalence. TSS compares the number of correct forecasts, minus those attributable to random guessing, to that of a hypothetical set of perfect forecasts. Like kappa, TSS takes into account both omission and commission errors, and success as a result of random guessing, and ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random (Allouche et al. 2006).

An ensemble model was created by first rejecting poorly performing algorithms with relatively extreme low AUC values and then averaging the predictions of the remaining algorithms, weighted by their AUC. To identify poorly performing algorithms, AUC values were converted into modified z-scores based on their difference to the median and the median absolute deviation across all algorithms (Iglewicz & Hoaglin, 1993). Algorithms with $z < -2$ were rejected. In this way, ensemble projections were made for each dataset and then averaged to give an overall suitability, as well as its standard deviation. The

projections were then classified into suitable and unsuitable regions using the ‘minROCdist’ method, which minimizes the distance between the ROC plot and the upper left corner of the plot (point (0,1)).

We also produced limiting factor maps for Europe following Elith et al. (2010). For this, projections were made separately with each individual variable fixed at a near-optimal value. These were chosen as the median values at the occurrence grid cells. Then, the most strongly limiting factors were identified as the one resulting in the highest increase in suitability in each grid cell.

Results

The ensemble model suggested that suitability for *Broussonetia papyrifera* was most strongly determined by Minimum temperature of the coldest month (Bio6), accounting for 31.6% of variation explained, followed by Mean temperature of the warmest quarter (Bio10) (26.6%), Climatic moisture index (CMI) (22.6%) and Human influence index (HII) (19.2%) (Table 1, Figure 3).

Table 1. Summary of the cross-validation predictive performance (AUC, Kappa, TSS) and variable importance of the fitted model algorithms and the ensemble (AUC-weighted average of the best performing algorithms). Results are the average from models fitted to 10 different background samples of the data.

Algorithm	AUC	Kappa	TSS	Used in the ensemble	Variable importance (%)			
					Minimum temperature of the coldest month (Bio6)	Mean temperature of the warmest quarter (Bio10)	Climatic moisture index (CMI)	Human influence index (HII)
GLM	0.916	0.589	0.734	no	32	27	17	24
GAM	0.924	0.601	0.736	yes	35	26	17	23
GBM	0.929	0.620	0.744	yes	34	20	18	28
RF	0.886	0.543	0.725	no	28	30	27	16
Maxent	0.924	0.604	0.737	yes	27	34	33	6
Ensemble	0.928	0.617	0.741		32	27	23	19

Figure 3. Partial response plots from the fitted models. Thin coloured lines show responses from the algorithms in the ensemble, while the thick black line is their ensemble. In each plot, other model variables are held at their median value in the training data. Some of the divergence among algorithms is because of their different treatment of interactions among variables.

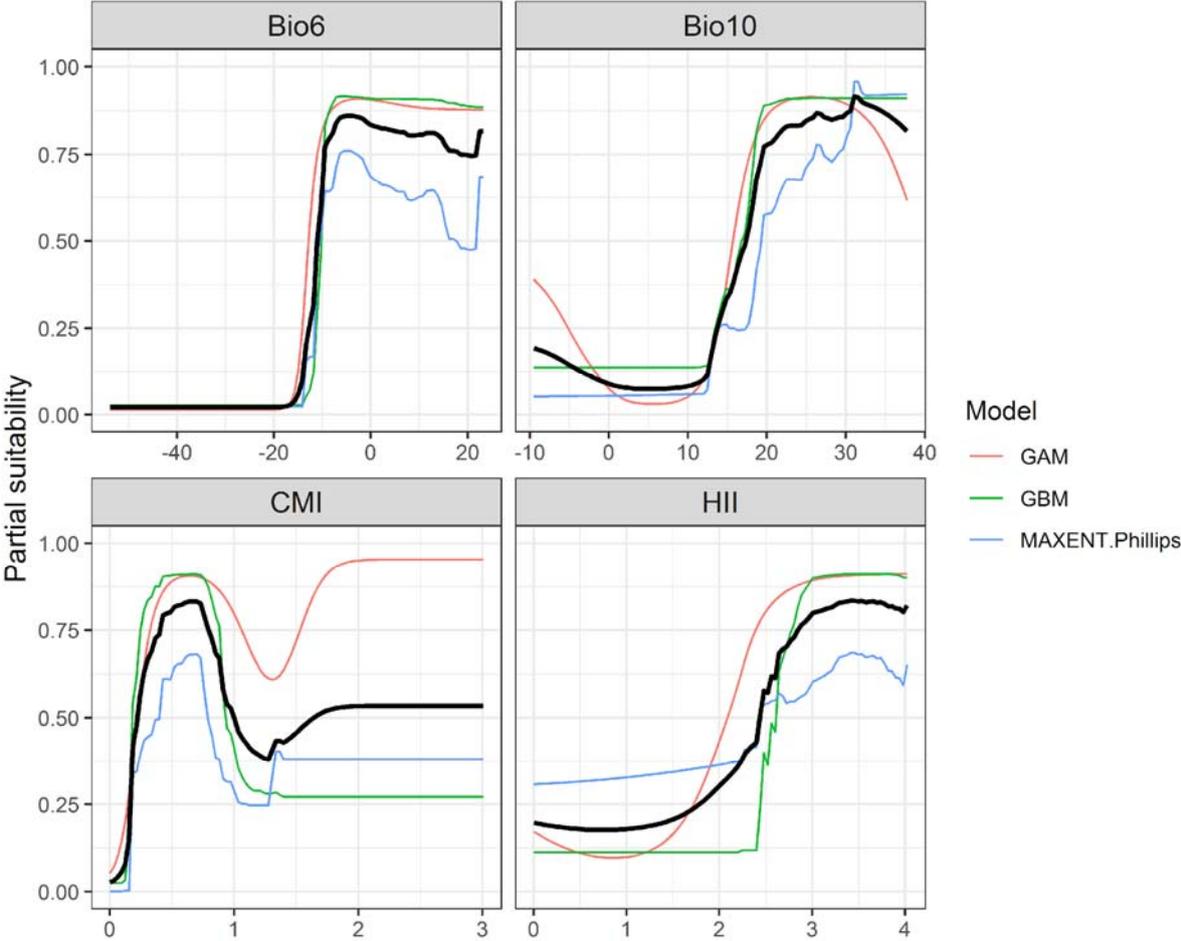


Figure 4. (a) Projected global suitability for *Broussonetia papyrifera* establishment in the current climate. For visualisation, the projection has been aggregated to a 0.5 x 0.5 degree resolution, by taking the maximum suitability of constituent higher resolution grid cells. Values > 0.38 may be suitable for the species. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

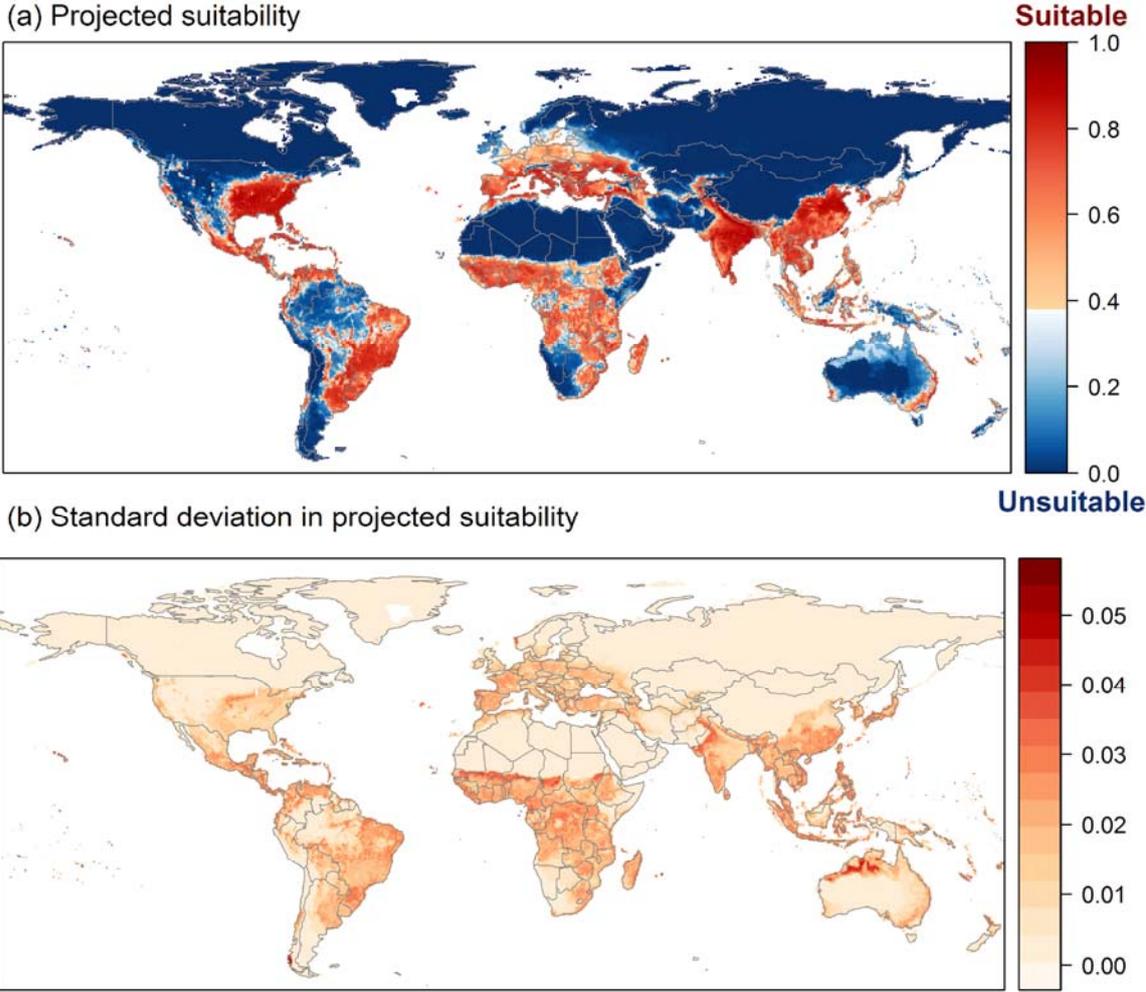


Figure 5. (a) Projected current suitability for *Broussonetia papyrifera* establishment in Europe and the Mediterranean region. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

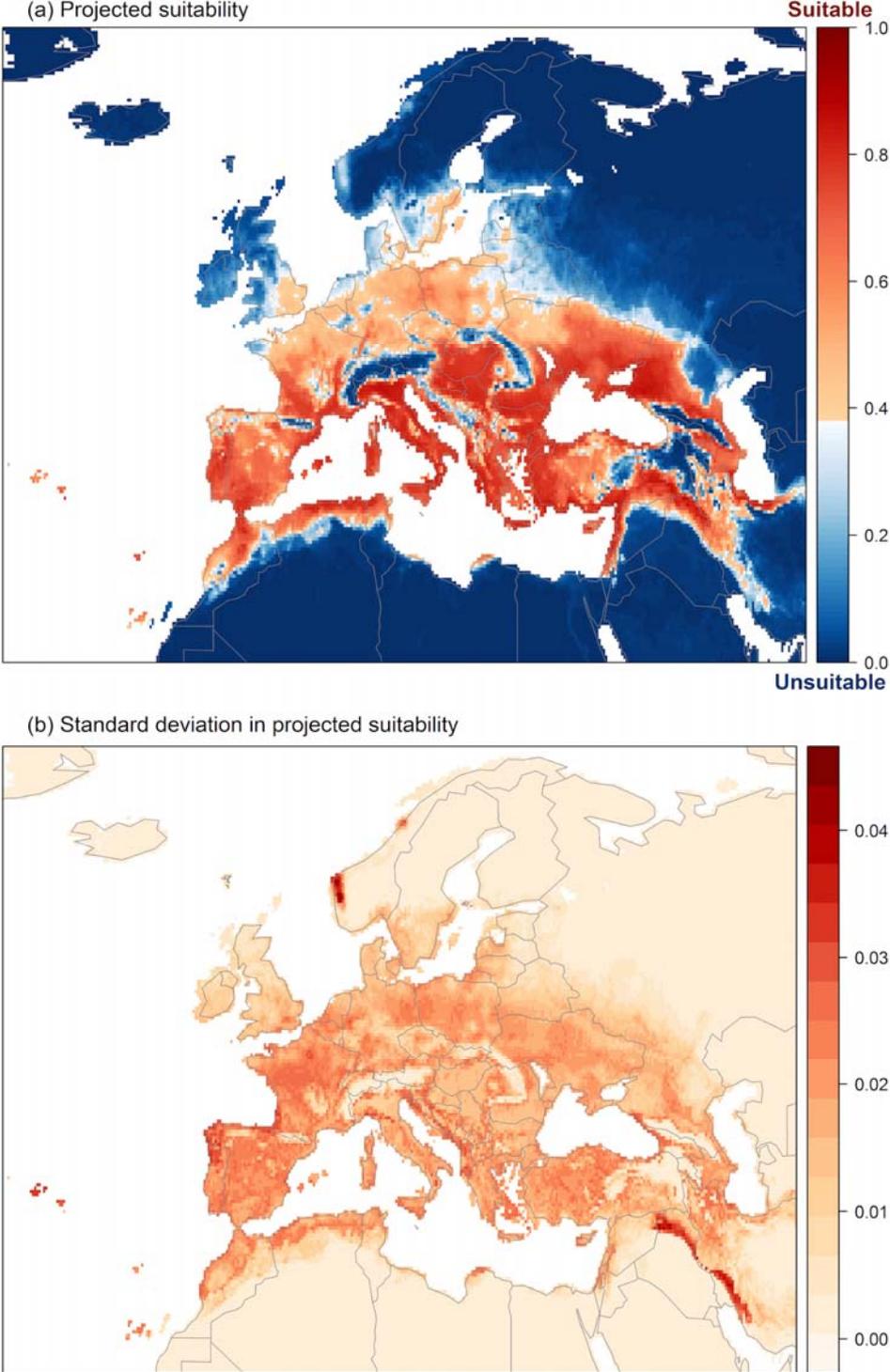


Figure 6. The most strongly limiting factors for *Broussonetia papyrifera* establishment estimated by the model in Europe and the Mediterranean region in current climatic conditions.

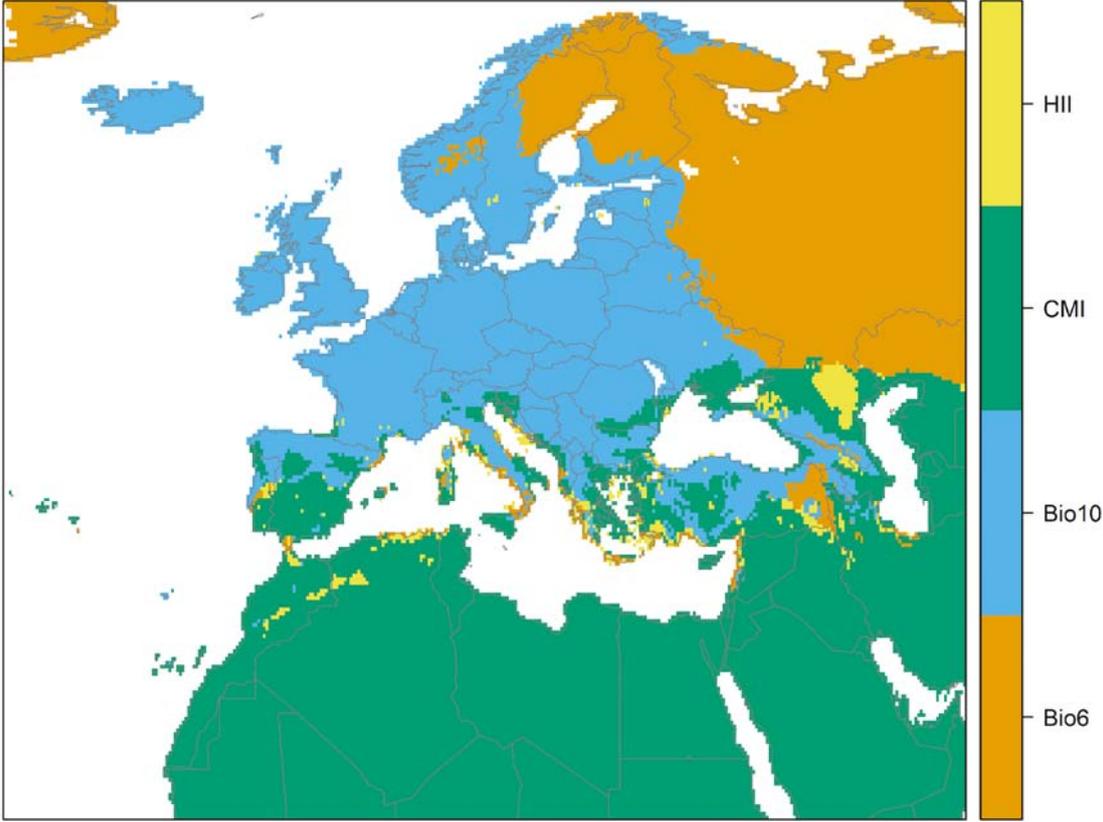


Figure 7. (a) Projected suitability for *Broussonetia papyrifera* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP2.6, equivalent to Figure 5. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

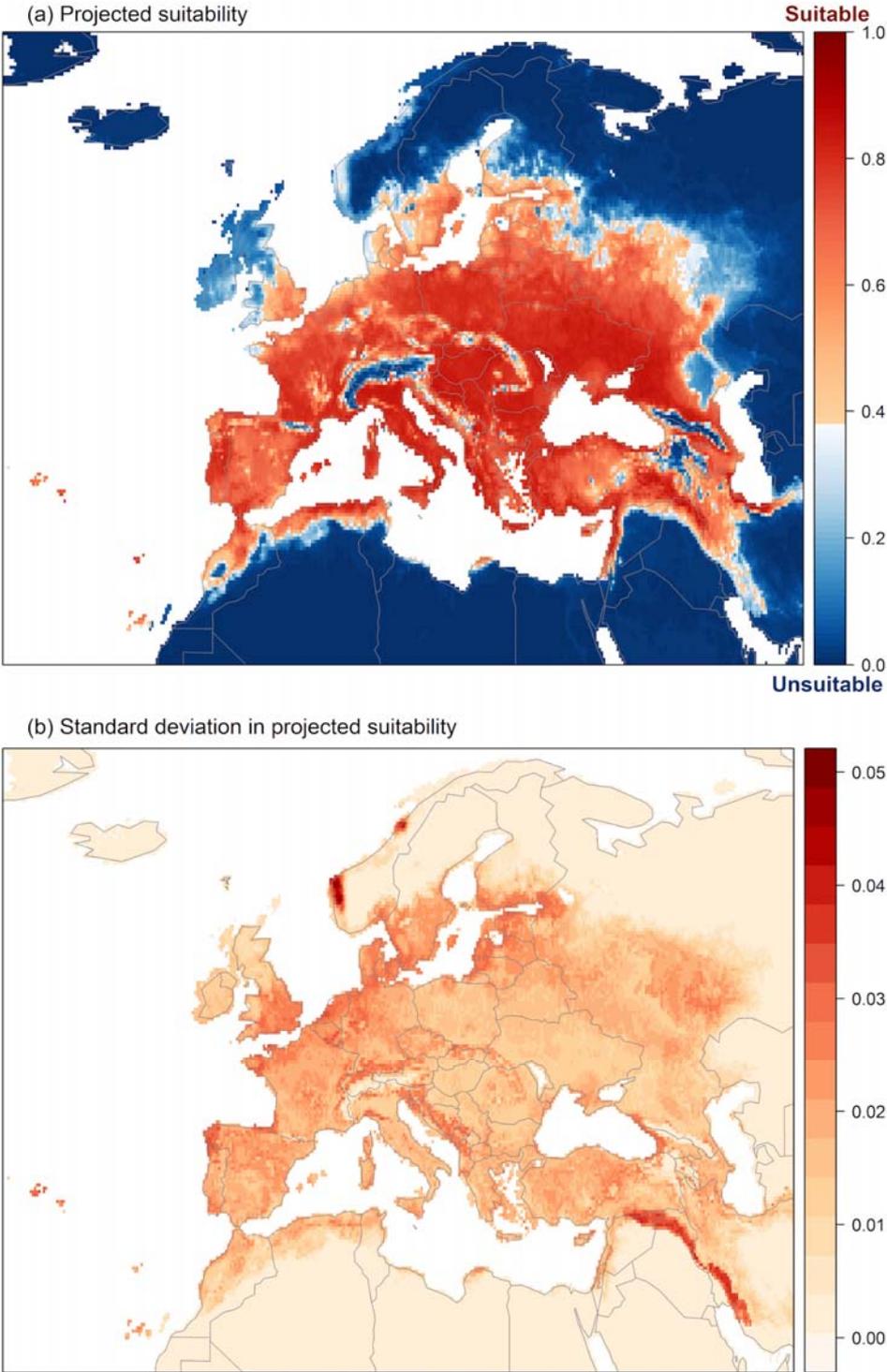


Figure 8. (a) Projected suitability for *Broussonetia papyrifera* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP4.5, equivalent to Figure 5. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

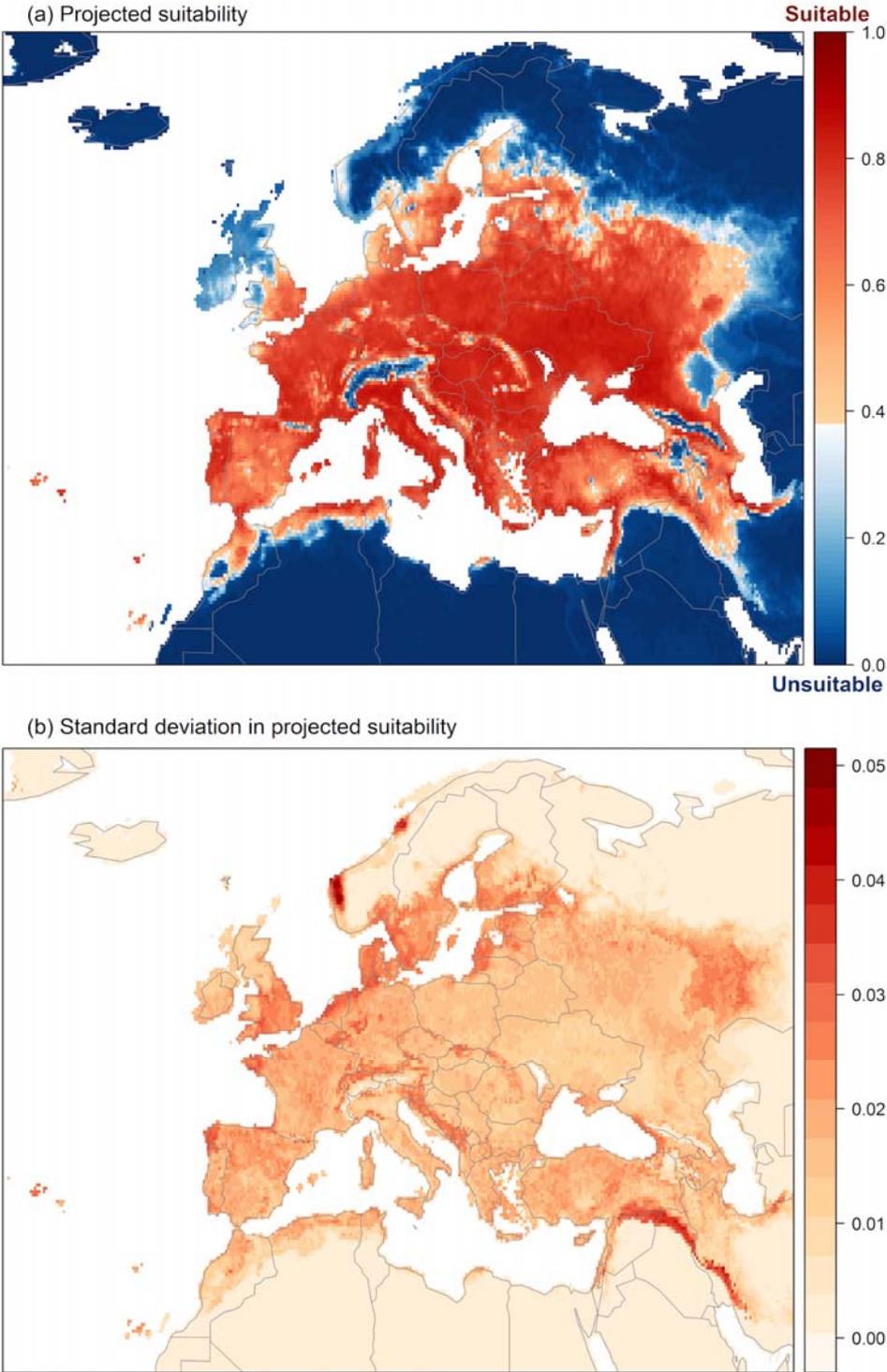


Figure 9. Variation in projected suitability for *Broussonetia papyrifera* establishment among Biogeographical regions of Europe (<https://www.eea.europa.eu/data-and-maps/data/biogeographical-regions-europe-3>). The bar plots show the proportion of grid cells in each region classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. The location of each region is also shown. The Arctic and Macaronesian biogeographical regions are not part of the study area, but are included for completeness.

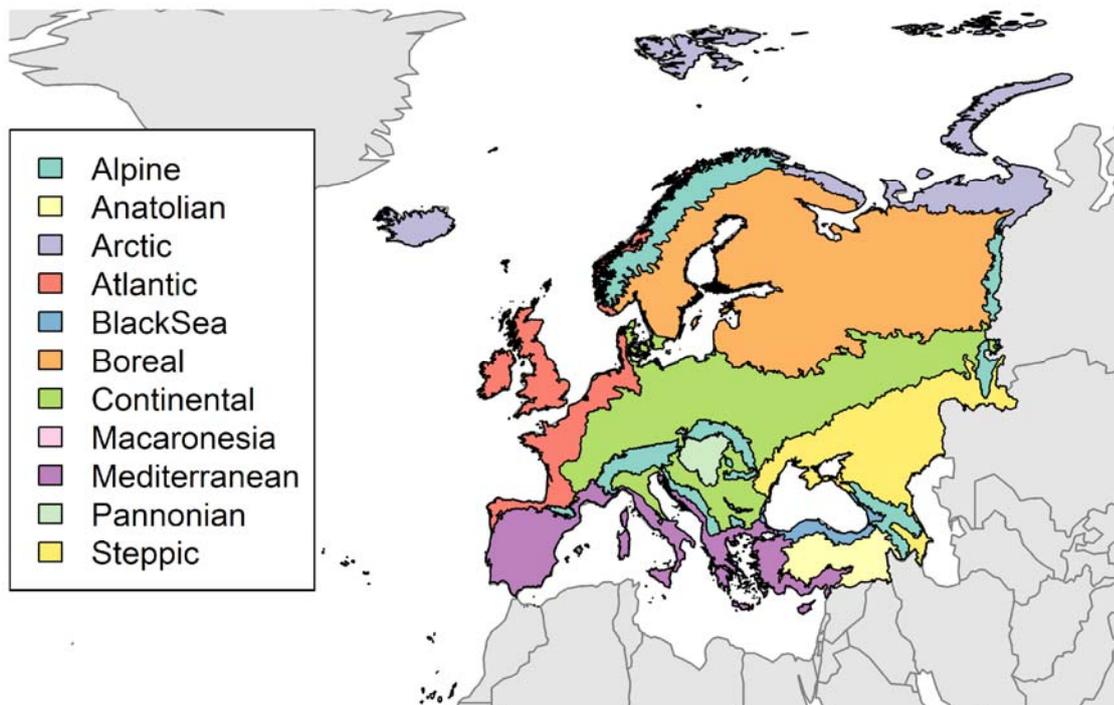
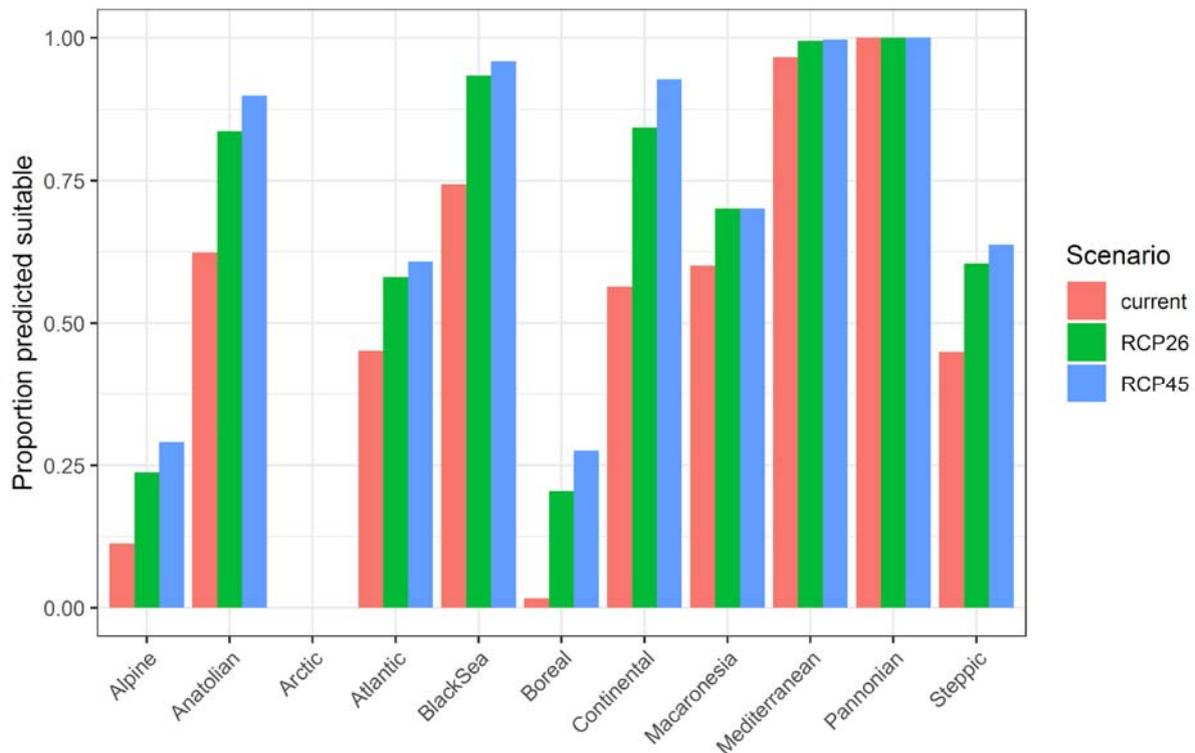


Table 2. Variation in projected suitability for *Broussonetia papyrifera* establishment among Biogeographical regions of Europe (numerical values of Figure 9 above). The numbers are the proportion of grid cells in each region classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. The Arctic and Macaronesian biogeographical regions are not part of the study area, but are included for completeness.

Biogeographical region	Current climate	RCP2 6	RCP4 5
Alpine	0.11	0.24	0.29
Anatolian	0.62	0.84	0.90
Arctic	0.00	0.00	0.00
Atlantic	0.45	0.58	0.61
BlackSea	0.74	0.93	0.96
Boreal	0.02	0.21	0.28
Continental	0.56	0.84	0.93
Macaronesia	0.60	0.70	0.70
Mediterranean	0.97	0.99	1.00
Pannonian	1.00	1.00	1.00
Steppic	0.45	0.60	0.64

Figure 10. Variation in projected suitability for *Broussonetia papyrifera* establishment among European Union countries and the UK. The bar plots show the proportion of grid cells in each country classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. Malta has been excluded because the Human Influence Index dataset lacks coverage for Malta.

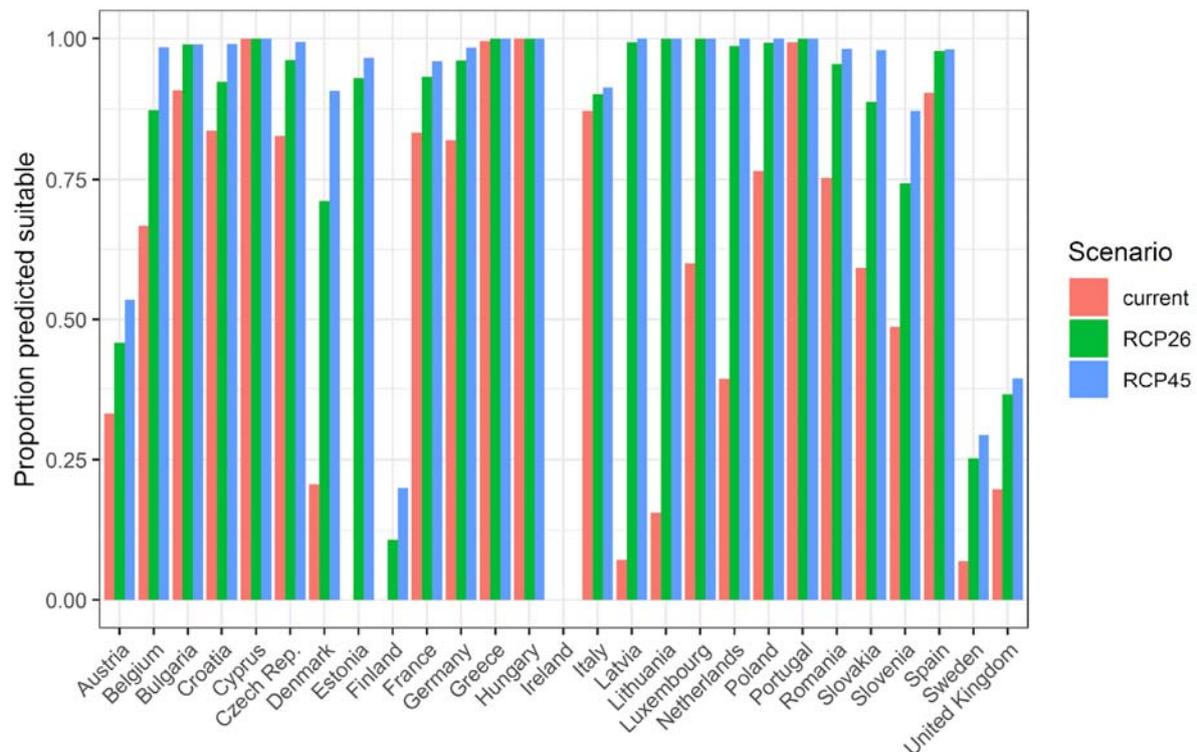


Table 3. Variation in projected suitability for *Broussonetia papyrifera* establishment among European Union countries and the UK (numerical values of Figure 10 above). The numbers are the proportion of grid cells in each country classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. Malta has been excluded because the Human Influence Index dataset lacks coverage for Malta.

Country	Current climate	RCP2 6	RCP4 5
Austria	0.33	0.46	0.54
Belgium	0.67	0.87	0.98
Bulgaria	0.91	0.99	0.99
Croatia	0.84	0.92	0.99
Cyprus	1.00	1.00	1.00
Czech Rep.	0.83	0.96	0.99
Denmark	0.21	0.71	0.91
Estonia	0.00	0.93	0.96
Finland	0.00	0.11	0.20
France	0.83	0.93	0.96
Germany	0.82	0.96	0.98
Greece	1.00	1.00	1.00
Hungary	1.00	1.00	1.00
Ireland	0.00	0.00	0.00
Italy	0.87	0.90	0.91
Latvia	0.07	0.99	1.00
Lithuania	0.16	1.00	1.00
Luxembourg	0.60	1.00	1.00
Netherlands	0.39	0.99	1.00
Poland	0.77	0.99	1.00
Portugal	0.99	1.00	1.00
Romania	0.75	0.95	0.98
Slovakia	0.59	0.89	0.98
Slovenia	0.49	0.74	0.87
Spain	0.90	0.98	0.98
Sweden	0.07	0.25	0.29
United Kingdom	0.20	0.37	0.40

Caveats to the modelling

To remove spatial recording biases, the selection of the background sample from the accessible background was weighted by the density of Tracheophyta records on the Global Biodiversity Information Facility (GBIF). While this is preferable to not accounting for recording bias at all, it may not provide the perfect measure of recording bias.

There was substantial variation among modelling algorithms in the partial response plots (Figure 3). In part this will reflect their different treatment of interactions among variables. Since partial plots are made with other variables held at their median, there may be values of a particular variable at which this does not provide a realistic combination of variables to predict from.

Other variables potentially affecting the distribution of the species, such as types of land cover were not included in the model.

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Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention"

Contract No 07.0202/2019/812602/ETU/ENV.D.2¹

Name of organism: *Callinectes sapidus* Rathbun, 1896

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Risk Assessment Area: The risk assessment area is the territory of the European Union 27 and the United Kingdom, excluding the EU-outermost regions

Peer review 1: Lucrezia Cilenti, Dr., Institute of Marine Science- (IRBIM- CNR), Lesina, Italy.

Peer review 2: Jack Sewell, The Marine Biological Association, Plymouth, UK

Date of completion: 15/10/2020

Date of revision: 15/10/2021

¹ This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA). A number of amendments have been introduced to ensure compliance with Regulation (EU) 1143/2014 on IAS and relevant legislation, including the Delegated Regulation (EU) 2018/968 of 30 April 2018, supplementing Regulation (EU) No 1143/2014 of the European Parliament and of the Council with regard to risk assessments in relation to invasive alien species (see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>).

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SECTION A – Organism Information and Screening

A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?

including the following elements:

the taxonomic family, order and class to which the species belongs;

This risk assessment covers one species, *Callinectes sapidus* Rathbun, 1896 a decapod crustacean belonging to:

- Portunidae (Family)
- Decapoda (Order)
- Malacostraca (Class)
- Arthropoda (Phylum)

Vernacular names: Blå svømmekrabbe (DK), blue crab, Atlantic blue crab (EN), Chesapeake blue crab (EN regional), crabe bleu (FR), Blaukrabbe (DE), Γαλαζοκάβουρας = galázios kávousas (GR), Cangrejo azul (ES), granchio nuotatore (IT), siri-tingasiri-azul (PT)

Mostly used name in commerce: American blue crab

Callinectes sapidus belongs to the family Portunidae which includes 18 genera (WoRMS Editorial board, 2020). The genus *Callinectes* alone includes 15 species, 3 distributed in the eastern Pacific Ocean (*C. arcuatus*, *C. bellicosus*, and *C. toxotes*), 5 in the eastern Atlantic (*C. amnicola*, *C. danae*, *C. gladiator*, *C. pallidus*), 7 in western Atlantic (*C. affinis*, *C. bocourti*, *C. exasperatus*, *C. ornatus*, *C. rathbunae*, *C. sapidus*, *C. similis*) and one – *C. marginatus* – occurring on both sides of the Atlantic (GBIF - , Global Biodiversity Information Facility, <https://www.gbif.org/>, accessed 29/06/2020)

Most common SYNONYMS

- *Lupa hastata* (Say 1817)
- *Portunus diacantha* (Latreille 1825) (synonym)
- *Lupa diacantha* (Milne-Edwards 1834)
- *Callinectes hastatus* (Ordway 1883)
- *Callinectes diacanthus* Latreille, 1825 (name suppressed)
- *Callinectes sapidus acutidens* Rathbun, 1896 (junior synonym)

A2. Provide information on the existence of other species that look very similar [that may be detected in the risk assessment area, either in the environment, in confinement or associated with a pathway of introduction]

Include both native and non-native species that could be confused with the species being assessed, including the following elements:

- other alien species with similar invasive characteristics, to be avoided as substitute species (in this case preparing a risk assessment for more than one species together may be considered);
- other alien species without similar invasive characteristics, potential substitute species;
- native species, potential misidentification and mis-targeting

Response: Worldwide, *C. sapidus* can be confused with other *Callinectes* species (*C. bocourti*, *C. maracaiboensis*, *C. rathbunae*, *C. ornatus*, *C. danae*) occurring in the same native distribution area that have the front of carapace with 4 more or less well-developed teeth (2 frontal teeth in *C. sapidus*). The inner pair of teeth are clearly smaller than the outer pair in *C. danae* and they are rudimentary in *C. ornatus*. It can be also confused with West Atlantic origin *Callinectes exasperatus* (Gerstaecker, 1856), that has been reported once from the Atlantic Spain (Cuesta et al., 2015).

In European waters it can be confused with another alien species namely the blue swimming crab *Portunus segnis*/*Neptunus pelagicus* also of economic importance in the RA area. This American blue crab was in fact often mistaken for *Portunus segnis* (Forskål, 1775) and often had been called *Neptunus pelagicus* or *Portunus pelagicus* (Linnaeus, 1758) until the *P. pelagicus* species complex was revised (Lai et al., 2010). As an example, one of the first records (Adriatic Sea: Giordani Soika, 1951) of *C. sapidus* was initially identified as *Neptunus pelagicus*. Evans & Schembri in Crocetta et al. (2015) have recently revisited the taxonomic identity of a crab recorded in Maltese waters over thirty years ago by Schembri & Lanfranco (1984), identified at the time as *C. sapidus*, and ascribed it to *Portunus segnis*.

Short description of *Callinectes sapidus*

Callinectes sapidus is a decapod crustacean of the family Portunidae. The species is characterised by a carapace wider than long (more than twice including the antero-lateral spines). Front (excluding inner orbital angles) bearing 2 obtuse to acuminate, broadly triangular teeth with sinuous inner margins longer than outer margins; 9 anterolateral, acuminate teeth, the larger one at the lateral corner long and sharp-pointed, and having a dorsal carina produced to the metagastric area; the telson is lanceolate, longer than wider. Chelipeds are strong with granular ridges; fingers have strong irregular cutting teeth; merus has 3 strong inner spines increasing in size distally; the two last segments of the fifth legs are flattened in form of paddles. The colour is greyish, bluish to brownish green dorsally. Diverse coloured tints present dorsally on the carapace ornamentation (spines or tubercles) and legs. Males with fingers on chelae blue, orange in mature females. In males, carapace widths (CW) and lengths (CL) up to 20.9 and 9.1 cm, respectively; in females, CW and CL up to 20.4 and 7.5 cm, respectively. CW range in mature females from 5.5 to 20 cm (FAO fact sheet, 2020).

Callinectes sapidus can be distinguished from *P. segnis*, another invasive species in the Mediterranean, by its front with two prominent triangular teeth, tips of male 1st pleopods not bent. In *Portunus* the basalantennal segment is narrow, antennal flagellum not excluded from orbit; front quadridentate. *Portunus segnis* is easily distinguished from *C. sapidus* by the prominent inner spine on cheliped carpus, triangular abdomen in males (see Figure 1). See also <http://www.bluecrab.info/identification.html>

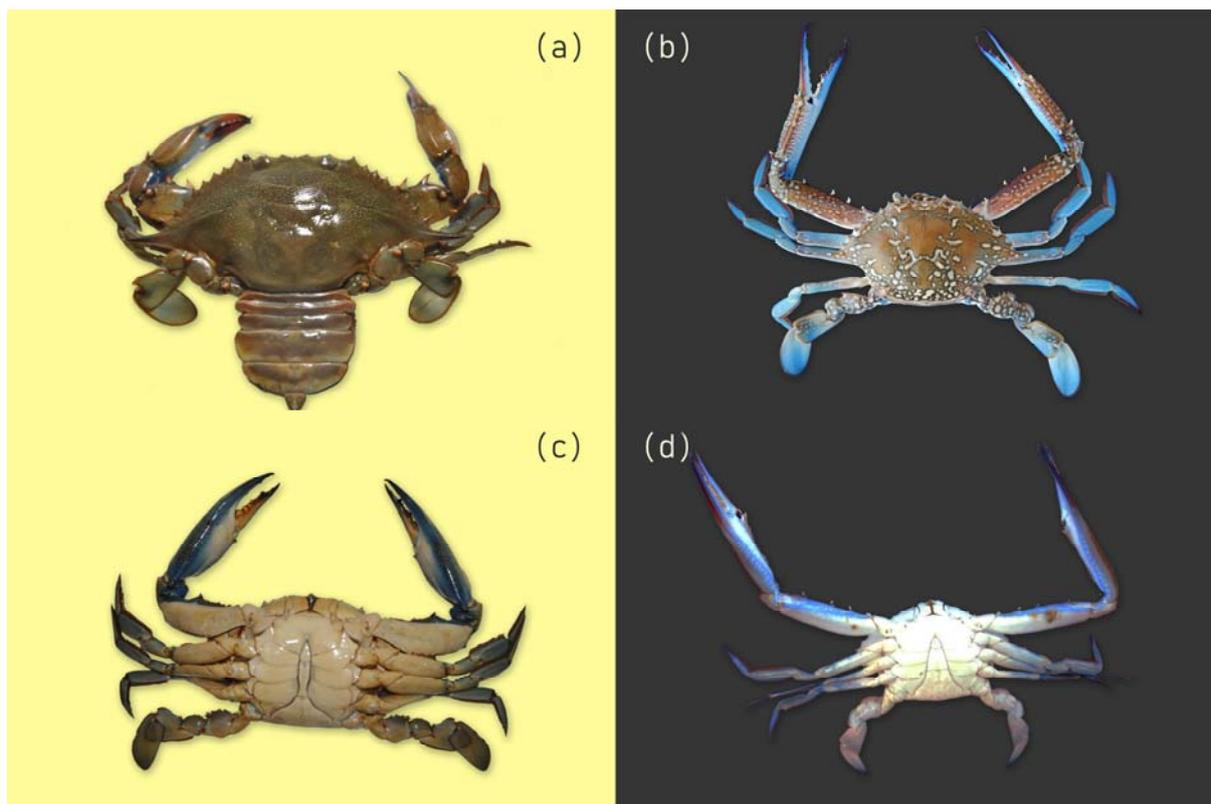


Figure 1. a) *Callinectes sapidus* dorsal side front (female); b) Upper: *Portunus segnis* dorsal side (sex not identified); c) *Callinectes sapidus* ventral side (male); d) *Portunus segnis* ventral side (male). Photo credit for *C. sapidus*: Hocein Bazairi, Morocco; photo credit for *P. segnis* Radhouan El Zrelli.

A3. Does a relevant earlier risk assessment exist? Give details of any previous risk assessment, including the final scores and its validity in relation to the risk assessment area.

Response: Although no formal risk assessment has been conducted for the species in the RA area, Perdikaris et al. (2016) applied the Marine Invertebrate Invasiveness Screening Kit (MIISK) risk screening tool for *C. sapidus*, concluding that this was a high-risk invasive species for western Greece. The MIISK score was based on the responses to 49 questions related to topics such as domestication/cultivation, climate and distribution, invasiveness in non-native range, undesirable traits, feeding guild, reproduction, dispersal mechanisms and persistence attributes (<http://www.cefas.defra.gov.uk/our-science/ecosystems-and-biodiversity/non-native-species/decisionsupport-tools.aspx>). A similar protocol (AS-ISK) was applied for the River Neretva Estuary (Eastern Adriatic Sea: Croatia and Bosnia– Herzegovina) by Glamuzina et al. (2021), who identified *C. sapidus* as one of the highest risk species for the region.

An initial risk assessment focusing on potential future NIS introduced by ships and taking into account the different salinity regimes in Nordic ports has included *C. sapidus* among target species that can potentially survive the conditions of the Nordic coastal waters including the outer parts of the Baltic Sea (Gollasch & Leppäkoski, 1999).

Horizon scanning to create a watch list of alien crab species that could establish along South African rocky shores under present-day and future temperature scenarios, included *C. sapidus* among potential candidates to be introduced by in all South African ecoregions (Swart & Robinson, 2019).

Roy et al. (2019) included *C. sapidus* among Invasive Non-Native Species with high likelihood of arrival, establishment and impacts within Gibraltar.

A4. Where is the organism native?

including the following elements:

- an indication of the continent or part of a continent, climatic zone and habitat where the species is naturally occurring
- if applicable, indicate whether the species could naturally spread into the risk assessment area

Response: The species is native to coastal habitats of the western Atlantic, apparently with a disjunct distribution encompassing temperate, subtropical, and tropical climatic zones, with a northern population from the United States and Canada (Nova Scotia, Maine, and northern Massachusetts) to Venezuela, and a southern population from Bahia, Brazil, to Argentina (Williams, 1984; Kennedy & Cronin, 2007). Estuaries and other transitional systems are the elective habitats of the species; however, during its life cycle it can be found also under fully marine conditions, as pregnant females move from brackish habitats to open sea environments for spawning (Hines, 2007). Accordingly, the species is generally found at shallow depths in the range 0 - 30/50 m, even though it has been sampled at depths comprised between 160 and 170 m (OBIS, 2020).

A5. What is the global non-native distribution of the organism outside the risk assessment area?

Response: Outside the risk assessment area *C. sapidus* has been reported in the Pacific Ocean and in the Mediterranean Sea. Specifically, the species has been reported from Japan since 1975 (Sakai, 1976; Milikin & Williams, 1984). Several specimens, all females, have been trapped in Kaneohe Bay, Hawaii since around 1985 (Eldredge, 1994). This crab has been transported to Hawaii since 1967 for human consumption; the majority of shipments originate from Louisiana. Speculatively, the crabs in Kaneohe Bay were purchased by an individual who released them in a personal attempt to have them available for commercial purposes. Only two records are reported (Castro, 2011, citing Stephenson, 1976 and Eldredge, 1994).

Callinectes sapidus, was collected occasionally in San Francisco Bay and elsewhere along the central California coast, released by private individuals (Cohen & Carlton, 1995; although listed in Jensen (1995), blue crabs are not believed to be established in San Francisco Bay). No recent records do exist of *C. sapidus* in the San Francisco Bay or in California.

The species is present in the Mediterranean Sea in a number of non EU countries. In particular there are records from Montenegro (Zenetos et al., 2010; Azzurro et al., 2019), Albania (Beqiraj & Kashta, 2010; Azzurro et al., 2019), Bosnia and Herzegovina (Dizdarević et al., 2016), Turkey (Holthuis, 1961; Kocatas, 1971), Syria (Hasan, 2018), Lebanon (George & Athanassiou, 1965), Israel (Holthuis & Gottlieb, 1955), Egypt (Banoub, 1963), Tunisia (Katsanevakis et al., 2020), Algeria (Benabdi et al.,

2019), Morocco (El-Ouamari & Bazairi in Chartosia et al., 2018; Taybi & Mabrouki, 2020). Also present and spreading in the Azov-Black Sea basin (Ukraine, Russia, Georgia, and Turkey) (Shaverdashvili & Ninua, 1975, Diripasko et al., 2009, Pashkov et al., 2012, Yağlıoğlu et al., 2014, Ceylan Y. 2020, Stefanov, 2021).

In addition, a record reported in inaturalist (<https://www.inaturalist.org>) and gbif (<https://www.gbif.org/>) from Benin, SE Atlantic is likely a misidentification of *Callinectes amnicola*. Records from the Pacific Mexico and Costa Rica are likely to be misidentifications of *C. bellicosus* or *C. arcuatus*. Records from Australia, New Zealand, Thailand, and the Philippines are deemed as misidentifications of portunids of the *Portunus segnis* complex (Mancinelli, personal observation).

A6. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species been recorded and where is it established? The information needs to be given separately for recorded (including casual or transient occurrences) and established occurrences. “Established” means the process of an alien species successfully producing viable offspring with the likelihood of continued survival².

A6a. Recorded: List regions

Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

- Greater North Sea, incl. the Kattegat and the English Channel, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea.

A6b. Established: List regions

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

- Greater North Sea, incl. the Kattegat and the English Channel, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea.

Response (6a): Reported.

- Baltic Sea: in the catchment basin of the Baltic Sea (Lake Dąbie, Poland) (Czerniejewski et al., 2020);
- North-east Atlantic Ocean: a questionable record from France in 1900 (Bouvier, 1901) and since 1932 in the Netherlands (Den Hartog & Holthuis, 1951). Most recent records from the English channel (Pezy et al., 2019)
- Mediterranean Sea: since 1947 and possibly since 1935 in the Aegean Sea (Serbetis, 1959); since 1949 in the Adriatic. See Mancinelli et al. (2017a) for an exhaustive review of the distribution and ecological impacts of the species in the Mediterranean Sea, as well as of potential control strategies.

² Convention on Biological Diversity, Decision VI/23

- Black Sea: In Bulgaria – a few records from 1967 to 2005 in Varna Bay (Bulgurkov, 1968, Zaitsev 1998, Uzunova 2016), among which one female with eggs (2005, Uzunova 2016). Since 2006, the species has expended its range along the entire Bulgarian Black Sea coast (Stefanov 2021). In Romania – single records with a tendency to become common species (Petrescu et al., 2000, Papadopol & Curișcă, 2016). There are some recent records from the southern areas and northern basin, but only from non EU countries. They refer mostly to isolated captures of adult individuals from Turkey (e.g. Yağlıoğlu et al., 2014; Ak et al., 2015; Aydin, 2017).

Marine subregions:

- Greater North Sea, incl. the Kattegat and the English Channel: since 1932 in the Netherlands (Den Hartog & Holthuis, 1951). First time in the English Channel in 1973 and in 1974 in the Bay of Seine (Benerville-sur-Mer) (Vincent, 1999).
- Bay of Biscay and the Iberian Coast: observed for the first time in 1900 along the Atlantic coast of France at Rochefort (Bouvier, 1901), and then in 1960 at Le Verdon-sur-Mer (Amanieu & Le Dantec, 1961).
- Western Mediterranean Sea: first record from Livorno port in 2005 (Bisconti & Silvi, 2005).
- Adriatic Sea: since 1949 (Giordani Soika, 1951) many records ever since.
- Ionian Sea, Central Mediterranean Sea: since 2004 from Taranto Gulf (Cecere et al., 2016): common.
- Aegean-Levantine Sea: since 1947 (possibly 1935 according to fishers) (Serbetis, 1959): common.
- Black Sea: In Bulgaria – a few records from 1967 to 2005 in Varna Bay (Bulgurkov, 1968, Zaitsev 1998, Uzunova 2016), among which one female with eggs (2005, Uzunova 2016). Since 2006, the species has expended its range along the entire Bulgarian Black Sea coast (Stefanov 2021). In Romania – a few records that refer to isolated individuals (Petrescu et al., 2000, 2010).

Response (6b): Established.

- Greater North Sea: Until today blue crabs were recorded from coastal waters of several North Sea European countries (The Netherlands, Denmark, United Kingdom, Germany) but the species is considered as established in France (Pezy et al., 2019) and Belgium (OBIS, 2020)
- Bay of Biscay and the Iberian Coast: Following the first observations in 1900 along the Atlantic coast of France at Rochefort (Bouvier, 1901), and then in 1960 at Le Verdon-sur-Mer (Amanieu & Le Dantec, 1961), the blue crab was observed for the first time in 1978 in the Tagus estuary (western Portugal) (Gaudêncio & Guerra, 1979). This species is also present in the Guadalquivir estuary (southwest Spain) at least since 2002 (Castejón & Guerao, 2013); in the Gijon coast in 2004 (Cabal et al., 2006); Ría de Vigo (Bañón et al., 2016); the Sado estuary in 2009; however anecdotal reports indicate the presence of this species at least since the mid-1990's (Ribeiro & Veríssimo, 2014). Morais et al. (2019) documented its establishment in Formosa lagoon and the Guadiana estuary, while Vasconcelos et al. (2019) confirmed its establishment in the southern coast of Portugal.
- Western Mediterranean Sea: Recently, different scientific papers and communications reported its arrival in several locations in the western Mediterranean Sea such as France (Labruno et al., 2019) and Sardinia (Italy) (Piras et al., 2019; Culurgioni et al., 2020); the coastal lagoons of Ebro Delta

(Spain) (Castejón & Guerao, 2013), the estuary of the Segura river (Spain) (González-Wangüemert & Pujol, 2016), the lagoons of eastern Corsica (France) (Garrido et al., 2018; Noël, 2017) and the Balearic Islands (Spain) (Garcia et al., 2018).

- Adriatic Sea: After its first record in Venice lagoon (Giordani Soika, 1951), it has been widely reported from various locations in the north Adriatic: Florio et al. (2008), Manfrin et al. (2015; 2016); central Adriatic: Castriota et al. (2012); east Adriatic: Croatia: Dulčić et al. (2008; 2011), Mancinelli et al. (2016); Albania: Beqiraj & Kashta (2010), Milori et al. (2017). Online questionnaires administered to recreational fishers revealed that it is widely distributed in the Adriatic (Cerri et al., 2020).
- Ionian Sea, Central Mediterranean Sea: *Callinectes sapidus* has spread rapidly in the Italian Ionian (Gennaio et al., 2006) and the Greek Ionian coast (Perdikaris et al., 2016).
- Aegean-Levantine Sea: becoming naturalized on northern Greece (Kevrekidis & Antonadou, 2018)
- Black Sea: data of spreading and presence of females with eggs in the Bulgarian coast (Uzunova, 2016, Stefanov, 2021) and Turkish coast (Ceylan 2020, Gül et al., 2021).

A7. In which biogeographic region(s) or marine subregion(s) in the risk assessment area could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A7a. Current climate: List regions

A7b. Future climate: List regions

With regard to EU biogeographic and marine (sub)regions, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (7a): Winter temperatures appear to limit the distribution of the species, reflecting the thermal tolerance of the megalopa stage, as well the sensitivity of juveniles and mature females to water temperatures < 3°C (Rome et al., 2005; Hines et al., 2010). Combining physiological tolerances and distribution modeling results, presented in Qu 2.1, Qu 2.10, the following assessments are made (See also Figure 2 and Annexes VIII and IX for details).

Baltic Sea: unlikely, high confidence (western Baltic – low winter temperatures)
Greater North Sea: likely, medium confidence (localized establishment, southern North Sea)
Celtic Seas: unlikely, medium confidence (localized establishment).
Bay of Biscay and the Iberian coast: very likely, high confidence
Mediterranean Sea: very likely, high confidence
Black Sea: likely, medium confidence. Very likely, high Confidence in the Sea of Marmara.

Response (7b): Combining physiological tolerances and distribution modeling, aspects of climate change most likely to affect future distribution were considered as an increase in minimum and maximum Sea Surface Temperatures (SST), according to scenario RCP4.5 for 2050/2070. The methodology for the developed models is described in Annex IX.

Baltic Sea: unlikely, high confidence (except for the western Baltic - likely)
Greater North Sea: very likely, high confidence
Celtic Seas: moderately likely, medium confidence. However, the Severn Estuary and some coastal areas in Britany, France (included in the Celtic Sea region) look highly suitable and include several shipping ports and other potential points of introduction.
Bay of Biscay and the Iberian coast: very likely, high confidence
Mediterranean Sea: very likely, high confidence
Black Sea: likely, medium confidence. However, establishment is very likely with high confidence in the Sea of Marmara due the the environmental conditions and proximity to the North -East Aegean where *C. sapidus* is already invasive.

A large body of literature from the native range strongly suggests that *C. sapidus* needs salinities above 20 psu to complete its larval development. Nevertheless, in the Black Sea with a surface salinity range of 14-19 psu, the increasing number of records and the recent findings of gravid females indicate that the species has likely developed adaptations that allow establishment of self-sustaining populations. The medium confidence score of this assessment is motivated by the fact that there is still not enough information on larval survival in the Black Sea and a lack of reports from upstream areas where one would expect to find juveniles and young males. See also Risk of Establishment section.

A8. In which EU Member States has the species been recorded and in which EU Member States has it established? List them with an indication of the timeline of observations. The information needs be given separately for recorded and established occurrences.

A8a. Recorded: List Member States

A8b. Established: List Member States

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden

The description of the invasion history of the species shall include information on countries invaded and an indication of the timeline of the first observations, establishment and spread.

Response (8a):

Belgium: Besides the first record from Oostende (Adema, 1991: no collection date) several records are included in OBIS (2020)

Bulgaria: Single records from 1967 to 2005 in Varna Bay (Bulgurkov, 1968, Zaitsev 1998, Uzunova 2016), among which one female with eggs (2005, Uzunova 2016). Since 2006, the species has expended its range along the entire Bulgarian Black Sea coast (Stefanov 2021)

Croatia: several records since 2004 (Dulčić et al., 2008; 2011; Mancinelli et al., 2016; Azzurro et al., 2019; Cerri et al., 2020)

Cyprus: since 1964 (Demetropoulos & Neocleous, 1969)

Denmark: scattered records since 1951 (Wolff, 1954; Tendal & Flintegaard, 2007; Nehring et al., 2008)-casual

France: first observations in 1900 along the Atlantic coast of France at Rochefort (Bouvier, 1901), and then in 1960 at Le Verdon-sur-Mer (Amanieu & Le Dantec, 1961). In the Mediterranean France since 1962 (Galil et al., 2002)

Germany: scattered records since 1964 (Kühl, 1965; see also Nehring et al., 2008; Nehring, 2011). The first record of a fertilized female blue crab, in 2008 (Nehring & Meer, 2010)

Greece: since 1947 (Serbetis, 1959). Widespread in the North Aegean (Kevrekidis & Antoniadou, 2018) and in the Ionian coasts and lagoons (Perdikaris et al., 2016; Mancinelli et al., 2017a)

Italy: since 1949 (Giordani Soika, 1951)

Netherlands: scattered records since 1932 (Den Hartog & Holthuis, 1951)

Poland: first record in 2018 from the western part of Lake Dąbie, at the mouth of the Duńczyca Canal (Czerniejewski et al., 2020)- casual

Portugal: since 1978 in Tagus estuary (Gaudêncio & Guerra, 1979)

Romania: scattered records since 1999 (Petrescu et al., 2000; Papadopol & Curlișcă, 2016)-casual

Spain: first reported in the estuary of the Guadalquivir in 2002 (Cabal et al., 2006); in the Cantabrian Sea in 2004 (Cabal et al., 2006); and in the Ebro delta in 2012 (Castejón & Guerao, 2013)

United Kingdom: a single record in 1975 (Ingle, 1980) - The first British record of *C. sapidus* was trawled off Littlestone-on-Sea, Kent, in September 1975 (Ingle, 1980). On 24 February 2010 a live adult female crab was caught in the Fal estuary, south of Turnaware point (P.F. Clark pers. comm.).

Response (8b):

Bulgaria: Single records from 1967 to 2005 in Varna Bay (Bulgurkov, 1968, Zaitsev 1998, Uzunova 2016), among which one female with eggs (2005, Uzunova 2016). Since 2006, the species has expended its range along the entire Bulgarian Black Sea coast (Stefanov 2021) – considered established (Stefanov 2021)

Greece: since 1947 (Serbetis, 1959). Widespread in the North Aegean (Kevrekidis & Antoniadou, 2018) and in the Ionian coasts and lagoons (Perdikaris et al., 2016; Mancinelli et al., 2017a) - established

Italy: the species is currently distributed in northern, central, and southern Adriatic Sea, and in northern Tyrrhenian Sea (Cilenti et al., 2015; Mancinelli et al., 2017b; Manfrin et al., 2015; 2016; Suaria et al., 2017) while it has recently spread in Sardinian (Italy) (Piras et al., 2019; Culurgioni et al., 2020) and E. Sicily (Ionian Sea: Katsanevakis et al., 2020) - established

Croatia: several records since 2004 (Dulčić et al., 2008; 2011; Mancinelli et al., 2016; Azzurro et al., 2019; Katsanevakis et al., 2020). Online questionnaires administered to recreational fishers revealed that it is widely distributed in Croatian coastal waters (Cerri et al., 2020) - established

France: first observations in 1900 along the Atlantic coast of France at Rochefort (Bouvier, 1901), and then in 1960 at Le Verdon-sur-Mer (Amanieu & Le Dantec, 1961). A well established population in the English Channel was reported by Pezy et al. (2019). Zibrowius reported the occurrence of *C. sapidus* in the Mediterranean Berre lagoon (France) in 1962 (Galil et al., 2002) while Labruno et al. (2019) reported it from eleven lagoons and three sites in the Gulf of Lions (French Mediterranean) after 2016, including one ovigerous female – considered established

Spain: Sporadic records from the Atlantic Spain since 2002 [estuary of the Guadalquivir in 2002 (Cabal et al., 2006); Gijón in 2004 (Cabal et al., 2006); Ría de Vigo (Bañón et al., 2016)]. The first records of the blue crab on the Mediterranean coastline are from Murcia (Abelló, 2010) and the Ebro delta in 2012 (Castejón & Guerao, 2013). Currently, it is considered an established species on the Spanish Mediterranean coastline in the Ebro River, in many coastal lagoons in Valencia and in the Mar Menor lagoon in Murcia (Servicio de Vida Silvestre, 2016; Mancinelli et al., 2017a). Fuentes et al. (2019) reported its establishments in the Fluvià River and estuary and sporadic record in the Muga and Ter rivers. In addition, a rapid expansion in the coastal lagoons of the Balearic Islands was reported by Garcia et al. (2018) and Box et al. (2020).

Portugal: Along the Portuguese coast, previous occurrences of *C. sapidus* were reported in the western coast, namely for the Tagus estuary (Gaudêncio & Guerra, 1979) and the Sado estuary (Ribeiro & Veríssimo, 2014), followed by very recent records in the southern coast, for both the Guadiana estuary and the Ria Formosa lagoon (Morais et al., 2019), while Vasconcellos et al. (2019) confirmed its establishment in the southern coast of Portugal.

Belgium: Since the 1990s it seems that *C. sapidus* has become permanently established in the Belgian Scheldt estuary Reported as common in AquaNIS (Gollasch, 2015).

Germany: establishment success questionable. The case for accepting that *C. sapidus* is now established in German water is presented by Nehring et al. (2008) because a female mature specimen was captured but Gollasch (2015) reports it as rare.

Netherlands: scattered records since 1932 (Den Hartog & Holthuis, 1951)

Since the 1990s it seems that *C. sapidus* has become permanently established in the Dutch Westerschelde estuary and adjacent waters as well as in the Noordzeekanaal between Amsterdam and the North Sea (ICES, 2001; Wolff, 2005; A. Gittenberger pers. commun) but AquaNIS (Gollasch, 2015) reports it as non established.

A9. In which EU Member States could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A9a. Current climate: List Member States

A9b. Future climate: List Member States

With regard to EU Member States, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)?

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (9a): See Annex VIII: Bulgaria, Greece, Cyprus, Malta, Italy, Croatia, Slovenia, France, Spain, Portugal, Belgium, Germany, the Netherlands.

Response (9b): In addition to Mediterranean countries (Greece, Cyprus, Malta, Italy, Croatia, Slovenia, France, Spain) and Portugal where it is already established, based on the temperature limits of the species distribution (see Qu. 2.1), and given the ongoing oceanographic variations in north Atlantic Ocean (Smeed et al., 2018), climate warming is expected to favour the spread of *C. sapidus* towards northern European waters (France, Belgium, Germany, the Netherlands and additionally Denmark, Sweden and the United Kingdom) under both RCP2.6 and RCP4.5. Despite the high habitat suitability predicted by environmental modelling procedures (see Annex VIII), low salinities may represent a limiting factor for the establishment of the species in the Baltic Sea countries (Estonia, Finland, Lithuania, Latvia).

A10. Is the organism known to be invasive (i.e. to threaten or adversely impact upon biodiversity and related ecosystem services) anywhere outside the risk assessment area?

Response: In its native (Atlantic USA) *C. sapidus* is the best-known predator of cultured clams and oysters, being responsible for much of their mortality in the region. It opens shellfish with its claws, by chipping the edge of a valve, or forcing the valves apart. Predation rates can be quite high (140 oysters (size 15 mm shell length) crab-/day) (Eggleston, 1990).

Outside the RA area it is established and exploited in Turkey and Egypt.

In Egypt and in particular in Bardawil lagoon where the species comprises 19% to 42% of the total lagoon production (Abdel Razek et al., 2016; Mehanna et al., 2019), there are some alleged implications that *C. sapidus* is involved in changes in the ecosystem and biological balance. For more details on impacts in Egypt and Turkey see Qu 4.2.

A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea

Response:

Mediterranean: No specific information on the ecological impact assessment is available for the blue crab in Italy. In North Aegean (Thermaikos Gulf and Papapouli Lagoon) *C. sapidus* was found to prey on a wide variety of species including economically important molluscs, fishes, and crustaceans, indicating a substantial potential impact on fisheries and aquaculture in the region. Observation showed that over 6 years (2009–2014), the blue crabs became dominant in Papapouli Lagoon at the expense of the native commercially fished crab *Carcinus aestuarii* Nardo, 1847 according to fisheries data (Kampouris et al., 2019).

North-east Atlantic: Established in Portugal and France but not invasive according to Jensen (2010). However, and despite the lack of documented impacts, the rapid expansion of *C. sapidus* in Atlantic Spain, Portugal and France as indicated in recent publications and by the number of i-naturalist records, suggest a large increase in population and spread since the 2010, which implies invasiveness. In particular, recent sales at the wholesale market, reveal a rapid westward expansion of *C. sapidus* along the southern coast of Portugal. The potential impacts of this species on local ecosystems and fishing/harvesting resources in Portugal are discussed in Vasconcelos et al. (2019).

A12. In which EU Member States has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden

Response:

Greece: Since its first record in Greek water (Serbetis, 1959), *C. sapidus* has expanded its distribution in most Greek Seas, becoming abundant and exploited in coastal lagoons of Northern Aegean Sea (Kevrekidis & Antoniadou, 2018), Ionian Sea (Perdikaris et al., 2016) and N. Kriti (landing reported at <https://www.okaa.gr/>). Its feeding habits and foraging behavior have been studied in the risk assessment area by Kampouris et al. (2019) who presented evidence that in northern Greece the blue

crab preys on a wide range of species, including economically important fish, molluscs and crustaceans. For more details see response to Qu. 4.2.

Italy: The rate of expansion is indicative of invasiveness. *Callinectes sapidus* is characterized by an almost ubiquitous distribution in the Adriatic Sea, with records occurring in the northern, central and southern sectors of the basin (Mancinelli et al., 2017a and literature cited; see also Cerri et al., 2020). In addition, established populations (including juveniles and pregnant females) have been recorded in the southern Adriatic and in the Ionian Sea (e.g., Lesina Lagoon: Florio et al., 2008 and Cilenti, personal communication; Salento Peninsula: Carrozzo et al., 2014; Mancinelli et al., 2013a; 2017a, 2017c) while in other areas of the basin occurrences refer only to adult individuals of both sexes. Fishermen from the Lesina Lagoon consider the species a nuisance, given its high abundance, null economic value, ability to tear nets and other fishing devices, coupled with the fact that once captured it eats or damages other captured fish and invertebrate species of commercial value (Cilenti, personal communication). Furthermore, for populations from the Salento Peninsula CN stable isotope analysis indicated for the species a high trophic plasticity, emphasizing a potentially high impact on multiple trophic levels of invaded food webs related with the local availability of trophic resources, ontogeny, and seasonal drivers (Carrozzo et al., 2014; Mancinelli et al., 2013a, 2017c). *Callinectes sapidus* has been recorded also in peninsular Italy in northern Tyrrhenian Sea (Mancinelli et al., 2017a; Suaria et al., 2017) while occurrences in central (e.g., Sabaudia and Fogliano lagoons) and in southern Tyrrhenian transitional habitats (e.g. Alento River mouth) are testified by unpublished records and citizen science observations (Cilenti, unpublished data; Cerri et al., 2020). The blue crab is currently expanding its range in Sicily (Giacobbe & Scaduto, 2019 and literature cited) and in the Sardinia Island, in particular in coastal lagoons of the western sector of the Island (Piras et al., 2019; Culurgioni et al., 2020).

Spain: After the review by Mancinelli et al. (2017a), summarizing multiple records of the species concentrated along the Valencian coasts, a number of additional occurrences has been recorded, in Atlantic waters as well as in the Mediterranean Sea, including the Balearic Island (Garcia et al., 2018; Fuentes et al., 2019 and literature cited; Vasconcelos et al., 2019; Box et al., 2020). Established populations have been indicated in the Albufera lagoon and in the Ebro River delta (Garcia et al., 2018; Mancinelli, unpublished data). Specifically, in the Ebro Delta negative impacts have been assessed for *C. sapidus* on multiple fish and invertebrate species, including some of economic interest (e.g., *Anguilla anguilla* a CITES listed with vulnerable populations throughout Europe) (Mancinelli, unpublished data). It has been documented that in Ebro Delta bays, it poses an important risk for the cultivation of Mediterranean mussel (*Mytilus galloprovincialis*) and Pacific oyster (*Crassostrea gigas*) (Prado et al., 2020).

A13. Describe any known socio-economic benefits of the organism.

including the following elements:

- Description of known uses for the species, including a list and description of known uses in the risk assessment area and third countries, if relevant.
- Description of social and economic benefits deriving from those uses, including a description of the environmental, social and economic relevance of each of those uses and an indication of associated beneficiaries, quantitatively and/or qualitatively depending on what information is available.

If the information available is not sufficient to provide a description of those benefits for the entire risk assessment area, qualitative data or different case studies from across the risk assessment area or third countries shall be used, if available.

Response: The species is of major interest to fisheries in temperate, tropical and subtropical waters of the Western Atlantic (Kennedy & Cronin, 2007; Mendonça et al., 2010). Specifically, it supports large valuable commercial and recreational hard- and soft-shell fisheries in the temperate areas of the Atlantic and Gulf coasts of the USA (Texas, Florida, Louisiana, New York, New Jersey); in 2013 the capture production was estimated at 74,495 tons, corresponding with a commercial and recreational asset valued at approximately US\$185 million (Mancinelli et al., 2017b).

There is no detailed information on the relative abundance of the species in the risk assessment area. The bulk of the literature reports on findings of one to few individuals. Nevertheless, locally, *C. sapidus* has established populations that support fisheries productions, a fact that is indicative of its population densities in the area. The expansion of the Atlantic blue crab into the Mediterranean basin has led to the development of fisheries focused on the species, such as in northern Greece (Kevrekidis & Antoniadou, 2018). In Spain, in 2016, the Spanish Ministry of Fisheries included *C. sapidus* in the list of commercial fishing species (BOE-A-2016-3357). In the Ebro Delta (NW Spain) total landings for the period 2016-2018 amounted to approximately 30 tones, while in 2017 *C. sapidus* sales represented 81% of the total lagoon fisheries sales (López and Rodon, 2018). The average price in 2017 was 3.94 €/kg and the profit from the species in the region of Catalonia reached 100,000 €. In Portugal, in the Olhão seafood market in April 2019, amounts of 10 kg per day are being sold (Vasconcelos et al., 2019).

In northern Greece blue crab specimens were rarely reported from fisheries and annual landings were very low until 2007. Since then, increased abundance has been reported from the northern Aegean Sea sustaining local scale fishery. In particular 84t were reported in 2010 from Thermaikos and as an average 13 t from Vistonikos (Kevrekidis & Antoniadou, 2018). In 2019 the total production was estimated to 64 t. The average wholesale price ranged from 0.58 to 4.7 euro per kg depending on the area (fisheries statistics), while the retail price reached 10 euro per kg.

SECTION B – Detailed assessment

Important instructions:

- In the case of lack of information the assessors are requested to use a standardized answer: “No information has been found.”
- With regard to the scoring of the likelihood of events or the magnitude of impacts see Annexes I and II.
- With regard to the confidence levels, see Annex III.
- Highlight the selected response score and confidence level in **bold** but keep the other scores in normal text (so that the selected score is evident in the final document).

1 PROBABILITY OF INTRODUCTION AND ENTRY

Important instructions:

- **Introduction** is the movement of the species into the risk assessment area (it may be either in captive conditions and/or in the environment, depending on the relevant pathways).
- **Entry** is the release/escape/arrival in the environment, i.e. occurrence in the wild
- Introduction and entry may coincide for species entering through pathways such as “corridor” or “unaided”, but it also may differ. If different, please consider all relevant pathways, both for the introduction into the risk assessment area and the entry in the environment.
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used (see Annex IV). For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document³ and the provided key to pathways⁴.
- For organisms which are already present (recorded or established) in the risk assessment area, the likelihood of introduction and entry should be scored as “very likely” by default.
- Repeated (independent) introductions and entries at separate locations in the risk assessment area should be considered here (see Qu. 1.7).

Qu. 1.1. List relevant pathways through which the organism could be introduced into the risk assessment area and/or enter into the environment. Where possible give details about the specific origins and end points of the pathways as well as a description of any associated commodities.

For each pathway answer questions 1.2 to 1.7 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 1.2a, 1.3a, etc. and then 1.2b, 1.3b etc. for the next pathway.

In this context a pathway is the route or mechanism of introduction and/or entry of the species.

The description of commodities with which the introduction of the species is generally associated shall include a list and description of commodities with an indication of associated risks (e.g. the volume of trade; the likelihood of a commodity being contaminated or acting as vector).

³ <https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf>

⁴ <https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf>

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 1.2-1.9.

Pathway name: A number of potential pathways/vectors have been identified in the literature, each with a different degree of support. They are here listed in order of importance:

A. TRANSPORT -STOWAWAY – ship/boat ballast water/sediment

Dispersal of larvae by ballast tanks of ships is likely with medium confidence level. Release through ballast water release is suspected as introduction vector because in its native range *C. sapidus* is found in high numbers next to well travelled shipping routes while in the RA area it is predominantly recorded in port regions where ballast water discharges do frequently occur (Galil, 2006; Nehring, 2011). Specifically, most of the early records of the species along the Atlantic coasts of Europe, including the first one in the harbour of Rochefort (France; Bouvier, 1901) and the first record in Dutch waters (Amsterdam harbour; Den Hartog & Holthuis, 1951) are concentrated in locations close to commercial ports (Nehring, 2011). Direct evidence was supplied in Gollasch (1996, cited in Nehring, 2011), reporting on three living mature specimens found in ballast tanks. Moreover, the larval development of *C. sapidus* lasts from about 37–69 days (Costlow & Bookhout, 1959), long enough to make vessel transport plausible.

B. TRANSPORT -STOWAWAY -ship/boat hull fouling

This is considered as one of the two most likely pathways/vectors of introduction in European Seas and is almost certainly responsible for records, within the confines of small marinas where only yachts and small fishing craft are berthed (Bodrum (Turkey), Porto Grande (Malta) (Ulman et al., 2017). Moreover, in Japan Otani (2004) considers that *C. sapidus* to have been introduced via hull fouling rather than via ballast water. Nehring (2011) suggested that the high affinity of adult (with the exclusion of pregnant females) and juvenile blue crabs to brackish water make them unsuitable for crossing oceanic waters on ships' hulls. No information is available on the presence of larval stages (zoeas or megalopae) of the species associated to hulls.

C. TRANSPORT-STOWAWAY (other: Floating debris)

Floating debris has been acknowledged as a raft for a number of brachyurans occurring in coastal habitats (Zaouali et al. 2007; Tutman et al., 2017a). Given the affinity of *C. sapidus* for brackish habitats, it seems unlikely that transport in marine litter is a potential pathway for transfer of *C. sapidus* into the North Sea from Mexico following the Gulf Stream. It is possible that individual crabs can cling to floating objects and drift with currents across the Atlantic, or become trapped in marine litter – e.g. several invertebrate species of NW Atlantic origin have been found washed up on European shores trapped in bait pots.

D. ESCAPEE FROM CONFINEMENT- including releases (live food for such species)

The species could have been introduced on purpose or accidentally from USA native habitats because live crustaceans (e.g. *Homarus americanus*) are imported in many European countries for human consumption. This practice is ongoing, and, at least for Germany, may include *C. sapidus* (ICES, 2006). In the USA blue crabs are regarded as a special delicacy (Williams, 1984). It is possible that still alive specimens were thrown overboard during cruises or in ports. Blue crab does appear to be available to buy live in the UK and the advert suggests possibly from the USA (see <https://readingqualityfish.co.uk/lobster-crabs/>) meaning that there may be the potential for intentional or accidental release once purchased or if unsold.

Another potential pathway (RELEASE IN NATURE- Fishery in the wild) is not discussed because it is no longer considered as an active (potential) pathway. According to Nehring (2011) unconfirmed records in Turkey dating back to 1930's are attributed to intentional introduction. This could explain the documented chronological distribution pattern (Aegean since 1947), Adriatic since 1949 (separate introduction event) but also the population establishment success (establishment first in the Aegean). However, considering the current policies regarding imported species for mariculture in the RA area, it is highly unlikely that a new introduction will occur from outside the RA area. This is a potential pathway for further spreading of the species within European Seas.

A final potential pathway (RELEASE IN NATURE -other intentional release) is also not discussed but may result in releases of large numbers of individuals at a time. We refer to intentional releases with reference to religious or other 'ethical' releases. such as of Buddhist 'fangsheng' "life release" ritual (e.g. <https://www.iflscience.com/environment/buddhists-fined-over-20000-for-releasing-invasive-species-into-sea-in-life-release-ritual/>.)

A. TRANSPORT-STOWAWAY (ship/boat ballast water)

Qu. 1.2a. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional	CONFIDENCE	low
	unintentional		medium
			high

Qu. 1.3a. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely	CONFIDENCE	low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response: The lowest estimates of the volumes of ballast water taken-up, transferred and discharged into world oceans each year are around 10 billion tonnes (Interwies & Khuchua, 2017), whereas just one cubic metre of ballast water may contain from 21 up to 50,000 zooplankton specimens (Locke et al., 1991; Gollasch, 1997) and a heavy bulk carrier can carry up to more than 130,000 tonnes of ballast water (GloBallast, 2009). *Callinectes sapidus* is widespread and supports commercial fisheries along much of the east USA coast, from Rhode Island to Florida and from Louisiana to Texas (Mizerek, 2012; Sutton & Wagner, 2007). It is a very fecund species, with females releasing 1 to 2 million but up to 8 million eggs usually once in their lifetime. While data on ballasts discharges are not available from EU organizations (EMSA, ECSA, ESPO) or other international relevant organisations, it is evident from the above information that the potential for high numbers of *C. sapidus* larvae to travel along this pathway is high. In fact, mature specimens of *C. sapidus* have been found in ballast sediment of a vessel from Baltimore sampled in Bremerhaven in 1993 and also from a vessel from the east coast of north America sampled in Rotterdam (Gollasch et al., 2002 and Gollasch pers. comm). Nevertheless, and despite the frequent presence of Brachyuran larvae in ballast water (e.g. Cohen & Carlton, 1997; Gollasch, 2002), the paucity of studies directly documenting the presence of *C. sapidus* larvae in ballast water indeed lowers the confidence of this assessment.

Qu. 1.4a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Transfer and survival of adults in ballast tanks were documented from vessels arriving to a German and a Dutch port (Gollasch, 1996; Gollasch et al., 2002; Gollasch pers. comm). As far as larvae are concerned, direct evidence of survival is lacking. However, the larval development of *C. sapidus* lasts from about 37–69 days (Costlow & Bookhout, 1959), long enough to make survival during vessel transport plausible. Furthermore, larvae can live under a wide range of chemical-physical environmental conditions (Hill et al., 1989) and certainly under the temperatures that prevail in ballast water during a transatlantic trip from the native region, considering that ballast water temperature follows the ambient sea surface temperature (Gollasch et al., 2000). According to the analysis of Seebens et al. (2013) and based on a combination of traffic volume, distance to the most connected regions and environmental similarity, the North-West Atlantic is the major source region for invasions to Northern European Seas. Given the high propagule pressure and survival rate of larval stages (see Qu. 2.7), *C. sapidus* is characterised by a high dispersion potential, considerable probability of survival in ballast waters, and high potential of establishment.

Qu. 1.5a. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Regulation (EU) No 1143/2014 (EU, 2014) on the prevention and management of the introduction and spread of invasive alien species recognises the International Maritime Organization (IMO) Ballast Water Management Convention (BWMC) as one of the possible management measures for species of Union concern. This entered into force in September 2017 and requires ships in international traffic to apply ballast water management measures, such as ballast water exchange (D-1 standard for an interim period) and fulfil a certain discharge standard (D-2 standard according to the ship specific application schedule). The latter generally requires the installation of a certified ballast water treatment device, which enables sterilization to avoid transfer of ballast water mediated species.

Ballast Water Exchange (BWE) can drastically reduce (by 80-95% - Ruiz & Reid, 2007) the concentration of live organisms in ballast water but has limitations to its application such that it is not always possible to practice (see David et al., 2007). Full compliance with the BWMC D2 standard on the other hand can decrease live zooplankton to undetectable levels and be highly effective against adult forms in ballast sediments as well (for a review see Tsolaki & Diamadopoulos, 2009) Its full implementation is expected by 2024 and this is expected to be sufficient to make survival of *C. sapidus* in ballast tanks unlikely. Until then, and although some of the global fleet has already installed BW treatment devices its likelihood to survive current practices is considered likely.

Qu. 1.6a. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The probability of observing the initial introduction event, particularly at the larval or early life stages is minimal without targeted monitoring surveys at introduction hotspots (Andersen et al., 2014) can increase the likelihood of early detection.

After September 2017, when the BWMC comes into effect and gradually starts being implemented, detection of larval stages in ballast water may be also possible. eDNA methodologies are currently being actively explored by a number of EU States (ICES, 2017) as tools for the detection of NIS in introduction

hotspot water samples and ballast water and can greatly aid the early detection even of larval stages (Darling et al., 2017).

Qu. 1.7a. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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Response: Widespread, if the ecological requirements of the species are considered. Ports in northern and western Europe are often situated in or near estuaries that exhibit major fluctuations in diurnal, monthly, and seasonal environmental condition. Blue crabs utilize all salinity regimes of an estuary in order to complete their life cycle (Guillory et al., 2001). Repeated introductions may also occur in ports and marinas.

Qu. 1.8a. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: If ballast water exchange occurs in open seas rather than in coastal areas, transfer of planktonic larvae to suitable substrate will be hampered. If, however ballast water is released in ports, estuaries or other coastal areas then introduction will be dependent on availability of suitable habitat.

Management measures implemented so far (i.e. BWE) have not proven adequate to prevent the introduction of this and other marine invasive species in EU marine waters. With the recent ratification of the BWMC (September 2017), compliance with the D2 standard is expected to greatly reduce the likelihood of introductions of *C. sapidus* in Europe with ballast water. Until then it is considered likely that the species can be introduced in the RA area via this pathway.

B. TRANSPORT-STOWAWAY (ship/boat hull fouling)

Qu. 1.2b. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: According to CBD (2014) and the guidelines fouling is an unintentional pathway

Qu. 1.3b. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Charybdis japonica* a species belonging to the same family (Portunidae) is included among the Common Hull Fouling Invasive Species by IMO (<http://www.imo.org/en/OurWork/Environment/Biofouling/Pages/Common-Hull-Fouling-Invasive-Species.aspx>). Although living specimens of *C. sapidus* were never found on ships' hulls, the presence of two alien crab species, *Panopeus africanus* A. Milne-Edwards, 1867 and *Pilumnopus africanus* (de Man, 1902), recently recorded in the nearby Ría de Arousa and introduced by vessel hull fouling (Cuesta et al., 2016) gives also credit to this vector of introduction (Bañon et al., 2016). In Japan hull fouling is considered the most plausible mechanism of its introduction rather than ballasts (Otani, 2004). In addition, sea-chests are recognized as an alternative mechanism for transfer of *C. sapidus* at juvenile stage (Otani, 2004). In fact, many decapod species have been found inside ships' sea-chests (Coutts & Dodgshun, 2007; Frey et al., 2014), where they can enter as juveniles or settle as larvae and sustain themselves on the rich fouling communities that develop in these niche areas. No information is

available on the propagulate pressure e.g. estimated volume or number of individuals / propagules, or frequency of passage through this pathway.

Qu. 1.4b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Crabs associated with vessel biofouling are usually restricted to heavily fouled vessels with mature fouling communities, and these are generally rare (MAF, 2011). For the reproductive biology of the species please see Qu 2.7. Considering the biological characteristics of the organism (i.e. it withstands wide temperature range and salinity range), it is considered possible that juvenile *C. sapidus* can maintain a viable population within the fouling communities on ships hulls, sufficient for a new introduction. However, no specific information on the propagule pressure of *C. sapidus* on ships hulls was found.

Qu. 1.5b. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Mobile organisms such as the blue crab can be transported for weeks or months amongst the fouling assemblage in, for example, empty shells of barnacles, or by sheltering in densely fouled areas of the hull (Gollasch, 2002). *Callinectes sapidus* was recorded in the Mediterranean within the confines of a small marina where only yachts and small fishing craft are berthed (Ulman et al., 2017).

Implementing practices to control and manage biofouling can greatly assist in reducing the risk of the transfer of invasive aquatic species. While the International Convention on the Control of Harmful Anti-Fouling Systems on Ships, 2001 (AFS Convention) addresses anti-fouling systems on ships, its focus is on the prevention of adverse impacts from the use of anti-fouling systems and the biocides they may contain, rather than preventing the transfer of invasive aquatic species.

Given the mobile nature of the species and swimming ability, there is a good chance that individuals would ‘drop off’ the vessel as it is removed from the water, rendering dry-docking no less likely to result in release to the wild than in water cleaning. The guidelines are voluntary and apply to all ships.

Commercial ship-owners have a strong interest in having their vessels cleaned in order to decrease their fuel consumption but dry-docking frequency is determined by performance (fuel consumption below a certain threshold) and can range from 0.5-5 years (Bohn et al., 2016). With respect to small leisure craft, Gittenberger et al. (2017) found that, although the majority (64%) of boat owners in the Netherlands haul their boats out of the water and clean them at least once a year, practices vary widely from harbour to harbour, with dry-docking/cleaning prevalence varying between 6% and 95%. This level of implementation allows for plenty of opportunities for organisms such as *C. sapidus* to be transferred via hull fouling.

Qu. 1.6b. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species is unlikely to be detected upon introduction, unless thorough inspections of hull fouling communities are carried out, which is currently not a routine practice. Even then, because, as part of the fouling community, it is likely to be hidden within other fouling macro-organisms, the likelihood of detection via visual inspections is medium. In order to reach GES targets with reference to Descriptor D2, most EU states are already designing or implementing national/regional NIS-targeted monitoring programmes (e.g., see ICES (2017) for national reports in France, Germany, Sweden, Denmark, Norway). Monitoring should focus on introduction hotspots (e.g. ports, marinas) and this will increase the likelihood of detection of *C. sapidus* entering the RA area through hull fouling (see ICES viewpoint by Galil et al., 2019).

Qu. 1.7b. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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Response: Widespread, if the ecological requirements of the species are considered. Ports in northern and western Europe are often situated in or near estuaries that exhibit major fluctuations in diurnal, monthly, and seasonal environmental condition. Blue crabs utilize all salinity regimes of an estuary in order to complete their life cycle (Guillory et al., 2001). In the Mediterranean repeated introductions may also occur in ports and marinas via shipping (ballasts and fouling).

Qu. 1.8b. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Brachyuran crabs have been associated with fouling communities on ships hulls and particularly with niche areas, even though usually only a small number of specimens are found. Propagule pressure (per year) is not expected to be high but need not necessarily be high for successful establishment. For example, if gravid adult females travel this way, one individual could represent very high propagule numbers (clutch size can vary between $1-8 \times 10^6$ eggs, see Qu. 2.7 for more details). Management measures such as frequent hull cleaning have the potential to greatly reduce the risk of introduction. However, effectiveness will depend on hull cleaning frequency and the methodologies employed; and measures are not mandatory. In relation to *C. sapidus*, even though transfer on ships hulls has not been documented for the species, it was considered as a highly likely pathway for its introduction to Japan (Otani, 2004), based on shipping patterns and types of commodities transported, supported by evidence of numerous decapod species found within sea-chests. Thus, this pathway is considered moderately likely for the introduction of the species in the RA area.

C. TRANSPORT-STOWAWAY (other: Floating debris)

Qu. 1.2c. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Qu. 1.3c. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication

- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Marine litter and floating debris have been considered a potential transport vector of non-indigenous species. It is possible that individual crabs can cling to floating objects and drift with currents across the Atlantic Ocean. Larvae, megalopae and juveniles of Portunidae, including Callinectes spp., are very abundant on floating algae in the northeast Atlantic region, as well as the east Pacific (Thiel & Gutow, 2005 and references therein). In a survey of the Atlantic, Barnes & Milner (2005) reported that mega-anthropogenic debris are particularly numerous in the North Atlantic around Europe. Holmes et al. (2015) discuss findings in trans Atlantic drift material, particularly ‘scotty pots’ used in the fishing industry in the USA and which trap otherwise mobile organisms. Most of the species described cohabit with *C. sapidus*. From these results it can be concluded that marine litter can significantly alter the biotic composition of coastal ecosystem, representing a shelter for invasive alien species and diverse natives. With reference to the Mediterranean in particular, while specific information could not be found, primary introductions of alien species in the Mediterranean Sea by rafting on floating litter through the Gibraltar strait are considered plausible, although within Brachyura the most commonly reported species are pelagic (Katsanevakis & Crocetta, 2014). Information on the propagule pressure of *C. sapidus* through this pathway was not found.

Qu. 1.4c. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Species that are considered likely to be transported with hull fouling over long trans-oceanic voyages are considered possible to survive on floating debris as well. Probability of survival will depend on the organism, the overall fouling community (mostly in terms of food availability) and the travelling time at sea. Transatlantic journeys of this type may be very short (e.g. in the range of a few weeks, see Holmes et al., 2015) or in some cases extremely long (months to years), depending on currents and wind (Thiel & Haye, 2006). Even though juveniles and adults of *C. sapidus* have been shown to survive periods of starvation of at least 30 and 60 days respectively (Wang & Stickle, 1986; Belgrad & Grifen,

2016), there is very high uncertainty regarding the likelihood of survival of transported propagules via this pathway which is sporadic and unpredictable.

Qu. 1.5c. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: This would primarily depend on marine debris management in the source location also (USA) as once in the water, the process is largely influenced by natural processes. The issues resulting from plastic waste in the marine environment have highlighted a general failure to control this pollutant on both land and at sea. (Raubenheimer & McIlgorm, 2018). There are no specific management practices for marine debris in EU regional seas that can affect the survival of rafting species during transport on floating debris. Relevant legislative tools regulating management practices of plastic litter in the EU are the Port Reception Facility (PRF) Directive and the Marine Strategy Framework Directive (MSFD) none taking into consideration the association of non indigenous species to it (Chen, 2005). Therefore, in the unlikely event that *C. sapidus* naturally survives the journey from the West Atlantic to the coasts of Europe on floating objects (see previous question), it is likely to remain alive and reach the shore

Qu. 1.6c. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Considering that floating debris and marine litter are not regularly examined for fouling biota it may go undetected. However, since it is an easily recognized animal when mature it is relatively easy to detect it, if present. Post settlement and younger individuals may be less detectable. Also depends on the size and complexity (available refuge) of the particular item of debris – some larger, structurally complex items covered in goosebarnacles for example can easily hide crabs such as *Planes* spp. It is noteworthy that given the amount of coastline in Europe and random nature of strandings of litter, an effective monitoring programme covering the whole RA would be almost impossible.

Qu. 1.7c. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	Isolated widespread ubiquitous	CONFIDENCE	low medium high
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Response: All natural sink areas can receive floating debris (Rech et al., 2016) such that rafting *C. sapidus* could get stranded all along the risk assessment area.

Qu. 1.8c. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species could arrive from the Gulf of Mexico on floating debris to Northern Europe e.g. Scotland assisted by the Gulf Stream. Holmes et al. (2015) discuss how and why living Western Atlantic US coastal molluscan species have been found washed up in SW England with marine debris. Moreover, paucity of information on the volume, frequency of occurrence and circulation patterns of floating debris in EU regional seas decreases the confidence level of the assessment.

D. ESCAPEE FROM CONFINEMENT- including releases of live food for such species”

Qu. 1.2d. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	Introduction: unintentional		high
	Entry: intentional/unintentional		high

The species could have been introduced on purpose from USA native habitats because live crustaceans (e.g. *Homarus americanus*) are imported in many European countries for human consumption. Blue crab appears to be available to buy live in the UK and the advert suggests possibly from the USA meaning that there may be the potential for intentional or accidental release once purchased or if unsold.

Furthermore, according to Nehring (2011), records of intact but boiled specimens on the Dutch North Sea coast (Wolff 2005), seem to indicate that *C. sapidus* is consumed aboard vessels, and it is possible that leftovers (boiled or live specimens) were thrown overboard.

Qu. 1.3d. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species could have been introduced on purpose or accidentally released from holding tanks because live blue crabs have been imported in many European countries for human consumption. It is believed that this practice is ongoing (ICES, 2006) although with the current presence of the species in the RA area it appears more likely that any intentional intrductions would originate in established European populations. In the USA blue crabs are regarded as a special delicacy (Williams, 1984). It is possible that still alive specimens were thrown overboard during cruises or in ports.

Qu. 1.4d. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Likely to survive. No reproduction is possible for blue crabs traded alive due to the low temperatures at which they are transported.

Likely to survive transport during live food trade operations (e.g. Ribeiro & Verissimo (2014) found live blue crabs, allegedly obtained from Greece, being sold at an Asian goods supermarket in Lisbon) but moderately likely to survive entry into the environment, depending on the point of entry and the local conditions. For example, recently, one of the authors (GM) witnessed an incident of accidental drop-off from a pallet of several 10-Kg packs containing live *C. sapidus* into the sea at the port of Brindisi (SE Italy), while being unloaded from a merchant ship. While such incidents could result in the survival of escaped individuals, they are expected to be rare and infrequent. Once live specimens are delivered to markets, escape into the urban environment is unlikely to lead to survival.

Qu. 1.5d. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: There are currently Regulations (e.g. Regulation (EU) 2017/625 (EU, 2017), Commission Delegated Regulation (EU) 2019/625 (EU, 2019) and guidelines concerning live food trade (see https://ec.europa.eu/food/safety/international_affairs/trade/import_animal_en), but are oriented towards the protection of human health and not transport or translocation of species.

If *C. sapidus* is frozen before being packed for transport, the procedure determines the death of the specimens, thus survival rates equal 0. If it is live food trade then it is moderately likely. Same for Qu 1.4d as there are no dedicated management measures for accidental or deliberate releases.

Qu. 1.6d. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Only large sized animals are transferred which are easily detected if looked for. *Callinectes sapidus* is easy recognizable, and trading companies are aware of its specific characteristics (see Qu.

1.2d). Thus, the possibility that it can be misidentified and imported into the risk assessment area is very unlikely

Qu. 1.7d. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	Isolated widespread ubiquitous	CONFIDENCE	low medium high
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Response: As mentioned in Qu. 1.4d, trade of live *C. sapidus* imposes a relatively low transport time. To date, the species is imported by ship, thus points of introduction and entry into the environment are primarily represented by harbors and ports. No information is available on trading of the species in the risk assessment area by flight.

Qu. 1.8d. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species could have been introduced on purpose or accidentally released from holding tanks because live blue crabs are imported in many European countries for human consumption. While the practice might still be ongoing, it seems more likely that introductions for live food trade will originate in the RA area. Nevertheless, this is a plausible pathway for additional introductions which cannot be discarded, even if the survival of the escaped/released individuals is highly uncertain.

Qu. 1.9. Estimate the overall likelihood of introduction into the risk assessment area or entry into the environment based on all pathways and specify if different in relevant biogeographical regions in current conditions.

Provide a thorough assessment of the risk of introduction in relevant biogeographical regions in current conditions: providing insight in to the risk of introduction into the risk assessment area.

RESPONSE	very unlikely	CONFIDENCE	low
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	unlikely moderately likely likely very likely		medium high
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Response: *C. sapidus* is already present in parts of the risk assessment area therefore the likelihood of introduction/entry is “very likely” by default (see guidelines). The spatial and temporal history of invasion indicates multiple introduction events at different locations (Mediterranean Sea, North Sea, Black Sea, Iberian Coast and the Bay of Biscay). A variety of pathways have been proposed, with existing evidence indicating that ship-mediated vectors (ballast and fouling) are the most likely means of introduction. Accidental escape and intentional release from the live food trade has also been suggested as a possible pathway and is considered still active, albeit less likely to contribute high propagule pressure. In a few cases such as of Buddhist ‘fangsheng’ animal releases may involve releases of large numbers of individuals at a time.

Introductions via rafting on floating debris, while possible, are not considered a likely event. Further introductions are very likely, given the high shipping traffic between the native area (east US coast) and EU ports and the ability of the species to survive during transport. Full implementation of the BWMC by 2024 is expected to greatly reduce the likelihood of introduction via ballast water.

Mediterranean, Iberian & Bay of Biscay, North Sea: it is already introduced and established very likely – high confidence.

Black Sea: already introduced and established. New introduction events likely with high confidence. Unaided introduction and establishment in the Sea of Marmara very likely.

Baltic Sea: unlikely – medium confidence.

Celtic Seas: likely - low confidence.

Greater North Sea: very likely – high confidence

Qu. 1.10. Estimate the overall likelihood of introduction into the risk assessment area or entry into the environment based on all pathways in foreseeable climate change conditions?

Thorough assessment of the risk of introduction in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of introduction (e.g. change in trade or user preferences)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely introduction within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Future climate change conditions are not anticipated to significantly change the likelihood of introduction of *C. sapidus* through the above-mentioned pathways. Potential donor areas are not predicted to greatly expand/contract (see modelling results – global projected distribution – RCP2.6 and RCP4.5).

2 PROBABILITY OF ESTABLISHMENT

Important instructions:

- For organisms which are already established in parts of the risk assessment area or have previously been eradicated, the likelihood of establishment should be scored as “very likely” by default.
- Discuss the risk also for those parts of the risk assessment area, where the species is not yet established.

Qu. 2.1. How likely is it that the organism will be able to establish in the risk assessment area based on similarity of climatic and abiotic conditions in its distribution elsewhere in the world?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The native range of *C. sapidus* in western Atlantic spans two continents, extending almost continuously from New England to Argentina (Millikin & Williams, 1984; Nehring, 2011), i.e., from 38.6S to 57.7N. Accordingly, the species experiences latitudinal variations in water temperature comprised between 0°C in winter at the extremes of its range and 32-33°C in transitional systems of the Caribbean area (Rodríguez, 2001; Johnson, 2015). In addition, populations in temperate areas generally experience comparable seasonal variations: in Chesapeake Bay, where one of the most abundant population in the USA is established, water temperatures range between 3 and 32°C (e.g., Shiah & Ducklow, 1994).

In its introduced range in the EU, *C. sapidus* is already established in the Mediterranean in Eastern Atlantic from Portugal to Belgium, and in the Black Sea (Bulgaria) under oceanographic and climatic conditions comprised within the abovementioned ranges (see Annex VIII). Similar climatic conditions characterize other quadrants of the RA area currently not colonized by the blue crab in the Black Sea region, and (to a lesser extent) the Celtic Seas region.

Winter temperatures appear to limit the native distribution of the species at high latitudes in both hemispheres, reflecting the thermal tolerance of the megalopa stage, as well the sensitivity of juveniles and mature females to water temperatures < 3°C (Rome et al., 2005; Hines et al., 2010). Thus, a northward expansion, as that observed in native ranges, should be expected also in European waters. Nevertheless, as with many other decapod species, the pelagic larval stages of *C. sapidus* have stricter abiotic requirements. Thus, in laboratory experiments *C. sapidus* larvae completed their development to the first zoeal stage at a minimum salinity of 20.1 psu (Costlow & Bookhout, 1959). This threshold is supported by field studies, where *C. sapidus* zoeae have been collected at salinities down to 20 psu in Chesapeake Bay (Sandifer, 1973) and the Mississippi Sound (Perry, 1975). Surface salinities in the Baltic Sea (beyond the western Baltic) and the Black Sea are lower than this threshold, most likely posing a limit for establishment in these marine regions. Temperatures suitable for spawning and larval development are reported as between 19-29°C (Hill et al., 1989). Additionally, hatching of fertilised eggs from a locally captured specimen was reported by Nehring & Meer (2010) in a German aquarium at 19°C, which is a normal ambient summer temperature for the German Wadden Sea. Such temperatures are unlikely to be encountered in most of the North Sea (east UK coast) and the Celtic

Seas, where establishment under current conditions seems unlikely and even under future climate change will probably remain localised.

However, temperature and salinity alone may not be the only limiting factors for the establishment of the species. A number of unsuccessful attempts of intentional introductions in Hawaii and California suggest that other factors such as the availability of brackish conditions during crucial stages of the biological cycle may play an important role for the establishment of *C. sapidus* populations. For further details and an extensive discussion on abiotic requirements and likelihood of establishment in the different EU marine regions see Annexes VIII and IX.

Qu. 2.2. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area? Consider if the organism specifically requires another species to complete its life cycle.

RESPONSE	very isolated isolated moderately widespread widespread ubiquitous	CONFIDENCE	low medium high
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Response: *Callinectes sapidus* has a complex life cycle, encompassing a larval phase of 8 zoeal stages and a post-larval megalopa, followed by 16-20 juvenile instars, before molting to maturity, followed by mating and egg production (Hines, 2007). Accordingly, habitat use shifts markedly from brackish to open sea habitats as life stages disperse and migrate across the salinity gradient of the estuary or other transitional environments to offshore. Specifically, mating takes place in oligohaline and mesohaline regions of estuaries (Epifanio, 2003) where females produce a series of egg batches. They incubate them for 14-17 days, during which time they migrate to areas of higher salinity at the mouths of estuaries, where they release them (Tankersley et al., 1998). Larvae hatch and are advected onto the continental shelf. Post-larvae use tidal currents to re-enter brackish environments and then settle preferentially in seagrass beds (Forward et al., 2004), where they metamorphose and grow for approximately seven instars to 20 mm juveniles. Juveniles then disperse into lower salinity, shallow nursery habitats, where they grow to maturity and mate. Thus, transitional habitats such as estuaries and lagoons represent the primary habitats of *C. sapidus*; in addition, seagrass beds play a crucial role as nursery areas for the juvenile stages of the species. Transitional systems are widespread throughout the risk assessment area (see, for example, distribution maps of transitional systems and river mouths available at <https://www.eea.europa.eu/data-and-maps/data/wise-wfd-spatial-3>) and in the vast majority are associated with seagrass beds (see <https://data.unep-wcmc.org/datasets/7>).

Qu. 2.3. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: For the risk assessment area no information is available on competitive interactions with representatives of native benthic assemblages, even though possible negative effects due to competition or intra-guild predation have been suggested with other brachyurans (Gennaio et al., 2006; Mancinelli et al., 2013a; Kampouris et al., 2019). In invaded habitats, *C. sapidus* is likely to interact with a diverse group of brackish brachyurans including *Pachygrapsus marmoratus*, *Carcinus aestuarii*, and *C. maenas*. In native US habitats, where *C. maenas* is invasive, the blue crab is acknowledged to represent one of the factors controlling the southward expansion of the green crab (de Rivera et al., 2005). Thus, the blue crab may result, in particular once reached the adult stage, a competitor and even an intra-guild predator showing superior characteristics in terms of size and aggressiveness as compared with most if not all native brachyuran taxa, at least those residing in brackish habitats of the RA area. However, an important regulative role may be played by temperature: in outdoor mesocosm experiments, Rogers et al. (2018) indicated that at low temperatures, green crabs had a competitive advantage and intra-guild predation by blue crabs on green crabs was low. At high temperatures, size-matched blue and green crabs were competitively similar, while large blue crabs had a competitive advantage, and intra-guild predation on green crabs was high. Thus, depending on the specific oceanographic conditions within the RA area (e.g., Mediterranean Sea vs. Eastern Atlantic) the competitive interactions with *C. maenas* and the congeneric *C. aestuarii* in the Mediterranean basin may result in different impacts on the establishment of the blue crab.

Qu. 2.4. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?

RESPONSE	N/A very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Several marine invertebrate and vertebrate predators of *C. sapidus* have been recorded in its native habitats, including conspecifics; in addition, juvenile blue crabs are included in the diet of predatory birds such as cranes and herons (Hines & Ruiz, 1995; Guillory & Elliot, 2001; Hines, 2007). However, there is no evidence that any of them exerts significant top-down controls on the abundance of blue crab populations (Seitz et al., 2003). Some of them occur also in the risk assessment area [e.g., turtles (*Lepidochelys kempii*), sharks (*Carcharhinus plumbeus*), bonyfish (e.g. *Lobotes surinamensis*, *Pomatomus saltatrix*, *Dicentrarchus labrax*), mammals (*Tursiops truncatus*)]. Their impacts on *C. sapidus* in invaded environments is still unexplored, and no information is available on whether they may exert a significant control on the species.

Parasites such as the dinoflagellate *Hematodinium perezii* have been demonstrated to represent a significant cause of mortality in blue crab populations under laboratory conditions (Shields & Overstreet, 2003; Huchin-Mian et al., 2018). With only two exceptions (Pagliara & Mancinelli, 2018; Czerniejewski et al., 2020) the pathogens associated with *C. sapidus* in the RA area have to date received no attention. Pagliara & Mancinelli, (2018) found that none of the [parasitized] crabs appeared lethargic, or showed clear signs of an on-going disease in terms of behavioral or exoskeleton anomalies. In

European invaded habitats, *H. perezii* has been detected in crustaceans from the North Sea (Small et al., 2006); thus, the possibility exists that *C. sapidus* may be infected also in invaded habitats in eastern Atlantic; however, it is unlikely that this may represent a factor limiting or reducing its establishment.

Another parasite that may represent a pathogenic risk for *C. sapidus* is the ciliate *Orchitophrya stellarum*, which was found to infect and cause high mortality to *C. sapidus* kept under laboratory conditions in Chesapeake Bay (Miller et al., 2013). *Orchitophrya stellarum* is native to the RA area but it requires a port of entry (i.e. a wound) to the crabs' body and has not been observed parasitizing wild populations, either in the species' native range (Messick & Shields, 2000; Miller et al., 2013), or in the invaded range, according to existing information. It is thus considered unlikely to prevent further establishment of *C. sapidus* in the RA area.

Qu. 2.5. How likely is the organism to establish despite existing management practices in the risk assessment area? Explain if existing management practices could facilitate establishment.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The organism is already established in Europe, thus existing management practices related to introduction pathways/vectors could not prevent the entry into the environment of sufficient propagules for establishment. Ballast Water Exchange and Treatment have the potential to reduce propagule pressure and, consequently, the rate of establishment due to primary introductions, these management practices however are not in full effect yet. Other management practices such as the removal of fouling species can reduce establishment potential. On the other hand, management of the natural environment (e.g. MPAs with no-take zones), is likely to limit early detection and removal by stakeholders, affording some protection to early populations.

Qu. 2.6. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: While eradication of marine species might be theoretically feasible if the species enters a new area and is rapidly identified when only a small number of individuals are present, in practice this does not apply to *C. sapidus*. The crabs preferentially prey during the night, when they show a high mobility. However, they can feed also during the day; in addition, during the day they do not hide, thus they can be detected and captured e.g., by hand nets. Despite the large size and easily recognizable morphology of the species, *C. sapidus* responds to low winter temperatures by burrowing in sediments, a life habit which severely hampers visual detection. If a blue crab population is already established,

complete removal would necessitate exhaustive field surveys, involvement of researchers, stakeholders (e.g., fishermen) as well as citizens, and the adoption of multiple collection strategies. However, given the high fecundity of females (see Qu. 2.7), it is very likely that even a considerable reduction in the number of pregnant females may not result in appreciable effects on the abundance of populations. Several examples from native as well as invaded habitats testify that significant negative variations in blue crab abundances but not eradication have been achieved only after prolonged overfishing (Mancinelli et al., 2017b).

Qu. 2.7. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?

including the following elements:

- a list and description of the reproduction mechanisms of the species in relation to the environmental conditions in the risk assessment area
- an indication of the propagule pressure of the species (e.g. number of gametes, seeds, eggs or propagules, number of reproductive cycles per year) of each of those reproduction mechanisms in relation to the environmental conditions in the risk assessment area.
- If relevant, comment on the likelihood of establishment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in establishment whereas for others high propagule pressure (many thousands of individuals) may not.
- If relevant, comment on the adaptability of the organism to facilitate its establishment and if low genetic diversity in the founder population would have an influence on establishment.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Callinectes sapidus* life cycle encompasses a planktonic larval phase of 8 zoeal stages and a post-larval megalopa, followed by 16-20 juvenile benthic instars, before molting to maturity, followed by mating and egg production (Jivoff et al., 2007; Hines, 2007). In native habitats, male and female crabs reach maturity in 1 to 2 years after 18-19 and 19-20 postlarval molts, respectively (Millikin & Williams, 1984). Depending on local temperature and salinity conditions, size at maturity varies considerably, with carapace widths (CW) ranging between 120 and 180 mm in males and 112 and 130 mm in females (Olm III & Bishop, 1983; Olsen et al., 2016). In invaded habitats, females are reported to reach maturity between 118 mm (Beymelek Lagoon, Turkey: Sumer et al., 2013) and 120 mm CW (Iskenderun Bay, Turkey: Türeli et al., 2018); ovigerous females captured in the Salento Peninsula (Puglia region, SE Italy) measured from 154 to 166 mm CW (Mancinelli et al., 2013a), while those captured in the English Channel had a CW between 172 and 178 mm (Pezy et al., 2019).

In native temperate habitats, the reproductive period of *C. sapidus* is seasonal (May-September) while in tropical regions reproduction occurs year-round although there can be seasonal peaks in activity (Millikin & Williams, 1984). A late spring-summer reproductive period is confirmed in invaded Italian, Greek, and Turkish habitats (Mancinelli et al., 2013a; Sumer et al., 2013; Kevrekidis & Antoniadou, 2018). Males can reproduce several times during the year, while females reproduce only once, while in the soft shell stage; in addition females are capable of storing sperm (Millikin & Williams, 1984). The reproductive output depends on the size/age of the females; clutch size generally varies between 1.7 and 2×10^6 eggs per female, with a maximum of 8×10^6 (Millikin & Williams, 1984; Jivoff et al., 2007). In

invaded habitats, fecundities between 0.8 and 1×10^6 (Salento Peninsula, SE Italy: Mancinelli et al., 2013a) and between 0.7 and 4.7×10^6 eggs per female have been reported (Turkey: Türeli et al., 2018). Zoeal stages can survive and complete development in 32-43 days under optimal temperature and salinity conditions (25°C and 30 PSU); under the same conditions, the megalopa gains competency and undergoes metamorphosis in 6 - 12 days (Millikin & Williams, 1984; Jivoff et al., 2007). Mortality from hatching to the megalopa stage under field conditions in native habitats has been estimated between -0.07 and -0.32 per day (McConaugha, 1992). Given the high propagule pressure and survival rate of larval stages, *C. sapidus* is characterized by high potential of establishment. The availability of habitats with appropriate characteristics of salinity and submerged vegetation is likely to regulate and reduce the actual establishment of populations in invaded habitats.

No information is to date available, either from native or invaded areas, as to whether low genetic diversity in a blue crab founder population may influence its establishment success.

Qu. 2.8. If the organism does not establish, then how likely is it that casual populations will continue to occur?

Consider, for example, a species which cannot reproduce in the risk assessment area, because of unsuitable climatic conditions or host plants, but is present because of recurring introduction, entry and release events. This may also apply for long-living organisms.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Adult *C. sapidus* can tolerate a wide range of abiotic conditions, both climatic- and habitat related, even though the availability of transitional environments with appropriate salinity gradients is a crucial prerequisite for the completion of the species' life cycle (Hines, 2007). However, survival at high latitudes in temperate zones is limited, as combinations of cold water temperatures (< 3°C) and low salinity (< 8 PSU) can cause markedly increased mortality, especially for small juveniles and mature females (Hines et al., 2010 and literature cited). Thus, if adult individuals are recurrently released in habitats unsuitable only for reproduction and larval survival, occasional populations will likely occur for some time periods, given also the life span of the species (up to 6 years: McMillen-Jackson & Bert, 2004 and literature cited). This is also testified by some intentional releases attempted in Hawaii and California: even though they were unsuccessful, they determined repeated records that were temporarily included in local lists of non-indigenous species.

Qu. 2.9. Estimate the overall likelihood of establishment in the risk assessment area under current climatic conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under current climatic conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in current conditions: providing insight in the risk of establishment in (new areas in) the risk assessment area.

RESPONSE	very unlikely unlikely moderately likely	CONFIDENCE	low medium high
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	likely very likely		
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Response: *Callinectes sapidus* is already established in parts of the risk assessment area therefore the likelihood of establishment is “very likely” by default. Based on recent records (e.g., Ribeiro & Verissimo, 2014; Bañón et al., 2016; Morais et al., 2019; Pezy et al., 2019; Vasconcelos et al., 2019; Czerniejewski et al., 2020) and the occurrence of suitable environmental conditions and habitats, the establishment of *C. sapidus* is very likely but localised in the Greater North Sea (i.e. Normandy coast and southern North Sea) and in the Bay of Biscay and the Iberian Coast, primarily its southern parts. Furthermore, based on the increasing frequency of records in the Black Sea and the findings of gravid females in the past decade (Uzunova 2016; Stefanov 2021; Gül et al., 2021), establishment has already occurred in this region. Similarly, a burgeoning number of records published in the last decade indicates that a further increase in the establishment of blue crab populations in the whole Mediterranean Sea and in particular in the northwestern, southwestern (e.g., Balearic Sea) and central (e.g., Tyrrhenian Sea) sectors of the basin is very likely (e.g., Garcia et al., 2018; Suaria et al., 2017; Piras et al., 2019; Box et al., 2020).

Qu. 2.10. Estimate the overall likelihood of establishment in the risk assessment area under foreseeable climate change conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under foreseeable climate change conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of establishment (e.g. increase in average winter temperature, increase in drought periods)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely establishment within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Based on the temperature limits of the species distribution (see Qu. 2.1), and given the ongoing oceanographic variations in north Atlantic Ocean (Smeed et al., 2018), climate warming is expected to favour the spread of *C. sapidus* towards northern European waters. The spread is also expected to occur since the blue crab demography (in terms of length of the seasons for reproduction, settlement, and growth, growth rates, maturation/generation times, reproductive output) is markedly

affected by the seasonal cycle of temperature (Hines et al., 2010). In this context, for native areas Johnson (2015) highlighted in the last decades a northward expansion of *C. sapidus* to the Gulf of Maine and Nova Scotia, 500 km north of its historic range. Conversely, there are no published predictions for invaded European habitats besides the output of the modelling efforts presented in Annex VIII, clearly showing an increase in suitability of northern European waters under both RCP2.6 and RCP4.5 (fig. 9). However, as already mentioned (Qu. 2.1), temperature alone may result an inadequate predictor of the blue crab future distribution. The articulated - and to date hard to be delineated - effects of climate change on i) sea level, and in turn on the extent and biological characteristics (e.g., seagrass beds) of transitional environments crucial for the completion of early life stages ii) salinity ranges in these environments, as affected by changes in continental freshwater discharges iii) stratification and related effects on the occurrence and extent of hypoxic zones are to be necessarily taken into consideration (Elliott et al., 2019).

3 PROBABILITY OF SPREAD

Important instructions:

- Spread is defined as the expansion of the geographical distribution of an alien species within the risk assessment area.
- Repeated releases at separate locations do not represent continuous spread and should be considered in the probability of introduction and entry section (Qu. 1.7).

Qu. 3.1. How important is the expected spread of this organism within the risk assessment area by natural means? (List and comment on each of the mechanisms for natural spread.)

including the following elements:

- a list and description of the natural spread mechanisms of the species in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of spread discussed in relation to the species biology and the environmental conditions in the risk assessment area.

The description of spread patterns here refers to the CBD pathway category “Unaided (Natural Spread)”. It should include elements of the species life history and behavioural traits able to explain its ability to spread, including: reproduction or growth strategy, dispersal capacity, longevity, dietary requirements, environmental and climatic requirements, specialist or generalist characteristics.

RESPONSE	minimal	CONFIDENCE	low
	minor		medium
	moderate		high
	major		
	massive		

Natural dispersal across borders from neighbouring countries, where the species has been established (for example Spain, France, Belgium) by planktotrophic larvae dispersed with oceanic currents, as well as by swimming adults. Considering the high fecundity, the long pelagic life of its larvae and the swimming ability of *C. sapidus*, which can travel from 10s to 100s of kilometers according to tagging experiments (e.g. west coast of Florida - Guillory et al., 2001 and references therein), the potential for natural dispersal is very high, particularly in the Mediterranean, the Black Sea and the south/south-western Iberian peninsula (Vasconcelos et al., 2019), where climatic conditions are more favourable for longer periods. Nevertheless, it is expected that natural dispersal will likely be much more limited along the Atlantic coast of W. Europe due to the lower temperatures prevailing there. For example, a new study currently in review indicates that despite the multiple records along the west and north of the Iberian Peninsula the species does not seem to have achieved wide-spread establishment yet and doubts the possibility of migrant individuals (Izquierdo Gomez, 2021). On the other hand, in the English Channel, two ovigerous females were captured in offshore locations, off the Normandy coast at depths of 10 and 12m (Pezy et al., 2019). Thus, under future climate conditions, considerable natural dispersal cannot be discarded even for western European locations, as indicated by the isotherm maps in Annex VIII.

Qu. 3.2a. List and describe relevant pathways of spread other than "unaided". For each pathway answer questions 4.3 to 4.9 (copy and paste additional rows at the end of this section

as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 4.3a, 4.4a, etc. and then 4.3b, 4.4b etc. for the next pathway.

including the following elements:

- a list and description of pathways of spread with an indication of their importance and associated risks (e.g. the likelihood of spread in the risk assessment area, based on these pathways; likelihood of survival, or reproduction, or increase during transport and storage; ability and likelihood of transfer from the pathway to a suitable habitat or host) in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of spread for each pathway discussed in relation to the species biology and the environmental conditions in the risk assessment area.
- All relevant pathways of spread (except “Unaided (Natural Spread)”, which is assessed in Qu. 4.1) should be considered. The classification of pathways developed by the Convention of Biological Diversity shall be used (see Annex IV).

A. TRANSPORT - STOWAWAY – ship/boat ballast water

Dispersal of larvae by ballast tanks of ships is likely with medium confidence level (see Qu. 3.4a, 3.5a). Ballast water release is suspected as introduction and spread vector because blue crabs were predominantly found in port regions where ballast water discharges do frequently occur (Galil, 2006). Due to the sheer volume of ballast water in global shipping and the large amount of *C. sapidus* larvae potentially taken up, the rate of spread via this pathway is likely to be rapid (Qu. 3.9a)

B. TRANSPORT - STOWAWAY -ship/boat hull fouling

This is considered as one of the two most likely pathways of spread and is almost certainly responsible for records, within the confines of a small marinas where only yachts and small fishing craft are berthed (Bodrum (Turkey), Porto Grande (Malta) (Ulman et al., 2017). Even though the number of propagules per vessel is not expected to be high, the amount of maritime traffic between the harbours and marinas of the RA area is such that a moderate rate of spread, at least, may be expected through this pathway (Qu 3.4b, 3.5b, 3.9b).

C. ESCAPEE FROM CONFINEMENT- Pet / aquarium / terrarium species (including live food for such species). The species could be accidentally released from holding tanks because live blue crabs have been imported in many European countries for human consumption. Blue crab does appear to be available to buy live in the UK (see <https://readingqualityfish.co.uk/lobster-crabs/>) and other EU countries (e.g. Portugal, see Ribeiro & Verissimo (2014)), meaning that there may be the potential for intentional or accidental release once purchased or if unsold., particularly with reference to religious or other ‘ethical’ releases. (see Qu 3.4c, 3.5c)

D. RELEASE IN NATURE- Fishery in the wild

Intentional introduction by **commercial enterprises** is likely but with a low confidence level. Aquaculture transfers has been attempted in California (Cohen & Carlton, 1995), and Hawai (Eldredge, 1994) which however were not successful. According to Nehring et al. (2008) unconfirmed records in Turkey dating back to 1930’s are attributed to intentional introduction. This could explain the documented chronological distribution pattern (Aegean since 1947), Adriatic since 1949 (separate introduction event) but also the population establishment success (establishment first in the Aegean). In fact, deliberate introductions for harvesting purposes are increasingly hypothesised, as the species continues spreading in the western Mediterranean and gains commercial importance, e.g. the possibility was raised by Fuentes et

al. (2019) and by Box et al. (2020) for two different Spanish locations, but such hypotheses are very difficult to verify (see also Qu 3.4d, 3,9d).

E. TRANSPORT-STOWAWAY (other: Floating debris)

Although it seems unlikely, transport in marine litter is a potential pathway for transfers of *C. sapidus*. It is possible that individual crabs can cling to floating objects and drift with currents within the RA area. (see Qu. 3.4e, 3.5e). Due to the stochastic nature of these events and the patchy distribution of potentially suitable recipient habitats, spread via this pathway is likely to proceed at a slow rate.

F. TRANSPORT-CONTAMINANT

Shellfish, such as oysters, mussels, scallops, cockles and clams are grown in open systems and *Callinectes sapidus* that feed on them could be transported between sites throughout the RAA. However, the risk of importation of contaminated bivalve is low since a) transportations are now carefully regulated to minimize introduction to member states via movements of shellfish between sites; and b) only big animals feed on these species (Prado et al., 2020) and they could not go unnoticed during inspection. Therefore this pathway is not analysed further.

A. TRANSPORT -STOWAWAY – ship/boat ballast water

Qu. 3.3a. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional	CONFIDENCE	low
	unintentional		medium
			high

Regional vessel movements are more likely than those from outside the RAA to pass over suitable habitat where any larvae released in transit may be able to survive. Particularly small vessels which may take routes close to the coast and shelter in estuaries or bays overnight or in bad weather.

Qu. 3.4a. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely	CONFIDENCE	low
	unlikely		medium

	moderately likely likely very likely		high
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Response: For reproductive output and ship ballast volume and potential larval concentration, see Qu. 1.3a. With respect to spread of the organism within the EU, transshipment operations constitute the main maritime traffic that will act as the vector for spread. Important transshipment hubs are situated along the southern Mediterranean (serving the rest of the Mediterranean and the Black Sea) and the Le Havre-Hamburg range, serving the UK, the Baltic and Scandinavia (Notteboom & de Langen, 2015). Moreover, recreational boats operating across European ports may transfer *C. sapidus* from one regional sea to another, from a country to another. Noteworthy are also larger passenger/ car ferries which operate in most regions and use ballast water tanks – currently often unchecked, untreated and unregulated with member states permitted under ballast water convention to allow exemptions to apparently low risk inter-regional shipping routes. Considering *C. sapidus* larvae are favoured by water temperatures encountered throughout the Mediterranean and the south Iberian Peninsula, it is considered likely that sufficient numbers can be transferred with ballast water along this pathway, although direct evidence of this is lacking. Nevertheless, mature specimens of *C. sapidus* have been found in ballast sediment of a vessel from Baltimore sampled in Bremerhaven in 1993 and also from a vessel from the east coast of north America sampled in Rotterdam (Gollasch et al., 2002 and Gollasch pers. comm) and Brachyuran larvae are found frequently in ballast water (e.g. Cohen & Carlton, 1997; Gollasch, 2002),

Qu. 3.5a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: See Qu. 1.4a. If larvae can survive trans-oceanic trips, they are likely to survive transfer within EU marine areas which are shorter trips.

Qu. 3.6a. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: As in Qu.1.5a. Worth noting is that some of the regulations and requirements under the Ballast Water Convention may be relaxed for shorter, low-risk shipping routes under regional exemption options.

Qu. 3.7a. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE		CONFIDENCE	
	very unlikely		low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response: As in Qu. 1.6a

Qu. 3.8a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE		CONFIDENCE	
	very unlikely		low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response: If ballast water exchange occurs in open seas rather than in coastal areas, transfer of planktonic larvae to suitable substrate will be hampered. If, however, untreated ballast water is released in ports, estuaries or other coastal areas, then establishment will be dependent on availability of suitable habitat. Considering (a) the breadth of habitat that characterizes the species; (b) the wide distribution of such habitats in the RA; and (c) the documented records inside/near harbours (e.g. Bouvier, 1901; Tortonese, 1965; Cabal et al., 2006; Nehring, 2011), there is a high likelihood that *C. sapidus* can transfer to a suitable habitat after release with ballast water.

Qu. 3.9a. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area. (please provide quantitative data where possible).

RESPONSE		CONFIDENCE	
	very slowly		low
	slowly		medium
	moderately		high
	rapidly		

	very rapidly		
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Response: The overall potential rate is the product of the interaction of several factors, such as the intensity of shipping transport, the frequency of ballast water discharge, the origin of ships - together with many other biotic and abiotic factors related with the conditions of ballast waters as well as those characterizing the location where ballast waters are discharged. Based on the existing populations, their distribution and the intensity of maritime traffic within the RA area, *Callinectes sapidus* has the potential to spread rapidly via ballast water transfers, particularly in the Mediterranean Sea, the Iberian coast/Bay of Biscay and to a lesser extent the Greater North Sea.

B. TRANSPORT-STOWAWAY (ship/boat hull fouling)

Qu. 3.3b. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional	CONFIDENCE	low
	unintentional		medium high

Regional vessel movements are more likely than those from outside the RAA to pass over suitable habitat where any larger individuals detached in transit may be able to survive. Particularly small vessels which may take routes close to the coast and shelter in estuaries or bays overnight or in bad weather.

Qu. 3.4b. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely	CONFIDENCE	low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response: Although not reported from a ship hull its presence in marinas (Ulman et al., 2017) gives credit to this vector of introduction. For the reproductive biology of the species please see Qu 2.7. Considering the biological characteristics of the organism (withstand wide temperature range and

salinity range), it is considered possible that *C. sapidus* can settle as a competent larva or a juvenile and maintain a viable population within the fouling communities on ships hulls and especially niche areas, sufficient for a new introduction.

In addition to merchant vessels travelling all year round, with thousands of trips per year between the ports of the RA area (Seebens et al., 2013), recreational boats (mostly cruise boats) can also transport the species on their hulls and niche areas. The world cruise tourism represented as Equivalent Fulltime Navigation Vessel (EFNV) index, allocated hot spots of activity of the world cruise fleet. According to EFNV, in 2015 the Mediterranean Sea was the second visited areas (EFNV=36.71) after the North Atlantic (EFNV=45.53), while the Norwegian Sea (11.76) and North Sea (6.47) played an important role (Vicente-Cera et al., 2020).

Qu. 3.5b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: As in Qu 1.4.b. The likelihood of survival is higher due to shorter distances and reduced likelihood of passing through unfavourable conditions

Qu. 3.6b. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Consider answer in 1.5b, but also consider that management practices are likely to be reduced for 'local' travel with potential stepping stone effects resulting from short, unregulated and unmonitored journeys.

Qu. 3.7b. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely	CONFIDENCE	Low Medium
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	moderately likely likely very likely		high
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Response: Shorter journey may mean smaller size on arrival and increased number of surviving young and even less likelihood of detection than 1.6b

Qu. 3.8b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Early benthic life stages of *C. sapidus* can be detached and inhabit soft and hard bottom communities or seagrass/eelgrass beds in the recipient area. Considering (a) the breadth of habitat that characterizes the species; (b) the wide distribution of such habitats in the RA; and (c) the documented records inside/near harbours (e.g. Bouvier, 1901; Tortonese, 1965; Cabal et al., 2006; Nehring, 2011), there is a high likelihood that *C. sapidus* can transfer to a suitable habitat.

Qu. 3.9b. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: Hull fouling is a plausible, albeit not documented, mechanism of spread of *C. sapidus* in the RA area. It can be mediated by large merchant vessels, passenger ships, recreational and fishing boats as well as other small vessels. Even though the number of propagules per vessel is not expected to be high, the amount of maritime traffic between the harbours and marinas of the RA area is such that a moderate rate of spread, at least, may be expected through this pathway.

C. ESCAPEE FROM CONFINEMENT- Pet / aquarium / terrarium species (including live food for such species)

Qu. 3.3c. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional and unintentional	CONFIDENCE	low medium high
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Response: The import of species to be kept as live food is an intentional pathway. Its escape in the marine environment however is unintentional. On the other hand, its release such as ethical release or disposal of leftover stock is deliberate.

The economic interest in *C. sapidus* has progressively increased in Mediterranean countries in the last decade. *Callinectes sapidus* captured in Greek waters is exported to Italy at least since 2016 (Mancinelli, personal observation); no information is available on traded quantities. According with Sea-Ex Trade Seafood Directory (<http://www.trade-seafood.com/>) live *C. sapidus* is actually exported from Turkey by North Aegean Seafood Ltd (www.northaegeanseafood.com) and from Greece by Blue Crab P.C. (<http://www.bluecrab.gr/>), Mediterranean Crabs (<http://www.mediterraneancrabs.com>), and Dimoudis Bros. S.A. (<http://www.dimoudis.gr>). No information is available on exports from Italy or Spain.

Crabs that are not being sold/consumed locally end up discarded in the environment (an incident has been recently documented in Lake Kastoria, North-West Greece, <https://www.facebook.com/eppkast/posts/2840062492781951>). In addition, trading of live specimens may also be related with unintentional release episodes: recently, one of the authors (GM) witnessed an incident of accidental drop-off from a pallet of several 10-Kg packs containing live *C. sapidus* into the sea at the port of Brindisi (SE Italy), while being unloaded from a merchant ship. Blue crabs originated from Greece and were transferred to refrigerated trucks for delivery to local distributors for the live sea-food market.

Qu. 3.4c. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely	CONFIDENCE	Low medium high
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	very likely		
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Response: It depends on the accidental or deliberate events. *Callinectes sapidus* is traded alive for alimentary purposes; accordingly, in principle even though exported in relatively high quantities, it should be expected that all specimens are killed soon after the arrival in the recipient country and not released alive.

Even though blue crabs of both sexes are traded, females carrying egg sponges are generally discarded. Accordingly, direct propagules release is a remote possibility. On the other hand, if specimens of both sexes survive after accidental release until mating, even at low abundances it is likely that this may set the basis for the establishment of a population, also given the high fecundity of female blue crabs (see section establishment).

Qu. 3.5c. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: As mentioned in Qu. 1.3d, live *C. sapidus* are traded at low temperatures. Under natural conditions, the species can survive to temperature close to 0°C entering a state of quiescence (Millikin & Williams, 1984) even though for prolonged periods this can cause an increase in populations' mortality (Hines, 2007). Thus, survival during transport is likely, given that the duration of the journey is limited to 24-48 h (Mancinelli, personal observation). Obviously, longer transport times would imply that the species is traded frozen.

No reproduction is possible for blue crabs traded alive.

Qu. 3.6c. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: There are currently Regulations (e.g. Regulation (EU) 2017/625, Commission Delegated Regulation (EU) 2019/625) and guidelines concerning live food trade (see https://ec.europa.eu/food/safety/international_affairs/trade/import_animal_en), but are oriented towards

the protection of human health and not transport or translocation of species. If *C. sapidus* is frozen before being packed for transport, the procedure determines the death of the specimens, thus survival rates equal 0.

Existing laws in member states prohibiting the release of non-native species have been used to prosecute those undertaking large-scale, intentional releases of non-native crabs and lobster for religious reasons (see previous article link). However, these are not preventative and there is great scope for avoidance of prosecution and illegal but un-regulated actions. The incident noted in the previous section by the authors illustrates also that management practices are insufficient to prevent release during transit.

Qu. 3.7c. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Only large sized animals are traded and they are likely to easily detected if looked for. However, if large numbers are released in estuarine areas not regularly surveyed nor frequented by divers etc and where soft sediment is available in which crabs could hide, then they can be undetected. *C. sapidus* is easy recognisable and trading companies as well as fishermen in most parts of the world and consumers are aware of its specific characteristics (see Qu. 1.2d). Thus, the possibility that it can be misidentified and imported into the risk assessment area is unlikely.

Qu. 3.8c. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Accidental release may occur during e.g., landing operations (as described in Qu. 3.3c) thus there is a moderately likely possibility of release through this pathway. For example, one of the authors (GM) witnessed an incident of accidental drop-off from a pallet of several 10-Kg packs containing live *C. sapidus* into the sea at the port of Brindisi (SE Italy), while being unloaded from a merchant ship. Blue crabs originated from Greece and were transferred to refrigerated trucks for delivery to local distributors for the live sea-food market.

As live blue crabs are transported at low temperatures in mesh bags or similar containers, once in seawater at ambient temperature specimens may become active, tear the mesh and spread in the

environment. It is also possible that unwanted or leftover specimens from the live food trade are disposed of inappropriately in the environment, e.g. thrown overboard from vessels (Nehring et al., 2008). This is most likely to take place in shallow coastal waters e.g. ports often sheltered in estuarine sites where habitat is most suitable.

Qu. 3.9c. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area. (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: Given the increasing commercial interest in *C. sapidus*, it is moderately likely that the trade of the species, especially if not regulated, will progressively increase in the next few years. This will determine an increase of exporting countries offering the blue crab as a shellfish product, as well as in the traded volumes, and in the points of introduction. This will in turn increase the probability that the species is introduced in the risk assessment area by this pathway.

D. RELEASE IN NATURE- Fishery in the wild

Qu. 3.3d. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: Considering the high commercial value of the species, it has been postulated that intentional introductions aiming to create a population for fisheries purposes were/are a possibility (Nehring, 2011). These hypotheses mainly referred to the early presence of the species in the Aegean Sea, which according to some authors, is suspected since as early as 1935 (Enzenroß et al., 1997). This narrative could not be verified but similar incidents remain a possibility.

Qu. 3.4d. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely very likely	CONFIDENCE	low medium high
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Response: Since this is only speculation, no actual information was found for introductions in the RA area. The disjunct distribution of the species in areas where it is already commercially exploited could point to intentional releases for harvesting purposes. For example, an intentional human introduction is suspected in inland waters of Catalonia, in a waterbody without fluvial connectivity with the sea (Fuentes et al., 2019), however it is unclear if this incident is associated with an intent to start a breeding population. In fact, deliberate introductions for harvesting are increasingly speculated on, as the species continues spreading in the western Mediterranean and gains commercial importance, e.g. the possibility was raised by Box et al. (2020) following the appearance of *C. sapidus* in Ibiza and Formentera, Balearic Islands, however this is very difficult to verify. A hypothesis about the frequency of such events cannot be made but if/when such incidents occur, they will definitely involve a large number of individuals at various lifestages, including gravid females to be released over a long period of time to ensure a viable population. Overall though this is unlikely to be done legally due to restrictions on the introduction, in reality, any 'black market' introductions would be very unpredictable.

Qu. 3.5d. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: It is assumed that if organisms are transported for the sole purpose of starting a population for fishery purposes, then it will be ensured that they are kept alive.

Qu. 3.6d. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The management practice most relevant in this case is Council Regulation (EC) No 708/2007 concerning use of alien and locally absent species in aquaculture. According to the regulation, all aquaculture operators who intend to introduce an alien species or translocate a locally absent species must first apply for a permit from the competent authority of the Member State where the transfer will take place. The Regulation specifies the information to be provided by the applicant and the type of assessment that the competent authority must perform before granting the permit.

Callinectes sapidus is not included in the list of Annex IV of Council Regulation 708/2007 which defines the species that are exempted and can be moved without any risk assessment or quarantine; as such, it would have to be risk assessed if transferred between Member States but it is still likely that it could be transported after a positive decision regarding its commercial harvesting. However, the regulation does not apply to movements of locally absent species within the Member States “except for cases where, on the basis of scientific advice, there are grounds for foreseeing environmental threats due to the translocation, Art. 2 para. 2.“, which means that the species could easily spread within Member States without any additional procedures/restrictions.

Qu. 3.7d. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Callinectes sapidus* is easy recognisable and trading companies as well as fishermen and consumers in most parts of the world are aware of its specific characteristics. Thus, the possibility that it can be misidentified and transferred within the risk assessment area undetected is very unlikely, especially the large, mature individuals that would be transferred intentionally in order to establish a new fishery in the wild.

Qu. 3.8d. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely	CONFIDENCE	low medium high
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	likely very likely		
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Response: In the case of an intentional release for harvesting purposes, it is expected that the species will be transferred to a suitable habitat.

Qu. 3.9d. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area. (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: Not enough concrete information is available to assess the rate of spread via this pathway with any confidence. However, the increasing number of new locations for *C. sapidus* in the risk assessment area and neighbouring countries may be partly attributed to intentional releases for harvesting, especially considering the increasing commercial exploitation of the already established populations. A moderately rapid rate of spread is possible.

E. TRANSPORT-STOWAWAY (other: Floating debris)

Qu. 3.3e. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Qu. 3.4e. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Marine litter and floating debris have been considered a potential transport vector of non-indigenous species. In coastal waters the identification of sources, trajectories and deposition sites of marine litter is often hampered by the complex oceanography of shallow shelf seas. Oceanographic modelling revealed that the distribution of floating litter in the North Sea is largely determined by the site of origin of floating objects whereas the trajectories are strongly influenced by wind drag. (Gutow et al., 2018). In the Mediterranean, transport of fouling as rafting material has been demonstrated in the Tyrrhenian and Ligurian Seas (Aliani & Molcard, 2003). In a recent study of fouling organisms on litter discharge in the Gulf of Naples, it was found that Arthropoda colonized 7-10% of the total colonized items. They were mostly found on cotton ($13.33 \pm 13.33\%$) and on plastic ($8.32 \pm 4.22\%$), litter (Crocetta et al., 2020). In a study developed in Tjärnö (Sweden), at the entry of the Baltic Sea, the communities inhabiting coastal litter and natural substrates were monitored from eight sites. The results showed that more diverse communities were found on litter. In fact, 10 Arthropoda species were identified fouling on litter vs 2 species fouling on natural substrata. Non-indigenous species were attached mainly to non-plastic artificial materials (Garcia-Vazquez et al., 2018). Although relevant information was not found in the literature specifically for *C. sapidus*, larvae, megalopae and juveniles of Portunidae, including *Callinectes* spp., have been repeatedly reported rafting on floating algae in the northeast Atlantic region, as well as the east Pacific (Thiel & Gutow, 2005 and references therein). Furthermore, its documented history of attacking fishing nets makes it also a likely candidate to be transported by drifting fishing gear, particularly at the adult stage.

From these results it can be concluded that marine litter can significantly alter the biotic composition of coastal ecosystem, representing a shelter for invasive species and diverse natives.

Qu. 3.5e. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Species that are considered likely to be transported with hull fouling over long trans-oceanic voyages can, very likely, survive on floating debris as well. Probability of survival will depend on the fouling community and the travelling time at sea. As an example, Aliani & Molcard (2003) developed a numerical model showing that 45% of floating objects could reach the Ligurian coast from Corsica

(about 75 km) in 50 days. Gutow et al. (2018) estimated the average residence time of floating items in the German Bight that was largely independent of the wind drag coefficient to vary between 16 and 23 days. In contrast, in both the Skagerrak and the wider North Sea the average residence time of floating items declined continuously with increasing wind drag factor from about 33 to 2 days and from 79 to 48 days, respectively. According to CIESM (2014) more than 80% of alien species in the Mediterranean might have arrived on floating debris or used this vector for further dispersal.

Qu. 3.6e. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: There are no relevant management practices for marine debris in EU regional seas. EU is trying to tackle marine litter issues through the descriptor D-10 of Marine Strategy Framework Directive. The need to comply with MSFD regulations may require member states to begin managing litter more effectively through removal, trapping and avoiding incidents of lost gear and equipment to keep the D-10 indicator down, which might in turn reduce likelihood of surviving.

Qu. 3.7e. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Considering that floating debris and marine litter are not regularly examined for fouling biota it may go undetected. However, since it is an easily recognized animal when mature it is relatively easy to detect it, if present. Post settlement and younger individuals may be less detectable. Short journey time may mean less growth and smaller size at arrival which may decrease likelihood of detection more than transAtlantic drift.

Qu. 3.8e. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely	CONFIDENCE	low
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	unlikely moderately likely likely very likely		medium high
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Response: Marine litter is often washed up in areas with low flow and lagoons and estuaries can funnel debris, particularly travelling in coastal currents. Considering the disjunct distribution of suitable habitats with the necessary conditions for the species to complete its life cycle (i.e. estuaries, lagoons), it is considered moderately likely that it will find its way, via stranded floating debris, to these habitats.

Qu. 3.9e. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area. (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: The trajectory and the destination of a floating object strongly depend on its buoyancy and persistence at the sea surface (Thiel & Gutow, 2005). Loss of buoyancy and sinking of floating litter can be accelerated by biofouling when colonizing organisms enhance the specific gravity of an object (Ye & Andrady, 1991) especially in objects with a high surface:volume ratio (Chubarenko et al., 2016).

It is certainly possible for *C. sapidus* to spread throughout the regional seas hitchhiking on natural and man-made floating objects, with drifting fishing gear being a very likely candidate as its substrate. On the other hand, arrival at a suitable habitat is considered a less likely event due to the patchy distribution of lagoons and estuaries.

Key factors that would cause rate of spread to be very slow is the irregular and stochastic nature of the environmental conditions and events that would be needed for spread by this pathway to be successful. It is thus concluded that spread via this pathway will proceed at a slow rate. Paucity of information on the volume, frequency of occurrence and circulation patterns of floating debris in EU regional seas decreases the confidence level of the assessment.

Qu. 3.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?

RESPONSE	very easy easy with some difficulty difficult	CONFIDENCE	low medium high
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	very difficult		
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Response: Naturally dispersing organisms are very difficult to contain, especially species such as *C. sapidus*, with high fecundity, long pelagic larval duration and a strong swimming ability ((Millikin & Williams, 1984), which enhances their dispersal potential (See Qu. 2.7 for the biological characteristics of the species). In the case of *C. sapidus* in particular, the increasing commercial value of the already established populations adds to the potential for spread through intentional releases.

Qu. 3.11. Estimate the overall potential rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (indicate any key issues and provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in current conditions, providing insight in the risk of spread into (new areas in) the risk assessment area.

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: The increasing rate of appearance of new records, especially in the Mediterranean Sea, indicates a rapid rate of spread (see figure 3), which is most likely attributable to natural dispersal, although the contribution of other pathways of spread cannot be overlooked. In the Black Sea, human-assisted mechanisms of spread, particularly through shipping vectors and/or the live food trade and intentional releases for commercial exploitation are likely to maintain a moderately rapid rate of spread. In the Greater North Sea, due to the limited availability of suitable climatic conditions, anthropogenic pathways of spread are expected to be primarily responsible for the spreading of the species. Due to the high shipping traffic volume and the precedence of live food trade escape, a rapid rate of spread may be expected.

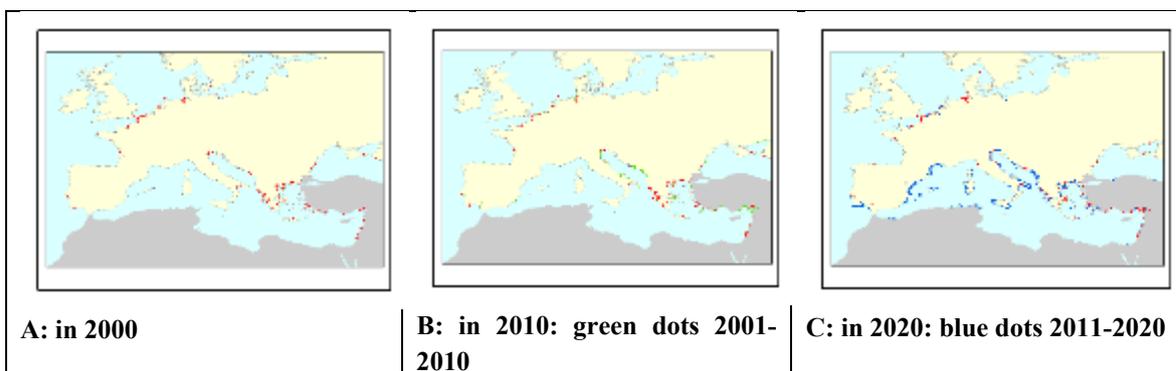


Figure 3. Distribution of *Callinectes sapidus* in the European Seas. (source Mancinelli et al. 2021)

Mediterranean Sea: rapidly, high confidence, all pathways involved

Bay of Biscay and the Iberian coast: rapidly, medium confidence, all pathways involved

Greater North Sea: rapidly, mostly due to shipping traffic and live food trade.

Black Sea: moderately rapidly, potentially due to intentional transfers for harvest, very rapidly in the Sea of Marmara due to unaided spreading

Celtic Seas: slowly, all pathways involved

Baltic Sea: slowly, all pathways involved

Qu. 3.12. Estimate the overall potential rate of spread in relevant biogeographical regions in foreseeable climate change conditions (provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if rates of spread are likely slowed down or accelerated.

RESPONSE		CONFIDENCE	
	very slowly		low
	slowly		medium
	moderately		high
	rapidly		
	very rapidly		

Response: The overall potential rate of spread is expected to remain rapid but this will likely apply to an even more extensive part of the RA area, which will, under future climate change, present the species with favourable conditions for larval development and dispersal. This is especially true for the western Iberian coast and the Bay of Biscay, as well as the southern North Sea coasts, which now present a marginal environment for *C. sapidus* but will offer more suitable conditions in a future warming scenario. This will further enhance the availability of propagules in these areas, and their subsequent uptake and spread through shipping vectors in particular. In the Celtic Sea, there will be some more possibilities for spread, due to natural dispersal, but these will still be limited by the low summer temperatures.

4. MAGNITUDE OF IMPACT

Important instructions:

- Questions 4.1-4.5 relate to biodiversity and ecosystem impacts, 4.6-4.8 to impacts on ecosystem services, 4.9-4.13 to economic impact, 4.14-4.15 to social and human health impact, and 5.16-5.18 to other impacts. These impacts can be interlinked, for example, a disease may cause impacts on biodiversity and/or ecosystem functioning that leads to impacts on ecosystem services and finally economic impacts. In such cases the assessor should try to note the different impacts where most appropriate, cross-referencing between questions when needed.
- Each set of questions starts with the impact elsewhere in the world, then considers impacts in the risk assessment area (=EU excluding outermost regions) separating known impacts to date (i.e. past and current impacts) from potential future impacts (including foreseeable climate change).
- Only negative impacts are considered in this section (socio-economic benefits are considered in Qu. A.7)
- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

Biodiversity and ecosystem impacts

Qu. 4.1. How important is the impact of the organism on biodiversity at all levels of organisation caused by the organism in its non-native range excluding the risk assessment area?

including the following elements:

- Biodiversity means the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems
- impacted chemical, physical or structural characteristics and functioning of ecosystems

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: There are no reported impacts on local biodiversity caused by the species in areas of its non-native distribution areas outside the Mediterranean (excluding the risk assessment area) such as Japan.

In the Mediterranean, there are some alleged implications that *C. sapidus* is involved in changes in the ecosystem and biological balance in Bardawil lagoon (Egypt), as the increasing abundance of the crab in conjunction with variations in the ecological conditions prevailing in the lagoon, have negatively affected native invertebrate and fish stocks (Mehanna et al., 2019). In particular, annual catches of benthic invertebrate (e.g., the shrimp *Metapenaeus stebbingi*) and fish species (soles of the genus *Solea*) have remarkably declined in the decade 2008-2018 (Mehanna et al., 2020); these evidences are consistent with the diet of the species as observed in invaded Greek waters (Kampouris et al., 2019).

The observed variations, however, cannot be unequivocally ascribed to the concomitant increase in the blue crab stock, due to recent variations in the abiotic conditions of the Bardawil Lagoon, changes in fishing gear, and the contemporaneous occurrence in the lagoon of the Lessepsian portunid *Portunus segnis*. In Turkey, *C. sapidus* has well established populations, yet, its impacts on biodiversity have not been thoroughly assessed; nevertheless, based on qualitative observations and reports from fishermen, it is considered a voracious predator and is expected to impact the structure and dynamics of benthic food webs through predation on native species (AS Ateş, pers. comm).

Qu. 4.2. How important is the current known impact of the organism on biodiversity at all levels of organisation (e.g. decline in native species, changes in native species communities, hybridisation) in the risk assessment area (include any past impact in your response)?

Discuss impacts that are currently occurring or are likely occurring or have occurred in the past in the risk assessment area. Where there is no direct evidence of impact in the risk assessment area (for example no studies have been conducted), evidence from outside of the risk assessment area can be used to infer impacts within the risk assessment area.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Stable isotope studies performed in the risk assessment area have confirmed *Callinectes sapidus* as an omnivore feeding at different trophic levels, interacting - either by predation or competition - with a variety of native organisms and thus affecting in multiple ways the structure and dynamics of invaded food webs (Carrozzo et al. 2014; Mancinelli et al., 2013a, 2016, 2017c). In addition, it is characterized by remarkable ontogenetic diet shifts (Mancinelli et al. 2017c). Based on these evidences, a considerable ecological impact can be already expected for the species, as invasive omnivores are particularly threatening to ecosystem function because of their ability to directly and indirectly influence multiple trophic levels through resource consumption (Lodge et al. 1994; Thompson et al. 2007; Tumolo & Flinn 2017).

Under field conditions, the feeding habits and foraging behavior of the species have been clearly assessed in Greek waters by Kampouris et al. (2019). In their account the authors indicated for the species an extensive list of prey items, including algae, polychaetes, mollusks (15 species), crustaceans (9 taxa) and a wide variety of fish (16 taxa). In a small lagoon in North Aegean Sea Greece (Papapouli Lagoon, Platamonas) an analysis of a short time-series of fisheries data (2009–2014), suggested that *C. sapidus* may have extirpated the native *Carcinus aestuarii* (Kampouris et al., 2019). Moreover, there are anecdotal records that *C. sapidus* had the same effect on *Cerastoderma glaucum* (Kampouris et al., 2019). Furthermore, an analysis of a time series of invertebrate and fish catches in the Ebro Delta (Spain) currently under submission (Clavero et al., 2020) indicated that from 2011 to 2019 severe impacts (roughly estimable in the range of 30-90% decreases in catches) were exerted by the spread and increase in abundance of *C. sapidus* in the area on a wide spectrum of organisms, including bivalves and

gastropods (10 species), the green crab *Carcinus aestuarii*, the common goby *Pomatoschistus microps*, the eel *Anguilla anguilla*, the sandsmelt *Atherina boyeri*, the seabass *Dicentrarchus labrax*, and the sea bream *Sparus aurata*. Concomitantly, the blue crab has been representing an increasing proportion in the lagoon fisheries captures (López and Rodon, 2018), and indication of a strong top-down control of local communities.

The strong preference of the species for bivalves has been confirmed by laboratory experiments. *C. sapidus* caught in Portugal when fed with commercial bivalves such as *Donax trunculus*, *Spisula solida*, *Chamelea gallina*, *Cerastoderma edule*, *Ruditapes decussatus* and *Mytilus galloprovincialis* revealed no species specific preference but preference to thin-shelled species that were easier to handle and fastest to break than the more hard-shelled species (Vasconcelos et al., 2019). These observations have been corroborated by laboratory trials performed using *C. sapidus* individuals captured in the Ebro Delta and different sizes of the bivalves *Mytilus galloprovincialis*, *Crassostrea gigas*, and *Corbicula fluminea*, and the gastropod *Pomacea maculata* (Prado et al., 2020).

Considering feeding observations (Kampouris et al., 2019) and stable isotope analyses (e.g. Mancinelli et al., 2013a; 2016; Carrozzo et al., 2014), the targeted prey of *C. sapidus* overlap with that of several native crustacean and fish species [e.g. *Sparus aurata* (Carrozzo et al., 2014); *Eriphia verrucosa*, *Pachygrapsus marmoratus* (Mancinelli et al., 2013a); *Gobius niger*, *Lithognathus mormyrus*, and *Zosterisessor ophiocephalus* (Mancinelli et al., 2016)]. The species is also suspected to outcompete indigenous species of crabs (e.g. Gennaio et al., 2006; Mancinelli et al., 2013a; Petović et al., 2018; Kampouris et al., 2019).

Bioturbation of seagrass beds, in particular during winter months when *C. sapidus* responds to low temperatures by burrowing in the bottom sediments and entering a state of quiescence, is a further, rather overlooked impact that may be exerted by the species on local biodiversity. In invaded habitats no quantitative studies have been performed to date (but see Kampouris et al. 2019 for some examples of burrowing activities); however, one of the authors (GM) has personally verified that in the Lesina and Acquatina lagoons (Puglia Region, SE Italy), both characterized by high abundances of *C. sapidus*, *Nanostera noltei* and *Cymodocea nodosa* beds presented at the end of winter – early spring anomalous unvegetated spots where the vegetation had been uprooted. Even though other natural or human-determined causes cannot be excluded, these evidences are consistent with observations from native habitats, where the species has been indicated to create pits and bare areas in seagrass stands (e.g., *Thalassia testudinum* Valentine et al., 1994; *Zostera* spp.: Townsend & Fonseca, 1998) see also Sturdivant et al. 2012). Moreover, besides consuming seagrass seedlings and live plant material, *C. sapidus* has also the potential to impact seagrass beds by bioturbation while ambushing, hiding for predators and/or overwintering (Hines, 2007).

These information from the RA area are fully consistent with those available for *C. sapidus* native habitats, where the blue crab has long been recognized to influence the structure and function of benthic food webs, either as a keystone species regulating carbon flows, or by reducing prey abundance and inducing trophic cascades (Mancinelli et al., 2017c). Accordingly, a major response and a high ecological impact (throughout all its ontogeny) on coastal ecosystems is expected for the species, particularly for the Mediterranean and the southern Iberian Peninsula. In the isolated locations in Atlantic Europe where *C. sapidus* has established populations no impacts have been reported to date, presumably because of the limited distribution and density of the populations (e.g. in Normandy, France and the Westerschelde, Netherlands).

No hybridization with other species has been reported (e.g. Millikin & Williams, 1984). In fact, with the only exception of an episodic record of *Callinectes exasperatus* in European waters (Cuesta et al., 2015) no congeneric species occur in the RA area.

Qu. 4.3. How important is the potential future impact of the organism on biodiversity at all levels of organisation likely to be in the risk assessment area?

See comment above. The potential future impact shall be assessed only for the risk assessment area.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: The species is expanding its distribution range throughout the RA area; in addition, the establishment of blue crab populations in invaded areas are characterized, as generally occurs for invasive species, by anomalous increases in the abundance of the species. Given that the strength of the interactions highlighted in Qu. 4.2 - either competitive or predatory - are by definition density-dependent, a major impact is expected for the species in the future. The response is estimated with a medium level of confidence, as several factors may influence the ultimate magnitude of the impact. For example, the blue crab is likely to become established in the Atlantic (e.g. Bay of Seine) where some pressure on the native green crab *Carcinus maenas* through predation and competition are expected (Pezy et al., 2019). Even though considerable negative effects have been highlighted for *C. sapidus* on the congeneric *C. aestuarii* in the Mediterranean Sea (see Qu. 4.2), the actual outcome of biotic interactions of the blue crab with *C. maenas* is characterized by a certain level of indeterminacy, as Rogers et al. (2018) indicated that at low temperatures, *C. maenas* in invaded US waters had a competitive advantage on *C. sapidus* and intra-guild predation by blue crabs on green crabs was low. Thus, future oceanographic conditions within the RA area may affect the ultimate impact of the species; monitoring the presence, establishment and potential expansion of the species in the warming North Sea, is of significance especially under the light of how future establishment of *C. sapidus* populations would affect the local biodiversity (Nehring et al., 2008). Given the lower temperatures in the North Sea and Celtic Seas, even under future climate conditions, it is not considered very likely that the invasion will be as strong and wide-spread as in the Mediterranean, nevertheless some predation impacts on native molluscs in particular can be expected. Furthermore, while there exists information on the trophic position of the species in the risk assessment area (for details see Qu. 4.2), the interactions with higher trophic levels, and thus with native predators that may control the abundance and expansion of blue crab populations, are to date virtually unexplored (Mancinelli et al., 2017a). Several recorded predators of *C. sapidus* in its natural distribution areas (see Palomares & Pauly, 2019), are also found in the risk assessment area (e.g. turtles (*Lepidochelys kempii*), sharks (*Carcharhinus plumbeus*), bonyfish (e.g. *Dicentrarchus labrax*, *Lobotes surinamensis*, *Pomatomus saltatrix*), mammals (*Tursiops truncatus*). Yet, their interactions have not been studied in invaded environments, thus no predictions can be made as regard with their potential response, in terms of abundance and diversity, to the establishment of *C. sapidus*.

Qu. 4.4. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism currently in the risk assessment area?

including the following elements:

- native species impacted, including red list species, endemic species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: The species has been recorded in lagoon areas that are of conservation value with regard to European and national nature conservation legislation sites, such as the Natura 2000 network and the Habitats Directive (lagoons listed in Annex I as priority habitats) and MPAs. In particular it has been reported from: Ugento, Salento peninsula-Lecce, Italy (Gennaio et al., 2006), Lesina and Varano lakes, Gargano National Park, Italy (Florio et al., 2008), Antinioti Lagoon, Corfu, Greece (Karachle in Bilecenoglu et al., 2013), s’Albufera de Mallorca Natural Park, Balearic Islands, Spain (Garcia et al., 2018), La Pletera salt marshes, part of the Natural Park “Montgrí, les Illes Medes i el Baix Ter”, Spain (Fuentes et al., 2019)] and The Ebro Delta Natural Park (Castejón & Guerao, 2013).

Currently, the overall decline in conservation value of these habitats has not been assessed, with only exception of the Ebro Delta area, where remarkable, community-wide negative impacts have been highlighted (Clavero et al. 2020). Given the impacts - verified and expected - of the species described in Qu. 4.2 and 4.3, major negative effects are likely to occur on the conservation value of invaded areas, as determined by changes induced on their functions and diversity. In particular, the high trophic plasticity and omnivorous trophic habits of the species imply that species of high conservation interest are likely to be included in its diet; in addition, seagrass beds may be threatened (listed under the code A5.53 in the EUNIS habitat type classification; related to habitat types 1110 and 1120, of the Annex I of the Habitats Directive) by bioturbation, as described in Qu 4.2.

With respect to the environmental status of the sites it has invaded, its impact could be related to the MSFD descriptors D1 (biodiversity), D2 (NIS), D3 (fishing), and D4 (food webs). Yet, the degree of *C. sapidus* impact on GES, based on the MSFD descriptors has not been quantitatively assessed. The only evident measured impact is presently the indicator D2 (criterion D2C1: number of new NIS per 6 years).

Qu. 4.5. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.4.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Warming and extent of suitable range could increase number of potentially suitable (hence vulnerable) estuaries, inlets and bays, mudflats, saltmarsh and seagrass beds. Provided the overall recorded (e.g. on biodiversity and habitat changes, impacts on the food webs) and perceived impacts of *C. sapidus* on biodiversity and ecosystems (see questions Qu 4.1-4.3), its presence and increased proportion in areas of conservation is likely to cause a decline in their overall value. Lower impacts may be expected in the North and Celtic Seas, where lower temperatures are likely to limit the magnitude of the invasive populations.

Ecosystem Services impacts

Qu. 4.6. How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?

- For a list of services use the CICES classification V5.1 provided in Annex V.
- Impacts on ecosystem services build on the observed impacts on biodiversity (habitat, species, genetic, functional) but focus exclusively on reflecting these changes in relation to their links with socio-economic well-being.
- Quantitative data should be provided whenever available and references duly reported.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Very little information has been found on the issue, as the species has very few established permanent populations in other non-native locations where it was observed (see also Qu 4.1). The only impact that could be identified is related to “provisioning” in Turkey and Egypt. In Turkey, the species has been exploited since the 1980’s, In south west Turkey, *C. sapidus* has been reported in 15 lagoonal systems, supporting important commercial fisheries (Ayas & Özogul, 2011) while in Bardawil Lagoon, Sinai (Egypt) where it is also exploited the species comprised 19% to 42% of the total lagoon production (Abdel Razek et al., 2016; Mehanna et al., 2019). However, impact on protected slow moving benthic fish species (e.g. *Gobius cobitus* protected in UK) and alteration of the quality of protected bird feeding habitat (e.g. estuarine mudflats and offshore mussel/ cockle beds important for duck species due to predation on bivalves) can be anticipated.

Qu. 4.7. How important is the impact of the organism on provisioning, regulating, and cultural services currently in the different biogeographic regions or marine sub-regions where the species has established in the risk assessment area (include any past impact in your response)?

- See guidance to Qu. 4.6.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: The main impact that could be identified is related to “provisioning”. The species has been responsible for destruction of fishing gear and the fish being caught on the nets (e.g. Dulčić et al., 2011; Katselis & Koutsikopoulos, 2017; Mancinelli et al., 2017a; Kampouris et al., 2019), but also it preys upon other exploited species as the Pacific oyster *Magallana gigas*, the blue mussel *Mytilus* spp. (Labrune et al., 2019; Prado et al., 2020). This leads to reduced fisheries yields and thus impact on food provisioning to the local communities, as in the case of suspected local (?) extirpation of *Carcinus aestuarii* and anecdotal records of effects on *Cerastoderma glaucum* (Kampouris et al., 2019).

Other impacts that could potentially occur relate to Regulation-maintenance services and Cultural services. Where dense populations of the species develop, their heavy predation on filter feeding organisms may affect water quality and transparency, triggering a cascade of effects on macrophyte abundance and nutrient cycles. This potentially may affect (i) mediation of waters or toxic substances and (ii) hydrological cycle and water flow regulation. Additionally, damage to nets and fishing rods can reduce the amenity value to recreational fishing, impacting cultural services in the form of physical and experiential interactions with the natural environment. Such impacts however are still speculative as no concrete information could be found on these issues.

Qu. 4.8. How important is the impact of the organism on provisioning, regulating, and cultural services likely to be in the different biogeographic regions or marine sub-regions where the species can establish in the risk assessment area in the future?

- See guidance to Qu. 4.6.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: The main impact that could be identified is related to “provisioning”. The species is known to prey on various types of organisms, including economically important bivalves (Kampouris et al., 2019; Labrune et al., 2019). For this reason, its presence, and recent establishment (GM, pers. observ.) in the lagoons of north Italy, where clam aquaculture is one of the main activities, raise concerns about

future impacts of the bivalve production (e.g. Manfrin et al., 2015). Moreover, its feeding habits are alarming regarding the effects they might have on fisheries production of locally important resources (e.g. Vasconcelos et al., 2019). For example, in the Venice Lagoon and in the Lesina Lagoon (Puglia region) *Carcinus aestuarii* is a valuable shellfish product and established populations of *C. sapidus* in the area might impact the fisheries of *C. aestuarii* (GM, per. obser.). In the Atlantic and Continental biogeographic regions where the magnitude of establishment is still under consideration (see also ANNEX VIII), the anticipated pressure on commercial species (e.g. *Carcinus maenas*, *Magallana gigas*; Nehring et al., 2008, Pezy et al., 2019) is expected to be significant.

Impacts on Regulation-maintenance as well as Cultural services, as outlined in Qu. 4.7, may also occur.

Economic impacts

Qu. 4.9. How great is the overall economic cost caused by the organism within its current area of distribution (excluding the risk assessment area), including both costs of / loss due to damage and the cost of current management.

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the world these should be reported here. The assessment of the potential costs of / loss due to damage shall describe those costs quantitatively and/or qualitatively depending on what information is available. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage. As far as possible, it would be useful to separate costs of / loss due to the organism from costs of current management.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: In the Bardawil lagoon (Egypt), inconclusive evidences (see Qu. 4.2) suggest that the presence, establishment, and increase in abundance of *C. sapidus* had impacts on economically important fish species (Mehanna et al., 2019; 2020 and references therein). Before the 1990’s, the lagoon had a significant production of high valued fish species (e.g. seabream, flathead grey mullet and thinlip grey mullet). Yet in the early 1990’s, a period that coincides with the establishment of *C. sapidus* in the area, the fish catches declined significantly, with the crustacean ones – including *C. sapidus* as well as *Portunus segnis* catches -rising as high as 60% of the total yield production of Bardawil Lagoon, a fact that is also related to other factors as: change in the ecological conditions of the lagoon, uncontrolled fish exporting policy and overexploitation of resources (Mehanna et al., 2019). In order to mitigate the losses from the fish, *C. sapidus* is now an exploitable resource and it supports a valuable commercial fishery in Bardawil lagoon, mitigating the economic losses determined by the decrease observed in the stocks of other species of economic interest. In Turkey, *C. sapidus* has well established populations, yet, the economic impact on fisheries has not been assessed; nevertheless, fishers complain on gear damages and impacts on the native fish through predation and changing the food webs (AS Ateş, pers. comm).

Qu. 4.10. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism currently in the risk assessment area (include any past costs in your response)?

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the EU these should be reported here. Assessment of the potential costs of damage on human health, safety, and the economy, including the cost of non-action. A full economic assessment at EU scale might not be possible, but qualitative data or different case studies from across the EU (or third countries if relevant) may provide useful information to inform decision makers. In absence of specific studies or other direct evidence this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response

In general, the blue crab has been claimed to be responsible for destruction of fishing gear and partial consumption of fish being caught in the gears (e.g. Dulčić et al., 2011; Katselis & Koutsikopoulos, 2017; Tutman et al., 2017b; Fuentes et al., 2019; Kampouris et al., 2019); in addition, the species preys upon bivalve species of economic interest as the Pacific oyster *Magallana gigas*, the blue mussel *Mytilus* spp. (e.g. Labruno et al., 2019) as well as on cuttlefish eggs (Martins et al., 2018). This is expected to considerably reduce catches of species of economic interest, as observed in the Ebro Delta (Clavero et al., 2020). There is currently no assessment of economic costs/losses, present or future. Nevertheless, actions to estimate these losses have been planned in the framework of running scientific projects (e.g. InvasiBES – Gallardo et al., 2019). Yet unpublished results from this project indicate that some fishers from the Ebro Delta have changed their way of fishing in order to avoid encounters, or started to capture the blue crab. Similarly, the population decline in many bivalve species, probably associated with the population increase of *C. sapidus*, is affecting many shellfish harvesters in the labor market (R. Bernardo-Madrid, pers.comm., May 2021). It should be noted here that bivalve cultivation constitutes one of the main local economic activities in the region (Prado et al., 2020).

In an unpublished survey conducted in Greece using questionnaires regarding the socio-economic impacts of alien species (Galanidi, Zenetos, Gavriil & Karachle, unpub. data), with respect to *C. sapidus*, professional small-scale fishers provided the following information: (a) approximately 42.5% of the fishers (28 out of the 66) captured the species; (b) 12 of them changed their way of fishing in order to avoid encounters with the species and damages to the nets and their catches; (c) 61 fishers reported economic losses ranging from 50 to 6500 euros per year per person (mean=1366; median=850 euros). It should be noted that in some member states blue crab fisheries are or will be allowed (e.g., Greece, Spain), a fact that may provide a counterbalance to monetary losses due to impacts on fishing gear or native species of economic interest. For example, in the Neretva Estuary (Croatia), no estimates of losses due to e.g., damage of fishing gear by blue crabs have been estimated, while a considerable (estimated

in the range of 20-30%) decrease in the catches of fish species of economic interest determined after the invasion of *C. sapidus*; however, a total income of approximately 7000 € for the July-December period has been estimated from the marketing of the blue crab (Glamuzina et al., 2020).

Qu. 4.11. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.10.

RESPONSE	minimal	CONFIDENCE	low
	minor		medium
	moderate		high
	major		
	massive		

Response: There are currently no exact estimates of the economic costs/losses, present or future for the RA area, yet actions to estimate these losses are planned within the framework of scientific projects (e.g. InvasiBES – Gallardo et al., 2019). The estimates provided in Qu. 4.10 for Greece, where the species has a long history of establishment, can be taken as a reference and extended to other countries where the blue crab distribution is currently expanding.

Based on the existing literature on the feeding habits and potential competition of *C. sapidus* with mollusks, crabs and fish of commercial interest (see also Qu 4.2, 4.4, 4.7), it is very likely that the species will cause economic losses to both the fishery and mussel/oyster culture sectors. It should be noted that in some member states blue crab fisheries are or will be allowed (e.g., Greece, Spain), a fact that may help counterbalance any monetary losses due to impacts on native species of economic interest (see Qu. 4.10). The UK, government policy currently does not permit the exploitation of such fisheries, as in the case of *Eriocheir sinensis* that there is the current ban on exploiting it where established.

Qu. 4.12. How great are the economic costs / losses associated with managing this organism currently in the risk assessment area (include any past costs in your response)?

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	minimal	CONFIDENCE	low
	minor		medium
	moderate		high
	major		N/A
	massive		
	N/A		

Response: Currently, *C. sapidus* is not managed in the RA area, and no information is available on the issue. Accordingly, the economic costs related to management activities are expected to be negligible, with a high confidence.

Qu. 4.13. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.12.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No information has been found on the issue. Managing the species might be achieved through fishing, as a potential fisheries metier that will result to a new income source for the fishers. Nevertheless, this might be an option for some of the fishers and not for all of them (see also Qu 4.12). On the other hand, bivalve growers are expected to implement management measures to avoid/minimize predation losses by the blue crab. A variety of methods are available, including protective netting or cages, selecting seed size, stocking density etc. (e.g. see Grosholz & Ruiz, 2002) and their cost and effectiveness will depend on the species and cultivation method. Furthermore, a ban of imports or restrictions in the movement of shellfish seed/stock from infected areas could have potentially significant economic implications for bivalve producers.

It should be noted however, that bioaccumulation of heavy metals and other chemical contaminants (e.g. Türkmen et al., 2006; Mutlu et al., 2011; Genc & Yilmaz, 2015; Zotti et al., 2016; Çoğun et al., 2017) may also limit the potential for fisheries to be used as a management method, where incursions occur in industrialised water bodies with poor water quality.

Social and human health impacts

Qu. 4.14. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism for the risk assessment area and for third countries, if relevant (e.g. with similar eco-climatic conditions).

The description of the known impact and the assessment of potential future impact on human health, safety and the economy, shall, if relevant, include information on

- illnesses, allergies or other affections to humans that may derive directly or indirectly from a species;
- damages provoked directly or indirectly by a species with consequences for the safety of people, property or infrastructure;
- direct or indirect disruption of, or other consequences for, an economic or social activity due to the presence of a species.

Social and human health impacts can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Within the risk assessment area, the species poses a threat to fishers, due to its aggressive behavior that could lead to injuries especially when they are trying to remove it from the fishing gear (e.g. Milori et al., 2017; Galanidi, Zenetos, Gavriil & Karachle, unpub. data).

Based on a recent questionnaire-based survey on recreational fishers (Cerri et al., 2020) in Italy, Croatia and Montenegro, the perceived impacts of the species on fisheries and the environment were not clear for half of the participants. Only a small percentage could see negative impacts, whereas there was only a minority that anticipated positive impacts. Nevertheless, the survey clearly showed that the higher the abundance of the species, the more intense is the perception of *C. sapidus* as a threat to fisheries and the environment.

In its native area of distribution, several studies on the presence of heavy metals in various tissues have been conducted. It has been found that the accumulation of heavy metals varies between different areas and the accumulation is different in the various tissues examined (e.g. Adams & Engel, 2014 and references therein). Moreover, as humans consume *C. sapidus*, bioaccumulation of heavy metals is considered an important issue on human health (Lincoln et al., 2011). *Callinectes sapidus* has been found to be a carrier of *Vibrio cholerae* strains and has been related to outbreaks of human cholera in the USA (e.g. Welsh & Sizemore, 1985; Huq et al., 1986; Hill et al., 1989; Nehring, 2011). Yet, proper storing, processing and cooking will prevent any threats on human health (Hill et al., 1989). Therefore, should appropriate measures be taken, the likelihood of such incidences within the risk assessment area, could be considered as low.

Several studies exist on heavy metal bio-accumulation of *C. sapidus* from the environment (e.g. Türkmen et al., 2006; Mutlu et al., 2011; Genc & Yilmaz, 2015; Zotti et al., 2016; Çoğun et al., 2017), yet impact is considered as negligible.

Qu. 4.15. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism in the future for the risk assessment area.

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	minimal minor moderate major	CONFIDENCE	low medium high
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	massive		
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Response: Given that in its native distribution the species has been related to outbreaks of human cholera (see Qu 4.14), *C. sapidus* could have negative effects on tourism and human health in the risk assessment area. Moreover, despite the fact that intoxication of heavy metal bioaccumulation in *C. sapidus* has not been recorded yet in the RA area, it might still be a threat to human health, and therefore harvesting of the species for consumption should be monitored and follow procedures similar to those for shellfish products (Zotti et al., 2016).

Other impacts

Qu. 4.16. How important is the organism in facilitating other damaging organisms (e.g. diseases) as food source, a host, a symbiont or a vector etc.?

RESPONSE	minimal	CONFIDENCE	low
	minor		medium
	moderate		high
	major		
	massive		

Response: Several diseases, parasites, and commensals have been recorded to infect *C. sapidus* in its native range in the East coasts of the USA (for an extended account see Millikin & Williams, 1984, Flowers et al., 2016, and references therein). In the risk assessment area, apart from the unconfirmed record of the parasitic dinoflagellate *Hematodinium* sp. in Italy (Mancinelli et al., 2013b), nowadays several bacteria, parasites and fungi are reported for the species (Aldik & Cengizler, 2017). Overall, 21 bacteria have been found in hemolymph samples, including four *Vibrio* species, yet not *V. cholerae* (Aldik & Cengizler, 2017). In areas of its native distribution *C. sapidus* is a carrier of *V. cholerae* (see also Qu 4.14). To date in the risk assessment area, no records of infected *C. sapidus* have been made, but the possibility cannot be ruled out. Moreover, nine parasites and two fungi have also been identified in the species (Aldik & Cengizler, 2017). Nevertheless, none of the parasites found in *C. sapidus* is alien, and impacts on other species by transfer of diseases from *C. sapidus* has not been reported. Czerniejewski et al (2020) report *Trichodina* sp. on a *C. sapidus* specimen from the Baltic Sea. Protozoa of the genus *Trichodina* are common parasites of gills and body surfaces of fish in Western Pomerania. Thus *C. sapidus* could act as a vector spreading this parasite in the reservoir and thus could contribute to its transmission to fish, especially because *Trichodina* sp. proliferates exponentially and in favorable conditions a single specimen can reproduce very quickly by division and start a new invasion.

Qu. 4.17. How important might other impacts not already covered by previous questions be resulting from introduction of the organism?

RESPONSE	minimal	CONFIDENCE	low
	minor		medium

	moderate major massive		high
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Response: No additional impacts could be identified.

Qu. 4.18. How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area?

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: The species in its native distribution areas has many natural predators (see Qu 2. 4, Qu. 4.1) as well as a number of parasites and diseases (see Qu 4.16). However, despite the fact that in the risk assessment area several of its natural predators exist (see also Qu. 4.3) and its contamination by parasites and diseases has been documented, their impact on *C. sapidus* has not been documented to date. Nevertheless, given the impacts of parasites and diseases on the wellbeing and status of *C. sapidus* stock in its native areas (e.g. Shields, 2003; CBSAC, 2014), such effects could be anticipated in the risk assessment area, and a reduction in the abundance and overall biomass might be observed.

Qu. 4.19. Estimate the overall impact in the risk assessment area under current climate conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, in current conditions.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: The bulk of information regarding *C. sapidus* impacts on various aspects (i.e. biodiversity, areas of protection, cultural elements, socio-economics) exists from the Mediterranean biogeographical region, where it has a long invasion history and such impacts are apparent. The species is a voracious predator, feeding on a wide range of native animal and vegetal items occurring in invaded marine environments (Kampouris et al., 2019). Stable isotope studies identify *C. sapidus* as an omnivore feeding at multiple trophic levels; accordingly, a high ecological impact is expected to be exerted on native

benthic assemblages throughout its ontogeny (e.g. Nehring, 2011; Mancinelli et al., 2016). Molluscan, crustacean, and fish species (including those of economic and/or conservation interest) are the groups that should experience the major predatory impact; besides predation on other species, *C. sapidus* is highly competitive for food resources, with a diet overlapping with that of native crustacean and fish species (Mancinelli et al., 2013a; Mancinelli et al., 2016). In particular, the blue crab is assumed to outcompete indigenous species of crabs (e.g. Gennaio et al., 2006; Mancinelli et al., 2013a; Petović et al., 2018; Kampouris et al., 2019), as confirmed by recent research (e.g. Kampouris et al., 2019). In addition, with respect to effects on habitats of conservation value, *C. sapidus* has also the potential to impact seagrass beds (listed under the code A5.53 in the EUNIS habitat type classification; related to habitat types 1110 and 1120, of the Annex I of the Habitats Directive) by bioturbation, even though only anecdotal information is currently available and no quantitative studies have been carried out to date in the RA area.

With respect to ecosystem services, the aggressive behavior of *C. sapidus* combined with its voracious character, makes it a pest for the fisheries and aquaculture sector. The species has been recognized as responsible for destruction of fishing gear and reduced fisheries yields. Moreover, its aggressiveness and size poses a threat to the health and safety of fishers, as captured crabs might cause injuries especially when they are manipulated during retrieval of fishing gears. However, the perceived impacts of the species on fisheries and the environment of recreational fishers (Cerri et al., 2020: Italy, Croatia and Montenegro) are not yet clear.

To-date, the impact of *C. sapidus* to human health in the RA area is not assessed. In its native distribution area some relation of the species with outbreaks of human cholera (e.g. Welsh & Sizemore, 1985; Huq et al., 1986; Hill et al., 1989) has been reported. Heavy metal bioaccumulation of the species in the Mediterranean has been studied, yet low levels of contamination have been measured in the RA area (Zotti et al., 2016), and the potential impact on human health is to date considered negligible. Finally, *C. sapidus* could act as a vector spreading a parasite such as *Trichodina* sp. in reservoirs and thus could contribute to its transmission to fish, especially because *Trichodina* sp. proliferates exponentially and in favorable conditions a single specimen can reproduce very quickly by division and start a new invasion (Czerniejewski et al., 2020).

It should be noted that impacts from the sparse and isolated populations in the North Sea have not been reported and are not likely to have been significant due to the low population sizes.

Qu. 4.20. Estimate the overall impact in the risk assessment area in foreseeable climate change conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, under future conditions.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: A predicted increase in seawater temperatures under foreseeable climate change conditions is anticipated to lead to a northward expansion/establishment of the species, extending the areas at risk

from localized strong populations and associated impacts in the Mediterranean and increasing the respective risks in Atlantic Europe and the Black Sea.

Provided the overall recorded and perceived impacts of *C. sapidus* on biodiversity and ecosystems (Qu. 4.18 and 4.19), its presence and further expansion in national nature conservation legislation sites (areas of conservation) is likely to cause a significant decline in their overall value (e.g. biodiversity loss due to competition with native species and/or predation; seagrass bed degradation due to bioturbation). In the Atlantic, under the light of population establishment (e.g. Bay of Seine: Pezy et al., 2019) questions are rising regarding the future impacts of *C. sapidus* and the significance of the anticipated pressure on (a) local biodiversity (b) native brachyurans (e.g. *Carcinus maenas* or *Hemigrapsus sanguineus*), (c) crustacean and bivalve species of economic interest (e.g. *Carcinus maenas*, *Magallana gigas*). Similarly, in the Mediterranean, the spread of the species in the Adriatic basin and recent establishment in lagoons and other transitional waters of north Italy, where clam and mussel aquaculture together with *Carcinus aestuarii* harvest represent activities of prime economic interest, raise serious concerns about future impacts, concerns that are beginning to be shared with other countries such as Spain experiencing a burst in the occurrence and abundance of the species. Finally, in the Black Sea area, the future establishment of thriving populations of *C. sapidus* is expected to impact on all levels of biodiversity, yet the effects on the structure of native organisms' communities is not considered to be of important magnitude (e.g. for the Romanian littoral, see Skolka & Preda, 2010).

RISK SUMMARIES			
	RESPONSE	CONFIDENCE	COMMENT
Summarise Introduction and Entry*	very unlikely unlikely moderately likely likely very likely	low medium high	<i>Callinectes sapidus</i> has already been introduced in the RA area, presumably multiple times at different locations (Mediterranean Sea, North Sea, Black Sea, Iberian Coast and the Bay of Biscay). A variety of pathways have been proposed, with existing evidence indicating that ship-mediated vector (ballast waters and sediment) is the most likely means of introduction. Transfer in ship hulls is also a plausible, yet undocumented mode of introduction. Accidental escape from tanks or other packing where it is kept alive via the live food trade has also been suggested as a possible pathway and is considered still active, albeit less likely to contribute high propagule pressure. Similarly, introductions via rafting on floating debris, while possible, are not considered a likely event. Further introductions are very likely, given the high shipping traffic between the native area (east US coast) and EU ports and the ability of the species to survive during transport. Full implementation of the BWMC by 2024 is expected to greatly reduce the likelihood of introduction via ballast water.
Summarise Establishment*	very unlikely unlikely moderately likely likely very likely	low medium high	<i>Callinectes sapidus</i> is already established in the RA area, with locally abundant populations, particularly in the Mediterranean Sea and the southern Iberian coast. The species has also established localised populations along the southern North Sea coast and appears to be established in the Black Sea. Under current climate conditions, further establishment is very likely in the western Mediterranean, further along the Iberian coast & the Bay of Biscay, while more local populations are also likely to establish in the Greater North Sea. Establishment in the Baltic Sea is expected to be prevented by low salinities and low winter temperatures, and in the

			Celtic Sea by low summer temperatures, conditions which prevent successful larval development. Under future climate conditions, further establishment may be expected in the North Sea and the southern Celtic Seas, where an increase in summer temperatures will likely allow larval survival over a wider area. Colonisation of the western Baltic may also be possible.
Summarise Spread*	very slowly slowly moderately rapidly very rapidly	low medium high	The increasing rate of appearance of new records, especially in the Mediterranean Sea, indicates a rapid rate of spread, which is most likely attributable to natural dispersal, although the contribution of other pathways of spread cannot be overlooked. In the Black Sea, human-assisted mechanisms of spread, particularly through shipping vectors and/or the live food trade and intentional releases for commercial exploitation are likely to maintain a moderately rapid rate of spread. In the Greater North Sea, due to the limited availability of suitable climatic conditions, anthropogenic pathways of spread are expected to be primarily responsible for the spreading of the species. Due to the high shipping traffic volume and the precedence of live food trade escape, a rapid rate of spread may be expected.
Summarise Impact*	minimal minor moderate major massive	low medium high	The species has a significant impact on biodiversity and ecosystem services (especially fisheries and aquaculture), mainly due to its size, aggressiveness, omnivorous feeding habits, and high adaptability to coastal habitats characterized by a wide range of salinities and temperatures. Moreover, its ethology (e.g. ambushing) and ecology (e.g. borrowing to avoid low temperatures during winter months) may exert negative impacts on seagrasses. These impacts have been demonstrated in the Mediterranean Sea, where it has been present for a long time and evidence exists, confirming and extending a wide literature available from native areas. In the future, the above

			mentioned impacts are anticipated in other areas where the species will expand its distribution.
Conclusion of the risk assessment (overall risk)	low moderate high	low medium high	<i>Callinectes sapidus</i> has already been established in the RA area (Mediterranean Sea, Iberian Coast and the Bay of Biscay, Black Sea, North Sea very localised) while it has exhibited invasive behaviour in the Mediterranean only. A variety of pathways have been implicated, with existing evidence indicating that ship-mediated vectors (ballast waters and sediment) is the most likely means of introduction. Transfer in ship hulls is also a plausible, yet undocumented mode of introduction. Accidental escape from the live food trade, introductions via rafting on floating debris, are also considered as potential pathway vectors. The aforementioned pathways along with natural dispersal are expected to lead to more local populations likely to establish in the Greater North Sea. Establishment in the Baltic Sea is expected to be prevented by low salinities and temperature. Under future climate conditions, further establishment may be expected in the North Sea and the southern Celtic Seas whereas colonisation of the western Baltic may also be possible. Considering its predatory and aggressive characteristics <i>C. sapidus</i> has a moderate impact on biodiversity and ecosystem services (especially fisheries and aquaculture), and thus it is considered a high-risk species for the EU.

*in current climate conditions and in foreseeable future climate conditions

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On-line resources

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DISTRIBUTION SUMMARY

Please answer as follows:

- Yes if recorded, established or invasive
- if not recorded, established or invasive
- ? Unknown; data deficient

The columns refer to the answers to Questions A5 to A12 under Section A.

For data on marine species at the Member State level, delete Member States that have no marine borders.

In all other cases, provide answers for all columns.

Member States and the United Kingdom

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Belgium	yes	yes	Yes	Yes	-
Bulgaria	yes	yes	Yes	-	-
Croatia	yes	yes	Yes	Yes	-
Cyprus	-	-	Yes	Yes	-
Denmark	yes	-	-	Yes	-
Estonia	-	-	-	-	-
Finland	-	-	-	-	-
France	yes	yes	Yes	Yes	-
Germany	yes	-	Yes	Yes	-
Greece	yes	yes	Yes	Yes	yes
Ireland	-	-	-	-	-
Italy	yes	yes	Yes	Yes	yes
Latvia	-	-	-	-	-
Lithuania	-	-	-	-	-
Malta	-	-	Yes	Yes	-
Netherlands	yes	-	Yes	Yes	-
Poland	yes	-	-	-	-
Portugal	yes	yes	Yes	Yes	-
Romania	yes	-	-	-	-
Slovenia	-	-	Yes	Yes	-
Spain	yes	yes	Yes	Yes	yes
Sweden	-	-	-	Yes	-
United Kingdom	yes	-	-	Yes	-

Marine regions and subregions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Baltic Sea	yes	no	yes	no	no
Black Sea	yes	yes	yes	yes	no
North-east Atlantic Ocean					
Bay of Biscay and the Iberian Coast	yes	yes	yes	yes	No potentially invasive in Portugal on southern coast
Celtic Sea	no	no	no	yes	no
Greater North Sea	yes	yes (localised)	yes (localised)	yes	no
Mediterranean Sea					
Adriatic Sea	yes	yes	yes	yes	yes
Aegean-Levantine Sea	yes	yes	yes	yes	yes
Ionian Sea and the Central Mediterranean Sea	yes	yes	yes	yes	yes
Western Mediterranean Sea	yes	yes	yes	yes	yes

ANNEX I Scoring of Likelihoods of Events

(taken from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Description	Frequency
Very unlikely	This sort of event is theoretically possible, but is never known to have occurred and is not expected to occur	1 in 10,000 years
Unlikely	This sort of event has occurred somewhere at least once in the last millenium	1 in 1,000 years
Moderately likely	This sort of event has occurred somewhere at least once in the last century	1 in 100 years
Likely	This sort of event has happened on several occasions elsewhere, or on at least once in the last decade	1 in 10 years
Very likely	This sort of event happens continually and would be expected to occur	Once a year

ANNEX II Scoring of Magnitude of Impacts

(modified from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Biodiversity and ecosystem impact	Ecosystem Services impact	Economic impact (Monetary loss and response costs per year)	Social and human health impact, and other impacts
	<i>Question 5.1-5</i>	<i>Question 5.6-8</i>	<i>Question 5.9-13</i>	<i>Question 5.14-18</i>
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected ⁵	Up to 10,000 Euro	No social disruption. Local, mild, short-term reversible effects to individuals.
Minor	Some ecosystem impact, reversible changes, localised	Local and temporary, reversible effects to one or few services	10,000-100,000 Euro	Significant concern expressed at local level. Mild short-term reversible effects to identifiable groups, localised.
Moderate	Measureable long-term damage to populations and ecosystem, but reversible; little spread, no extinction	Measureable, temporary, local and reversible effects on one or several services	100,000-1,000,000 Euro	Temporary changes to normal activities at local level. Minor irreversible effects and/or larger numbers covered by reversible effects, localised.
Major	Long-term irreversible ecosystem change, spreading beyond local area	Local and irreversible or widespread and reversible effects on one / several services	1,000,000-10,000,000 Euro	Some permanent change of activity locally, concern expressed over wider area. Significant irreversible effects locally or reversible effects over large area.
Massive	Widespread, long-term population loss or extinction, affecting several species with serious ecosystem effects	Widespread and irreversible effects on one / several services	Above 10,000,000 Euro	Long-term social change, significant loss of employment, migration from affected area. Widespread, severe, long-term, irreversible health effects.

⁵ Not to be confused with “no impact”.

ANNEX III Scoring of Confidence Levels

(modified from Bacher et al. 2017)

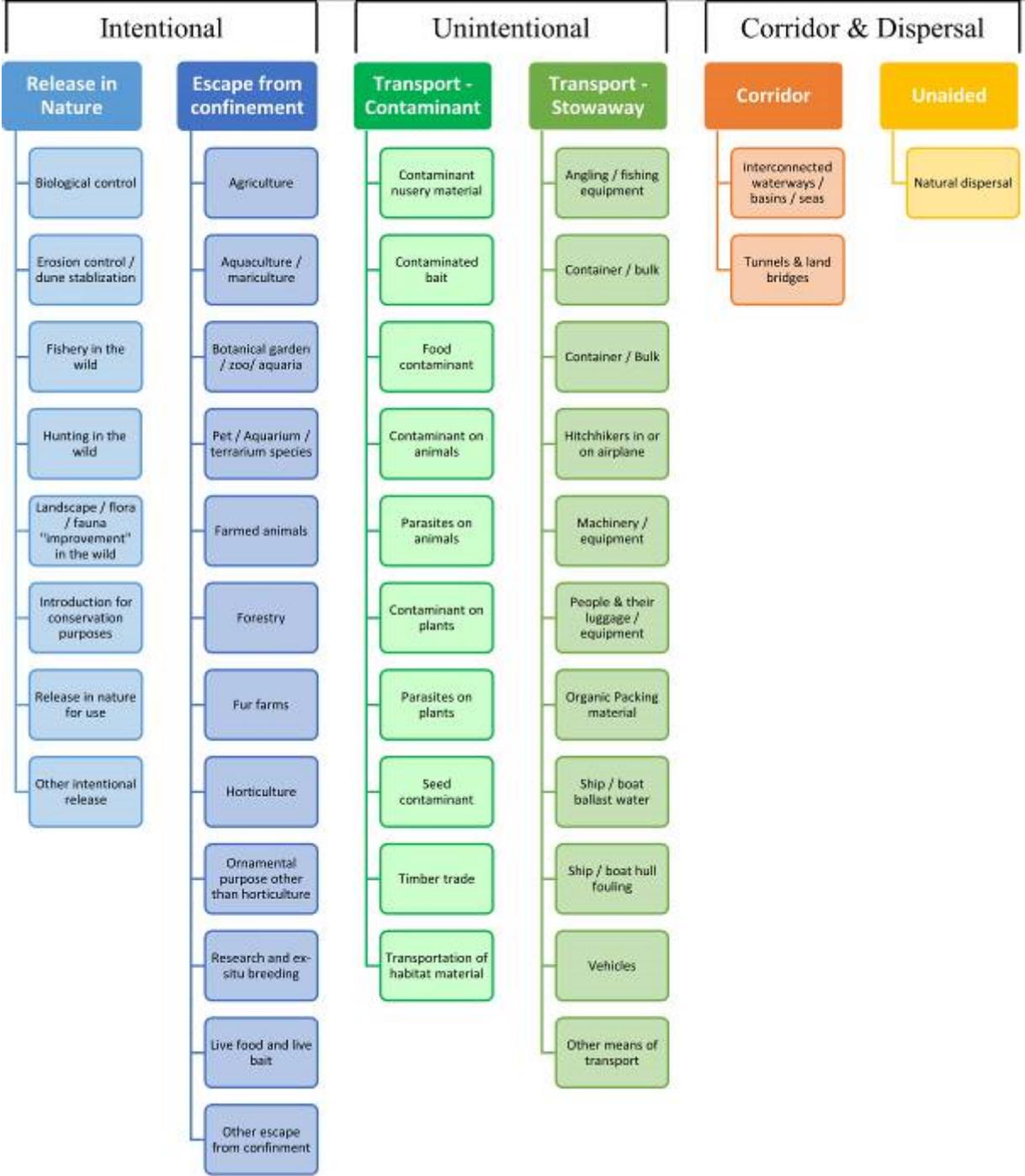
Each answer provided in the risk assessment must include an assessment of the level of confidence attached to that answer, reflecting the possibility that information needed for the answer is not available or is insufficient or available but conflicting.

The responses in the risk assessment should clearly support the choice of the confidence level.

Confidence level	Description
Low	There is no direct observational evidence to support the assessment, e.g. only inferred data have been used as supporting evidence <i>and/or</i> Impacts are recorded at a spatial scale which is unlikely to be relevant to the assessment area <i>and/or</i> Evidence is poor and difficult to interpret, e.g. because it is strongly ambiguous <i>and/or</i> The information sources are considered to be of low quality or contain information that is unreliable.
Medium	There is some direct observational evidence to support the assessment, but some information is inferred <i>and/or</i> Impacts are recorded at a small spatial scale, but rescaling of the data to relevant scales of the assessment area is considered reliable, or to embrace little uncertainty <i>and/or</i> The interpretation of the data is to some extent ambiguous or contradictory.
High	There is direct relevant observational evidence to support the assessment (including causality) <i>and</i> Impacts are recorded at a comparable scale <i>and/or</i> There are reliable/good quality data sources on impacts of the taxa <i>and</i> The interpretation of data/information is straightforward <i>and/or</i> Data/information are not controversial or contradictory.

ANNEX IV CBD pathway categorisation scheme

Overview of CBD pathway categorisation scheme showing how the 44 pathways relate to the six main pathway categories. All of the pathways can be broadly classified into 1) those that involve intentional transport (blue), 2) those in which the taxa are unintentionally transported (green) and 3) those where taxa moved between regions without direct transportation by humans and/or via artificial corridors (orange and yellow). **Note that the pathways in the category “Escape from confinement” can be considered intentional for the introduction into the risk assessment area and unintentional for the entry into the environment.**



ANNEX V Ecosystem services classification (CICES V5.1, simplified) and examples

For the purposes of this risk assessment, please feel free to use what seems as the most appropriate category / level / combination of impact (Section – Division – Group), reflecting information available.

Section	Division	Group	Examples (i.e. relevant CICES “classes”)
Provisioning	Biomass	Cultivated terrestrial plants	<p>Cultivated terrestrial plants (including fungi, algae) grown for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from cultivated plants, fungi, algae and bacteria for direct use or processing (excluding genetic materials); Cultivated plants (including fungi, algae) grown as a <u>source of energy</u></p> <p><i>Example: negative impacts of non-native organisms to crops, orchards, timber etc.</i></p>
		Cultivated aquatic plants	<p>Plants cultivated by in- situ aquaculture grown for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from in-situ aquaculture for direct use or processing (excluding genetic materials); Plants cultivated by in- situ aquaculture grown as an <u>energy source</u>.</p> <p><i>Example: negative impacts of non-native organisms to aquatic plants cultivated for nutrition, gardening etc. purposes.</i></p>
		Reared animals	<p>Animals reared for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from reared animals for direct use or processing (excluding genetic materials); Animals reared to provide <u>energy</u> (including mechanical)</p> <p><i>Example: negative impacts of non-native organisms to livestock</i></p>
		Reared aquatic animals	<p>Animals reared by in-situ aquaculture for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from animals grown by in-situ aquaculture for direct use or processing (excluding genetic materials); Animals reared by in-situ aquaculture as an <u>energy source</u></p> <p><i>Example: negative impacts of non-native organisms to fish farming</i></p>
		Wild plants (terrestrial and aquatic)	<p>Wild plants (terrestrial and aquatic, including fungi, algae) used for <u>nutrition</u>; <u>Fibres and other materials</u> from wild plants for direct use or processing (excluding genetic materials); Wild plants (terrestrial and aquatic, including fungi, algae) used as a <u>source of energy</u></p>

			<p><i>Example: reduction in the availability of wild plants (e.g. wild berries, ornamentals) due to non-native organisms (competition, spread of disease etc.)</i></p>
		Wild animals (terrestrial and aquatic)	<p>Wild animals (terrestrial and aquatic) used for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from wild animals for direct use or processing (excluding genetic materials); Wild animals (terrestrial and aquatic) used as a <u>source of energy</u></p> <p><i>Example: reduction in the availability of wild animals (e.g. fish stocks, game) due to non-native organisms (competition, predations, spread of disease etc.)</i></p>
	Genetic material from all biota	Genetic material from plants, algae or fungi	<p><u>Seeds, spores and other plant materials</u> collected for maintaining or establishing a population; Higher and lower plants (whole organisms) used to <u>breed new strains or varieties</u>; Individual genes extracted from higher and lower plants for the <u>design and construction of new biological entities</u></p> <p><i>Example: negative impacts of non-native organisms due to interbreeding</i></p>
		Genetic material from animals	<p>Animal material collected for the purposes of maintaining or establishing a population; Wild animals (whole organisms) used to breed new strains or varieties; Individual genes extracted from organisms for the design and construction of new biological entities</p> <p><i>Example: negative impacts of non-native organisms due to interbreeding</i></p>
	Water ⁶	Surface water used for nutrition, materials or energy	<p>Surface water for <u>drinking</u>; Surface water used as a material (<u>non-drinking purposes</u>); Freshwater surface water, coastal and marine water used as an <u>energy source</u></p> <p><i>Example: loss of access to surface water due to spread of non-native organisms</i></p>
		Ground water for used for nutrition, materials or energy	<p>Ground (and subsurface) water for <u>drinking</u>; Ground water (and subsurface) used as a material (<u>non-drinking purposes</u>); Ground water (and subsurface) used as an <u>energy source</u></p>

⁶ Note: in the CICES classification provisioning of water is considered as an abiotic service whereas the rest of ecosystem services listed here are considered biotic.

			<i>Example: reduced availability of ground water due to spread of non-native organisms and associated increase of ground water consumption by vegetation.</i>
Regulation & Maintenance	Transformation of biochemical or physical inputs to ecosystems	Mediation of wastes or toxic substances of anthropogenic origin by living processes	<u>Bio-remediation</u> by micro-organisms, algae, plants, and animals; <u>Filtration/sequestration/storage/accumulation</u> by micro-organisms, algae, plants, and animals <i>Example: changes caused by non-native organisms to ecosystem functioning and ability to filtrate etc. waste or toxics</i>
		Mediation of nuisances of anthropogenic origin	<u>Smell reduction</u> ; <u>noise attenuation</u> ; <u>visual screening</u> (e.g. by means of green infrastructure) <i>Example: changes caused by non-native organisms to ecosystem structure, leading to reduced ability to mediate nuisances.</i>
	Regulation of physical, chemical, biological conditions	Baseline flows and extreme event regulation	<u>Control of erosion rates</u> ; <u>Buffering and attenuation of mass movement</u> ; <u>Hydrological cycle and water flow regulation</u> (Including flood control, and coastal protection); <u>Wind protection</u> ; <u>Fire protection</u> <i>Example: changes caused by non-native organisms to ecosystem functioning or structure leading to, for example, destabilisation of soil, increased risk or intensity of wild fires etc.</i>
		Lifecycle maintenance, habitat and gene pool protection	<u>Pollination</u> (or 'gamete' dispersal in a marine context); <u>Seed dispersal</u> ; <u>Maintaining nursery populations and habitats</u> (Including gene pool protection) <i>Example: changes caused by non-native organisms to the abundance and/or distribution of wild pollinators; changes to the availability / quality of nursery habitats for fisheries</i>
Pest and disease control		<u>Pest control</u> ; <u>Disease control</u> <i>Example: changes caused by non-native organisms to the abundance and/or distribution of pests</i>	
		Soil quality regulation	<u>Weathering processes</u> and their effect on soil quality; <u>Decomposition and fixing processes</u> and their effect on soil quality <i>Example: changes caused by non-native organisms to vegetation structure and/or soil fauna leading to reduced soil quality</i>

		Water conditions	<p>Regulation of the <u>chemical condition</u> of freshwaters by living processes; Regulation of the chemical condition of salt waters by living processes</p> <p><i>Example: changes caused by non-native organisms to buffer strips along water courses that remove nutrients in runoff and/or fish communities that regulate the resilience and resistance of water bodies to eutrophication</i></p>
		Atmospheric composition and conditions	<p>Regulation of <u>chemical composition</u> of atmosphere and oceans; Regulation of <u>temperature and humidity</u>, including ventilation and transpiration</p> <p><i>Example: changes caused by non-native organisms to ecosystems' ability to sequester carbon and/or evaporative cooling (e.g. by urban trees)</i></p>
Cultural	Direct, in-situ and outdoor interactions with living systems that depend on presence in the environmental setting	Physical and experiential interactions with natural environment	<p>Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through <u>active or immersive interactions</u>;</p> <p>Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through <u>passive or observational interactions</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that make it attractive for recreation, wild life watching etc.</i></p>
		Intellectual and representative interactions with natural environment	<p>Characteristics of living systems that enable <u>scientific investigation</u> or the creation of traditional ecological knowledge;</p> <p>Characteristics of living systems that enable <u>education and training</u>;</p> <p>Characteristics of living systems that are resonant in terms of <u>culture or heritage</u>;</p> <p>Characteristics of living systems that enable <u>aesthetic experiences</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have cultural importance</i></p>
	Indirect, remote, often indoor interactions with living systems that do not require	Spiritual, symbolic and other interactions with natural environment	<p>Elements of living systems that have <u>symbolic meaning</u>;</p> <p>Elements of living systems that have <u>sacred or religious meaning</u>;</p> <p>Elements of living systems used for <u>entertainment or representation</u></p>

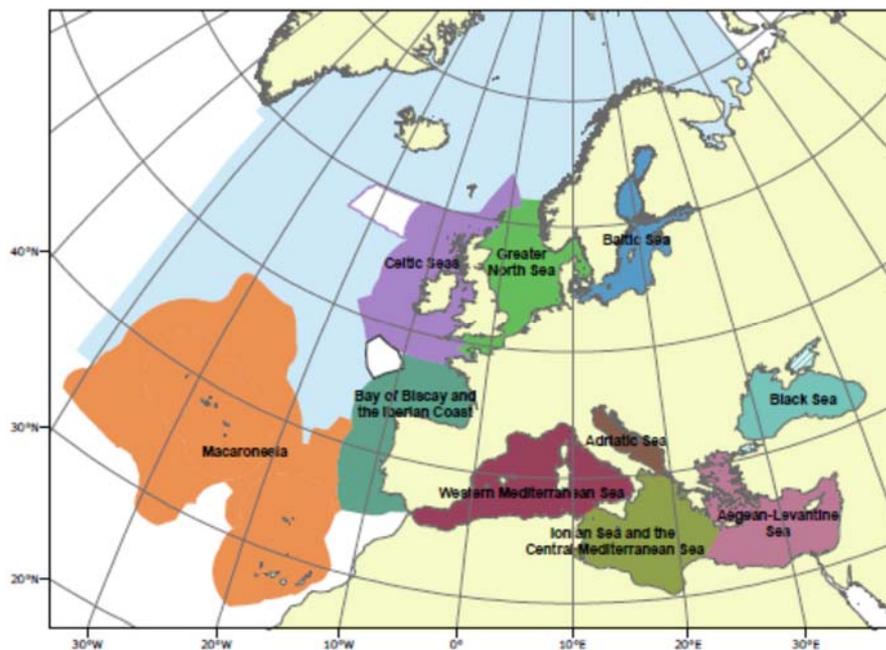
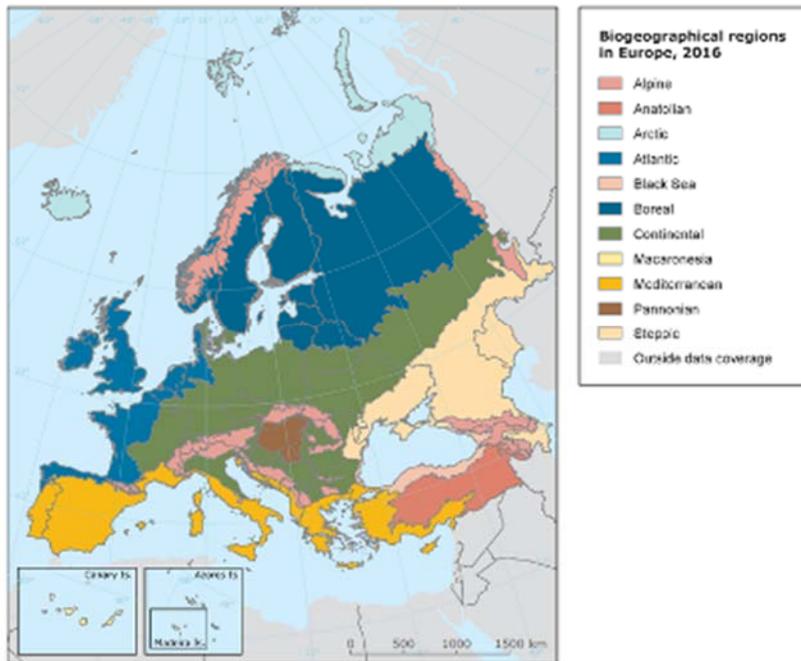
	presence in the environmental setting		<i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have sacred or religious meaning</i>
		Other biotic characteristics that have a non-use value	<p>Characteristics or features of living systems that have an <u>existence value</u>;</p> <p>Characteristics or features of living systems that have an <u>option or bequest value</u></p> <p><i>Example: changes caused by non-native organisms to ecosystems designated as wilderness areas, habitats of endangered species etc.</i></p>

ANNEX VI EU Biogeographic Regions and MSFD Subregions

See <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2>
http://ec.europa.eu/environment/nature/natura2000/biogeog_regions/

and

<https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions-1/technical-document/pdf>



ANNEX VII Delegated Regulation (EU) 2018/968 of 30 April 2018

see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>

ANNEX VIII *Callinectes sapidus* abiotic requirements and their availability in the Risk Assessment area

The native range of *C. sapidus* in western Atlantic spans two continents, extending almost continuously from New England to Argentina (Millikin & Williams, 1984; Nehring, 2011), i.e., from 38.6S to 57.7N. Accordingly, the species experiences latitudinal variations in water temperature comprised between 0°C in winter at the extremes of its range and 32-33°C in transitional systems of the Caribbean area (Rodríguez, 2001; Johnson, 2015). In addition, populations in temperate areas generally experience comparable seasonal variations: in Chesapeake Bay, where one of the most abundant population in the US is established, water temperatures range between 3 and 32°C (e.g., Shiah and Ducklow, 1994). Winter temperatures appear to limit the native distribution of the species at high latitudes in both hemispheres, reflecting the thermal tolerance of the megalopa stage, as well the sensitivity of juveniles and mature females to water temperatures < 3°C (Rome et al., 2005; Hines et al., 2010). Thus, a northward expansion, as that observed in native ranges, should be expected also in European waters. Nevertheless, as with many other decapod species, the pelagic larval stages of *C. sapidus* have stricter abiotic requirements compared with the benthic adult and juveniles. Thus, in laboratory experiments *C. sapidus* larvae completed their development to the first crab at a minimum salinity of 20.1 psu (Costlow & Bookhout, 1959). This threshold is supported by field studies, where *C. sapidus* zoeae have been collected at salinities down to 20 psu in Chesapeake Bay (Sandifer, 1973) and the Mississippi Sound (Perry, 1973). Surface salinities in the Baltic Sea (beyond the western Baltic) and the Black Sea are lower than this threshold, most likely posing a limit for establishment in these marine regions. This is supported by the current lack of establishment of the species there, despite the repeated occurrence of adult specimens during the past few decades, particularly in the Black Sea (see section A of the RA for details). Casual populations are not unlikely to persist, given wider physiological tolerances and the lifespan of the adults. This is a provisional assessment because the Black Sea, with salinities in the range of 14-19 psu, presents a marginal environment for the species and a possible adaptation to slightly lower salinities cannot be discounted –

Temperatures suitable for spawning and larval development are reported as between 19-29°C in the native range and in the laboratory (Hill et al., 1989). Additionally, hatching of fertilised eggs from a locally captured specimen was reported by Nehring & van der Meer (2010) in a German aquarium at 19°C, which is a normal ambient summer temperature for the German Wadden Sea.

Thus, limiting factors for establishment were considered as:

Average surface temperature of the coldest month > 3°C

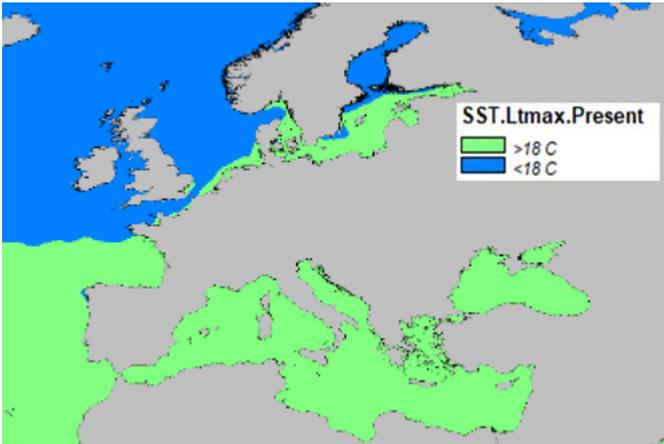
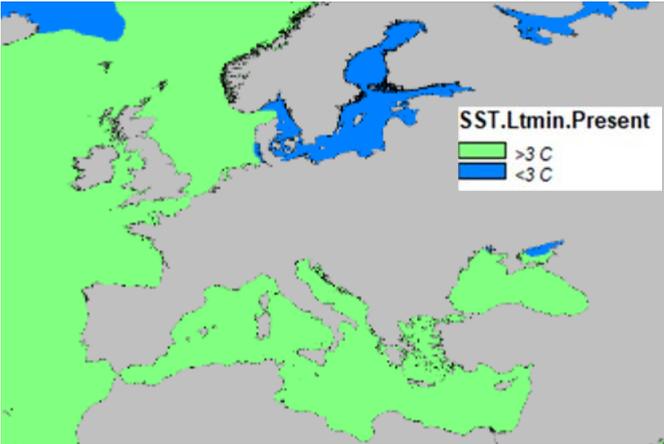
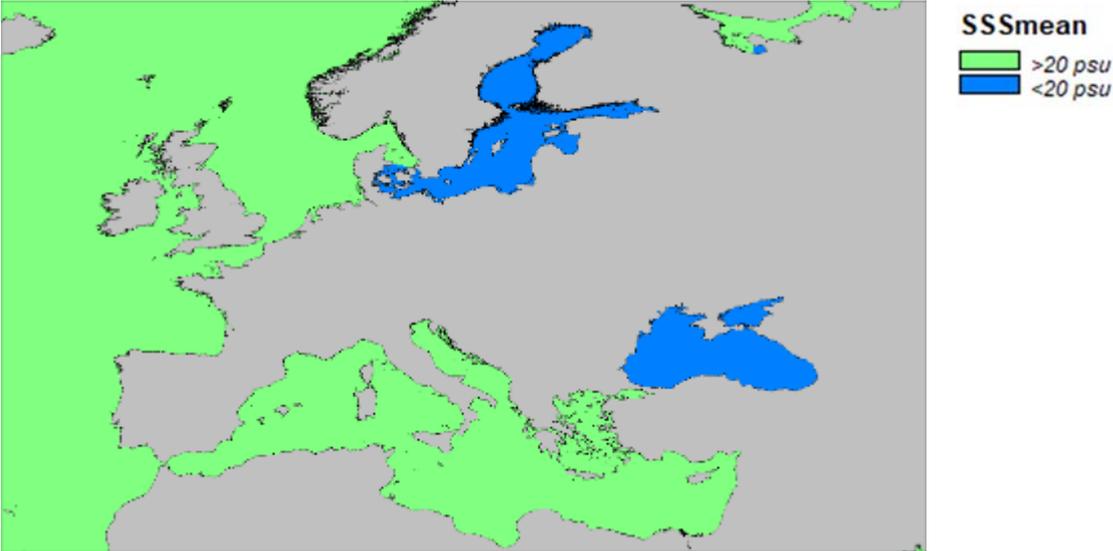
Average surface temperature of the warmest month > 18°C

Mean surface salinity > 20 psu

These limits are visualised in the maps below, prepared with data retrieved from BIO-ORACLE (Tyberghein et al., 2012, Assis et al. 2017).

Concerning the discrepancies between the produced binary maps and the predictions from the SDM

(see previous/following Annex), they are tentatively attributed to the following reasons; i) the high suitability of the low salinity marine subregions as predicted in the model is believed to be driven by an appreciable number of records in the Black Sea, which however refer to isolated captures of adult individuals. Additionally, small scale salinity variations are not likely to be reflected in the predictor data layer, which is modelled itself and not highly resolved, ii) the overwhelming contribution of the parameters bathymetry and maximum temperature to the model potentially masks the effect that minimum temperature can have on the predicted distribution of the species in areas where both parameters can act as limiting factors (i.e. the western Baltic Sea).



Accordingly, and taking into account the documented presence of the species currently in the RA area, the risk of establishment in the different marine subregions is assessed as follows:

Present climatic conditions

Baltic Sea: unlikely, high confidence (western Baltic – low winter temperatures)

Greater North Sea: likely, medium confidence (localized establishment, southern North Sea)

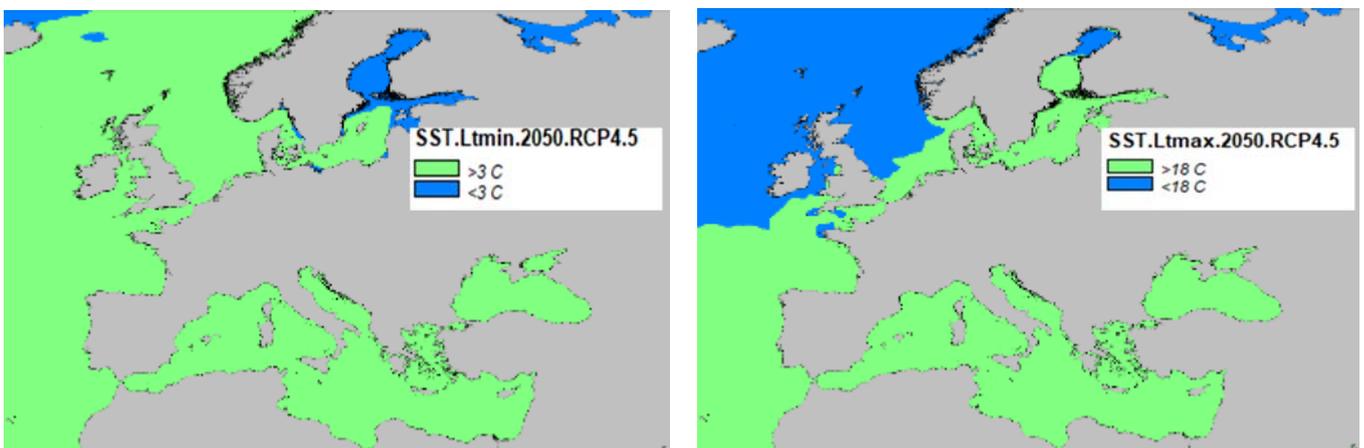
Celtic Seas: unlikely, medium confidence

Bay of Biscay and the Iberian coast: very likely, high confidence

Mediterranean Sea: very likely, high confidence

Black Sea: unlikely/moderately likely, medium confidence

Future climatic conditions



Baltic Sea: unlikely, high confidence (except for the western Baltic = likely)

Greater North Sea: very likely, high confidence

Celtic Seas: moderately likely, medium confidence

Bay of Biscay and the Iberian coast: very likely, high confidence

Mediterranean Sea: very likely, high confidence

Black Sea: unlikely/moderately likely, low confidence

ANNEX IX Species distribution model

Projection of environmental suitability for *Callinectes sapidus* establishment in Europe

Björn Beckmann, Argyro Zenetos, Giorgio Mancinelli, Marika Galanidi, Paraskevi Karachle and Dan Chapman

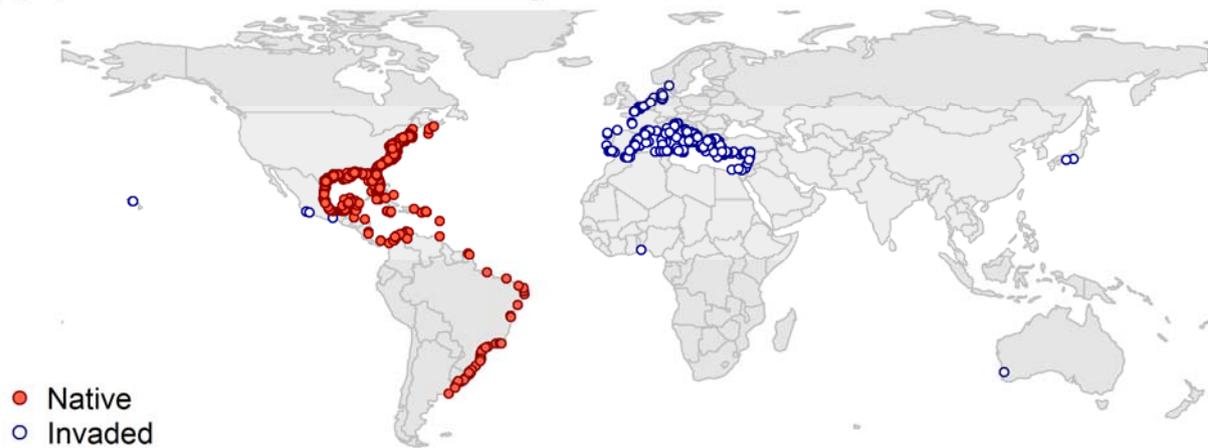
Aim

To project the suitability for potential establishment of *Callinectes sapidus* in Europe, under current and predicted future climatic conditions.

Data for modelling

Species occurrence data were obtained from a recently published dataset of global occurrences of *Callinectes sapidus* comprising verified records from the Global Biodiversity Information Facility (GBIF) (23678 records), the Ocean Biogeographic Information System (OBIS) (834 records), the Biodiversity Information Serving Our Nation database (BISON) (784 records), iNaturalist (44 records), and a further 549 records from 177 publications (Mancinelli et al. 2020). We removed any occurrence records where the georeferencing was too imprecise or outside of the coverage of the predictor layers. We also removed any records at locations with sea depths below -1000m, and any with mean sea surface salinity values below 20 PSU, because these are likely to represent dispersing individuals, not established populations. The records were gridded at a 0.25 x 0.25 degree resolution for modelling, yielding 820 grid cells with occurrences (Figure 1a). As a proxy for recording effort, the density of Decapoda records held by GBIF was also compiled on the same grid (Figure 1b).

(a) Species distribution used in modelling



(b) Estimated recording effort (log10-scaled)

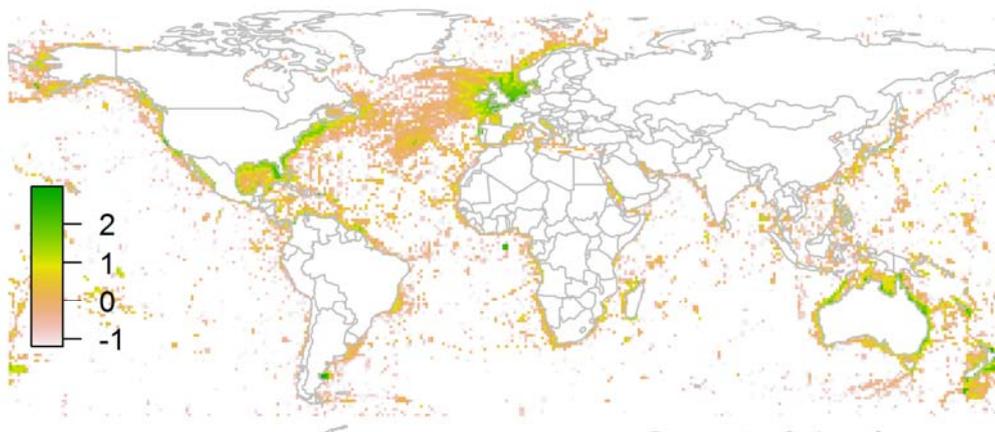


Figure 1. (a) Occurrence records obtained for *Callinectes sapidus* and used in the modelling, showing native and invaded distributions. (b) The recording density of Decapoda on GBIF, which was used as a proxy for recording effort.

Predictors describing the marine environment were selected from the ‘Bio-ORACLE2’ set of GIS rasters providing geophysical, biotic and environmental data for surface and benthic marine realms (Tyberghein et al., 2012, Assis et al. 2017), originally at 5 arcminute resolution (0.083 x 0.083 degrees of longitude/latitude) and aggregated to a 0.25 x 0.25 degree grid for use in the model.

Based on the biology of *Callinectes sapidus*, the following variables were used in the modelling:

- Maximum long-term temperature (templtmax_ss)
- Minimum long-term temperature (templtmin_ss)
- Mean salinity (salinitymean_ss)
- Mean bathymetry (bathymean)

All parameters (except depth) are measured at the sea surface.

To estimate the effect of climate change on the potential distribution of *Callinectes sapidus*, equivalent modelled future conditions for the 2070s under the Representative Concentration Pathways (RCP) 2.6 and 4.5 were also obtained. These represent low and medium emissions scenarios, respectively. Projections for the 2070s were calculated as averages of projections for the 2040s and 2090s (which are the time periods available on Bio-ORACLE).

Species distribution model

A presence-background (presence-only) ensemble modelling strategy was employed using the BIOMOD2 R package version 3.4.6 (Thuiller et al., 2020, Thuiller et al., 2009). These models contrast the environment at the species' occurrence locations against a random sample of the global background environmental conditions (often termed 'pseudo-absences') in order to characterise and project suitability for occurrence. This approach has been developed for distributions that are in equilibrium with the environment. Because invasive species' distributions are not at equilibrium and subject to dispersal constraints at a global scale, we took care to minimise the inclusion of locations suitable for the species but where it has not been able to disperse to (Chapman et al. 2019). Therefore the background sampling region included:

- The area accessible by native *Callinectes sapidus* populations, in which the species is likely to have had sufficient time to disperse to all locations. Based on presumed maximum dispersal distances, the accessible region was defined as a 1000km buffer around the native range occurrences (the Pacific Ocean was excluded from this area because *Callinectes sapidus* occurs only on the Atlantic coasts of North and South America and historically could not readily disperse to the Pacific side); AND
- A 150km buffer around the non-native occurrences, encompassing regions likely to have had high propagule pressure for introduction by humans and/or dispersal of the species; AND
- Regions where we have an *a priori* expectation of high unsuitability for the species so that absence is assumed irrespective of dispersal constraints (see Figure 2). The following rules were applied to define a region expected to be highly unsuitable for *Callinectes sapidus* at the spatial scale of the model:
 - Maximum long-term temperature (templtmax_ss) < 18°C
 - Minimum long-term temperature (templtmin_ss) < 3°C
 - Mean salinity (salinitymean_ss) < 20 PSU

Altogether, 4.8% of occurrence grid cells were located in the unsuitable background region.

Within the unsuitable background region, 10 samples of 5000 randomly sampled grid cells were obtained. In the accessible background (comprising the accessible areas around native and non-native occurrences as detailed above), the same number of pseudo-absence samples were drawn as there were presence records (820), weighting the sampling by a proxy for recording effort (Figure 2).

Figure 2. The background from which pseudo-absence samples were taken in the modelling of *Callinectes sapidus*. Samples were taken from a 1000km buffer around the native range and a 150km buffer around non-native occurrences (together forming the accessible background), and from areas expected to be highly unsuitable for the species (the unsuitable background region). Samples from the accessible background were weighted by a proxy for recording effort (Figure 1(b)).

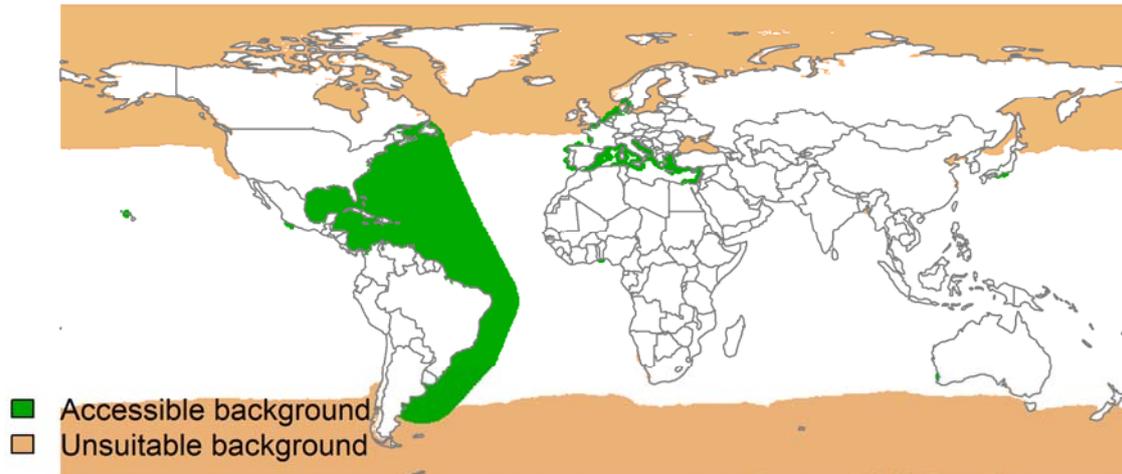


Figure 2. The background from which pseudo-absence samples were taken in the modelling of *Callinectes sapidus*. Samples were taken from a 1000km buffer around the native range and a 150km buffer around non-native occurrences (together forming the accessible background), and from areas expected to be highly unsuitable for the species (the unsuitable background region). Samples from the accessible background were weighted by a proxy for recording effort (Figure 1(b)).

Each dataset (i.e. combination of the presences and the individual background samples) was randomly split into 80% for model training and 20% for model evaluation. With each training dataset, five statistical algorithms were fitted with the default BIOMOD2 settings and rescaled using logistic regression, except where specified below:

- Generalised linear model (GLM)
- Generalised boosting model (GBM)
- Generalised additive model (GAM) with a maximum of four degrees of freedom per smoothing spline
- Random forest (RF)
- Maxent

Since the total background sample was larger than the number of occurrences, prevalence fitting weights were applied to give equal overall importance to the occurrences and the background. Normalised variable importance was assessed and variable response functions were produced using BIOMOD2's default procedure.

Model predictive performance was assessed by the following three measures:

- AUC, the area under the receiver operating characteristic curve (Fielding & Bell 1997). Predictions of presence-absence models can be compared with a subset of records set aside for model evaluation (here 20%) by constructing a confusion matrix with the number of true positive, false positive, false negative and true negative cases. For models generating non-dichotomous scores (as here) a threshold can be applied to transform the scores into a dichotomous set of presence-absence predictions. Two measures that can be derived from the confusion matrix are sensitivity (the proportion of observed presences that are predicted as such,

quantifying omission errors), and specificity (the proportion of observed absences that are predicted as such, quantifying commission errors). A receiver operating characteristic (ROC) curve can be constructed by using all possible thresholds to classify the scores into confusion matrices, obtaining sensitivity and specificity for each matrix, and plotting sensitivity against the corresponding proportion of false positives (equal to 1 - specificity). The use of all possible thresholds avoids the need for a selection of a single threshold, which is often arbitrary, and allows appreciation of the trade-off between sensitivity and specificity. The area under the ROC curve (AUC) is often used as a single threshold-independent measure for model performance (Manel, Williams & Ormerod 2001). AUC is the probability that a randomly selected presence has a higher model-predicted suitability than a randomly selected absence (Allouche et al. 2006).

- Cohen's Kappa (Cohen 1960). This measure corrects the overall accuracy of model predictions (ratio of the sum of true presences plus true absences to the total number of records) by the accuracy expected to occur by chance. The kappa statistic ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random. Advantages of kappa are its simplicity, the fact that both commission and omission errors are accounted for in one parameter, and its relative tolerance to zero values in the confusion matrix (Manel, Williams & Ormerod 2001). However, Kappa has been criticised for being sensitive to prevalence (the proportion of sites in which the species was recorded as present) and may therefore be inappropriate for comparisons of model accuracy between species or regions (McPherson, Jetz & Rogers 2004, Allouche et al. 2006).
- TSS, the true skill statistic (Allouche et al. 2006). TSS is defined as sensitivity + specificity - 1, and corrects for Kappa's dependency on prevalence. TSS compares the number of correct forecasts, minus those attributable to random guessing, to that of a hypothetical set of perfect forecasts. Like kappa, TSS takes into account both omission and commission errors, and success as a result of random guessing, and ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random (Allouche et al. 2006).

An ensemble model was created by first rejecting poorly performing algorithms with relatively extreme low AUC values and then averaging the predictions of the remaining algorithms, weighted by their AUC. To identify poorly performing algorithms, AUC values were converted into modified z-scores based on their difference to the median and the median absolute deviation across all algorithms (Iglewicz & Hoaglin, 1993). Algorithms with $z < -2$ were rejected. In this way, ensemble projections were made for each dataset and then averaged to give an overall suitability, as well as its standard deviation. The projections were then classified into suitable and unsuitable regions using the 'minROCDist' method, which minimizes the distance between the ROC plot and the upper left corner of the plot (point (0,1)).

We also produced limiting factor maps for Europe following Elith et al. (2010). For this, projections were made separately with each individual variable fixed at a near-optimal value. These were chosen as the median values at the occurrence grid cells. Then, the most strongly limiting factors were identified as the one resulting in the highest increase in suitability in each grid cell.

Results

The ensemble model suggested that suitability for *Callinectes sapidus* was most strongly determined by Mean bathymetry (bathymean), accounting for 54% of variation explained, followed by Maximum long-term temperature (templtmax_ss) (40.4%), Minimum long-term temperature (templtmin_ss) (3.3%) and Mean salinity (salinitymean_ss) (2.3%) (Table 1, Figure 3).

Table 1. Summary of the cross-validation predictive performance (ROC, Kappa, TSS) and variable importance of the fitted model algorithms and the ensemble (AUC-weighted average of the best performing algorithms). Results are the average from models fitted to 10 different background samples of the data.

Algorithm	AUC	Kappa	TSS	Used in the ensemble	Variable importance (%)			
					Mean bathymetry (bathymean)	Maximum long-term temperature (templtmax_ss)	Minimum long-term temperature (templtmin_ss)	Mean salinity (salinitymean_ss)
GLM	0.977	0.802	0.917	yes	40	58	1	1
GAM	0.986	0.825	0.928	yes	41	56	1	1
GBM	0.995	0.896	0.948	yes	47	51	1	1
RF	0.996	0.915	0.959	yes	73	17	7	3
Maxent	0.985	0.866	0.917	yes	68	21	6	5
Ensemble	0.996	0.908	0.948		54	40	3	2

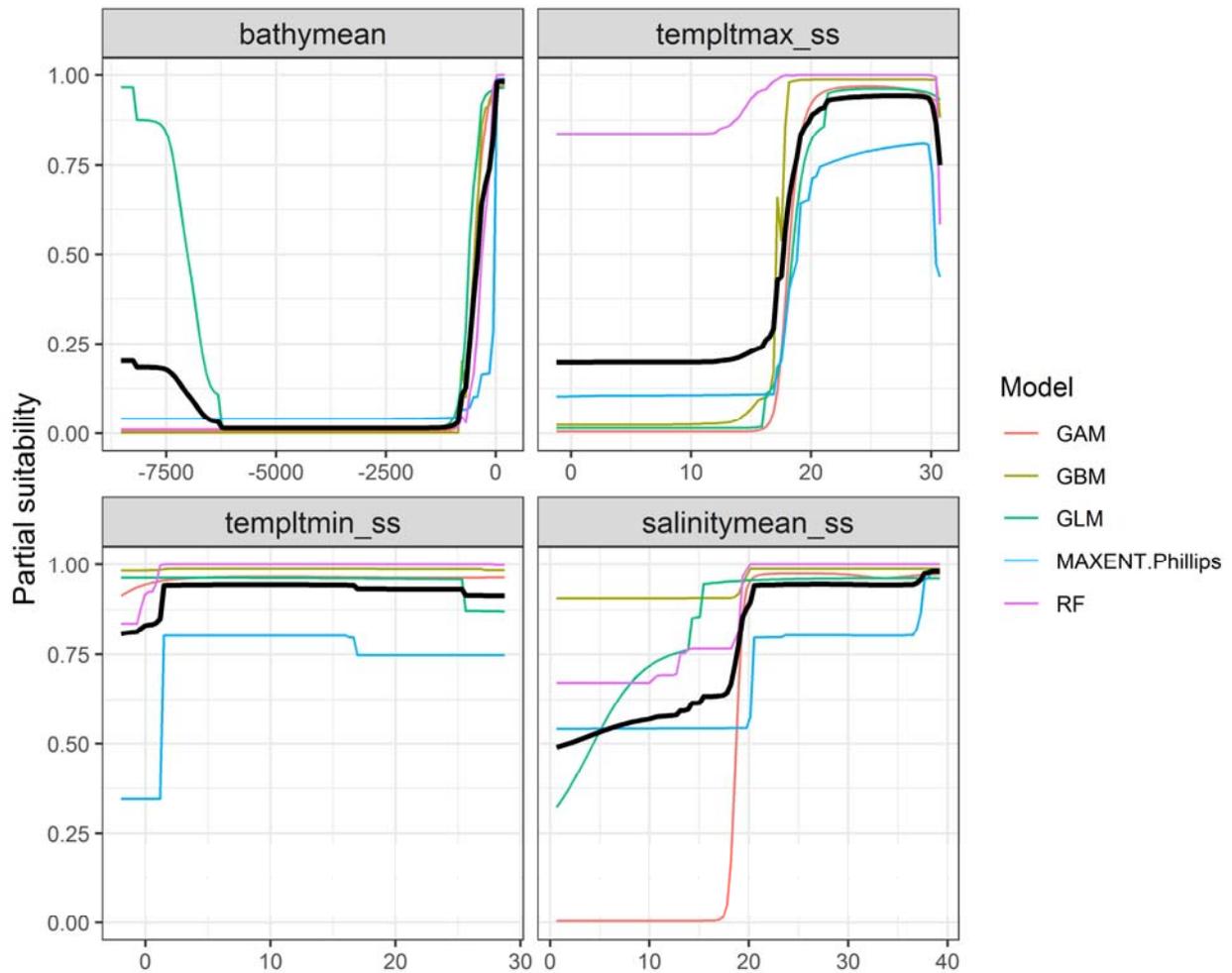
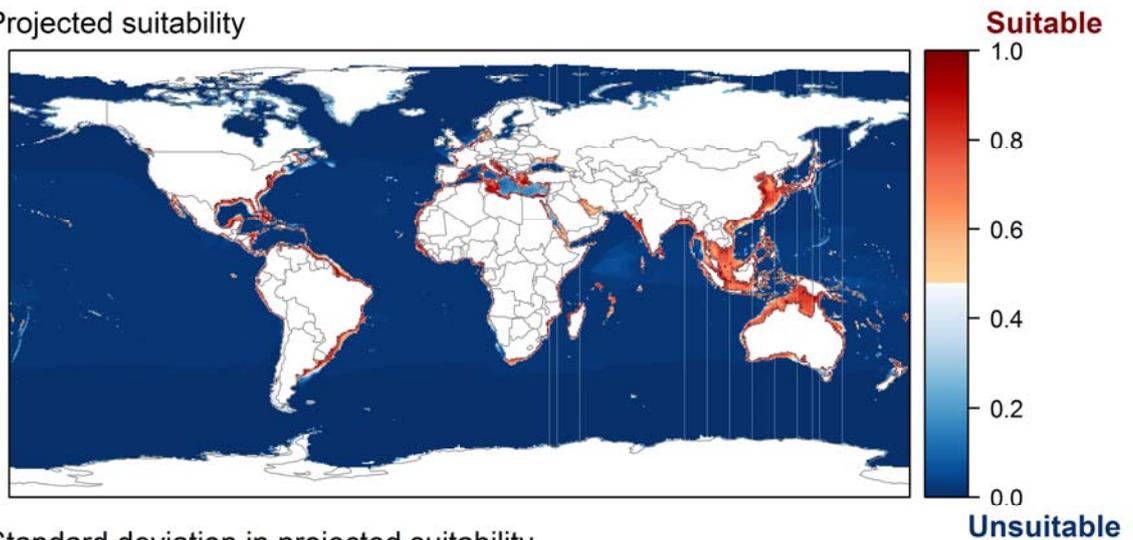


Figure 3. Partial response plots from the fitted models. Thin coloured lines show responses from the algorithms in the ensemble, while the thick black line is their ensemble. In each plot, other model variables are held at their median value in the training data. Some of the divergence among algorithms is because of their different treatment of interactions among variables.

(a) Projected suitability



(b) Standard deviation in projected suitability

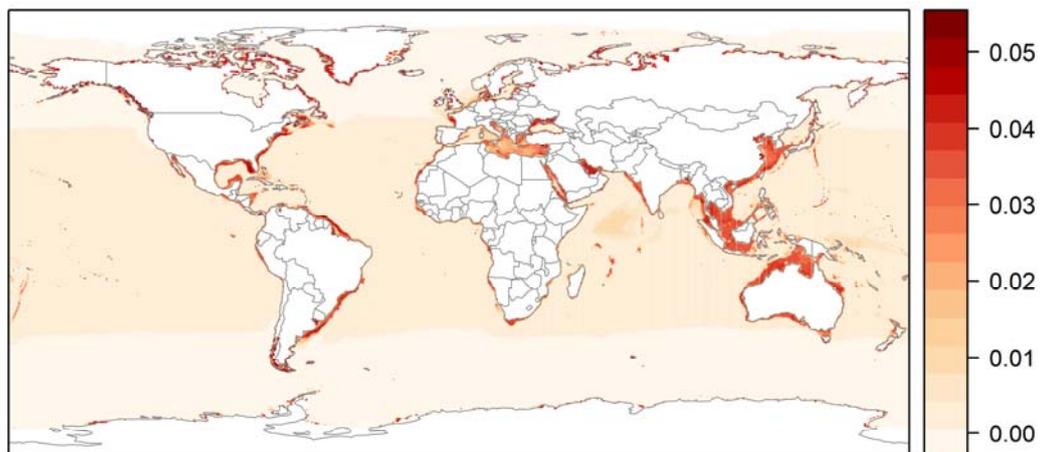


Figure 4. (a) Projected global suitability for *Callinectes sapidus* establishment in the current climate. For visualisation, the projection has been aggregated to a 0.5 x 0.5 degree resolution, by taking the maximum suitability of constituent higher resolution grid cells. Values > 0.48 may be suitable for the species. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

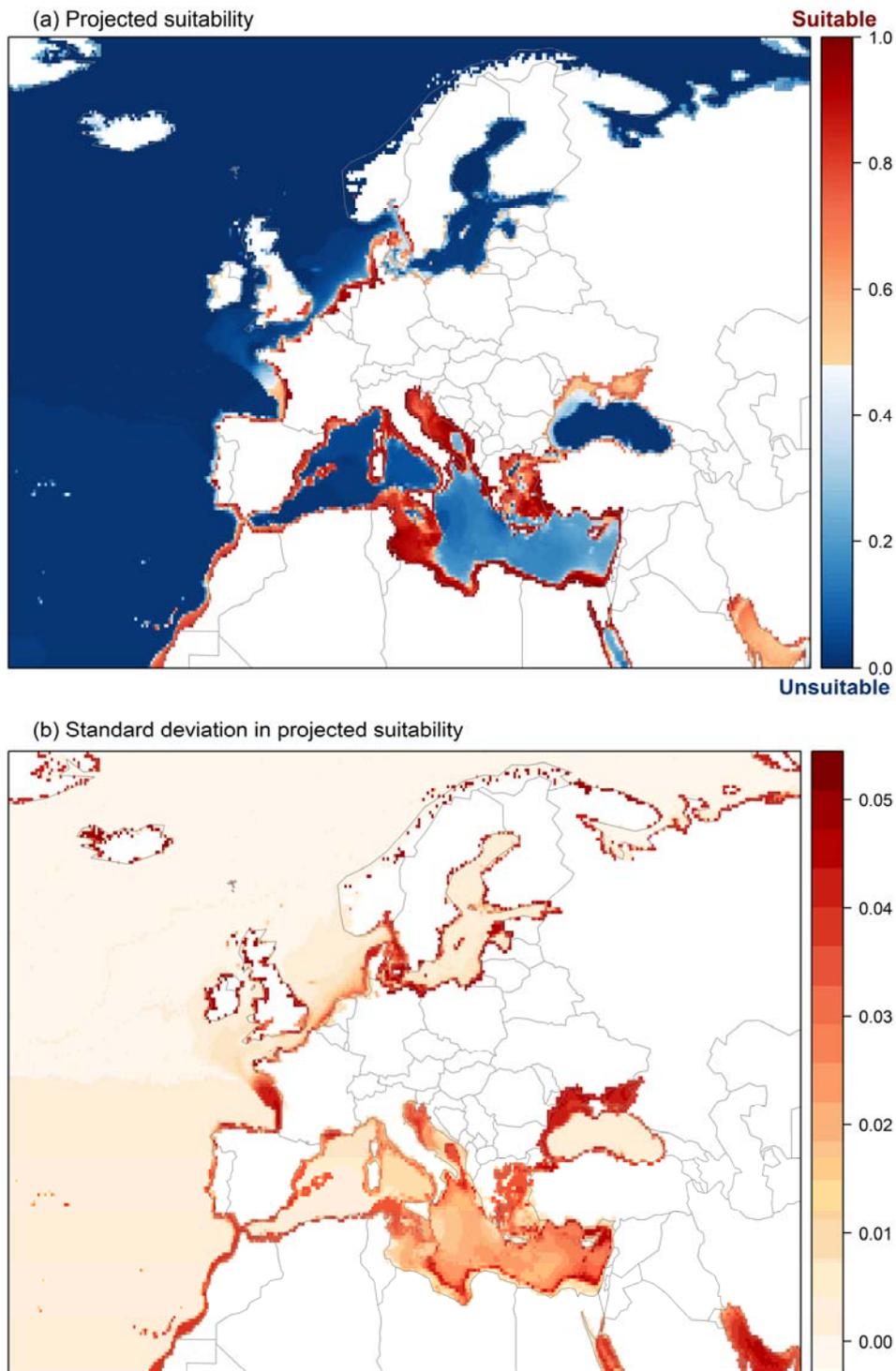


Figure 5. (a) Projected current suitability for *Callinectes sapidus* establishment in Europe and the Mediterranean region. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

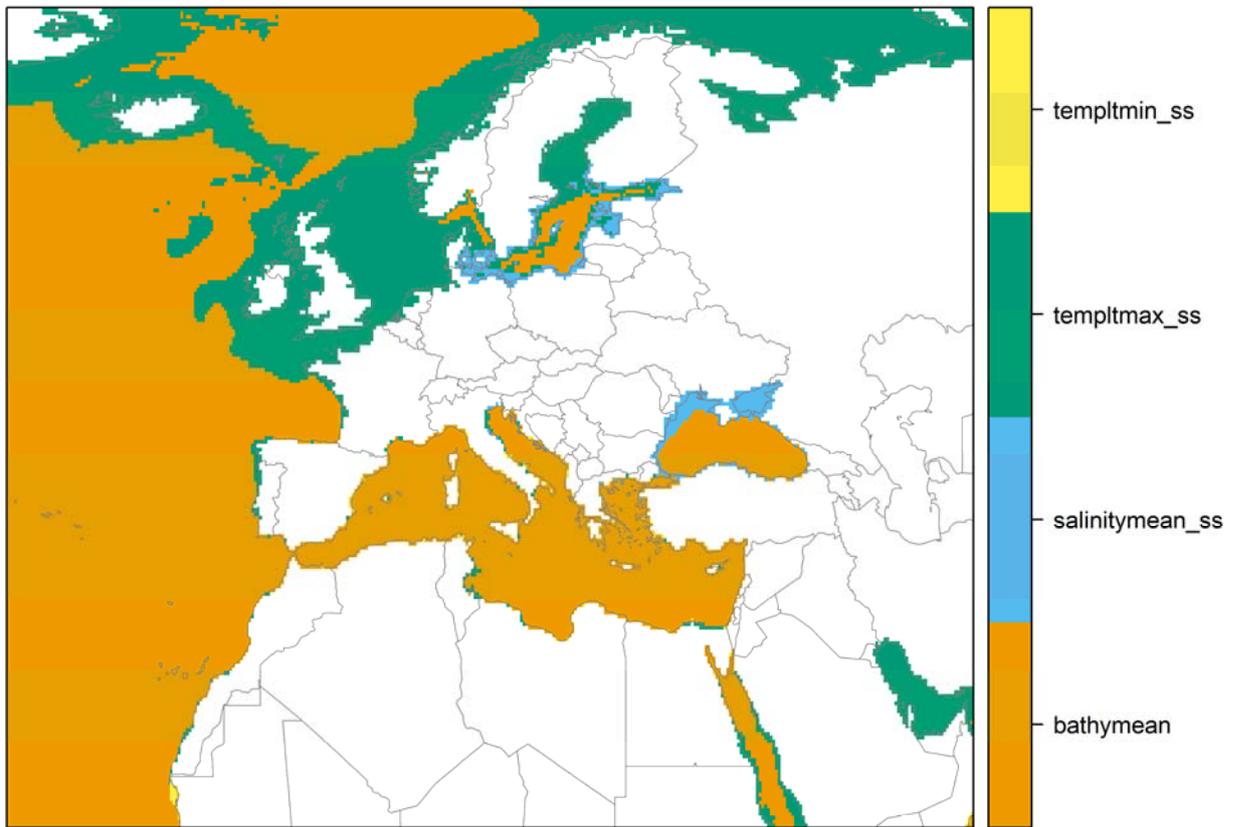


Figure 6. The most strongly limiting factors for *Callinectes sapidus* establishment estimated by the model in Europe and the Mediterranean region in current climatic conditions.

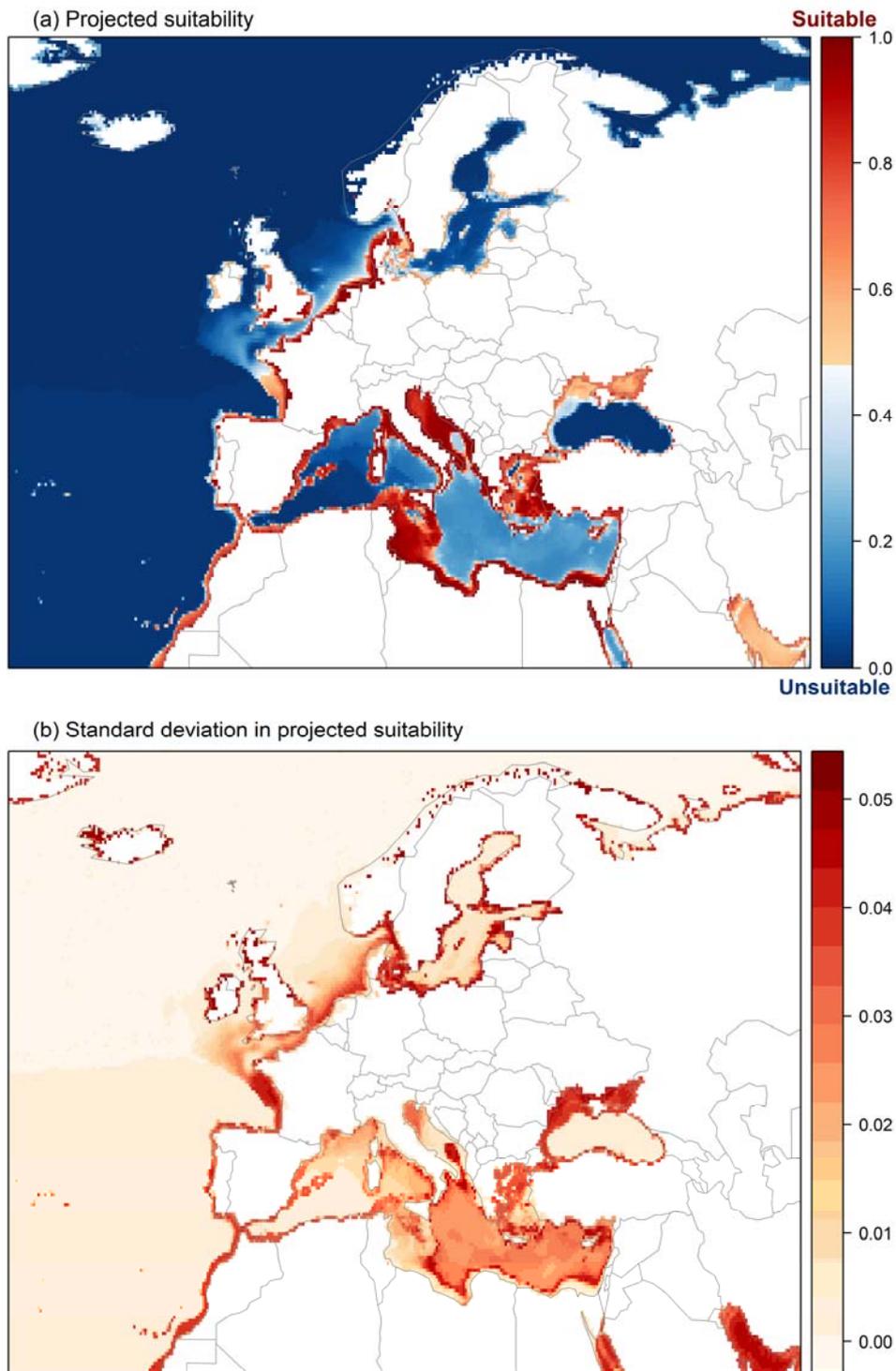


Figure 7. (a) Projected suitability for *Callinectes sapidus* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP2.6, equivalent to Figure 5. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

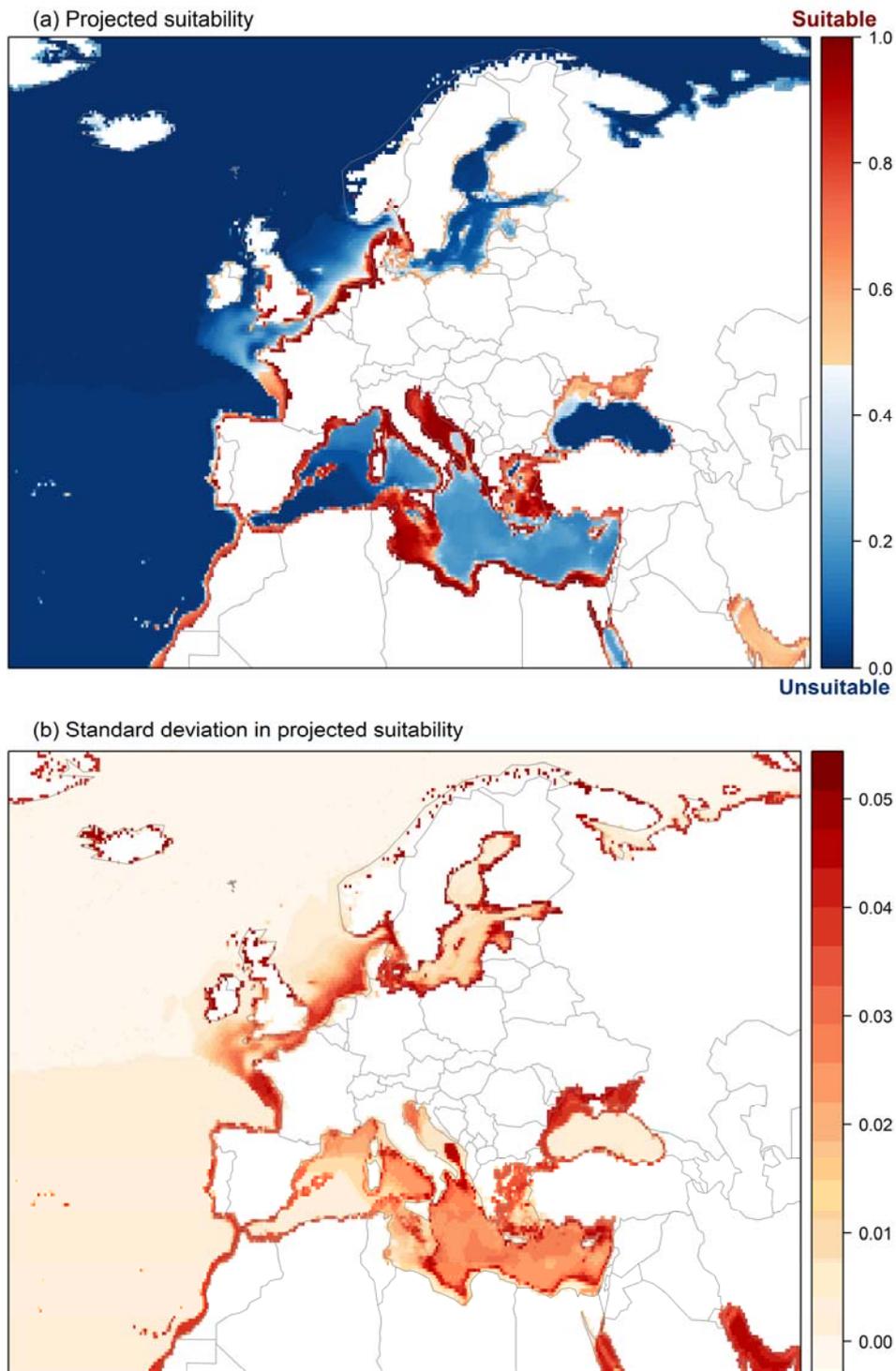


Figure 8. (a) Projected suitability for *Callinectes sapidus* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP4.5, equivalent to Figure 5. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

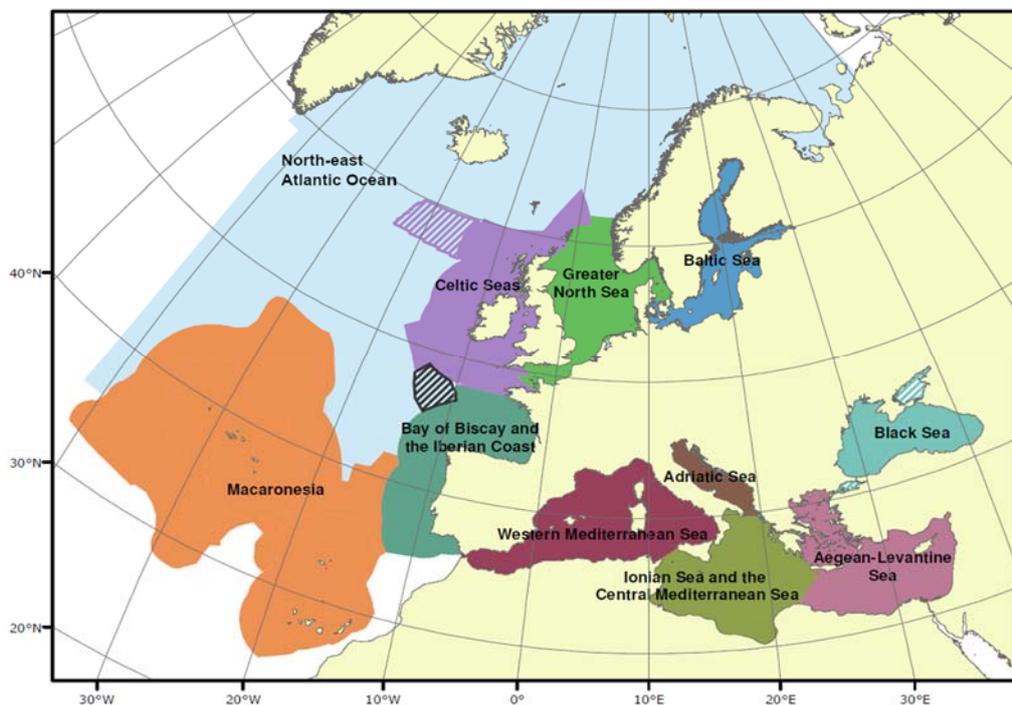
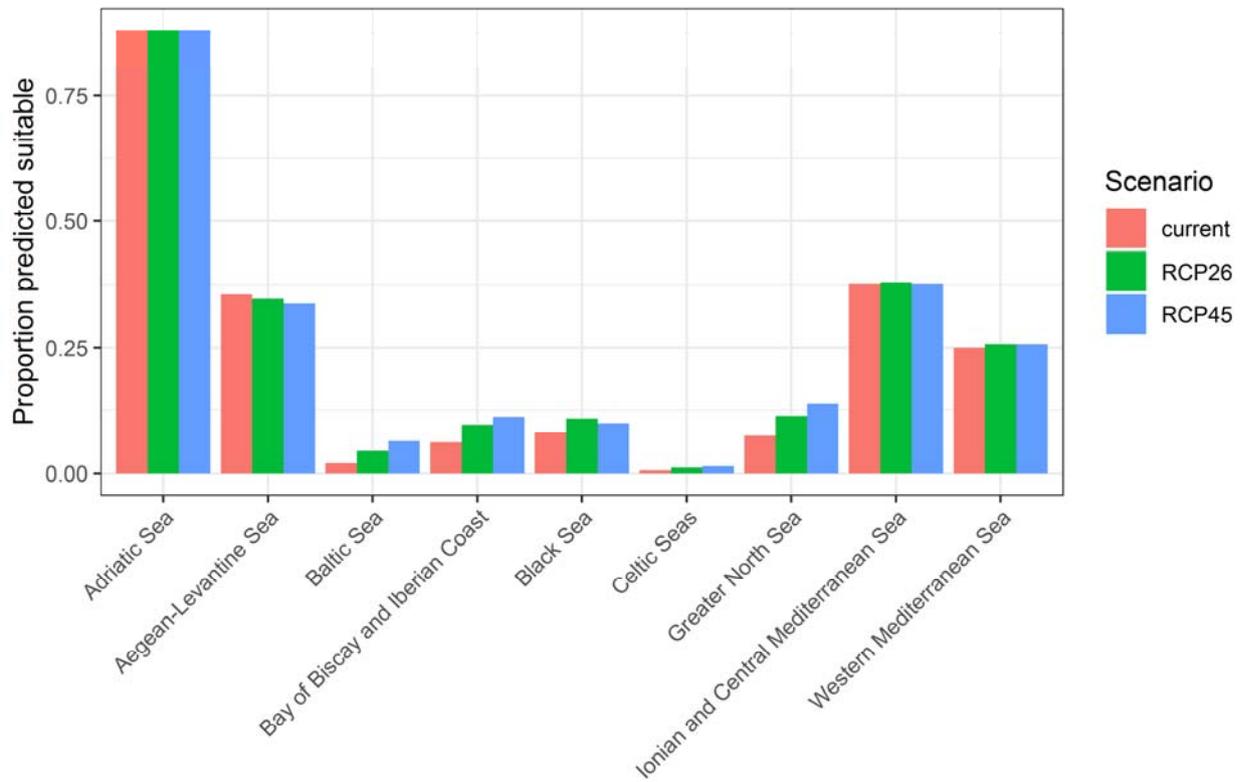
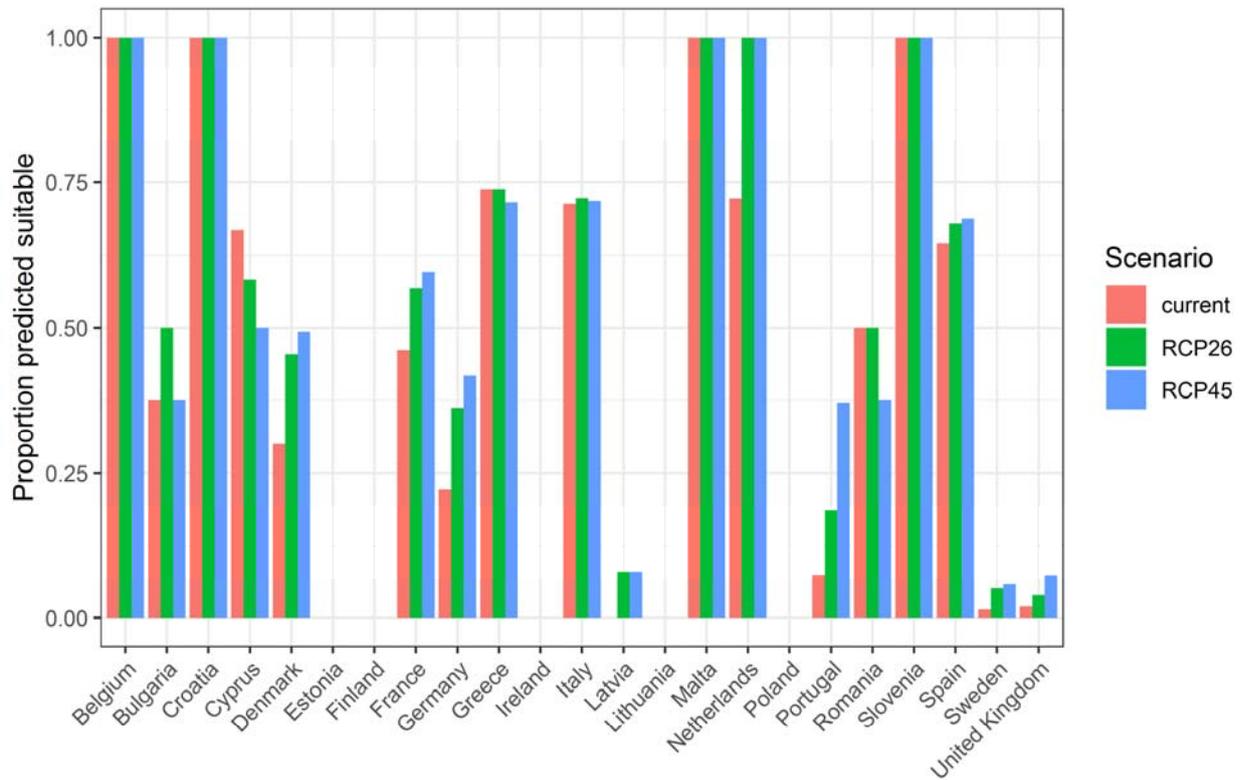


Figure 9. Variation in projected suitability for *Callinectes sapidus* establishment among marine subregions of Europe. The bar plots show the proportion of grid cells in each region classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. The location of each region is also shown. Macaronesia is excluded as it is not part of the study area.

Table 2. Variation in projected suitability for *Callinectes sapidus* establishment among marine subregions of Europe (numerical values of Figure 9 above). The numbers are the proportion of grid cells in each region classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios.

Region	current climate	RCP26	RCP45
Adriatic Sea	0.88	0.88	0.88
Aegean-Levantine Sea	0.36	0.35	0.34
Baltic Sea	0.02	0.04	0.06
Bay of Biscay and Iberian Coast	0.06	0.10	0.11
Black Sea	0.08	0.11	0.10
Celtic Seas	0.01	0.01	0.01
Greater North Sea	0.07	0.11	0.14
Ionian and Central Mediterranean Sea	0.38	0.38	0.38
Western Mediterranean Sea	0.25	0.26	0.26



12-nautical-mile national waters of European Union countries and the UK

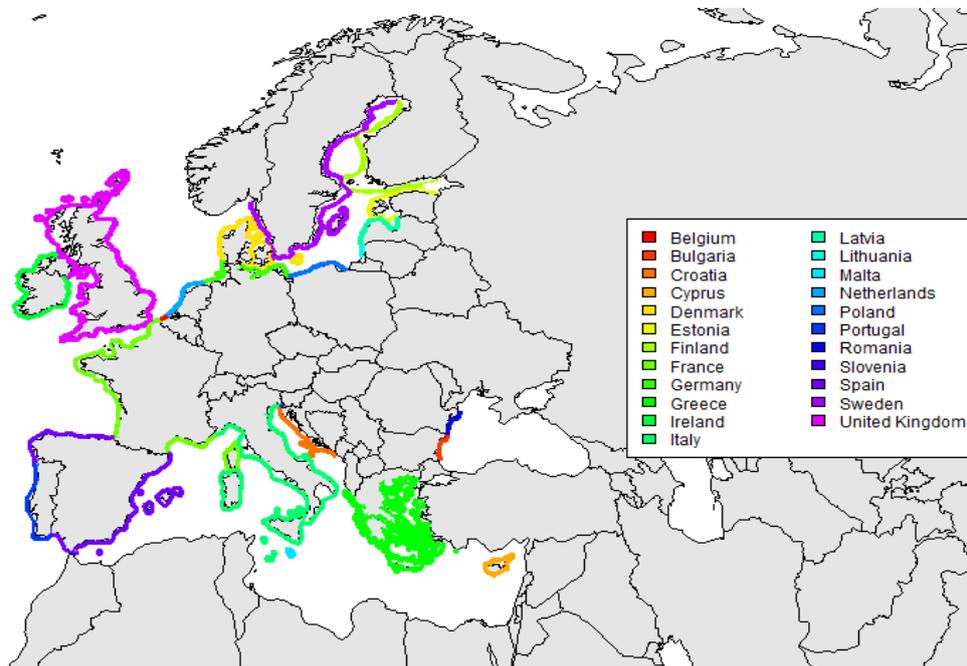


Figure 10. Variation in projected suitability for *Callinectes sapidus* establishment among the 12-nautical-mile national waters of European Union countries and the UK. The bar plots show the proportion of grid cells in each country's waters classified as suitable in the current climate and projected climates for the 2070s under two RCP emissions scenarios. The location of each region is also shown

Table 3. Variation in projected suitability for *Callinectes sapidus* establishment among 12-nautical-mile national waters of European Union countries and the UK (numerical values of Figure 10 above). The numbers are the proportion of grid cells in each country’s waters classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios.

Country	current climate	RCP26	RCP45
Belgium	1.00	1.00	1.00
Bulgaria	0.38	0.50	0.38
Croatia	1.00	1.00	1.00
Cyprus	0.67	0.58	0.50
Denmark	0.30	0.45	0.49
Estonia	0.00	0.00	0.00
Finland	0.00	0.00	0.00
France	0.46	0.57	0.60
Germany	0.22	0.36	0.42
Greece	0.74	0.74	0.72
Ireland	0.00	0.00	0.00
Italy	0.71	0.72	0.72
Latvia	0.00	0.08	0.08
Lithuania	0.00	0.00	0.00
Malta	1.00	1.00	1.00
Netherlands	0.72	1.00	1.00
Poland	0.00	0.00	0.00
Portugal	0.00	0.03	0.03
Portugal	0.00	0.00	0.00
Portugal	0.07	0.19	0.37
Romania	0.50	0.50	0.38
Slovenia	1.00	1.00	1.00
Spain	0.04	0.04	0.04
Spain	0.64	0.68	0.69
Sweden	0.01	0.05	0.06
United Kingdom	0.02	0.04	0.07

Caveats to the modelling

To remove spatial recording biases, the selection of the background sample from the accessible background was weighted by the density of Decapoda records on the Global Biodiversity Information Facility (GBIF). While this is preferable to not accounting for recording bias at all, it may not provide the perfect measure of recording bias.

There was substantial variation among modelling algorithms in the partial response plots (Figure 3). In part this will reflect their different treatment of interactions among variables. Since partial plots are made with other variables held at their median, there may be values of a particular variable at which this does not provide a realistic combination of variables to predict from.

Other variables potentially affecting the distribution of the species, such as underwater vegetation were not included in the model.

Additional references of Annex IX

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Revision of the Risk assessment of the North American beaver (*Castor canadensis*), as based on Member State comments

Introduction

This document explains how the comments received through the Scientific Forum and the consultation of stakeholders on the **Risk assessment of the North American beaver (*Castor canadensis*)** by Hollander et al. (2017)¹ have been considered.

Disclaimer: The authors of the present document have prepared this note with the only purpose of processing the Member States comments to the original Risk Assessment by Hollander et al. (2017), at the request of the European Commission. The present document does not represent an endorsement of the statements and scores given within the original document. Since the focus of this document was limited to the Member States comments, it cannot be considered as a comprehensive revision of the original document, whose contents remain the sole responsibility of the original authors.

Name of species assessed: <i>Castor canadensis</i> Kuhl, 1820
Date of completion of this document: 14/01/2021
Member States that provided comments: <ul style="list-style-type: none">• Austria (AT)• Czech Republic (CZ)• Finland (FI)• France (FR)• Hungary (HU)• Luxembourg (LU)
Stakeholder organizations that provided comments: none.

Outline

The first part of this document highlights how the comments from Member States have been dealt with, in particular the general comments. Also, the few comments that were not processed are discussed in this part, providing explanations for not processing them.

The second part of this document deals with the specific comments from Member States. The text should be read as an addendum and corrigendum to the original risk assessment (Hollander et al. 2017). The headings from the original assessment are repeated to facilitate direct comparison.

¹ Hollander H., van Duinen G.A., Branquart E., de Hoop L., de Hullu P.C., Matthews J., van der Velde G., Leuven R.S.E.W. (2017) Risk assessment of the alien North American beaver (*Castor canadensis*). © Department of Environmental Science, Radboud University, 6525 AJ Nijmegen, The Netherlands

References that were included in the original risk assessment are not repeated. New references are provided at the end of this document.

Part 1: Comments per Member State

This part deals with the general comments provided by the Member States, and the (few) comments that were not processed.

Austria (AT)

AT suggested a *minor revision*.

Comment from Member State: AT suggested to more clearly refer to the criteria for risk assessments set out by the Regulation, as it is sometimes difficult to find the necessary information.

Answer: The common elements of risk assessments are defined by Article 5 of the Regulation 1143/2014 of 22 October 2014. All elements are addressed in the assessment, though not necessarily in the prescribed order (Table 1).

Table 1 -- The elements of risk assessments as defined by Regulation 1143/2014, and the headings under which they are treated by Hollander et al. (2017)

Element	Headings
Art 5(1)(a) — <i>a description of the species with its taxonomic identity, its history, and its natural and potential range</i>	2.1, 2.2, 2.3
Art 5(1)(b) — <i>a description of its reproduction and spread patterns and dynamics including an assessment of whether the environmental conditions necessary for its reproduction and spread exist</i>	2.1, 2.3
Art 5(1)(c) — <i>a description of the potential pathways of introduction and spread of the species, both intentional and unintentional, including where relevant the commodities with which the species is generally associated</i>	2.4
Art 5(1)(d) — <i>a thorough assessment of the risk of introduction, establishment and spread in relevant biogeographical regions in current conditions and in foreseeable climate change conditions</i>	3.1, 3.2
Art 5(1)(e) — <i>a description of the current distribution of the species, including whether the species is already present in the Union or in neighbouring countries, and a projection of its likely future distribution</i>	2.2, 2.3
Art 5(1)(f) — <i>a description of the adverse impact on biodiversity and related ecosystem services, including on native species, protected sites, endangered habitats, as well as on human health, safety, and the economy including an assessment of the potential future impact having regard to available scientific knowledge</i>	2.5*
Art 5(1)(g) — <i>an assessment of the potential costs of damage</i>	2.5
Art 5(1)(h) — <i>a description of the known uses for the species and social and economic benefits deriving from those uses</i>	(2.5)

*But note: section 2.5.7 is more on climate change effects on distribution rather than impact

Comment from Member State: The risk assessment argues that climate change is expected to have no effect on the risks related to *C. canadensis*. AT points out that this argument is questionable, since some breeding parameters do relate to climate.

Answer: *C. canadensis* produces 1 litter per year, irrespective of latitude or climate, but the severity of winter weather is indeed considered an important factor influencing litter size (Jenkins & Busher 1979). In this respect, the rate of spread might indeed be increased under future winter conditions. However, the risk of spread was already considered maximal under the Harmonia⁺ and ISEIA scoring (Tables 3.1 and 3.2). The conclusion thus holds that climate change is not expected to alter the overall risk. As set out under 3.2.2, the ISEIA assessment was effectively repeated under the assumption of

future conditions. But since this gave the same results as when assuming current conditions, the table shows the results only once.

There is no dedicated species distribution model (SDM) for *C. canadensis* in Europe available. Alakoski et al. (2020) provide distribution models for Finland, but also highlight that the models have difficulty filtering out the invasion history. Therefore, to provide more insights if climate change might alter the overall risk, a dedicated SDM should be made. It should be mentioned, however, that a dedicated SDM is not required by Art. 5 of the Regulation (see Table 1).

Comment from Member State: AT points out that a risk assessment is available for Germany, based on the GABLIS method.

Answer: References to that assessment are included, and its results discussed (3.3, below).

Comment from Member State: AT suggests to explicitly list biogeographical regions and Member States in which the species is established, and is potentially able to establish in.

Answer: Although a bit scattered, this information is addressed under 2.2, 2.3.2 (current Member States); 2.3.4 (current regions); 2.3.4 and 2.3.5 (potential states and regions). The information is summarised in Tables 2 and 3 below. Not all Member States were explicitly considered by Hollander et al. (2017). As it is acknowledged that the distribution of *C. fiber* provides a useful proxy for the potential distribution of *C. canadensis* in Europe (2.3.4), the suitability for missing Member States is considered on that basis in Table 2 (Wróbel 2020). This leaves four Member States for which potential establishment of *C. canadensis* remains unascertained.

Table 2 – Summary of the status of *C. canadensis* in Member States of the European Union, and the United Kingdom

Member State	Recorded ¹	Currently established	Potential establishment	Currently invasive
Austria	Yes	No ²	Yes ^{4, 5, 6}	No
Belgium	Yes	Yes ^{2,3}	Yes ^{5, 6}	No
Bulgaria	No	No	Yes ⁶	No
Croatia	No	No	Yes ⁶	No
Cyprus	No	No	Unclear	No
Czech Republic	No	No	Yes ^{4, 6}	No
Denmark	No	No	Yes ^{5, 6}	No
Estonia	No	No	Yes ^{4, 6}	No
Finland	Yes	Yes ²	Yes ^{5, 6}	Yes
France	Yes	No ²	Yes ^{4, 5, 6}	No
Germany	Yes	Yes ^{2,3}	Yes ^{5, 6}	Yes
Greece	No	No	Unclear	No
Hungary	Yes	Unknown ²	Yes ^{5, 6}	No
Ireland	No	No	Yes ⁴	No
Italy	No	No	Yes ⁶	No
Latvia	No	No	Yes ^{4, 6}	No
Lithuania	No	No	Yes ^{4, 6}	No
Luxembourg	Yes	No ³	Yes ^{5, 6}	No ³
Malta	No	No	Unclear	No
Netherlands	No	No	Yes ^{4, 5, 6}	No
Poland	Yes	No ²	Yes ^{4, 5, 6}	No
Portugal	No	No	Unclear	No
Romania	No	No	Yes ⁶	No
Slovakia	No	No	Yes ^{4, 6}	No
Slovenia	No	No	Yes ⁶	No
Spain	No	No	Yes ⁶	No
Sweden	No	No	Yes ^{4, 5, 6}	No
United Kingdom	No	No	Yes ^{4, 6}	No

(1) See Table 2.3 (Hollander et al. 2017). (2) See Table 2.3 and heading 2.3.2 (Hollander et al. 2017). (3) Based on updated information, provided by Schley (2019). See the addition under 2.3.6 (below) for more information. (4) Cited under 2.3.4. (5) Cited under 2.3.5. (6) Based on the current presence of *Castor fiber* in the Member State, the latest overview of which is provided by Wróbel (2020).

Table 3 -- Summary of the status of *C. canadensis* in biogeographical regions of the European Union

Biogeographical region	Recorded ¹	Currently established	Potential establishment ^{2,3,4}	Currently invasive
Alpine	No	No	Yes ^{2,3,4}	No
Atlantic	Yes	No	Yes ^{2,3,4}	No
Black Sea	No	No	Yes ³	No
Boreal	Yes	Yes	Yes ^{2,3,4}	Yes
Continental	Yes	Yes	Yes ^{2,3,4}	Yes
Mediterranean	No	No	Yes ^{2,3,4}	No
Pannonian	Yes	No	Yes ^{2,3,4}	No
Steppic	No	No	Yes ³	No

(1) As Hollander et al. (2017) provide historic records at Member State level only, assigning them to biogeographic regions is somewhat complicated. (2) Based on informations given in Hollander et al. (2017: 2.3.4 and 2.3.5). (3) Based on the current presence of *Castor fiber*, the latest distribution map of which is provided by Halley et al. (2021). (4) Based on distributional data provided in GBIF (2020).

Other specific comments are dealt with in the second part of this document.

Czech Republic (CZ)

CZ suggested a *minor revision*.

Comment from Member State: CZ would prefer to see more information that allows for comparisons among *C. canadensis* and *C. fiber* (e.g. costs and impact). Also, more detail on how local managers can differentiate between the two species would be useful.

Answer: The risk assessment (Hollander et al. 2017) already extensively compares *C. canadensis* with *C. fiber* in terms of species characteristics (2.1), habitat (2.3) and impacts (2.5). Two new references give supplementing information on *C. fiber* management in Europe (see addition to 2.5.5 below).

Without the necessary expertise, there is no means for local managers to reliably distinguish the two species. Reliable methods are based on DNA or anal gland secretion (Rosell & Sun 1999), or skull morphometry (Jenkins & Busher 1979). See 2.4.

Other specific comments are dealt with in the second part of this document.

Finland (FI)

FI provided numerous specific comments, in large part with regard to northern areas, which are dealt with in the second part of this document.

France (FR)

FR considered the risk assessment *fit-for-purpose*. All specific comments are dealt with in the second part of this document.

Hungary (HU)

HU considered the risk assessment *fit-for-purpose*. There were no comments to process.

Luxembourg (LU)

LU suggested a *minor revision*.

Comment from Member State: LU noted that the RA is not in the template format of the European Commission, though all questions from the template have been addressed.

Answer: Regulation 1143/2014 does not require any specific template to be adopted. See above (AT) for the compliance with Regulation 1143/2014 and Delegated Regulation 2018/968.

Comment from Member State: LU comments that, when referring to the Western European population of *C. canadensis*, it is appropriate to use more specific geographic designators rather than referring to e.g. Germany, Belgium or France. More specifically, it is recommended to refer to the German states of Rhineland-Palatinate, North Rhine-Westphalia and Saarland; the Belgian region of Wallonia; and north-eastern France.

Answer: This is indeed important when considering the current distribution, scale of spread and impact, among others. Care has been taken to use more precise geographic designators, where useful, in the corrections and additions (see above and second part: summary, 2.2, 2.3, 2.4, 3.1, 5). At other occasions, it seems appropriate to stick to the (coarse) level of Member States.

Comment from Member State: LU points out that the RA puts the interaction among *C. canadensis* and *C. fiber* forward as a key potential impact. Yet, the practical implications for the combined management of both species are not addressed, if *C. canadensis* were to mingle with *C. fiber* e.g. at the river catchment level.

Answer: Indeed, regional initiatives on policy, management and communication may be complicated in situations where *C. fiber* and *C. canadensis* occur together, since the targets set for these species might be opposite to one another. A statement is added under heading 2.5.5.

Comment from Member State: LU points out that *C. canadensis* has not been introduced into Luxembourg, but spread spontaneously from Rhineland-Palatinate (Germany). LU accordingly questions the description of the species' European range as the "*introduced range*", since the species was not introduced to each of the Member States. Also, the species has been subject to dedicated eradication efforts in Luxembourg, which are incompletely covered in the original assessment. In fact, the species is now considered eradicated from the country.

Answer: Numerous additions and corrections on introduction and management in LU are included in the second part of this document. With regard to the use of "*introduced range*", it might indeed be better to speak of the "*non-native range*". Yet, both terms are well-established in the narrative of the Regulation 1143/2014, and considered synonyms.

Comment from Member State: The risk analysis with the ISEIA protocol yields a lower total score for areas with *C. fiber*, than for areas without *C. fiber*. This may be surprising, since the biggest estimated impact is the competition among these species.

Answer: In the ISEIA protocol, the category for impacts on native species does not separate negative impacts on *C. fiber* from those on other species in the same community (e.g. aquatic species). If *C. fiber* is present in the area, it already accounts for impacts on such species, and the remaining effect for *C. canadensis* is lowered. The same reasoning applies to the category for impacts on ecosystem functions. This leads to a lower total score. Additionally, the lower score might be a consequence of higher uncertainty (see p. 28 of the Risk Assessment).

Other specific comments are dealt with in the second part of this document.

Part 2: Specific comments

This part provides additions and corrections under the respective headings of the original assessment (Hollander et al. 2017). The Member State on whose comment it is based, is indicated between brackets.

Summary

Addition (AT): Apart from describing the endangered area by habitat types, it is useful to describe it by biogeographical regions. See Table 3 (above) for an overview of the regions with potential for establishment.

Addition (AT): The summary of the risk for *C. canadensis* in the EU may be a bit confusing on the scenarios that were scored with the Harmonia+ protocol, and those that were scored with the ISEIA protocol. We therefore provide an overview of the scenarios and their scores in Table 4.

Table 4 – An overview of the scores provided by Hollander et al. (2017)

Protocol	Harmonia+		ISEIA			
<i>C. fiber</i>	Without <i>C. fiber</i>		With <i>C. fiber</i>		Without <i>C. fiber</i>	
Conditions	Current	Future	Current	Future	Current	Future
Score	High (1.0)	High (1.0)	9 (of 12)	9 (of 12)	12 (of 12)	12 (of 12)

Correction (LU): It is incorrectly stated that *C. canadensis* has been introduced to Luxembourg, as the presence of the species is considered to be due to natural dispersal from Rhineland-Palatinate (Germany). The same is probably true for Belgium (Schley 2019).

Addition (FI): The Finnish population of *C. canadensis* was considered to comprise about 6.000 family groups in 2013 (Brommer et al. 2017). The population is viable, has generally spread westward, and is now partly sympatric with *C. fiber* (Alakoski et al. 2019, 2020).

2. Risk inventory

2.1 Species description

2.1.1 Nomenclature and taxonomic status

Addition (LU): The original reference for the separation of the two *Castor* species is Lavrov & Orlov (1973), and that for the recognition of the subspecies is Hall & Kelson (1959), both of which are referred to in Jenkins & Busher (1979).

2.1.2 Species characteristics

Correction (LU): The text under this heading supposes that *C. canadensis* is currently reproducing in Luxembourg. Yet, no reproduction has been observed for at least 5 years (Schley 2019).

Correction (LU): The oily substance Castoreum does not serve to waterproof the beaver's fur. (This is also not stated in the given reference of Jenkins & Busher 1979.) Castoreum is used for territorial marking, whereas the most likely candidate for waterproofing of the fur is the skin lipid squalene (Rosell 2002).

Addition (LU): No primary reference is provided for the breeding attempt between *C. canadensis* and *C. fiber* that has led to a stillborn kit. The reference is (probably) Lavrov (1996).

Correction (LU): It is probably more correct to state that North American beaver *sometimes* displaces the Eurasian species, rather than *often*, as the outcome of competition is unclear so far (Danilov et al. 2008, Alakoski et al. 2019).

Addition (FI): A recent reference that studied sympatrically occurring *C. canadensis* and *C. fiber* is Alakoski et al. (2019). Results from that study are discussed below under various headings.

Addition (FI): With regards to foraging behaviour, beavers also prefer birch (*Betula* spp.). Apart from aspen and willow species, banks of water bodies populated by birch thus also provide suitable habitat (Alakoski et al. 2019).

Addition (LU): For predation of American mink on beaver, references include Swank (1949) in Jenkins & Busher (1979), and Errington (1943). The first reference specifies that predation is on beaver kits, the second clarifies that mink frequently visit beaver lodges. The suggestion that European mink may predate on beaver only stems from analogy.

Addition (LU): As an additional source of natural mortality, heavy rainfall that leads to floods may cause newborn beavers to drown in the dens (Hoffmann 1967).

2.2 Probability of introduction

Correction (LU): It is incorrectly stated that *C. canadensis* has been introduced to Luxembourg. The presence of the species was due to natural dispersal from Rhineland-Palatinate (Germany). The first observation is from 2006 (not before). The last observation is from 2019, of one individual that was captured (Schley 2019). See below under 2.3.6 for an overview of captures.

Addition (FI): An additional reference for the population status in Finland (to be added in Table 2.3) is Brommer et al. (2017). The Finnish population of *C. canadensis* was considered to comprise about 6.000 family groups in 2013.

2.3 Probability of establishment

2.3.1 Current global distribution

Correction (LU): It is incorrectly stated that *C. canadensis* has been introduced to Luxembourg, as the presence of the species is considered to be due to natural dispersal from Rhineland-Palatinate (Germany). The same is probably true for Belgium (Schley 2019).

Correction (LU): Luxembourg should no longer be regarded as part of the non-native range, as the country is now considered free of *C. canadensis* (Schley 2019).

Addition (LU): As a reference for the natural range of *C. canadensis* in North America, Wikipedia (2016) should be replaced by Jenkins & Busher (1979).

Addition (LU): The statement that *C. canadensis* has left “*a swath of destruction*” (Hollander et al. 2017) in the Tierra del Fuego archipelago, is further elaborated under heading 2.5.1.

2.3.2 Current distribution in the European Union and neighbouring areas

Addition (LU): For France, the situation is discussed up to the reference of Dewas et al. (2012). Since then, hundreds of beaver samples have genetically been checked for *C. canadensis*, but all proved to be *C. fiber*. Such analyses are continued within the operation of France’s ‘beaver network’ (*Réseau Castor*; Bressan & Hurel 2018). The status as ‘eradicated’ thus holds.

Addition (LU): For Germany, the statement made by Parker et al. (2012) that *C. canadensis* was released in North Rhine-Westphalia is flawed: although the breeding farm housed both species, there is no proof that specimens of *C. canadensis* were involved in the releases (Schley 2019). Genetic investigations by the Senckenberg Museum in Frankfurt support that conclusion (Christiane Frosch, unpublished results). The only times *C. canadensis* was detected in North Rhine-Westphalia was in 2012 and 2014 on the southern border with Rhineland-Palatinate (where its presence is known). The specimens from 2012 disappeared without a trace, the 2014 specimen was caught (Schley 2019).

Addition (LU): The 2006 finding of a North American beaver on the Luxembourg-Germany border sparked a large-scale genetic study in the greater region of Wallonia (Belgium), northern France, Rhineland-Palatinate, southern North Rhine-Westphalia and Saarland (Germany), and Luxembourg. The result have not been published, though, but the relevant results are included in the following additions (Schley 2019).

Addition (LU): For Belgium, the statement of Dewas et al. (2012) that the known site in Wallonia could have been the source of spread in the area, is rather unlikely. Genetic analysis showed several sites in Wallonia, all of which were close to the border with Germany and Luxembourg. It is therefore more likely that they too spread from Rhineland-Palatinate (Germany, notably the Eifel-Zoo). Apart from that, all samples from elsewhere in Wallonia proved to be *C. fiber* (Schley 2019). This weakens the claim that the (illegal) releases of beavers from Bavaria into Belgium might separately have led to the presence of *C. canadensis* (Hollander et al. 2017: p. 18).

Correction (LU): For Luxembourg, it is incorrectly stated that *C. canadensis* has been introduced to Luxembourg, as the presence of the species is considered to be due to natural dispersal from Rhineland-Palatinate (Germany). Luxembourg is now considered free of *C. canadensis* (Schley 2019).

Correction (FI): For Finland, more recent information is provided by Alakoski et al. (2019) and Alakoski et al. (2020). There are three, rather than two, fronts where the distribution of *C. canadensis* and *C. fiber* have converged in Finland. These are the regions of Pirkanmaa, Etelä- Pohjanmaa and Lapland.

Hollander et al. (2017) suggest that it is expected that *C. canadensis* will spread towards Sweden from Finland. However, at present, it seems that *C. fiber* is spreading from Sweden to Finnish Lapland. It is currently unclear whether *C. canadensis* is still present in the region. Alakoski et al. (2020) and Halley et al. (2021) provide the latest maps for the known distribution of both species in Finland (as compared to the map shown in Figure 2.3).

Correction (FI): Hollander et al. (2017) state that “*in Finland, C. canadensis (...) seems to outcompete C. fiber in areas where the two species come into contact*”. Yet, for the Finnish river system where both species occur and that has been studied most extensively, the outcome of competition is not yet clear (Alakoski et al. 2019).

2.3.3 Habitat description and physiological tolerance

Addition (FI): With regard to the habitat of *C. fiber* as compared to *C. canadensis*, Alakoski et al. (2019) studied an area where both species occur, and found habitat use to be similar indeed. The most apparent difference between both species was the distance from agricultural areas to beaver lodges (with *C. fiber* occurring closer to agriculture than *C. canadensis*), and the volume of birch versus other deciduous trees (with *C. fiber* sites having comparatively more birch).

Correction (LU): The text under this heading suggests that it is unclear whether populations of *C. canadensis* are established in Luxembourg. However, there are currently no established populations (Schley 2019.).

Correction (LU): The subtitle “*Effects on establishment through competition or predation with other species*” (Hollander et al. 2017) should be “*Effects on establishment through competition with other species or predation*”. Under this subtitle, “*wolfs*” should be replaced by “*wolves*”.

2.3.4 Climate match and biogeographical comparison

Addition (CZ): As readers may want to consult the climate zone map (Figure 2.5) in greater detail, it is useful to inform that it is available online as a supplement to the article of Peel et al. (2007) at <https://hess.copernicus.org/articles/11/1633/2007/>. Beck et al. (2018) published an update of the climate zone map, that is downloadable from <http://www.gloh2o.org/koppen/>.

Correction (LU): The text under this heading suggests that *C. canadensis* currently occurs in Luxembourg. However, Luxembourg is now considered to be free of the species (Schley 2019).

2.3.5 Endangered areas

Addition (LU): The list with examples of Member States that make part of the endangered area can be supplemented with Mediterranean countries such as Spain, Croatia and Italy. The ability of *C. canadensis* to live in these countries is proven by the presence of *C. fiber*.

2.3.6 Influence of management practices

Correction (LU): The statement that “*there are no specific management practices for C. canadensis in the EU except for Scandinavia*” (Hollander et al. 2017) is not correct. The following information was provided by Schley (2019):

Following the discovery of the presence of *C. canadensis* in its border region, Luxembourg took the initiative to set up a group to deal with the issue. This group included representatives from Luxembourg; the Walloon Region (Belgium); the states of Rhineland-Palatinate, North Rhine-Westphalia and Saarland (Germany); the Lorraine Region (France); and the Netherlands. This group launched a large-scale genetic survey to delimit the geographic area colonized by *C. canadensis* and to investigate its origin, and decided on coherent management actions aimed at its eradication.

In Luxembourg, the eradication programme started in 2009. From 2009 to '14, 25 individuals were removed. From 2015 to '17, 6 were removed. From 2018 to '19, 1 was removed. In total, 12 sites were involved. Noteworthy, all individuals removed after 2014 were solitary at their site (Schley 2019). Luxembourg is now considered to be free of *C. canadensis*. The *C. fiber* population is growing rapidly, and every new site is tested genetically to confirm the species' identification.

In Wallonia, an eradication programme was launched in 2010, which initially consisted of trapping and euthanizing specimens. Since this method was very time consuming, it was decided to hunt the beavers instead. As of May 2019, a total of 43 Canadian beavers have been removed from 16 sites, all of which are situated on the watershed of the river Our along the country border. On two sites, there still seems to be activity (Schley 2019).

In Rhineland-Palatinate, where the problem originated from, the strategy is to capture and sterilize specimens, and release them again at the site of capture. This particular strategy does not follow the recommendation of the aforementioned beaver group, and was also heavily criticized by the Scientific Committee of the International Beaver Symposium of 2012.

In North Rhine-Westphalia, on the border with Rhineland-Palatinate, *C. canadensis* was detected by genetic testing in 2012 and again at another location in 2014. The beavers from 2012 disappeared without a trace, the single specimen from 2014 was caught. Other samples of beaver in the state, including more recent ones, turned out to be *C. fiber*, or were inconclusive so far.

In France, genetic testing of beaver samples for *C. canadensis* are continued within the operation the 'beaver network' (*Réseau Castor*; Bressan & Hurel 2018).

Addition (FI): In Finland, the hunting season runs from August 20 to April 30 for both *Castor* species. A license is needed to hunt *C. fiber* (with quota applying), but not *C. canadensis*. An analysis of census data over the period 1995-2013 suggested that *C. fiber* is numerically increasing in Finland, while *C. canadensis* is declining in numbers. It is very likely that hunting is contributing to these trends (Brommer et al. 2017).

Hollander et al. (2017) conclude that eradication in the European Union is difficult “*due to the similarity of morphological characteristics between C. canadensis and (...) C. fiber.*” In eastern Finland, the beavers are known to be exclusively *C. canadensis* (Alakoski et al. 2020), so that difficulty does not apply.

2.4 Pathways and vectors for spread within the EU

Correction (LU): The text under this heading involves Luxembourg as a potential source for further spread. However, Luxembourg is now considered to be free of the species (Schley 2019).

Addition (LU): It is stated that “*species identification is difficult without genetic analysis*” (Hollander et al. 2017). However, the use of anal gland secretion provides a helpful and reliable tool, too (Rosell & Sun 1999).

Addition (FI): In addition to the previous comment, also skull morphometry can be used to identify the species (Jenkins & Busher 1979).

2.5 Impacts

2.5.1 Environmental effects on biodiversity and ecosystems

Correction (LU): The word “*endemic*” must be replaced with “*native*”.

Addition (LU): With regard to the impact of beaver dams on water temperatures, a large-scale and long-term study has recently been published by Weber et al. (2017). They highlight that different complexities underly this relation, but generally found that increased dam and pond creation contributes to the moderation of daily temperature cycles (i.e., increasing minimum temperatures and decreasing maximum temperatures).

Addition (LU): A German review on the impact of *C. fiber* on biodiversity is provided by Sommer et al. (2019).

Addition (FI): When it comes to niche overlap among *C. fiber* and *C. canadensis* and the outcome of competition, Alakoski et al. (2019) provide a useful reference (see above; 2.3.2 and 2.3.3).

2.5.4 *Effects on public health*

Addition (FR): Giardiasis, for which *Giardia* is the causative agent, is popularly known as “beaver fever” (Tsui et al. 2018).

2.5.5 *Socio-economic effects*

Correction (LU): The text under this heading suggests that *C. canadensis* currently occurs in Luxembourg. However, Luxembourg is now considered to be free of the species (Schley 2019).

Addition (LU): The trapping data for Luxembourg and Belgium are outdated. An update is provided above (2.3.6).

Addition (LU): The text highlights the costs needed for *C. canadensis* control, where the species establishes. It deserves to be emphasized that control of *C. canadensis* can become complicated when the species establishes together with, or close to, *C. fiber*, as additional challenges in terms of species distinction and stakeholder communication arise in such situations.

Addition (CZ): As *C. canadensis* and *C. fiber* are very similar, it is of use to consider the costs of impact, and costs of management, for *C. fiber*. Campbell-Palmer et al. (2015) reviewed actual management options and associated costs across Europe. Additional references may be found in the overview of Brazier et al. (2021) on human–beaver conflicts.

2.5.6 *Effects on ecosystem services*

Addition (AT): In the table on ecosystem services linked to *C. canadensis* (Table 2.5), a ‘climate regulating service’ may be added. Notably, the increase in surface area of standing water following beaver establishment leads to increased emissions of methane (CH₄). The resulting methane flux is estimated to have increased 200-fold over the course of the 20th century (Whitfield et al., 2015).

3. Risk assessment

3.1 Risk assessment and classification with the Harmonia+ protocol

3.1.1 *Classification for the current situation*

Addition (LU): It is *more* than likely that the presence of *C. canadensis* in Rhineland-Palatinate (and at least formerly in adjacent regions) is linked to the Eifelzoo, as all specimens analyzed during a large-scale genetic study that also included the animals kept at that zoo at that time, were of the same haplotype. The beavers that replaced *C. canadensis* at the zoo after they were removed have genetically been shown to be *C. fiber* (Schley 2019).

Addition (AT): The probability that *C. canadensis* will be introduced into the EU by intentional actions was scored low in the Harmonia⁺ assessment (Table 3.1). The confidence was scored as medium, and not as high, the reason for which is not explained in detail. A medium confidence seems justified, though, since (illegal) releases as a game species or for “fauna improvement”, and confusion of both species in conservation reintroductions (as happened in the past) cannot be ruled out.

3.1.2 *Classification for future situation*

Addition (AT, FI): The severity of winter weather is considered a factor influencing litter size (Jenkins & Busher 1979). Although an increased rate of spread might thus be expected under future climate conditions, the overall risk score of *C. canadensis* remains the same, as the current risk of spread is already considered maximal.

3.2 Risk assessment and classification with the ISEIA protocol

3.2.1 *Classification for the current situation*

Addition (LU, FI): The text under this heading assumes that “*C. canadensis* is reported to build more dams” (Hollander et al. 2017). However, this point is not evident from scientific studies, and in practice depends very much on local conditions (e.g. Danilov & Fyodorov 2015).

3.3 Other available risk assessments

3.3.1 *EU Member States*

Addition (AT): Another risk assessment for EU Member States can be added to the overview. The risk of *C. canadensis* has been assessed for Germany (Nehring et al. 2015) using the GABLIS method (Essl et al. 2011). The assessment is much in line with the other European assessments. As a result of the assessment, the species is included in the Black list for Germany, and more specifically, the Action list for the country, which means that eradication is considered feasible and recommended (Nehring et al. 2015). The assessment for Germany, however, can not be extended to the entire EU without modifications.

Addition (LU): At the time of the ISEIA risk assessment in Luxembourg (Ries et al. 2014), *C. canadensis* was known to be present at four sites. Now, the country is considered free of the species (Schley 2019). Maintaining the same risk score, the species thus ends up on the Alert list (B0) instead of the Watch list (B1).

4. Discussion

4.2 Knowledge gaps and uncertainties

4.3 *Management*

Correction (LU): The statement that in France, “a small population of *C. canadensis* co-occurring with *C. fiber* was successfully eradicated” (Hollander et al. 2017), is somewhat misleading. According to Dewas et al. (2011), *C. fiber* was present in the Loire river and *C. canadensis* in a tributary to the Loire. The statement that “there are no existing *C. canadensis* management programs in the EU” is also incorrect, as discussed under 2.3.6.

5. Conclusions

Correction (LU): It is incorrectly stated that *C. canadensis* has been introduced to Luxembourg, as the presence of the species is considered to be due to natural dispersal from Rhineland-Palatinate (Germany). The same is probably true for Belgium (Schley 2019).

Correction (LU): The text suggests that the species is present in the greater border region of Belgium, Germany and Luxembourg. To specify, however, it currently seems to be present with certainty only in Rhineland-Palatinate (Germany, Schley 2019). For measures in North Rhine-Westphalia (Germany), Luxembourg and Belgium, see above (2.3.6).

Correction (FI): It is not really to be “*expected that the species will spread towards Sweden*” (Hollander et al. 2017). At present, it seems that *C. fiber* is spreading from Sweden to Finland, and no observations of *C. canadensis* near the Swedish border have recently been made (Alakoski et al. 2020). As the outcome of competition is not clear (yet), this does not take away concerns of a possible range expansion of *C. canadensis*, though.

Addition (FI): *C. canadensis* may benefit from milder winters, as the severity of winter weather is considered a factor influencing litter size (Jenkins & Busher 1979). See under 3.1.2 for a discussion.

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Revision of the Risk assessment of the Staff-vine (*Celastrus orbiculatus*), as based on Member State comments

Introduction

This document explains how the comments received through the Scientific Forum and the consultation of stakeholders on the **Risk assessment of the Staff-vine (*Celastrus orbiculatus*)** by Beringen et al. (2017)¹ have been considered.

Disclaimer: The authors of the present document have prepared this note with the only purpose to process the Member States comments to the original Risk Assessment by Beringen et al. 2017, on behalf of the European Commission. For the same reason, the present document does not represent an endorsement of the statements and scores given within the original document. Since the focus of this document was limited to the Member States comments, it cannot be considered as a revision of the original document, whose contents and bibliography have not been through a comprehensive check, and remain the sole responsibility of the original authors.

Name of species assessed: <i>Celastrus orbiculatus</i> Thunb.
Date of completion of this document: 01/03/2021
Member States that provided comments: <ul style="list-style-type: none">• Austria (AT)• Belgium (BE)• Denmark (DK)• Estonia (EE)• Germany (DE)• Poland (PL)• Romania (RO)
Stakeholder organizations that provided comments: none.

Outline

The first part of this document highlights how the comments from Member States have been dealt with. The second part of this document deals with the specific comments from Member States. The text should be read as an addendum and corrigendum to the original risk assessment (Beringen et al. 2017). The headings from the original assessment are repeated to facilitate direct comparison.

¹ Beringen R., van Duinen G.A., de Hoop L., de Hullu P.C., Matthews J., Odé B., van Valkenburg J.L.C.H., van der Velde G., Leuven R.S.E.W. (2017) Risk assessment of the alien Staff-vine (*Celastrus orbiculatus*). © Department of Environmental Science, Radboud University, 6525 AJ Nijmegen, The Netherlands

References that were included in the original risk assessment are not repeated. New references are provided at the end of this document.

Part 1: Comments per Member State

Austria (AT)

AT suggested a *minor revision*. Specific comments are dealt with in the second part of this document.

Belgium (BE)

BE considered the risk assessment was *fit-for-purpose* and had no additional comments.

Denmark (DK)

DK suggested a *major revision*. Specific comments are dealt with in the second part of this document.

Estonia (EE)

EE considered the risk assessment was *fit-for-purpose* and had no additional comments.

Germany (DE)

DE provided additional informations, which are dealt with in the second part of this document.

Poland (PL)

PL suggested a *minor revision*. Specific comments, especially on the distribution of the species in Poland, are dealt with in the second part of this document.

Romania (RO)

RO considered the risk assessment was *fit-for-purpose* and had no additional comments.

Part 2: Specific comments

This part provides additions and corrections under the respective headings of the original assessment (Beringen et al. 2017). The Member State on whose comment it is based, is indicated between brackets.

The following table provides details of where the common elements of risk assessments defined by Article 5 of the Regulation 1143/2014 of 22 October 2014 can be found in the *Celastrus orbiculatus* risk assessment.

Table 1 -- The elements of risk assessments as defined by Regulation 1143/2014, and the headings under which they are treated by Beringen et al. (2017)

Element	Headings
Art 5(1)(a) — <i>a description of the species with its taxonomic identity, its history, and its natural and potential range</i>	2.2.1
Art 5(1)(b) — <i>a description of its reproduction and spread patterns and dynamics including an assessment of whether the environmental conditions necessary for its reproduction and spread exist</i>	2.1.2
Art 5(1)(c) — <i>a description of the potential pathways of introduction and spread of the species, both intentional and unintentional, including where relevant the commodities with which the species is generally associated</i>	2.4
Art 5(1)(d) — <i>a thorough assessment of the risk of introduction, establishment and spread in relevant biogeographical regions in current conditions and in foreseeable climate change conditions</i>	3.1, 3.2
Art 5(1)(e) — <i>a description of the current distribution of the species, including whether the species is already present in the Union or in neighbouring countries, and a projection of its likely future distribution</i>	2.3.1, 2.3.2, 3.1.2
Art 5(1)(f) — <i>a description of the adverse impact on biodiversity and related ecosystem services, including on native species, protected sites, endangered habitats, as well as on human health, safety, and the economy including an assessment of the potential future impact having regard to available scientific knowledge</i>	2.5
Art 5(1)(g) — <i>an assessment of the potential costs of damage</i>	2.5
Art 5(1)(h) — <i>a description of the known uses for the species and social and economic benefits deriving from those uses</i>	2.5

Overarching comments from Germany

DE provided additional information from recent studies that should be included in the risk assessment. The following text details the comments and includes suggestions of text and its position in the risk assessment.

- In Germany, male and female plants occur together in the field. Seed formation and first spread were observed.

Page 10 (reproduction)

Flowers of *Celastrus* species frequently become unisexual through the abortion of male or female organs. Plants are often functionally dioecious (Brizicky 1964, Burnham & Santanna 2015). Occasionally monoecious plants, with both male and female flowers, are reported (Hou, 1955), and occasionally plants develop both unisexual and perfect flowers on the same plant (polygamodioecious) (Dreyer et al. 1987). In Germany, male and female plants occur together in the natural environment which enables reproduction and seed production (Alberternst & Nawrath 2018). The cultivar *C. orbiculatus* 'Hermaphroditus' is self-pollinating. The cultivar *C. orbiculatus* 'Diana' is a female clone and only sets fruit if a male clone is present locally. *C. orbiculatus* 'Hercules' is a male clone and is used as a pollinator for female plants (e.g., Marczyński 2016).

Page 21 (2.4.1. Dispersal potential by natural means)

- A zoochore spread by birds is to be assumed on the basis of the distribution pattern and the growth place.

Page 43 (probability of spread)

The unintentional distribution of plants from gardens into the wild, caused by berry eating birds can contribute to the spread of *C. orbiculatus*. In Germany, bird dispersal has been recorded at 400 m from

the parent plants (Alberternst & Nawrath, 2018). Additionally, the improper disposal of (bonsai) trees or decorations outdoors or in compost can contribute to spread.

- In Hesse similar habitats are already populated as in the USA, where the species is classified as invasive.

Page 16

In Europe, most sites are situated in or near urban areas. Therefore, it is not clear in which EU habitats *C. orbiculatus* will establish. The most likely areas for establishment are forest habitats on moist, fertile, neutral soils like 91E0: “Alluvial forests with *Alnus glutinosa* and *Fraxinus excelsior* (Alno-Padion, Alnion incanae, Salicion albae)” or 91F0 “Riparian mixed forests of *Quercus robur*, *Ulmus laevis* and *Ulmus minor*, *Fraxinus excelsior* or *Fraxinus angustifolia*, along major rivers (*Ulmenion minoris*)”.

A detailed survey in Hessen, Germany (Alberternst & Nawrath, 2018) recorded dominant stands with vegetative and generative regeneration. One stand originated from a nursery and has spread into an area protected under the EU Habitats Directive and classified as Annex I habitat type code 91E0. Another stand occurs in a nature protection area of light pine forest on sandy soils resembling 91T0. Regeneration and spread of the species was observed in three of the four stands. Another stand was found on railway land, where the plant covers unused tracks. Such mentioned habitats are similar to those where the species occurs in the USA.

Summary (p. 3/4)

Comment (AT): The authors assume that the applied risk assessment method (internet version of Harmonia+) is compliant with the criteria for RA derived from Reg 1143/2014. This review confirms this assumption in large parts; however, it also identified some gaps that need revision. While the risk assessment is very clear and well written in general, it is sometimes difficult to find the necessary information related to the specific criteria of the Regulation. It would be helpful to modify (or add) the Summary accordingly. The summary lacks explicitly clear information related to current and possible future occurrences with regard to Member States and biogeographical regions.

Answer: The following text should be included in the summary:

Celastrus orbiculatus currently occurs in ten EU countries (Austria, Belgium, the Czech Republic, Denmark, Germany, Latvia, Lithuania, the Netherlands, Poland, Sweden), and the United Kingdom. Under climate change, in the future, the entire Atlantic, Continental and southern Boreal biogeographical regions of Europe are likely to be suitable for the establishment of the species. Therefore, this has the potential to increase the future occurrence of *C. orbiculatus* to the following countries: Austria, Belgium, Bulgaria, Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, the Netherlands, Poland, Romania, Slovakia, Slovenia, Sweden, Spain, and the United Kingdom.

Comment (AT): The summary lacks indications for the associated levels of uncertainty of the scorings.

Answer: The only score that is detailed in the summary section is the overall score, which is medium. This score was produced with the Harmonia system which does not provide an uncertainty score associated with the overall score.

Comment (DK): On p. 3, it is stated that the report also includes a risk assessment of *C. orbiculatus* focussed on the Netherlands, we question whether it is the right place to publish a national risk assessment.

Answer: The inclusion of the statement is a decision of the authors and does not impact the results of the risk assessment and therefore there is no change suggested.

Comment (DK): On p. 3 it is stated that “Suitable habitats in the EU are most likely forests on moist, fertile, and neutral soils...”, from the sections 2.3.3 and 2.3.4 it is not clear why e.g. urban sites, former agricultural fields, reforested agricultural fields and sand dunes are not suitable habitats in the EU as they are in other parts of the its introduced range. Furthermore, the RA (p. 29) states that “the species thrives best in recently disturbed habitats” and forests may not be regarded as recently disturbed.

Answer: The sentence should be amended to read:

“The most suitable habitats in the EU are most likely forests on moist, fertile, and neutral soils...”

Species description (p. 7)

Comment (DK): There are difficulties in distinguish *C. orbiculatus* from *C. scandens*, except when the species are fertile. The species, however, forms a “seedling bank” (mentioned on p. 12) that may last for many years especially in forest ecosystems (shaded habitats). This problem should be mentioned because it also have implications for early identification and eradication. Furthermore, *C. orbiculatus* from *C. scandens* hybridize (Pooler et al. 2002). The Ra needs to mention problems separating hybrids from parental species.

Answer: On page 9 of the RA, it is clearly stated that folding of the leaves is a useful identifying characteristic and therefore it is not correct to say the species can only be told apart when they are fertile. Additionally, there is no evidence that hybrids occur in the risk assessment area, and therefore identifying characteristics of hybrids and parent species is not required.

2.1.2 Species characteristics (p. 10)

Reproduction:

Comment (DK): It should be mentioned in the section on reproduction that *C. orbiculatus* requires gaps in the canopy for sexual reproduction (flowering and fruiting) as mentioned on p. 21 (reference to study by Silveri et al. (2001).

Answer: The paragraph should be amended to read:

Gaps in the canopy are required for sexual reproduction, flowering and fruiting (Silveri et al. 2001).

2.2 Probability of introduction (p. 13)

Comment (DK): It is stated that *C. orbiculatus* has been cultivated for many years in several member states”. How many? And which?

Answer: This is a general statement which is then further elaborated in the remaining text of section 2.2 giving the information Denmark requests.

2.3 Probability of establishment (p. 13)

Comment (AT): An explicit answer to which Member States and biogeographical regions are suitable under current and foreseeable future conditions is missing. This information should also be provided in the Summary.

Answer: The following text should be included:

Celastrus orbiculatus currently occurs in ten EU countries (Austria, Belgium, the Czech Republic, Denmark, Germany, Latvia, Lithuania, the Netherlands, Poland, Sweden), and the United Kingdom. Under climate change, in the future, the entire Atlantic, Continental and southern Boreal biogeographical regions of Europe are likely to be suitable for the establishment of the species. Therefore, this has the potential to increase the future occurrence of *C. orbiculatus* to the following countries: Austria, Belgium, Bulgaria, Czech Republic, Denmark, Estonia, Finland, France, Germany, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, the Netherlands, Poland, Romania, Slovakia, Slovenia, Sweden, Spain, and the United Kingdom.

Table 2 – Summary of the status of *C. orbiculatus* in Member States of the European Union, and the United Kingdom

Member State	Recorded	Currently established	Potential establishment	Currently invasive
Austria	Yes	Yes (locally)	Yes	No
Belgium	Yes	Yes (locally)	Yes	No
Bulgaria	No	No	Yes	No
Croatia	No	No	No	No
Cyprus	No	No	No	No
Czech Republic	Yes	No	Yes	No
Denmark	Yes	Yes (locally)	Yes	No
Estonia	No	No	Yes	No
Finland	No	Yes -No	Yes	No
France	No	No	Yes	No
Germany	Yes	Yes	Yes	No
Greece	No	No	No	No
Hungary	No	No	Yes	No
Ireland	No	No	Yes	No
Italy	No	No	Yes	No
Latvia	Yes	Yes (locally)	Yes	No
Lithuania	Yes	Yes	Yes	Yes
Luxembourg	No	No	Yes	No
Malta	No	No	No	No
Netherlands	Yes	Yes (locally)	Yes	No
Poland	Yes	Yes	Yes	Yes
Portugal	No	No	No	No
Romania	No	No	Yes	No
Slovakia	No	No	Yes	No
Slovenia	No	No	Yes	No
Spain	No	No	Yes	No
Sweden	Yes	Yes (locally)	Yes	No
United Kingdom	Yes	Yes (locally)	Yes	No

Table 3 -- Summary of the status of *C. orbiculatus* in biogeographical regions of the European Union

Biogeographical region	Recorded	Currently established	Potential establishment	Currently invasive
Alpine	Yes	Yes	Yes	No
Atlantic	Yes	Yes	Yes	No

Black Sea	No	No	No	No
Boreal	Yes	Yes	Yes	Yes
Continental	Yes	Yes	Yes	Yes
Mediterranean	No	No	No	No
Pannonian	No	No	No	No
Steppic	No	No	No	No

2.3.2 Current distribution in the European Union (p. 14/15)

Addition (PL): *Celastrus orbiculatus* is currently recorded in the central segment of the Międzyrzecz Reinforced Region and its expansion has been observed near Panzerwerkes (Purcel, 2010, 2011).

Comment (DK): Six records in Denmark (Hartvig, P. 2015 Atlas Flora Danica, Gyldendal, København. See also GBIF². First record 1981. Map p. 14 need correction.

Answer: The maps cannot be updated within this document due to copyright issues.

Comment (DK): In Denmark, *C. orbiculatus* is also found in woodland (Hartvig, 2015).

Answer: The first paragraph on page 15 should be amended to include the following sentence at the end of the paragraph: In Denmark, the species is also found in woodland (Hartvig 2015).

2.3.3 Habitat description and physiological tolerance (p. 15/16)

Comment (DK): In Denmark, the species is found in forest habitats (coniferous forest, thicket, park), along railways and in urban areas (Hartvig 2015).

Answer: The second paragraph on page 16 should be amended to include the text below at the end of the paragraph: In Denmark, the species is found in forest habitats (coniferous forest, thicket, park), along railways and in urban areas (Hartvig 2015).

2.3.4. Climate match and bio-geographical comparison (p. 17)

Endangered areas:

Comment (AT): The statement that the area with suitable climate will expand northward (in 2.3.4) needs be expanded with information on possible changes in the biogeographic regions.

Answer: The paragraph should be amended to read:

Based on expert opinion (authors of the current risk assessment), under climate change, in the future, the entire Atlantic, Continental and southern Boreal biogeographical regions of Europe are likely to be suitable for the establishment of the species.

² https://www.gbif.org/occurrence/search?country=DK&taxon_key=8104460

2.4.1 Dispersal potential by natural means (p. 21/22)

Comment (DK): We wonder why starlings are the only seed eating birds mentioned as potentially contributing significantly to spread of the species? Other berry-eating birds may serve as vector as well?

Answer: Starlings are the only specific bird species mentioned in the risk assessment as this is where there is evidence. Throughout the RA, berry eating birds are mentioned often with the example of Starlings, therefore confirming that other bird species may spread seed.

Comment (DK): Although the scoring might be right, the fact that “to date the species has not shown considerable spread at the current recorded locations in Europe” should be discussed and reflected in the section on spread.

Answer: The first paragraph in the spread section (p. 31) can be amended as follows:

It should be noted, that to date the species has not shown considerable spread at the current recorded locations in Europe. Reasons for this could include that the propagule pressure is not currently that high, or the species is in its lag phase.

2.5. Impacts (p. 22)

Comment (DK): Referring to Gederaas et al. (2012) (the Norwegian species database) is not a valid reference for the mentioned biodiversity effects. Furthermore, the reference indicate that *C. orbiculatus* is a low risk species, i.e. the reference is not correctly cited.

Answer: The reference to Gederaas et al. (2012) can be deleted from the reference list as it is only cited in the above case. The paragraph can be amended to read:

Fryer (2011) regard *C. orbiculatus* as a severe invasive species, mainly because of detrimental interactions with indigenous endangered or rare species and endangered or rare habitats.

Comment (DK): Referring to Glastonbury Partners in Planting (GPIP 2013) is not a valid reference for effects of the species.

Answer: The reference to GPIP (2013) can be deleted from the reference list as it is only cited in the above case and can be replaced by Fryer (2011).

Comment (DK): It should be noted that the observations by Lutz (1943) are made on the related species *C. scandens*. Just above the photo (Fig. 2.10) it is stated that: “Hardwoods are more commonly damaged than conifers because the relatively dense canopy of conifers offers unfavourable light conditions for *C. orbiculatus* (Lutz 1934)”. Looking into another study cited in the RA (Ellsworth et al. 2004b) it is, however, found that survival and growth the first year did not differ between 2%, 28% and 100% sun, at the second season growth and biomass were greater in the 100% and 28% sun treatments than 2% sun...” and furthermore “The ability of *C. orbiculatus* to survive under deep shade despite its slow growth implies that intact forest are vulnerable to invasion and that established understory populations should be controlled before harvesting or thinning the forest”

Answer: The paragraph should be amended as follows:

For *C. scandens*, hardwoods are more commonly damaged than conifers because the relatively dense canopy of conifers offers unfavourable light conditions [for the species to establish] (Lutz 1943). This is also likely to be the case for *C. orbiculatus* due to similarities in form, function and requirements. *Celastrus orbiculatus* can persist in forest understories in the USA for a number of years and following a disturbance in the canopy (i.e. tree or limb falls causing canopy gaps), rapid growth can occur

(Patterson, 1973). *Celastrus orbiculatus* “uses a ‘sit and wait’ invasion strategy” (Greenberg *et al.*, 2004).

Comment (DK): The study by Dreyer *et al.* (1987) should not be used as reference for “the competition and hybridization effects of introduced *C. orbiculatus*”. This study does not include hybridization but tells that “this is being investigated”. The study by Pooler *et al.* (2002) found in the reference list, however, deals with hybridization and vigour of the interspecific seedlings. This study should be mentioned here.

Answer: In the paragraph on page 23, Dreyer *et al.* (1987) is not referring to hybridization but in fact the latter sentence. Pooler *et al.* (2002) can however be added to the paragraph.

Comment (DK): Zaya *et al.* (2015) is cited for the conclusion that interspecific hybrids between *C. orbiculatus* and *C. scandens* do not have increased invasiveness and vigour. The study by Pooler *et al.* (2002), however, found that seedlings from interspecific crosses had less dormancy and were more vigorous than the intraspecific seedlings. This should be discussed.

Answer: In the paragraph on page 35 the following text should be amended to read:

It should be noted that Pooler *et al.* (2002) found that seedlings from interspecific crosses had less dormancy and were more vigorous than the intraspecific seedlings.

Comment (DK): The study by Leicht-Young and Pavlovic (2012) is not correctly cited. It does not support that “*C. orbiculatus* has the ability to move out from the forest into the open dune habitats”. Actually, it states (p. 172) “In full sun, we have often observed *C. orbiculatus* growing under the shade of shrubs, tree saplings, or grapes with ramets extending out into open habitats. These ecotonal habitats are where the threat to *C. pitcheri* is likely greatest from *C. orbiculatus*, because without some type of cover for *C. orbiculatus* to establish under, this species does not grow as well in wide open dunes where *C. pitcheri* is also present”.

Answer: In the paragraph on page 35 the following text should be amended to read:

Along Lake Michigan. *C. orbiculatus* has the ability to encroach into the dune habitat which may be a threat to the endangered *Cirsium pitcheri* (Leicht-Young & Pavlovic 2012).

Comment (DK): Looking at effects on cultivated plants (chap. 2.5.2), effects on socio-economy (chap. 2.5.5) and effects on provisioning services (chap. 2.5.6) seem to be an overrepresentation of the same impact.

Comment (DK): As commented for section on impacts, there seem to be an over-assessment of effects on plant crops/provisioning services.

Answer: This is a common problem with evaluating ecosystem services. We don’t have information on how the authors of the risk assessment weighted these scores and therefore cannot suggest a revision. The overall conclusion of the RA are nevertheless not affected.

Comment (DK): Section 2.5.4 Effects on public health states that no information regarding the effects of *C. orbiculatus* was found, however WOS have numerous citations of studies of the efficacy of extracts from the species against gastric cancer. The section on Provisioning services (P. 26) also document use in the treatment of poisoning, infectious disease and as an antidote for snakebites. This should also be mentioned here.

Answer: A risk assessment details negative effects. Although benefits are detailed as a requirement of the Regulation, they are not included in the scoring of this section.

Comment (DK): The study by Pavlovic *et al.* 2016. Oriental bittersweet (*Celastrus orbiculatus*): Spreading by fire. *Forest Ecology and Management* 364, 183-194) clearly documents that fire is not a

good measure for control of *C. orbiculatus*. This needs to be reflected in the section on Physical measures. Pavlovic et al. (2016) also tested other methods such as cutting and herbicide application and the study should be mentioned in these paragraphs as well.

Answer: In the paragraph on page 26 the following text should be amended to read:

Physical measures

Prescribed burning is not considered a viable option for control. It is likely that *C. orbiculatus* is actually favored by fire due to rapid growth in response to opening the canopy and the large nutrient flushes that usually occur after fires (Pavlovic et al. 2016). However, if fire management is properly applied, the year's crop of seeds would be essentially eliminated (Leicht-Young et al. 2013). Cutting and herbicide application can be used to control the species (Pavlovic et al. 2016).

Comment (DK): Section 2.5.6 Effects on ecosystem services have very few references. The part on Provisioning services could be supplemented by numerous references on use extracts against gastric cancer.

Answer: The risk assessment scores negative impacts not positive impacts.

Comment (DK): The section on Supporting services should refer to the study by Leicht-Young et al. 2009 (found in the reference list) on effects of *C. orbiculatus* on soil composition and processes.

Answer: In the paragraph on page 28 the following text should be amended to read:

The species has a negative effect on ecosystem nutrient cycling due to the plant's ability to function like a nutrient pump (Leicht-Young et al. 2009). Minerals become available to other species as the *C. orbiculatus* litter layer degrades easily and enriches the soil (Leicht-Young et al. 2009).

3. Risk assessment (p. 29ff)

Comment (DK): There is little reasoning and justification of the statements and scoring of the species in the risk assessment section. One needs to seek around in the scientific section. That is not optimal.

Answer: The structure of the risk assessment cannot be addressed by this document.

3.1 Risk assessment and classification with Harmonia+ (p. 30)

Comment (AT): Table 3.1. does not correspond to the recommended pathway terminology (while Table 2.4 does).

Answer: In Table 3.1 the following text could be inserted between the line:

A06. Probability of introduction by natural means

Escape from confinement

A07. Probability of introduction by unintentional human actions

However, since Table 3.1. refers to the Harmonia+ protocol, no change to the wording of the categories should be made.

Comment (AT): Why the medium score for probability of intentional human actions (A08) was given a “high confidence”? According to the explanation in the text (3.1.1.), in comparison with A06 and A07, a “medium” confidence would fit better.

Answer: Under this pathway, the species is the commodity itself, and therefore any movement and planting of the species in the RA area can lead to the medium risk of entry. The confidence of high remains supported. It should be noted that no other reviewers highlighted this as an issue.

Comment (AT): Why is the confidence for establishment in habitats only “medium” and for climate “high”? There seems to be no difference in the explanation in 3.1.1. I think both should be scored identical as “medium”. Detailed information on probability of spread is equally uncertain, and was scored with “medium” confidence.

Answer: The paragraph on establishment (p. 29) should be amended to read:

The confidence differs though (habitats: medium and climate: high) which reflects the uncertainty of the species finding a suitable habitat and difference in habitats of the species in North America compared to the EU. For climate, the Köppen-Geiger system shows a strong suitability.

Environment: biodiversity and ecosystems (p. 31)

Comment (DK): The conclusion that *C. orbiculatus* cause medium effect on native species (“at worst, severe population declines in species that are not of conservation concern or limited population declines in species that are of conservation concern”) might be right but it could not be drawn based on the information given in chapter 2.5.1. Chapter 2.5.1 needs to be elaborated to support the conclusion.

Answer: The current score is considered appropriate and the point was not raised by other reviewers. Section 2.5.1 is detailed giving numerous examples to support a ‘medium’ impact.

Comment (DK): The risk of adverse effects on ecosystem integrity due to changes to biotic properties is estimated to medium. Again, that might be right but virtually no references are given on such effects. Even though, the risk estimates are based on professional knowledge the confidence should be “low” as effects on ecosystem biotic parameters might be highly variable.

Answer: Based on the fact that there are no supporting references, the suggestion to change the confidence score to low is reasonable.

Ecosystem services (p. 32)

Comment (DK): The scoring of effects on provisioning services as moderately negative should maybe be re-evaluated, as effects on timber might be severe. The outweighing of positive effect on pharmaceuticals, natural medicine and bio-chemicals against negative effects on timber production is not really a good idea.

Answer: Only negative effects are evaluated and thus, the sentence ‘The positive effect of the species on bio-chemicals, natural medicines, and pharmaceuticals was outweighed by its negative effect on timber production’ should be removed from the paragraph.

Comment (DK): Effect on plant crops should be mentioned in a section on effects on socio-economy, which is missing? Why does the section on “Plant crops” not include effects on forest? Consequences for other crops are more speculative as it needs structures such as trees, shrubs, tree saplings, or grapes as support but may have ramets extending out into open habitats. Therefore, the score “medium impact” might be an overestimation if forest is not included.

Answer: An additional sentence should be added to the paragraph on plant crops on p. 32:

Additionally, *C. orbiculatus* can have negative impacts on tree species in forest systems, potentially killing trees by smothering them.

3.1.1 Classification for current situation (p. 29)

Species introduction

Comment (DK): The only pathway described is escape from cultivation/horticulture. On p. 29 the RA states that “Introductions via natural pathways are expected to occur less than once every 30 years, because it is highly unlikely that the species will enter the EU via earth-moving activities”. Is this statement based on the knowledge that the species does not form a persistent seedbank (p. 12)? (This is an example that the reviewer need to seek around in the RA to find the relevant information, which is not optimal).

Answer: This statement is more related to the fact that soil as a pathway into the EU is mainly closed due to phytosanitary regulations. The paragraph should be amended to read:

Introductions via natural pathways are expected to occur less than once every 30 years, because it is highly unlikely that the species will enter the EU via earth-moving activities (due to the pathway of soil into the EU being mainly closed and regulated).

Comment (DK): *C. orbiculatus* is also recorded from Denmark (see below), therefore number of MS where the species has been recorded should be changed from “eight” to “nine”.

Answer: Denmark should be added as a country where the species has been recorded, but it remains eight MS, excluding the United Kingdom.

Comment (DK): The finding that “to date the species has not shown considerable spread at the current recorded locations in Europe” (p. 29) is not an argument for establishment (where it is put now). Actually, it should be discussed in the section on spread that scores the capacity of *C. orbiculatus* to disperse within the EU by natural means as high.

Answer I: The paragraph on establishment (p. 29) should be amended to read:

This criterion is scored with medium confidence considering that there is no relevant information available within the EU and that the species thrives best in recently disturbed habitats.

Answer II: The paragraph on spread (p. 31) should be amended to read:

It should be noted, that to date the species has not shown considerable spread at the current recorded locations in Europe. Reasons for this could include that the propagule pressure is not currently that high, or the species is in its lag phase.

Comment (DK): What is the conclusion about probability of introduction to the EU’s wild habitats being medium (between 1 and 9 events per decade) based on?

Answer: It appears to be expert opinion of the authors as documented information is not available.

3.1.2 Classification for future situation (p. 33)

Comment (AT): On the one hand the assessment (2.3.3, also 2.3.4 and 2.5.7) states that temperature/climate is relevant (... climate change may render high-elevation sites increasingly vulnerable ...), but on the other hand (3.1.2) expects no effects on the risk scores (based on a conservative climate change scenario). This expectation should be backed up with some arguments. Is the argument still valid under more severe (>2°C) climate change scenarios?

Answer: It must be assumed that uncertainty increases, but the argument seems still valid under more severe scenarios.

3.2 Risk assessment and classification with ISEIA-protocol (p. 35)

Comment (DK): Only the study by Zaya et al. (2015) that found no evidence that invasiveness increases is cited in the section on adverse impacts on native species. The study by Pooler et al. (2002), however, showed increased vigour of hybrids.

Answer: Pooler et al. (2002) should be added in the paragraph on page 35 (see above).

5. Conclusions (p. 42)

Comment (AT): The RA is not organized in a question-answer-way, which makes it sometimes difficult to find the relevant information. An extended summary page with the relevant information (according to the requirements of the Regulation) would be helpful.

Answer: This is outside the scope of this document.

Comment (DK): As mentioned in the comments on the summary, the RA could be much improved if the structure was changed so sections on studies of relevant risks were put next to sections evaluating and scoring the respective risks. As it is now the reviewer/reader needs to do a lot of page turning in order to seek and eventually find the relevant information for the reasoning and the scoring.

Answer: This is outside the scope of this document.

Comment (DK): Including the Danish records, the species has been recorded in nine countries.

Answer: ~~It~~ Following more recent information, it is recorded in still eighteen EU Member States, and the United Kingdom.

Appendix 3 (p. 68)

Comment (DK): *C. orbiculatus* is present in Denmark

Answer: Appendix 3 should be amended to include Denmark

References

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[*Celastrus orbiculatus* \(Celastraceae\) in Europe. NeoBiota 56: 1-25](#)

Johnson LR & Handel SN 2016. Restoration treatments in urban park forests drive long-term changes in vegetation trajectories. *Ecological Applications*, 26, 940-956.

Patterson DT (1973) The ecology of Oriental bittersweet, *Celastrus orbiculatus*, a weedy introduced ornamental vine. Durham, NC: Duke University. 252 p. Dissertation

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Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention"
Contract No 07.0202/2019/812602/ETU/ENV.D.2¹

Name of organism: *Cortaderia selloana* (Schult. & Schult.f.) Asch. & Graebn.

Author(s) of the assessment:

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Risk Assessment Area: The risk assessment area is the territory of the European Union 27 and the United Kingdom, excluding the EU-outermost regions.

Peer review 1: John Lambrinos, Department of Horticulture Oregon State University, Corvallis, USA

Peer review 2: Helmut Kudrnovsky, Umweltbundesamt/Environment Agency Austria, Vienna, Austria

Date of completion: 15/10/2020

Date of revision: 15/10/2021

¹This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA). A number of amendments have been introduced to ensure compliance with Regulation (EU) 1143/2014 on IAS and relevant legislation, including the Delegated Regulation (EU) 2018/968 of 30 April 2018, supplementing Regulation (EU) No 1143/2014 of the European Parliament and of the Council with regard to risk assessments in relation to invasive alien species (see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>).

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SECTION A – Organism Information and Screening

A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?

including the following elements:

- the taxonomic family, order and class to which the species belongs;
- the scientific name and author of the species, as well as a list of the most common synonym names;
- names used in commerce (if any)
- a list of the most common subspecies, lower taxa, varieties, breeds or hybrids

As a general rule, one risk assessment should be developed for a single species. However, there may be cases where it may be justified to develop one risk assessment covering more than one species (e.g. species belonging to the same genus with comparable or identical features and impact). It shall be clearly stated if the risk assessment covers more than one species, or if it excludes or only includes certain subspecies, lower taxa, hybrids, varieties or breeds (and if so, which subspecies, lower taxa, hybrids, varieties or breeds). Any such choice must be properly justified.

Response: This risk assessment covers one species, the Pampas grass *Cortaderia selloana* (Schult. & Schult.f.) Asch. & Graebn. Testoni and Villamil (2014) provided evidence that *Cortaderia jubata* (Lemoine ex Carrière) Stapf “represents only a portion of the morphologic variability of *C. selloana* s.l.” and should therefore be recognised as a subspecies of the latter (*Cortaderia selloana* subsp. *jubata* (Lemoine) Testoni and Villamil), and clearly distinguished from *Cortaderia selloana* subsp. *selloana* (the autonymic subspecies). In the most recent revision of the genus *Cortaderia*, Testoni and Linder (2017) upheld this reclassification. There is also considerable identification uncertainty in regions where both taxa are introduced (e.g. DiTomaso et al. 2003; DiTomaso et al. 2010; Houliston and Goeke 2017), and Lambrinos (2001) suggested that the floral traits of *C. selloana* in California have gradually become more similar to that of *C. jubata* over the previous 80 years. However, apart from the two aforementioned references in all of the literature, web references and databases assessed during the preparation of this PRA, *C. selloana* and *C. jubata* are still referred to at the species level. Moreover, regardless of the specific or sub-specific classification of *C. selloana* and *C. jubata* diagnostic morphological, reproductive and phenological characters are used to distinguish these two taxa (e.g. Houliston and Goeke 2017, Lambrinos 2001; Testoni and Linder 2017). This PRA therefore follows the previous nomenclature by Ascherson and Graebner (1900) for this taxon: *Cortaderia selloana* (Schult. & Schult.f.) Asch. & Graebn. [Syn. Mitteleur. Fl. [Ascherson & Graebner]. 2(1): 325].

“*Cortaderia jubata* (Lemoine ex Carrière) Stapf” is already listed as an invasive alien species of Union concern (second update of the Union list entered into force on 15 August 2019²), therefore, the focus of this risk assessment is especially on *Cortaderia selloana* (Schult. & Schult.f.) Asch. & Graebn. subsp. *selloana* [Syn. Mitteleur. Fl. 2(1): 325. 1900.] hereafter *C. selloana*.

Class: *Monocotyledons* (*Monocots*, *Liliopsida*)

Order: *Poales* Small (*sensu* APG IV, Chase et al. 2016)

Family: *Poaceae* (R. Br.) Barnhart, *nom. cons.* (=Gramineae Juss., *nom. alt.*)

Subfamily: *Danthonioideae* N.P.Baker and P.H.Linder

² Commission Implementing Regulation (EU) 2019/1262 of 25 July 2019 amending Implementing Regulation (EU) 2016/1141 to update the list of invasive alien species of Union concern, C/2019/5360, OJ L 199, 26.7.2019, p. 1–4.

Genus: *Cortaderia* Stapf, Gard. Chron. ser. 3. 22: 378 (1897), *nom. cons.*

Synonyms:

Arundo dioeca Spreng., Syst. Veg. (ed. 16) 1: 361. 1825 [1824], *nom. illeg.* (non Lour. 1790);

Arundo selloana Schult. & Schult.f., Mant. 3(1): 605. 1827; [Basionym];

Gynerium argenteum Nees, Agrost. Bras. 462. 1829, *nom. illeg.*;

Moorea argentea (Nees) Lemaire, Ill. Hort. 2: 14. 1855;

Cortaderia argentea (Nees) Stapf, Gard. Chron. ser. 3, 22: 396. 1897, *nom. illeg.*;

Cortaderia dioeca (Spreng.) Speg., Anales Mus. Nac. Buenos Aires 7: 194. 1902.

Common names and names used in commerce: carrizo de las pampas (Spanish, Castillian), carrizos de las pampas (Spanish, Castillian), gineri (Catalan), hierba de la pampa (Spanish, Castillian), hierba de las pampas (Spanish, Castillian), panpa-lezka (Euskera), planta de les plomes (Catalan), plomalls (Catalan), plomes (Catalan), plumeros (Asturian), plumeros (Catalan), plumeros (Spanish, Castillian); pampová tráva (Czech Republic); pampasgræs (Denmark); pampaheinä (Finnish); Cortaderia, erba della Pampa, erba delle Pampas (Italy); Pampasgräs (Sweden); Trawa pampasowa (Poland); iarba de Pampas (Romanian); Pampuzāle (Latvian), Ezüstös pampafű (Hungarian), (Euro+Med (2006-2020). Amerikanisches Pampasgras (German).

List of the most common subspecies, lower taxa, varieties, breeds or hybrids (note, no hybrids are known): There are many cultigens (a plant species or variety only known in cultivation) of *Cortaderia selloana*. A number of these cultigens were assessed in a genetic study by Okada et al. (2007): Most cultivars (a plant variety produced in cultivation by selective breeding) are propagated asexually by division or tissue culture to maintain morphological and phenological uniformity. Such clonally propagated cultigens are female or hermaphroditic (but functionally male) in sex expression. In addition, a few cultivars and most named selections (i.e. selected genotypes that do not have cultivar status) are propagated by seed and consist of both sexes.

The most common traded cultigens are: 'Albolineata' ('Silver Stripe'), 'Andes Silver', 'Aurolineata' ('Gold Band'), 'Blue Bayou', 'Candy Floss', 'Carnea', 'Cool Ice', 'Evita', 'Ivory Feathers', 'Golden Goblin', 'Monstrosa', 'Monvin', 'Patagonia', 'Pink Feather', 'Point du Raz', 'Pumila', 'Rendatleri' ('Carminea Rendatleri'), 'Silver Comet', 'Splendid Star', 'Sunningdale Silver', 'Usballata', 'White Feather' (Okada et al. 2007; Lucas 2011). However, although a few studies exist, it must be noted that no specific (genetic) study has attributed each of all the above listed cultigens to *Cortaderia selloana*. A dedicated DNA fingerprinting analysis would be required.

A2. Provide information on the existence of other species that look very similar [that may be detected in the risk assessment area, either in the environment, in confinement or associated with a pathway of introduction]

Include both native and non-native species that could be confused with the species being assessed, including the following elements:

- other alien species with similar invasive characteristics, to be avoided as substitute species (in this case preparing a risk assessment for more than one species together may be considered);
- other alien species without similar invasive characteristics, potential substitute species;
- native species, potential misidentification and mis-targeting

Response: According to the synoptic taxonomic study on the genus *Cortaderia* Stapf recently published by Testoni and Linder (2017) the *Selloana* group includes three morphologically and anatomically similar species, with both gynodioecious and apomictic breeding systems. They are easily distinguishable from other species in the genus *Cortaderia*: they form big tussocks 1.5 to 3 m in diameter and up to 4 m in height, and the leaf edges are strongly cutting. The panicles are large and plumose, very showy, and much larger than in most of the other species. The spikelets have 1-veined glumes, the lemmas are long-acuminate, with or without evident awns, unlobed, 3-veined and with long hairs only in female plants (hermaphrodites glabrous) (Testoni and Linder 2017). These three species in the *Selloana* group are *Cortaderia selloana* (Schult. & Schult.f.) Asch. & Graebn. s.l., *Cortaderia araucana* Stapf, and *Cortaderia speciosa* (Nees & Meyen) Stapf. According to Testoni and Linder (2017) *Cortaderia selloana* (Schult. & Schult.f.) Asch. & Graebn. s.l. includes two different subspecies, *Cortaderia selloana* (Schult. & Schult.f.) Asch. & Graebn. subsp. *selloana*, and *Cortaderia selloana* subsp. *jubata* (Lemoine) Testoni & Villamil [syn.: *Cortaderia jubata* (Lemoine) Stapf]. However, as stated in section A1, we will use the name *C. selloana* for *C. selloana* subsp. *selloana* in the whole RA, apart from a few parts in the text, such as here (A2), where we specifically cite and refer to Testoni and Linder (2017) synopsis.

It is generally agreed that specific morphological, reproductive and phenological characters are required to distinguish these two subspecies, but identification in the field might be challenging. According to Testoni and Linder (2017), *C. selloana* subsp. *selloana* can be diagnosed by the glumes about as tall as the basal lemma, and lemma without a distinct awn. The plants are generally larger than those of *C. araucana* and *C. speciosa*, and the panicles are larger (0.5 to 1 m long), laxer, and coloured white, pink or yellowish. The similar size of basal lemmas and glumes (6–15 mm) further separates *C. selloana* subsp. *selloana* from *C. araucana* (glumes 9–17 mm long, ca. $\frac{1}{2}$ length of basal lemmas), whereas the larger glumes separate it from *C. speciosa* (glumes 6–8 mm, ca. $\frac{3}{4}$ length of basal lemmas). The large size may also lead to confusion with *C. nitida*³, but it is easily separated by the larger and laxer panicles, 3-veined, awnless (mutic) lemmas that are glabrous on hermaphrodite plants; and female plants with tiny staminodes (Testoni and Linder 2017).

Cortaderia selloana was originally described as dioecious (de Vargas 1959; Connor 1965, 1983, under the name of *C. selloana*), but Astegiano et al. (1995) showed that it is functionally-gynodioecious (i.e., co-occurrence of plants with hermaphrodite flowers and plants with female pistillate flowers), and Testoni and Villamil (2014) recorded several populations with only pistillate individuals (so presumably apomictic) in central and northern Argentina. Both types of individuals (hermaphrodite plants and female plants) could be morphologically differentiated by their size and spike dimensions, resulting in less robust hermaphrodite plants with narrower spikes. Hermaphrodite plants are only pollen donors whereas female plants are responsible for the seed production (Doménech-Carbó et al. 2018).

According to Costas-Lippmann (1979), in its naturalized habitats in New Zealand, *C. selloana* is described as functionally gynodioecious. In cultivation in California, it is considered dioecious or imperfectly dioecious because only a few seeds are found in some predominantly staminate plants, while thoroughly pistillate individuals also occur (Costas-Lippmann 1976).

³ Within the *Nitida* group, *Cortaderia nitida* does approach the *Selloana* group by its large size, big plumose inflorescences, and especially by the lemma shape (Testoni and Linder 2017).

Cortaderia selloana presents the greatest morphological variability and geographical range in the genus. The morphological characterization is also complicated by interbreeding between natural populations and cultivated plants (Testoni and Linder 2017).

Cortaderia jubata is generally similar to *C. selloana*, and includes all the morphologically homogeneous apomictic populations of the Yungas region (Bolivia). It can be separated from *C. selloana* by the inflorescences which extend far beyond the foliage, and the pink, 75–90 cm long, very lax, pyramidal and nodding panicles. In Ecuador it is sympatric with *C. nitida*, from which it can be separated by its larger size and its spectacular pink panicles. They can also easily be distinguished by the leaves: in *Cortaderia jubata*, they are flat and folded V-shaped, while in *C. nitida* leaves are enrolled from both margins (Testoni and Linder 2017).

Cortaderia selloana and *Cortaderia jubata* have been described as readily distinguishable genetically by Houlston and Goeke (2017), although the amount of genetic variation present in each differs markedly. According to Houlston and Goeke (2017), this pattern is not surprising given the difference in breeding systems of the two species. An earlier study on the genetics of invasive *C. jubata* from around the world, Okada et al. (2007) suggested that *C. jubata* in New Zealand was almost clonal; a finding consistent with its apomictic reproductive mode (Houlston and Goeke 2017).

In addition to *Cortaderia selloana* and *Cortaderia jubata*, according to (Verloove 2020) another species in cultivation in Europe is the New Zealand endemic *Cortaderia richardii* (Endl.) Zotov (syn.: *Arundo richardii* Endl.) (a species not reviewed in the synopsis by Testoni and Linder 2017, which focuses on South American species). It has been reported as increasingly self-seeding in the British Isles, especially in Scotland (Clement 2005). Other *Cortaderia* species are traded as ornamental and are also available on-line (see above).

In the EU, two native species might cause potential misidentification and mis-targeting, also due to the fact that they thrive in similar habitats: in particular *Saccharum ravennae* (L.) L. (syn: *Tripidium ravennae* (L.) H.Scholz), and *Ampelodesmos mauritanicus* (Poir.) T.Durand & Schinz (both belonging to Poaceae). These two are also potential substitute species, limited to the MS (or to the regions of the MS) where they are native.

Other species of *Cortaderia* that are traded as ornamental plants are *Cortaderia jubata*, *Cortaderia fulvida*, *Cortaderia richardii*, *Cortaderia toetoe* (Lucas 2011).

A3. Does a relevant earlier risk assessment exist? Give details of any previous risk assessment, including the final scores and its validity in relation to the risk assessment area.

Response: There are a number of risk assessments available which in general rank *C. selloana* as an invasive species. All of the risk assessments below assess the species *C. selloana*. These documents include:

- The 2015 GB Non-native species Rapid Risk Assessment (NNS 2015). In this document it is reported that *C. selloana* is already present and well established in Great Britain. Records of the species have been increasing considerably in recent years and it seems likely that this trend will continue in the future. Environmental, economic and social impacts are discussed. *C. selloana* is ranked as a high risk alien plant to Great Britain.

- The National Biodiversity Data Centre Ireland (Kelly et al. 2013) assessed the risk of *C. selloana* for Ireland and scored the species 15. Species scored between 14-17 are species with a medium risk.
- For Spain, Gassó et al. (2010), applying the Australian Weed Risk Assessment tool calculated a score of 26. This would mean that the species would be rejected from import for Spain.
- Pacific Island Ecosystems at Risk (PIER, 2001), (Reject, Score: 24). The reject score indicates for Australia, the species is likely to be of high risk and should be rejected from import.
- The Big Island Invasive Species Committee (2020) (Hawaii, US) award a HPWRA score of 24-26 which results in the species being listed as an invasive species.
- United States Department of Agriculture - Animal and Plant Health Inspection Service (USDA-APHIS), February 18, 2014, RA result = high risk to the USA. The 2014 document remarks that Hawaiian researchers also obtained a result of 24 (high risk) when they evaluated this species with the Australian weed risk assessment system (UH 2013).
- UF/IFAS Assessment of Non-Native Plants in Florida's Natural Areas (University of Florida, 2020). Results = High Invasion Risk. This tool is a predicted tool that evaluates the risk of the species to the risk assessment area of Florida.
- Invasiveness Assessment in Victoria (Agriculture Victoria 2020) assesses plant invasiveness by evaluating the plants biology and ecological characteristics against criteria that encompass establishment requirements, growth rates and competitive ability, methods of reproduction and dispersal mechanisms. Pampas grasses (*C. jubata* and *C. selloana*) are declared under the Natural Resources Management Act, 2004 throughout the whole of the State of South Australia, so that cultivation, sale and movement of plants can be prevented.
- The Canadian Food Inspection Agency (CFIA) conducts weed risk analyses in accordance with international guidelines for pest risk analysis established by the International Plant Protection Convention (IPPC) of the Food and Agriculture Organization of the United Nations (FAO). The CFIA is preparing a weed risk analysis for *Cortaderia selloana*. However, the “Environmental” impact is considered the most important impact for this species.

All of the above risk assessments highlight that *Cortaderia selloana* poses a risk to areas throughout the world. In particular, the two assessments from Europe (Great Britain and Spain) detail a high risk.

A4. Where is the organism native?

including the following elements:

- an indication of the continent or part of a continent, climatic zone and habitat where the species is naturally occurring
- if applicable, indicate whether the species could naturally spread into the risk assessment area

Response: *Cortaderia selloana* is endemic to Argentina, Chile, central-west and southern Brazil and Uruguay (de Vargas 1959; Linder et al. 2010; Filgueiras et al. 2015; Testoni and Linder 2017; Flora Argentina 2020; Flora do Brasil 2020). In its native range, it is found in alluvial plains, and it is common to riparian areas, hydromorphic and sandy soils, humid dune slacks (de Vargas 1959; Monserrat et al. 2012; Prina et al. 2015; Oyarzabal et al. 2018), but in Argentina it can be found also in mountain areas above 2,400 m a.s.l. (Tucumán, Depto. Chicligasta, Serra de Aconquija) (Cabido et al. 2010; Testoni and Villamil

2014). In Argentina, the native range extends from the north of the country to Patagonia, Neuquén region, Río Negro and the Atlantic coast at Chubut region (Astegiano et al. 1995). In the native range a considerable association of *C. selloana* to fire disturbance is known (Sirolli and Kalesnik 2011). In Uruguay, on the Atlantic coast, the typical plant communities of humid dune slacks are called *pajonales* (Delfino and Masciadri 2005; Fagúndez and Lezama 2005). According to Flora do Brasil (2020) *Cortaderia selloana* is present in the following states: Distrito Federal, Goiás, Minas Gerais, Rio de Janeiro, São Paulo, Paraná, Rio Grande do Sul, Santa Catarina. It is found in several habitats and vegetation types in the Central Brazilian Savanna, and in the Atlantic Rainforest, including high altitude grasslands and coastal forest (*restinga*), but also in man-disturbed areas (Filgueiras et al. 2015). In Chile (de Vargas 1959) it is found in the Cordillera de Nahuelbuta region, and Contulmo area (Arauco), on sandy soils and riparian sites. De Vargas (1959), in her monographic work on the *Cortaderia* genus in Chile, described this species as also in cultivation, e.g. at Ñuñoa (Santiago).

The native range is primarily located in the warm temperate climates (Cfa Cfa = [Humid subtropical climate](#), Cfb [oceanic climate](#), Cwa [Monsoon-influenced humid subtropical climate](#)), extending northwards to the equatorial (Aw Tropical wet and dry or [savanna climate](#), Af, [Tropical rainforest climate](#): southern Brazil) and southwards to arid climates (BSk [Cold semi-arid climate](#), BWk [Cold desert climate](#)), according to the Köppen-Geiger climate classification.

C. selloana grows best in open ground in full sun but it can tolerate light shade.

Cortaderia selloana could not naturally spread from the native range to the risk assessment area.

A5. What is the global non-native distribution of the organism outside the risk assessment area?

Response:

Africa and Indian Ocean

Algeria	Introduced (cultivated)	Maire (1953)
Libya	Introduced (cultivated)	Maire (1953)
Egypt	Introduced (cultivated)	Ibrahim et al. (2016)
Morocco	Introduced (cultivated)	Maire (1953)
South Africa	Introduced and Invasive	Chippindall (1955) in Robinson (1984)
Tunisia	Introduced (cultivated)	Guillochon (1909)
Réunion	Introduced and Established	http://especies-exotiques-envahissantes.fr/espece/cortaderia-selloana/
Zambia	Introduced (cultivated)	Kew (2020)

Asia

China	Introduced (cultivated) Jiangsu, Taiwan, Zhejiang	Flora of China (2020)
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Georgia	Introduced	Duncan (1981)
Japan	Introduced and Established	Mito and Uesugi (2004)
Turkey	Introduced and Casual	Uludağ et al. (2017)
United Arab Emirates	Introduced (cultivated)	https://dgnurseries.com/product/cortaderia-selloana/
India	Introduced and Established	Mehra and Sharma (1975) https://indiabiodiversity.org/species/show/243218
Israel	Introduced (rare casual)	Dufour-Dror (2013)

Europe

Norway	Introduced (cultivated)	https://stavanger.gardenexplorer.org/taxon-21489.aspx
Russia	Introduced (cultivated)	pers comm. Yuliana Kulakova (2020)
Serbia	Introduced (cultivated)	https://www.inaturalist.org/observations/15736495
Ukraine	Introduced (cultivated)	pers comm. Yuliana Kulakova (2020)

North and Central America

United States (Alabama, California, Georgia, Louisiana, New Jersey, Oregon, Tennessee, Texas, Utah, Virginia, Washington)	Introduced and Established	USDA NRCS (2020)
Mexico	Introduced and Established	Beetle et al. (1987); Sánchez-Ken (2019)
Honduras	Introduced	Molina (1975)
Guatemala	Introduced	CONAP (2011)
Costa Rica	Introduced (cultivated)	Alvarado García et al. (2011)

South America

Colombia	Introduced and Casual	Giraldo-Cañas (2013)
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Oceania

Australia	Introduced and Invasive	Parsons and Cuthbertson (2001)
French Polynesia	Introduced and Invasive	CABI (2020)

New Zealand	Introduced and Invasive	Gosling et al. (2000)
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A6. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species been recorded and where is it established? The information needs be given separately for recorded (including casual or transient occurrences) and established occurrences. “Established” means the process of an alien species successfully producing viable offspring with the likelihood of continued survival⁴.

A6a. Recorded: List regions

A6b. Established: List regions

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

- Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea.

Comment on the sources of information on which the response is based and discuss any uncertainty in the response.

For delimitation of EU biogeographical regions please refer to <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2> (see also Annex VI).

For delimitation of EU marine regions and subregions consider the Marine Strategy Framework Directive areas; please refer to <https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions/technical-document/pdf> (see also Annex VI).

Response (6a): Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Steppic

It should be noted that *C. selloana* is likely to be present in confined environments (gardens, parks etc.) in all biogeographical regions within the risk assessment area as a garden ornamental but confirmed records are lacking.

Response (6b): Atlantic, Continental, Mediterranean.

A7. In which biogeographic region(s) or marine subregion(s) in the risk assessment area could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A7a. Current climate: List regions

A7b. Future climate: List regions

With regard to EU biogeographic and marine (sub)regions, see above.

⁴ Convention on Biological Diversity, Decision VI/23

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (7a): Atlantic, Continental, Mediterranean (Annex VIII).

Response (7b): Under future climatic conditions (projection: 2070: RCP 2.6), *C. selloana* can establish in the following biogeographical regions: Alpine, Atlantic, Black Sea, Continental, Boreal, Mediterranean, Pannonian and Steppic. Under the climatic projection of RCP 2.6 by 2070, *C. selloana* has a high potential to establish in the Mediterranean and Pannonian biogeographical regions. The Atlantic, Black Sea, Continental, and Steppic biogeographical regions all have a moderate likelihood of establishment. Establishment is also moderately likely outside the risk assessment area in the Anatolian and Macaronesian biogeographical regions. (Annex VIII).

The main aspects of climatic change which may influence the occurrence of *C. selloana* include increased minimum temperature of winter months, increased spring and summer temperatures and increased precipitation. Increased frequency of fire, as the result of increased temperatures, may promote the establishment of the species in natural areas where otherwise it would not be able to get a foothold. If climate change promotes local increased rainfall this may act to increase the spread of the species within the risk assessment area. If climate change promotes higher wind frequency and velocity this may act to spread seeds over longer distances than currently shown.

A8. In which EU Member States has the species been recorded and in which EU Member States has it established? List them with an indication of the timeline of observations. The information needs be given separately for recorded and established occurrences.

A8a. Recorded: List Member States

A8b. Established: List Member States

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden

The description of the invasion history of the species shall include information on countries invaded and an indication of the timeline of the first observations, establishment and spread.

Response (8a):

Member State	Status	Reference
Austria	Introduced (cultivated)	pers comm (Sven Follak, 2020)

Belgium	Introduced: casual in some areas and locally establishing in other areas.	Manual of the Alien Plants of Belgium (2021) Verloove F. (2012)
Bulgaria	Introduced (cultivated)	<i>Index seminum</i> 2018, University Botanic Garden Sofia
Croatia	Introduced (cultivated)	http://hirc.botanic.hr/vrt/DS_Zagreb_2015.pdf
Cyprus	Introduced (cultivated)	Meikle, R. D. (1985) - Flora of Cyprus 2 Kew
Czech Republic	Doubtfully Introduced (cultivated)	Probably planted in the zoological garden of Prague and available online in many web site in the Czech Republic
Denmark	Introduced (cultivated)	2017 Facebook page of the Botanisk Have, København (pampasgræs), close to the Palm House (Palmehuset).
Estonia	No records	
Finland	Introduced (cultivated)	https://www.exoticgarden.fi/Pampaheinae-White-Feather , https://kotiliesi.fi/paratiisi-takapihalla/kukkia-cottage-garden-henkeen-kesakukat-nauttivat-auringosta/ , https://putkinotkoblog.wordpress.com/2018/09/11/petty-mysten-pampaheina/
France	Introduced, Established and invasive	Muller (2004)
Corse (FR)	Introduced, Established and invsive	Jeanmonod, D. and Burdet, H. M. - Compléments au prodrome de la flore corse. Annexe 3 [Gamisans, J. and Jeanmonod, D.] Genève 1993. Jeanmonod, D. and Burdet, H. M. - Compléments au prodrome de la flore corse. Annexe 4. Flore analytique des plantes introduites en Corse [Natali, A. and Jeanmonod, D.] Genève 1996. <i>Jeanmonod et al (2010), Puddu et al (2016) Comité français de l'UICN (2020)</i>
Germany	Introduced and Casual	https://www.kp-buttler.de/florenliste/index.htm [Accessed May 2020]
Greece	Introduced (cultivated)	Diomedes Botanical Garden
Rhodos (GR)	Introduced and Casual	Galanos (2015)
Crete (GR)	Introduced (cultivated)	Dal Cin D'Agata et al. (2007)
Hungary	Doubtfully Introduced (cultivated)	https://www.disznovenywebaruhaz.hu/ezustos-pampafu_cortaderia-selloana
Ireland	Introduced, Established and Invasive	Nelson and Seaward (1981) https://species.biodiversityireland.ie/profile.php?taxonId=40779 Reynolds, S.C.P. (2002) A catalogue of alien plants in Ireland. National Botanic Gardens. Glasnevin, Dublin. Stace
Italy	Introduced, Established and Invasive	Galasso et al. (2018)

Sardinia (IT)	Introduced, Established and Invasive	Bacchetta et al. (2010); Galasso et al. (2018)
Sicily (IT)	Introduced, Established and Invasive	Galasso et al. (2018)
Latvia	Doubtfully Introduced (cultivated)	https://praktiski.la.lv/eksotiska-pampuzale-ka-kopt-so-augu
Lithuania	No records	
Luxembourg	No records	
Malta	Introduced (cultivated)	Cleghorn (1870), Domina and Mazzola (2008)
Netherlands	Introduced (cultivated)	Zeijlstra (1905)
Poland	Introduced (cultivated)	Urbański (2006)
Portugal	Introduced and invasive	Bejarano et al. (2011), Marchante et al. (2005)
Azores (PT)	Introduced	Hansen and Sunding (1993)
Madeira (PT)	Introduced	Hansen and Sunding (1993)
Romania	Introduced (cultivated)	Diaconescu (1979)
Slovakia	Introduced (cultivated)	Tomaškin et al. (2015)
Slovenia	Introduced (cultivated)	Martinčič (1999)
Spain	Introduced, Established and Invasive	Guinea (1953); Domènech and Vilà (2007)
Balearics (ES)	Introduced and Established	Moragues and Rita (2005)
Canary Islands (ES)	Introduced and Casual	La Palma island, Otto et al. (2008)
Sweden	Introduced (cultivated)	https://www.impecta.se/sv/artiklar/rott-pampasgras-pink-feather.html
United Kingdom	Introduced and Established	Smyth (1908)

Introduction to Europe dates back to the first half of the 19th Century. During Moore's curatorship of Glasnevin Botanic Gardens in Ireland (David Moore was born in Dundee on 23 April 1807), many new plants were introduced into cultivation in the British Isles through Glasnevin; among these was *Cortaderia selloana* from South America (Nelson and Seaward 1981). John Tweedie, a Scot resident in Buenos Aires, in 1839 sent some packages of seed collected in Argentina. The seeds were planted out in the open at Glasnevin in 1840. They produced flowering spikes for the first time in the autumn of 1842. Soon after, Moore sent plants and pressed specimens to the Royal Botanic Gardens, Kew (Stapf 1897; Nelson 1982). It was introduced, from the UK, to the Netherlands in 1848 in the Japanese garden at Clingendael, The Hague (Van der Eb-Brongersma 2012). *Cortaderia selloana* was introduced in Italy in 1850 (Saccardo, cited in Maniero 2000) and it features in the *Index seminum* 1906-1923 of the Botanic Garden of Modena (Delectus seminum horti botanici R. Universitatis Mutinensis). *Cortaderia selloana* was first introduced to France in the Montpellier Botanical Garden in 1857 and was commercialised after 1874 (Bossard et al. 2000). It was listed in the 1905 edition of the plant list of the Botanic Garden of Amsterdam (Zeijlstra

1905). The first record as escaped from cultivation for Spain dates back to Guinea (1953) in the gulf area of quien la Santander, and the first herbarium sample was collected by Loriente at Liencres (Cantabria) in 1969 (Herrera Gallastegui and Campos Prieto 2006). In Portugal the species was first mentioned as naturalised in 1955 (LIFE STOP Cortaderia, 2020).

Response (8b): As specified in the above table *C. selloana* is established in the following Member States: France, Ireland, Italy, Portugal, Spain; and in the United Kingdom.

In this case, adding a timeline for establishment of *C. selloana* is not possible as records do not indicate the year when the species first established.

A9. In which EU Member States could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A9a. Current climate: List Member States

A9b. Future climate: List Member States

With regard to EU Member States, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6 °C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0 °C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (9a): Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, France, Germany, Greece, Hungary, Ireland, Italy, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden; and in the United Kingdom (see Annex VIII).

Response (9b): Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden; and in the United Kingdom (see Annex VIII).

A10. Is the organism known to be invasive (i.e. to threaten or adversely impact upon biodiversity and related ecosystem services) anywhere outside the risk assessment area?

Response: *Cortaderia selloana* is recognized as an invasive pest in California (USA) where it can invade natural habitats and outcompete native biodiversity and has frequently occupied non-ruderal habitats over

the past 50 years (Lambrinos 2001). In the U.S., in addition to California, *C. selloana* is listed as a noxious weed in:

Washington State <https://www.nwcb.wa.gov/weeds/pampas-grass>

Hawaii: <https://dlnr.hawaii.gov/hisc/info/invasive-species-profiles/pampas-grass/>

In New Zealand, *C. selloana* is recorded as a weed of concern (invasive). It displaces native plant species and can create fire hazards.

Cortaderia selloana is recognised as one of the main priority invasive weeds in bushland reserves and native forestry in Tasmania (Young 1993). In Australia it replaces natural plant communities and excludes plants through shading and below ground competition since the root system of a single plant is estimated to occupy 103 cubic meters of soil (Parsons and Cuthbertson 2001).

Cortaderia selloana competes with and replaces native plants in South Africa (Wells et al. 1986). The aforementioned author highlights undesirable characteristics as competing for space, light, nutrients and water.

A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea

Response: Atlantic, Continental, Mediterranean

The endangered area includes natural and ruderal habitats in the Mediterranean biogeographical region.

A12. In which EU Member States has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden

Response: France, Ireland, Italy, Portugal, Spain; and in the United Kingdom.

The area endangered includes countries in the Mediterranean biogeographical region.

A13. Describe any known socio-economic benefits of the organism.

including the following elements:

- Description of known uses for the species, including a list and description of known uses in the risk assessment area and third countries, if relevant.
 - Description of social and economic benefits deriving from those uses, including a description of the environmental, social and economic relevance of each of those uses and an indication of associated beneficiaries, quantitatively and/or qualitatively depending on what information is available.
- If the information available is not sufficient to provide a description of those benefits for the entire risk assessment area, qualitative data or different case studies from across the risk assessment area or third countries shall be used, if available.

Response: *Cortaderia selloana* is a widely traded and used ornamental species. Ornamental use includes the use for fencing and protecting the garden or property, due to its sharp leaves (“green guardian”, Brigham 2002). The European Nursery Stock Association have provided quantitative data on the production and value of *C. selloana* in the horticulture trade in the risk assessment area. They detail:

- In the Netherlands circa 8 000 000 to 10 000 000 plants a year are grown at approximately 50 nurseries. We estimate the trade value approximately at 80 – 100 million Euro’s (based on consumer prices at garden centre’s and internet-sale),
- Approximately 20 -25 million plants a year of *C. selloana* are cultivated at nurseries in Europe, with an estimated value of 200 million Euro’s,
- Some new varieties of *Cortaderia selloana* are coming from ‘tissue culture’ these varieties can not be grown from seed. We estimate the number of plants coming from tissue culture at 2 – 3 million a year, with a value around 50 – 60 million Euro.

There is a trade in the dried plumes of the species. These are used for decorative purposes mainly indoors and are available via e-commerce. There is no quantitative data on the value in the trade of dried plumes.

Cortaderia selloana, due to its high phytolith (minute mineral particle formed inside the plant) production and its capacity to absorb silica soluble particles in plant tissues, has been used in constructed wetland systems and phytoremediation applications (e.g., Gholipour et al. 2020). In South Africa it has been used to dry up and stabilise the slimes dams in mines (Grillo and Venora 2011). There has also been some research on using *C. selloana* as fibre components in composite material (Jordá-Vilaplana et al. 2017).

SECTION B – Detailed assessment

Important instructions:

- In the case of lack of information the assessors are requested to use a standardized answer: “No information has been found.”
- With regard to the scoring of the likelihood of events or the magnitude of impacts see Annexes I and II.
- With regard to the confidence levels, see Annex III.
- Highlight the selected response score and confidence level in **bold** but keep the other scores in normal text (so that the selected score is evident in the final document).

1 PROBABILITY OF INTRODUCTION AND ENTRY

Important instructions:

- **Introduction** is the movement of the species into the risk assessment area (it may be either in captive conditions and/or in the environment, depending on the relevant pathways).
- **Entry** is the release/escape/arrival in the environment, i.e. occurrence in the wild
- Introduction and entry may coincide for species entering through pathways such as “corridor” or “unaided”, but it also may differ. If different, please consider all relevant pathways, both for the introduction into the risk assessment area and the entry in the environment.
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used (see Annex IV). For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document⁵ and the provided key to pathways⁶.
- For organisms which are already present (recorded or established) in the risk assessment area, the likelihood of introduction and entry should be scored as “very likely” by default.
- Repeated (independent) introductions and entries at separate locations in the risk assessment area should be considered here (see Qu. 1.7).

Qu. 1.1. List relevant pathways through which the organism could be introduced into the risk assessment area and/or enter into the environment. Where possible give details about the specific origins and end points of the pathways as well as a description of any associated commodities.

For each pathway answer questions 1.2 to 1.7 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 1.2a, 1.3a, etc. and then 1.2b, 1.3b etc. for the next pathway.

In this context a pathway is the route or mechanism of introduction and/or entry of the species.

The description of commodities with which the introduction of the species is generally associated shall include a list and description of commodities with an indication of associated risks (e.g. the volume of trade; the likelihood of a commodity being contaminated or acting as vector).

⁵ <https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf>

⁶ <https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf>

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 1.2-1.9.

The following introduction/entry pathways are identified for *Cortaderia selloana* and are discussed within this section. Pathways are detailed in order of importance. Pathway categories are in brackets.

Pathways are listed in order of importance

- (1): Horticulture (escape from confinement)**
- (2): Ornamental purpose other than horticulture: (escape from confinement)**
- (3): Landscape / flora / fauna (improvement in the wild)**
- (4): Transport of habitat material (soil, vegetation) (transport – contaminant)**
- (5): People and their luggage/equipment in particular tourism (transport - stowaway)**

Pathways considered but excluded from the risk assessment.

USDA (2014) detail that *C. selloana* seeds can attach to kiwi fruit destined for export. In New Zealand, kiwifruit (*Actinidia deliciosa*) have been intercepted with seeds of *C. selloana* attached to the fruit. Subsequently, these consignments have been rejected for export (Knowles and Tombleson 1987). This pathway was not considered for the risk assessment as there was no further evidence that this is an (active) pathway neither in New Zealand nor in the EU. In any case, phytosanitary measures at export and import in the risk assessment area would likely identify and remove contaminants before the fruit enters the risk assessment area.

Historically, *C. selloana* has been planted for erosion control and as a dune stabilizing plant (CABI 2020, GISD 2020, NNSS 2015, Di Tomaso et al. 2010). There is little evidence of use of the species for these aspects in the risk assessment area in recent times (e.g. last 30 years) but the aforementioned references, and in particular, Domènech et al. (2006) highlights that this has previously occurred in the risk assessment area. Due to the awareness of the invasiveness of the species and the overall public awareness of their impacts, planting of *C. selloana* for such purposes as erosion control and windbreaks is less attractive now, than historically. For example, EU funded projects such as LIFE STOP Cortaderia - Urgent measures for controlling the spread of Pampa Grass (*Cortaderia selloana*) in Atlantic area (see: <http://stopcortaderia.org/language/en/stop-cortaderia-en/>) act to raise awareness of the invasive behavior of the species. As there is no evidence that this is an active pathway, it is not considered further in this risk assessment.

Pathway name: (1) Horticulture (escape from confinement)

Qu. 1.2a. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	Unintentional intentional	CONFIDENCE	low medium high
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Response: Horticulture is the intentional introduction of the species into the risk assessment area for commercial culturing. *Cortaderia selloana* has been utilized as a garden ornamental species in the risk assessment area since the early to mid-1800s.

The Horticulture pathway should be applied to plants escaping from commercial culturing facilities (nurseries, greenhouses) (including the deliberate dumping of plant material) or during transport to/from the nursery trade. Therefore, this pathway includes the bulk shipment of live plants for nurseries and gardens centres, and the intentional introduction of seeds (and potentially rhizomes) for planting.

Qu. 1.3a. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year? including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species is a popular ornamental species (one of the most popular ornamental grass species in cultivation) and grown throughout the risk assessment area (Bacchetta et al. 2010; Missouri Botanical Garden 2020; NNSS 2015). Numerous suppliers are listed throughout EU Member states.

The pathway horticulture is the deliberate movement of the plant into the risk assessment area for use as an ornamental species. However, it should be noted that the frequency and volume of individuals entering the risk assessment area is potentially low as much of the material sold in nurseries and garden centres is likely to be propagated within the risk assessment area.

It is moderately likely that the species will enter the natural environment via this pathway. Potentially, if planted in nurseries and garden centres, plant material may escape from confined areas into the natural environment. Additionally, dumping of material into the natural environment may occur though unlikely with good horticulture industry practise.

If eradication measures are taken following dumping, there is the potential that the species can re-establish if it is dumped again. As one female plant can produce up to 1 million seeds, only a small population is needed to result in subsequent establishment. However, the fecundity depends on the gender composition of the population, and the sex ratios could be very skewed in a small population.

Qu. 1.4a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The pathway ‘Horticulture’ is the deliberate movement of plant material into the risk assessment area and as such plant material would be maintained and moved to ensure survival.

Three types of material can potentially enter the risk assessment area via this pathway (1) seed, (2) live plants and (2) rhizomes. Although seed import cannot be ruled out, it is unlikely as part of the horticulture pathway for the commercial production of the species.

Seed (caryopsis)

It is very likely that seeds (caryopsis) will survive along the pathway. It would not be possible for the species to reproduce or increase during transport and storage along this pathway. When dried, the seeds of *C. selloana* can remain viable approximately 12 months and therefore this will increase the chance of survival during transport. Seeds can germinate under a wide range of environmental conditions, yet germination rates can be improved under shading, in sandy soils and with high water availability (Domènech and Vilà, 2007). Seeds would need to be dumped on the ground substrate, or near to the ground surface to facilitate germination.

Live plants

Live plants will be moved along the pathway and packaged to promote their survival. It is unlikely the plants will reproduce or increase during their transport along this pathway. The end result of the pathway is the planting of the species in a suitable area (garden) to promote its growth. From this, seeds can enter the environment via escape/release.

Rhizomes

Rhizomes may be moved along the pathway and packaged to promote their survival. There is no data on the survival of the rhizomes but it is likely that they will survive during transportation as industry will ensure they are packaged appropriately to ensure survival. Rhizomes are robust and if placed near the soil surface, this can increase the chances of the rhizomes to germinate after they are dumped. If plant material is placed in areas where there is disturbance, there may be a greater chance that the species can survive in these habitats. Often dumping of plant material takes place in ruderal habitats.

Qu. 1.5a. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely	CONFIDENCE	low medium high
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	likely very likely		
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Response: Plant material is the commodity itself and it is deliberately moved for sale within the risk assessment area. Therefore, management practices before and during transport are not considered in this question. Management practices during storage at the end point (nursery and garden centre) may affect the survival of the species for example cleaning, temperature storage etc. However, the species is likely to survive.

Qu. 1.6a. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: It is unlikely that the organism will enter the risk assessment area undetected via this pathway as this concerns the intentional movement of plant material into the risk assessment area.

The species could enter the environment undetected (likely) as the species may escape from garden centres and nurseries via seed or the dumping of plant material. Although there is no direct evidence of actual movement of the species along this pathway it is highlighted in Pausas et al. (2006). Additionally, *C. selloana* is found growing in or around rubbish dumps in the Mediterranean region of the risk assessment area (Saura-Mas and Lloret, 2005).

An overall score of moderately likely has been given combining both of the scores for entry into the risk assessment area and entry into the environment.

Qu. 1.7a. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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Response: For live plant imports in the risk assessment area (introduction), each EU Member country has official entry points where plant material can be imported into. Therefore, these introduction points are widespread in the risk assessment area.

Cortaderia selloana can enter the environment from nurseries and gardens centres (the end point of this pathway) which are widespread within the risk assessment area.

Qu. 1.8a. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The pathway horticulture involves the movement of plant material for planting from outside of the risk assessment area via nurseries and garden centres.

This pathway is active and there is firm evidence that the species is being moved along the pathway into the risk assessment area and there is the potential for the species to enter the natural environment.

Live plant material may be imported into the risk assessment area for sale in the horticulture industry. However, it should be noted that this is probably more of a historic pathway as the species is likely to be propagated within the risk assessment area as the species can be easily grown from root fragments.

The overall likelihood of *C. selloana* entering the risk assessment area is scored as very likely as the species is already present.

Pathway name: (2) Ornamental purposes other than horticulture (escape from confinement)

Qu. 1.2b. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	Unintentional intentional	CONFIDENCE	low medium high
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Response: This pathway includes the two ornamental purposes as detailed below. These ornamental purposes involve the intentional introduction of the species, though entry into the natural environment would be unintentional. This pathway includes the movement of all plant parts (live plants, seed and rhizomes) into the risk assessment area. The pathway includes the movement of plant material via e-commerce.

For escape/entry into the environment, this pathway includes the dumping of plant material into the natural environment.

(1) Planting in private gardens (intentional introduction, unintentional entry)

Cortaderia selloana has been utilized as a garden ornamental species since the early to mid-1800s within the risk assessment area. The species is a popular ornamental species (one of the most popular ornamental grass species in cultivation) and grown throughout the risk assessment area (Bacchetta et al. 2010; Missouri Botanical Garden 2020; NNSS 2015). Domenech et al. (2005) highlighted clear evidence that *C. selloana*

invasion in Spain is highest in areas close to urban landscapes, indicating that planting the species can facilitate spread into other areas.

(2) Dried panicles (intentional introduction, unintentional entry)

Dried panicles of *C. selloana* are available via e-commerce both within the risk assessment area and from outside the risk assessment area from suppliers like eBay and Amazon.

Qu. 1.3b. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year? including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Fig. 1 An example of *Cortaderia selloana* seeds available via online suppliers.

Response:

Planting in private gardens

There is no information on frequency or volumes entering the risk assessment area or the environment.

The species is available for sale via e-commerce sites such as:

eBay https://www.ebay.com/b/cortaderia-selloana/bn_7024827254)

Amazon <https://www.amazon.com/PAMPAS-GRASS-WHITE-Cortaderia-selloana/dp/B00480FHXS>)

B&T Seeds: <https://b-and-t-world-seeds.com/carth.asp?species=Cortaderia%20selloana&sref=73>.

These sites will ship seeds from outside of the risk assessment area into the risk assessment area.

There are numerous suppliers listed on the e-commerce sites that advertise various color forms of the species, both full size and in dwarf form. Fig. 1 highlights examples which can be purchased via an online supplier in China. Seeds are also available via e-commerce from North America. These suppliers sell seeds directly to the buyer and are popular for buyers wanting different color forms.

Entry into the natural environment is very likely as the species will be planted in gardens, sometimes in close proximity to the natural environment. Wind dispersal can act to move the species from gardens into the natural areas.

If eradication measures are taken following planting, there is the potential that the species can re-establish if replanted. As one female plant can produce up to 1 million seeds, only a small population is needed to result in subsequent establishment.

Dried panicles

There is no information on frequency or volumes entering the risk assessment area or the environment.

Dried panicles of *C. selloana* are available via e-commerce both within the risk assessment area and from outside the risk assessment area from suppliers like eBay and Amazon. The panicles may be color dyed and sold as decorative plumes for indoor use: <https://lamboodrieddeco.com/new-in-coloured-pampas-grass/>

This pathway is highlighted within the risk assessment for *C. selloana* conducted by the USDA (2014).

Lambrinos (2001) highlights the historical production and export of *C. selloana* plumes in California (USA) in the late-1800s. Up to 1 000 000 plumes were exported each year throughout the United States.

For introduction into the risk assessment area, full, fluffy white plumes without seeds shedding would be the preferred state. It is likely that premature female inflorescences would be harvested (this was one of the “innovations” that helped spark the dried plume craze in the late 1880’s). If current commercial methods are the same, fertilization of the embryos and the viability of any seed should be near zero in a floral trade inflorescence. Of course, there may be errors, particularly since it depends on harvest timing. But in a commercial operation the quality control is probably pretty good. Therefore, for dried plumes the overall risk is low (John Lambrinos, pers. comm. 2020).

If eradication measures are taken following planting, there is the potential that the species can re-establish if replanted. As one female plant can produce up to 1 million seeds, only a small population is needed to result in subsequent establishment. The number of potential areas where the species can escape from increases the difficulty of eradication at a large scale.

Qu. 1.4b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Plant material is the commodity itself and it is deliberately moved for sale within the risk assessment area. Therefore, plant parts or whole parts are likely to survive along the pathway.

The following section lists the plant parts of concern:

Seed

It is very likely that seeds (caryopsis) will survive along the pathway. It would not be possible for the species to reproduce or increase during transport and storage along this pathway. When dried, the seeds of *C. selloana* can remain viable approximately 12 months and therefore this will increase the chance of survival during transport. However, DiTomaso et al. (2003) highlight that seeds typically survive for less than six months under field conditions.

Live plants

Live plants will be moved along the pathway and packaged to promote their survival. It is unlikely the plants will reproduce or increase during their transport along this pathway. The end result of the pathway is the planting of the species in a suitable area (garden) to promote its growth. From this, seeds can enter the environment via escape/release.

Rhizomes

Rhizomes may be moved along the pathway and packaged to promote their survival. There is no data on the survival of the rhizomes but it is likely that they will survive during transportation as industry will ensure they are packaged appropriately to ensure survival.

Dried plumes

Dried plumes are unlikely to contain viable seeds (1.3b) due to the timing of the harvest.

Qu. 1.5b. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Management practices before and during transport are very likely to facilitate the survival of the species as it is the commodity itself. Management practices during storage may affect the survival of the species and this could increase or decrease the length of time seed is viable. For example, temperature may affect the survival of the seed. As the panicles are delicate, care would be taken to transport the product and if seeds remain viable the seed can remain attached to the panicle. There would not be any management practices conducted along this pathway.

Management practices, i.e. removal and dumping, in gardens and waste disposal may increase the survival of the species and facilitate the reproduction as the species. CABI (2020) note that vegetative reproduction can occur when tillers are fragmented and receive adequate moisture. Therefore, management practices such as physical removal or cutting above and below ground material may lead to fragmentation of the

rhizome material. Thus, below ground plant material is likely to survive the movement and dumping when placed in a suitable habitat.

Other management practices (e.g. chemical control of weeds in gardens), may have an impact on the survival and reproduction of the species.

Qu. 1.6b. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: It is unlikely that the organism will be introduced into the risk assessment area undetected as this is the deliberate movement of plant material into the risk assessment area.

The species can enter the risk environment via natural spread (wind) and via the dumping of garden waste. If plant waste has mature flowers this would increase the likelihood to likely. Female plants can produce up to 1 million seeds, large numbers of propagules can enter the natural environment as a result of a single dumping of one plant, if the plant contains plumes that contain viable seeds. *C. selloana* can grow in dense stands where numerous plants would be managed and controlled and potentially dumped.

For dried plumes, it is unlikely that the species would be introduced into the risk assessment area undetected as the dried plumes are the commodity. However, the species could be introduced into the environment undetected if fragments of the dried material are accidentally moved. As the dried plumes are for use mainly in an indoor environment, it is unlikely that this would occur.

An overall score of moderately likely has been given combining both of the scores for entry into the risk assessment area and entry into the environment.

Qu. 1.7b. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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Response: For the introduction of seed imports and dried plumes via e-commerce, the entry points are widespread as anyone with an internet connection and a postal address can obtain seeds of the species from

outside the risk assessment area. Therefore, introduction points are widespread within the risk assessment area.

In 2016, 85% of European households had an internet connection. 189.8 million people had a broadband connection in Europe in 2016 (Statista, 2020). Private gardens associated with households are high. For example, data from 2014 shows that 58% of houses have a garden or outside space in France, 81% in the United Kingdom, 80.3% in Poland and 27% in Spain (Statista, 2020).

Qu. 1.8b. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Seed is the most likely plant material that would be imported into the risk assessment area and they have the highest likeliness of entry into the natural environment as they are wind dispersed. This introduction pathway is active and there is firm evidence that the species is being moved along the pathway into the risk assessment area. Especially, the species is already present (recorded or established) in the risk assessment area, hence the likelihood of introduction and entry should be scored as “very likely” by default.

For dried plumes, the overall likelihood of the species entering the risk assessment and enter into the environment is unlikely. This score reflects the fact that viable seeds are unlikely to be present, and it is for use in an indoor environment. Additionally, there is no evidence that viable seeds are moved along this pathway. As previously discussed, plumes are harvested before seed would reach maturity.

The score above is based on the worst case scenario (seeds).

Pathway name (3): Landscape / flora / fauna improvement in the wild

Qu. 1.2c. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: Introduction via this pathway is intentional. Entry into the environment is unintentional. The species may be planted on transportation networks and other semi-urban habitats but not intentionally in the natural environment.

Qu. 1.3c. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: There is no data on the frequency and volume of movement along this pathway.

It is very likely that the number of individuals introduced will be sufficient to originate a viable population that can escape from the origin over the course of one year. As already highlighted, a single female plant can produce up to 1 000 000 seeds in the first year of growth. These seeds are wind spread and seeds are robust and can germinate in a variety of soil types.

C. selloana has been planted in urban and semi-urban environments in the risk assessment area for a number of purposes including as (1) a boundary or barrier species for properties, or transportation route and (2) landscape improvement (Domènech et al. 2006).

In the Mediterranean region, the species has been repeatedly planted along transportation routes to hide roads from bordering properties. For example, in Galicia (North West Spain), *C. selloana* was planted along the main highways during the 1980s where it was used as an ornamental species along the side or central strip of the road. The species is commonly grown on roundabouts (Charpentier et al. 2020). Planting along urban transportation routes has aided its spread in the Mediterranean region (Domènech and Vilà 2007).

If eradication measures are taken following planting, there is the potential that the species can reestablish if replanted. As one female plant can produce up to 1 million seeds, only a small population is needed to result in subsequent establishment.

Qu. 1.4c. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: There is no data on the effect of movement along this pathway on the survival or reproduction of *C. selloana* along this pathway. This pathway involves the deliberate movement of plant material into the risk assessment area and as such plant material would be maintained and moved to ensure the survival of plant material.

Planting of live plants would be the most likely type of material used in this practice. Once planted in urban or semi-urban habitats, the species will be able to reproduce and increase.

Qu. 1.5c. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Along the pathway, management practices will not be applied to the species and existing practices are unlikely to affect the survival of the species as it will be deliberately planted.

Vegetation management along road networks may affect the survival of the species. This would include general vegetation management and along roads and roundabouts. Road works and management of the bank side vegetation may increase the survival of the species and increase its potential to survive in the environment as such management may increase disturbance which can promote the establishment of the species.

Qu. 1.6c. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: It is unlikely that the organism will be introduced into the risk assessment area undetected as this is the deliberate movement of plant material within the risk assessment area.

For entry into the environment, it is likely that *C. selloana* could enter the environment undetected as seed has the potential to escape from planted populations via wind movement. Any seeds that find a suitable habitat are likely to remain undetected until the plants develop into a more mature plant. In Spain and France the species has been shown to have spread from urban and semi-urban plantings (Charpentier et al. 2020; Pardo-Primoy and Fagúndez 2019).

Qu. 1.7c. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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Response: The possible entry points of introduction are widespread within the risk assessment area. Urban and semi-urban areas are widespread within the risk assessment area. Transportation networks are widespread throughout the risk assessment area.

Qu. 1.8c. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The overall likelihood of *C. selloana* entering the environment from the pathway landscape / flora / fauna improvement in the wild is very likely with a high confidence. The species can be planted as (1) a boundary or barrier species for properties, or transportation route and (2) landscape improvement (Domènech et al. 2006). There is a long history of planting *C. selloana* for this purpose in the risk assessment area.

Pathway name (4): Transport – Contaminant (transport of habitat material (soil, vegetation))

Qu. 1.2d. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: Entry via this pathway is unintentional movement of the species via the contamination of habitat material (soil and vegetation).

Qu. 1.3d. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year? including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: There is no information available on the volumes of movement along this pathway. The transport of topsoil, sand and or other contaminated habitat material with seed or rhizome could facilitate entry into the RA area. However, the pathway is mainly closed within the RA as there are prohibitions of the movement of soil into the EU from many countries.

Such material is likely to be placed in suitable habitats in the risk assessment area for the establishment of the species. Both seed and rhizome fragments can be introduced and enter the environment via this pathway.

Cortaderia selloana can produce a large biomass of rhizomes which may regenerate into viable plants. These rhizomes can be buried in soil and included in habitat material which is then transported both into the risk assessment area and within the risk assessment area.

Seed may be included in soil and habitat material. Although there is no evidence, seed may be less of a risk than rhizome material as the seeds would be less robust and their viability decreases over time.

Qu. 1.4d. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The seeds of the species are small and therefore they could survive during transport along this pathway. The species has been shown to germinate in a range of soil substrates which may aid survival along this pathway (Domènech and Vilà 2007). Rhizome material can remain viable within soil material.

Qu. 1.5d. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species is moderately likely to survive existing management practices along this pathway. However, there is no information available for this pathway.

Management practices such as those that cause disturbance (roadworks, development, laying new electric or water pipes) to the habitat are likely to facilitate the entry of the species into the environment.

Qu. 1.6d. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The seeds are relatively small and potentially can remain undetected within soil. Similarly, rhizomes can be viable at a small size and buried in soil and other material, making them difficult to detect.

Qu. 1.7d. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated	CONFIDENCE	low
	widespread		medium
	ubiquitous		high

Response: There are no specific data on the points of introduction or entry.

Points of introduction can include road routes which join countries and other international transportation hubs. These are widespread throughout the risk assessment area. Urbanization and the management of land is increasing through the risk assessment area.

Qu. 1.8d. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely	CONFIDENCE	low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response: Both seed and rhizome may be able to move through this pathway, and the commodity is placed within the environment where the propagules have the opportunity to establish. However, there is no direct evidence that the species has moved along this pathway and therefore it is scored moderately likely with a low uncertainty.

Pathway name (5): People and their luggage/equipment (in particular tourism)

Qu. 1.2e. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional	CONFIDENCE	low
	unintentional		medium
			high

Response: This pathway involves the unintentional introduction and entry into the environment of plant material (seeds or rhizomes) as a contaminant of people's luggage and their equipment.

Qu. 1.3e. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year? including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Seeds of *C. selloana* can be moved along this pathway as a contaminant of recreational equipment which can adhere to surfaces like Velcro or on hiking boots (Weeds of Southern Tasmania, n.d.). Recreationalists would need to be in the natural environment when the seeds are being dispersed by wind (autumn months) and the seeds would need to be transferred to the environment at a time when conditions are suitable for germination and growth. There is a low confidence on the number of propagules that may be transported along this pathway as this depends on a number of factors including walking close to monocultures of the species, the direction of wind, etc.

Qu. 1.4e. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The seeds of the species are small and could survive during transport along this pathway. The substrate would be conducive to maintain survival. The species would not reproduce along this pathway or increase.

Qu. 1.5e. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Careful methodical management practices coupled with inspection of boots and clothing would be needed to ensure that the species is not introduced.

Qu. 1.6e. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The seeds are small and potentially may potentially remain undetected within the tread of hiking boots and on other surfaces of recreational equipment. Additionally, the seeds may not be easily identifiable to non-botanists and thus may be overlooked.

Qu. 1.7e. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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Response: Introduction points into the risk assessment area can include airports, international railway stations but also road networks that join with non-EU countries. The entry points which can include any area that is used for recreational purposes in the EU.

Qu. 1.8e. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: A rating of moderately likely with a low confidence is given to this pathway. As previously mentioned, several factors can influence the movement along this pathway (wind, proximity of travellers to stands of *C. selloana*, etc.) and there is a number of unknowns, for example can seed attach to recreational equipment.

Qu. 1.9. Estimate the overall likelihood of introduction into the risk assessment area or entry into the environment based on all pathways and specify if different in relevant biogeographical regions in current conditions.

Provide a thorough assessment of the risk of introduction in relevant biogeographical regions in current conditions: providing insight in to the risk of introduction into the risk assessment area.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The overall likelihood of *C. selloana* being introduced into the risk assessment area is very likely with a high confidence especially because the species is already present (recorded or established) in the risk assessment area, hence the likelihood of introduction and entry should be scored as “very likely” by default. The species has been known to be introduced into the risk assessment area by Horticulture (escape from confinement), Ornamental purpose other than horticulture (e.g. planting the species along roads and for private gardeners). *C. selloana* has the potential of being dumped in the environment as the species produces a lot of above ground biomass which can be difficult to dispose of in the proper means. Dried plumes of the species are available for purchase and can be imported into the risk assessment area. People and their luggage/equipment (in particular tourism) (movement into the risk assessment area) can act to move the species into the environment if seeds are attached to recreational equipment.

As the highest occurrences of the species in the environment are in the Atlantic, Continental and Mediterranean biogeographical regions, it is these regions that would potentially see a higher likelihood of entry into the environment. The species distribution modelling shows that the boreal and alpine biogeographical regions are currently unsuitable for the establishment of the species and therefore the likelihood of the species traded for horticultural purposes in these regions is low.

Qu. 1.10. Estimate the overall likelihood of introduction into the risk assessment area or entry into the environment based on all pathways in foreseeable climate change conditions?

Thorough assessment of the risk of introduction in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of introduction (e.g. change in trade or user preferences)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely introduction within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The main aspects of climatic change which will influence the entry of *C. selloana* into the risk assessment area include increased minimum temperature of winter months, increased spring and summer temperatures.

Climate change (RCP 2.6 2070) will expand the potential distribution the species can establish in, making the species more available to gardeners. For example, the southern areas of Sweden may become more suitable for growing the species and the horticulture pathway may expand in this area to meet further demand. Ornamental plants traded for private gardens and landscape improvements may also see a higher volume and frequency.

2 PROBABILITY OF ESTABLISHMENT

Important instructions:

- For organisms which are already established in parts of the risk assessment area or have previously been eradicated, the likelihood of establishment should be scored as “very likely” by default.
- Discuss the risk also for those parts of the risk assessment area, where the species is not yet established.

Qu. 2.1. How likely is it that the organism will be able to establish in the risk assessment area based on similarity of climatic and abiotic conditions in its distribution elsewhere in the world?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Cortaderia selloana* is already established in the natural environment in the risk assessment area (France, Ireland, Italy, Portugal, Spain, United Kingdom). It is likely that further countries where the species could potentially establish are present within the RA area.

The SDM (Annex VIII) shows the potential distribution of the species based on the current occurrence of the species both in the risk assessment area and elsewhere in the world. The model shows that there is a large area of the EU that is suitable for the establishment of *C. selloana*.

In the native and introduced ranges (outside of the EU), *C. selloana* occurs predominantly in the Köppen-Geiger climate classifications of Cfa ([Humid subtropical climate](#)), Cfb (Temperate [oceanic climate](#)), CSb ([Warm-summer Mediterranean climate](#)) and Bsk ([Cold semi-arid climate](#)). MacLeod and Korycinska (2019) estimate that the percentage of 5-minute grid cells for each aforementioned climate category occurring in the EU are as follows: Cfa 6.31 %, Cfb 48.62 %, CSb 3.8% and Bsk 1.46. Based on this almost 60% of the EU plus the United Kingdom is climatically suitable.

Cortaderia selloana seeds have been shown to germinate under a range of temperatures from 15 °C to 30 °C. Within this range, 100% germination was demonstrated (Domènech and Vilà 2007). In northern, humid areas of Spain, population size can increase two-fold in a year (Herrera Gallastegui and Campos Prieto 2006). Seeds can germinate under a wide range of environmental conditions (light, soil types and varying water availability), however, shading, high levels of sand in soil and water availability all act to improved germinations rates (Domènech and Vilà 2008a).

Qu. 2.2. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area? Consider if the organism specifically requires another species to complete its life cycle.

RESPONSE	very isolated isolated	CONFIDENCE	low medium
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	moderately widespread widespread ubiquitous		high
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Response: In the risk assessment area, *C. selloana* is found in ruderal habitats (Domènech and Vilà 2007) and natural habitats. The species benefits from habitat disturbance which can promote the establishment (Pausas et al. 2006). *Cortaderia selloana* has established in urban and semi-urban habitats in the Mediterranean region, including industrial areas and waste/abandoned lands. The species can be found growing along transportation infrastructure including railways and road networks (where in the case of the latter the species has been deliberately planted).

The species can invade wetlands, coastal dune systems and coastal grasslands. Additionally, the species can grow in scrubland and shrubland (CABI 2020). It can grow along the side of rivers, also in the case of Mediterranean temporary rivers.

Cortaderia selloana does not need any other species to complete its life cycle. Seeds of *C. selloana* can withstand salt exposure. At 3% NaCl concentration seeds showed 90% viability. Seedling survival showed decreases with 2 and 3% NaCl. Laboratory tests conducted in Sardinia (Italy) on seeds collected from naturalised populations of *Cortaderia selloana* showed that salinity did not prevent seeds from germinating, but it affected germination rate and seedling vigour (Bacchetta et al. 2010). Seeds can germinate in a wide range of substrates which promote the establishment of the species in a wide range of habitats. An analysis of soil characteristics across 27 populations along a 300 km area, found soil N ranging from 0.03-0.3%, organic C from 0.09-4.6% and pH 7.4-8.5 (CABI 2020). The species can tolerate free draining soils and soaked soils (wetlands). *Cortaderia selloana* can also grow in light and medium soil textures (CABI 2020). In the introduced range, the proportion of juvenile plants has been shown to be positively correlated to percentage bare ground (Domènech and Vilà 2007).

Domènech and Vilà (2008a) showed that *C. selloana* is adapted to water stress as the species can maximize water uptake and minimize water loss when water becomes scarce. This can enable the species to establish in both wetlands and ruderal habitats where in the case of the latter water may be scarce. This factor can also act to help establishment in Mediterranean ecosystems where the climate is hot and dry and water availability can be limited.

C. selloana grows best in open ground in full sun but it can tolerate light shade. CABI (2020) highlights that in its introduced range, *C. selloana* can be found from sea level to around altitudes of 400 m.

Qu. 2.3. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Cortaderia selloana* is a highly competitive species which can form dense monodominant stands and outcompete native vegetation. *Cortaderia selloana* increases its density and colonizes semi-natural

areas in a short period of time, changing the vertical structure of the vegetation and competing with native plants (Sinnassamy 2001; Campos et al. 2004). Its invasion reduces plant species, life form richness and native species cover; the similarity of native species composition with non-invaded areas can decrease by more than 50% (Domènech and Vilà, 2008b). The plant is spreading along roads and within fragile dune ecosystems in Portugal (Marchante et al. 2005). In France it is recognised that its rapid growth allows it to form dense colonies, accumulating significant above and below ground biomass thereby competing with native plants for access to resources including light, space, nutrients and water to the detriment of these species (AME 2003).

Qu. 2.4. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?

RESPONSE	N/A very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: It is very likely that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area. *Cortaderia selloana* does not have any host specific natural enemies in the risk assessment area and there is no evidence that native organisms feed or infect the species in substantial levels to inflict any damage on the species.

Qu. 2.5. How likely is the organism to establish despite existing management practices in the risk assessment area? Explain if existing management practices could facilitate establishment.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: As detailed in section (3.2), the establishment of *C. selloana* is suited to disturbed habitats especially abandoned land (Domènech and Vilà 2007; Domènech et al., 2005). It is therefore likely that the current urbanization trend occurring in Europe may favour the establishment of the species.

Domènech et al. (2006) noted that the invasiveness of *C. selloana* decreased with distance from urban areas, highlighting that the source of many invasions is a result of propagule pressure from urban areas.

Qu. 2.6. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Wind dispersal of seed, and underground rhizomes which can spread vegetatively, can promote the survival of the species during eradication campaigns.

Although the seed is relatively short lived, and there is no long-lived seed bank (Company et al. 2019; Bacchetta et al. 2010), the species produces large amounts of small seeds which can colonise areas where eradication efforts could potentially miss (Company et al. 2019).

Qu. 2.7. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?

including the following elements:

- a list and description of the reproduction mechanisms of the species in relation to the environmental conditions in the risk assessment area
- an indication of the propagule pressure of the species (e.g. number of gametes, seeds, eggs or propagules, number of reproductive cycles per year) of each of those reproduction mechanisms in relation to the environmental conditions in the risk assessment area.
- If relevant, comment on the likelihood of establishment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in establishment whereas for others high propagule pressure (many thousands of individuals) may not.
- If relevant, comment on the adaptability of the organism to facilitate its establishment and if low genetic diversity in the founder population would have an influence on establishment.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Cortaderia selloana* has two reproductive mechanisms (sexual via seed and asexual via rhizomes). However, CABI (2020) citing (DiTomaso 2000) detail that vegetative reproduction hardly occurs in nature, though it is not clear if this US reference applies to the risk assessment area. The rhizomes are short and contribute to the gradual lateral growth of individual clumps. Old specimens in botanical gardens can get quite large in part through shoot growth from rhizomes along the outer edge of the clump. Often the center of old plants will die leaving a ring of new clones. But in contrast to some other species, new vegetative propagules develop far from the parent plant/clump. So in a practical sense almost all population growth at a location is from sexual reproduction. Seeds that are wind blown can increase the likelihood of propagules finding a suitable habitat in which to establish. Each female plant can produce up to 1 million seeds. According to CABI (2020) “It flowers in late summer, and hermaphrodite panicles appear 1 or 2 weeks before female panicles (Parsons and Cuthbertson 2001). Seed dispersal occurs in the autumn and seeds germinate in the early spring”. Thus, one flowering period occurs within the risk

assessment area in one year. The species can be long lived with references to the rhizomes being able to survive up to 40 years (Costas Lippmann, 1976). However, Moore (1994) suggest under normal conditions plants may live between 10 and 15 years. Parsons and Cuthbertson (2001) state: Rhizome fragments, severed during cultivation, tend to take root and establish new colonies wherever they are deposited. Grubbed crowns, carelessly dumped in tips and waste places, re-establish easily if moisture is available, and provide a potential seed source for future spread". CABI (2020) citing (DiTomaso 2000) detail that vegetative reproduction hardly occurs in nature, but plant propagation through the division of mature plants has traditionally been used for ornamental purposes.

There is no information on the establishment potential in relation to genetic diversity.

Qu. 2.8. If the organism does not establish, then how likely is it that casual populations will continue to occur?

Consider, for example, a species which cannot reproduce in the risk assessment area, because of unsuitable climatic conditions or host plants, but is present because of recurring introduction, entry and release events. This may also apply for long-living organisms.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: In areas where *C. selloana* is not present, casual populations may occur if propagule pressure is high in urban habitats or semi-urban habitats (Domènech *et al.*, 2005). Casual populations may occur in surrounding habitats. Casual populations are likely to occur in areas outside the optimum climatic conditions. For example, this may include areas in Figure 5 of Appendix 4 at the margin of suitable and unsuitable areas. Countries where casual populations may occur include Germany, Poland, Lithuania, Slovakia, Southern Sweden and the coastline of Norway.

Qu. 2.9. Estimate the overall likelihood of establishment in the risk assessment area under current climatic conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under current climatic conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in current conditions: providing insight in the risk of establishment in (new areas in) the risk assessment area.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The overall likelihood of *C. selloana* establishing in the risk assessment area is very likely with high confidence. The species is already established in the Mediterranean, Continental and Atlantic

biogeographical regions. Countries where the species is established include: France, Ireland, Italy, Spain, Portugal and the United Kingdom. Both the Mediterranean and Pannonian biogeographical regions have a high proportion of area suitable for the establishment of the species and thus further establishment is likely in these areas under the current climatic conditions. Likewise, the Macaronesia, Continental, Black Sea, and Atlantic biogeographical regions have a moderate proportion of area available for the establishment of the species. Within the EU, the boreal biogeographical region has a very low area available for establishment.

Qu. 2.10. Estimate the overall likelihood of establishment in the risk assessment area under foreseeable climate change conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under foreseeable climate change conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of establishment (e.g. increase in average winter temperature, increase in drought periods)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely establishment within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6 °C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0 °C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The overall likelihood of *C. selloana* establishing in the risk assessment area under future climatic conditions (2070 RCP. 2.6) is very likely with high confidence. The species is already established in the Mediterranean, Continental and Atlantic biogeographical regions. Both the Mediterranean and Pannonian biogeographical regions have a high proportion of area suitable to the establishment of the species and thus further establishment is likely in these areas under future climatic conditions. Some areas within the aforementioned, increase in sustainability. Likewise, areas of suitability are shown to increase in both area and local density in the following biogeographical regions: Macaronesia, Continental, Black Sea and Atlantic. Within the EU, southern areas of the Boreal biogeographical region show potential for establishment and small coastal areas of Norway (Alpine biogeographical region) under future climatic conditions (see Annex VIII).

The main aspects of climatic change which will influence the establishment of *C. selloana* include increased minimum temperature of winter months, increased spring and summer temperatures and increased

precipitation. Increased frequency of fire, as the result of increased temperatures, may promote the establishment of the species in natural areas where otherwise it would not be able to get a foothold.

3 PROBABILITY OF SPREAD

Important instructions:

- Spread is defined as the expansion of the geographical distribution of an alien species within the risk assessment area.
- Repeated releases at separate locations do not represent continuous spread and should be considered in the probability of introduction and entry section (Qu. 1.7).

Qu. 3.1. How important is the expected spread of this organism within the risk assessment area by natural means? (List and comment on each of the mechanisms for natural spread.)

including the following elements:

- a list and description of the natural spread mechanisms of the species in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of spread discussed in relation to the species biology and the environmental conditions in the risk assessment area.

The description of spread patterns here refers to the CBD pathway category “Unaided (Natural Spread)”. It should include elements of the species life history and behavioural traits able to explain its ability to spread, including: reproduction or growth strategy, dispersal capacity, longevity, dietary requirements, environmental and climatic requirements, specialist or generalist characteristics.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: There are two main reproductive modes for the natural spread of *Cortaderia selloana* (1) seed and (2) vegetative propagation via basal-stem-rhizome fragments.

via seeds:

It is interesting to note that historically both in the USA and the RA area, only female plants were imported for cultivation, and later seeds were traded which grew into hermaphrodite plants (Bossard et al. 2000; LIFE STOP Cortaderia, 2020).

Caryopsis and glumettes (hereafter seeds) of *C. selloana* are light and dispersed by wind and human activity (DiTomasto et al. 2010. Seeds are hairy (due to the presence of long hairs on lemmas, only in female plants) narrowly elliptical and small (0.6 mm by 2mm, Parsons and Cuthbertson 2001). From studies in the United States, WSNWCB (2014) state ‘Saura-Mas and Lloret’s (2005) research on wind dispersal of seeds found wind-affected seed from 1 to 20 meters away from the sources, and there was no effect at distances greater than 20 meters. The distance from the source plant significantly reduced the number of seeds reaching the soil’.

According to CABI (2009) ‘Seeds can disperse to at least 40 m away from mother plants, and local wind direction determines the spatial distribution of seedlings (Saura-Mas and Lloret 2005). Typically though, the effect of wind on seeds moved them relatively short distances (1-20 m) (Saura-Mas and Lloret 2005). However, NNS (2015) quote larger distance of wind dispersal, established seed producing plants in gardens, parks and outside cultivation can be sources for natural spread through the dispersal of the

lightweight seeds with distances to up to 25 km (Parsons and Cuthbertson 2001)'. Difference in wind speed, intensity, direction and physical barriers to the movement of seed, can all affect the distance seed will be able to travel. Wind currents produced by traffic should also be considered as a factor of seed spread where the species occurs along transportation routes. For the risk assessment region, the natural spread by wind should be considered for up to 40 m though in coastal areas this may be greater.

If planted, or if the species escapes into habitats in the risk assessment area, natural spread is likely to facilitate movement to suitable habitats as the seed can be carried over long distances. Locally, in the Mediterranean region, the volume of seed production will support spread as the species occurs in high numbers in this area. USDA (2014), citing Muyt (2001), highlight that seeds can spread in water bodies, but no evidence has been found for water dispersal of the seeds. USDA (2014) also detail that there is no evidence for bird or animal (external or internal) dispersal, however, CAL IPC (2020) detail that seed can adhere to the fur of animals.

Seed dispersal occurs late in the year following flowering and fruiting usually in October to mid-November in the Atlantic biogeographical region.

via rhizomes:

Natural spread via rhizome fragments is likely to be only local and over short distances. There is no exact information available on the size of a viable rhizome fragment. However, it is cited throughout the literature that fragments can be viable and regenerate from the node. Therefore, based on the knowledge of the authors of this risk assessment, it is possible that small rhizome fragments (e.g. 2-3 cm in length with a node) can produce a viable plant. CABI (2020) citing (DiTomaso 2000) detail that vegetative reproduction hardly occurs in nature.

Spread within the risk assessment area

Cortaderia selloana has shown a rapid range expansion within the Mediterranean region (Company et al. 2019). Bacchetta et al. (2010) highlight that much of the spread of the species in the Mediterranean regions is facilitated by the change in land use. Presumably, increased urbanisation has resulted in existing populations being disturbed, and along with a combination of natural dispersal and potential movement of contaminated habitat material, has promoted the spread of the species within the region. Much of this spread can be attributed to natural dispersal of seeds via wind movement, but also due to the propagule pressure within the region.

In Galicia (North West Spain), *C. selloana* spread along the main highways during the 1980s where it was used as an ornamental species along the side or central strip of the road. Therefore, wind currents produced by traffic should also be considered as a factor of seed spread where the species occurs along transportation routes. Domènech et al. (2006) assessed the invasion of *C. selloana* in Catalonia (NE Spain) between 1998 to 2003. The number of invaded fields increased 1.85 times during 5 years (from 12.05% in 1998 to 22.30% in 2003), at a mean rate of 0.37 fields per year (Domènech et al. 2006). Invasion in agricultural fields increased from 17 in 1998 to 40 in 2003, pastures increased from 18 to 27 from 1998 to 2003 and old fields increased from 5 to 7 from 1998 to 2003.

Qu. 3.2a. List and describe relevant pathways of spread other than "unaided". For each pathway answer questions 3.3 to 3.9 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 3.3a, 3.4a, etc. and then 3.3b, 3.4b etc. for the next pathway.

including the following elements:

- a list and description of pathways of spread with an indication of their importance and associated risks (e.g. the likelihood of spread in the risk assessment area, based on these pathways; likelihood of survival, or reproduction, or increase during transport and storage; ability and likelihood of transfer from the pathway to a suitable habitat or host) in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of spread for each pathway discussed in relation to the species biology and the environmental conditions in the risk assessment area.
- All relevant pathways of spread (except “Unaided (Natural Spread)”, which is assessed in Qu. 4.1) should be considered. The classification of pathways developed by the Convention of Biological Diversity shall be used (see Annex IV).

Spread pathways are detailed in order of importance

Ornamental purposes other than horticulture (escape from confinement)

This spread pathway is the intentional spread of the species for private gardening and urban and semi-urban plantings (roadsides and roundabouts for example). This pathway would be the most relevant for spread and include the highest movement of plant propagules.

Transport – Contaminant (transport of habitat material (soil, vegetation))

This spread pathway deals with the potential spread of contaminated habitat material. There is no quantitative evidence/ interceptions to support the movement of the species along this spread pathway but where urbanisation has occurred in the Mediterranean region the species has shown significant spread.

Transport – stowaway: Machinery/ equipment

This pathway details the potential spread of contaminated machinery and equipment. There is no quantitative evidence/ interceptions to support the movement of the species along this spread pathway but where urbanisation has occurred in the Mediterranean region the species has shown significant spread.

People and their luggage/ equipment (in particular tourism)

This pathway details the potential spread of contaminated luggage and equipment. There is no quantitative evidence/ interceptions to support the movement of the species along this spread pathway but it is considered as a potential pathway.

Pathway name: (1) Ornamental purposes other than horticulture (escape from confinement)

Qu. 3.3a. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	Unintentional intentional	CONFIDENCE	low medium high
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Response: *Cortaderia selloana* is a popular ornamental species within the risk assessment area and has been spread throughout the region as a garden ornamental species. Additionally, the species is planted in urban and semi-urban habitats (for example along roads and on roundabouts).

Qu. 3.4a. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Question A13 highlights that there are significant sales of the species within the risk assessment area (whole plants). Sales of the species can also be from seed. Seeds are sold in packets of multiple seeds. Plants can be purchased from garden centers within the risk assessment area and moved to gardens where they are planted. The species may also be dumped from waste garden material into the natural environment, though again, there is no details and the volume of movement or frequency.

Qu. 3.5a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: It is very likely that *C. selloana* plant material will survive during transport and storage along this pathway. A high seed viability would be an important factor for the suppliers to sell and continue to sell their stock. Once planted in a garden, the species would be planted and cared for so that the species grows and thrives. When dried, the seeds of *C. selloana* can remain viable approximately 12 months (USDA, 2014) and therefore this will increase the chance of survival during transport. Live plants will be moved along the pathway and packaged to promote their survival. It is unlikely the plants will reproduce or increase during their transport along this pathway.

Qu. 3.6a. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Cortaderia selloana* is very likely to survive existing management practises during spread. Large amounts of seed are produced. Management of urban and semi-urban habitats is likely to increase disturbance of the habitat, which is favoured by the species (Pausas et al. 2006). Urbanisation has been shown to facilitate the spread of the species in the Mediterranean region (Domènech and Vilà 2007). If dumped, the species is likely to be deposited in ruderal habitats and potentially on waste ground where management practices may be minimal and the native vegetation sparse, this may make the survival of the species in these areas more likely. Soil disturbance (which may result from management activities) has been shown to increase the survival and biomass of plants (Domènech and Vilà 2006).

Qu. 3.7a. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Seeds of the species are small and wind dispersed which can help to facilitate there spread undetected in the risk assessment area. Small rhizome fragments (e.g. 2-3 cm in length with a node) can produce a viable plant. These fragments may be spread via dumping of garden waste and therefore may be spread in the risk assessment area undetected.

Qu. 3.8a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Seeds of the species are wind dispersed and there is a high potential that seeds could move outside the confines of the garden before being planted. The large amount of seeds produced increases the likelihood of some seeds finding a suitable habitat. The species has been shown to spread to ruderal habitats (Domènech and Vilà 2007) and natural habitats. The presence of the species in urban and semi-urban

habitats in the Mediterranean region, including industrial areas and waste/abandoned lands highlights the species spread (Pardo-Primoy and Fagúndez 2019). In Galicia (North West Spain), *C. selloana* spread along the main highways during the 1980s where it was used as an ornamental species along the side or central strip of the road (Pardo-Primoy and Fagúndez 2019).

The species can invade wetlands, coastal dune systems and coastal grasslands. Additionally, the species can grow in scrubland and shrubland (CABI 2020). It can grow along the side of rivers, also in the case of Mediterranean temporary rivers. There is also the potential for transfer from the pathway during movement but this should be considered as unlikely as seed would be packaged.

Qu. 3.9a. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area. (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: *Cortaderia selloana* is a popular garden ornamental species which is sold and grows throughout the risk assessment area. It has also been planted in urban and semi-urban habitats. Spread has been recorded in Catalonia (NE Spain) between 1998 to 2003 where the number of invaded fields increased 1.85 times during 5 years (from 12.05% in 1998 to 22.30% in 2003), at a mean rate of 0.37 fields per year (Domènech et al. 2006). Invasion in agricultural fields increased from 17 in 1998 to 40 in 2003, pastures increased from 18 to 27 from 1998 to 2003 and old fields increased from 5 to 7 from 1998 to 2003. The species has also been shown to spread in industrial sites in Spain (Pardo-Primoy and Fagúndez 2019).

Pathway name: (2) Transport – Contaminant (transport of habitat material (soil, vegetation))

Qu. 3.3b. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: The spread of *C. selloana* via transport of habitat material (soil, vegetation) is the unintentional spread of the species within the risk assessment area. Both seed material and rhizome fragments can be spread via this pathway.

Qu. 3.4b. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: There is no information available on the volumes of movement along this pathway. However, individual female plants produce a high number of seeds which can become incorporated into soil and attached to vegetation material. Additionally, rhizomes can be present in the soil. Soil disturbance has been shown to increase the survival and biomass of plants (Domènech and Vilà 2006).

Qu. 3.5b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: There is no quantitative evidence for *C. selloana*, for the survival, reproduction or increase as a contaminant of habitat material. The seeds of the species are small and therefore they could survive during transport along this spread pathway. Habitat material would be suitable for the survival of the species over short periods. The species has been shown to germinate in a range of soil substrates which will aid survival along this spread pathway. Rhizome material can remain viable within soil material.

Qu. 3.6b. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Careful methodical management practices coupled with inspection would be needed to ensure that the species did not spread with contaminated soil and vegetation. This is often not feasible with such small seeds and small rhizome fragments.

Qu. 3.7b. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The seeds are relatively small and potentially can remain undetected within soil. Soil and other habitat material can be moved throughout the RA area and seeds can be spread within such material. Similar, rhizomes may be small and buried in soil and other material, making them difficult to detect.

Qu. 3.8b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: It would be likely that *C. selloana* can transfer to a suitable habitat if seed and rhizome material of the species is incorporated in soil. Topsoil and habitat material is often physically transferred to suitable habitats and thus it is very likely that the species will transfer to suitable habitats. This fact that the species is often recorded in urban development areas further supports the hypothesis that the species can be moved by soil and habitat material.

Qu. 3.9b. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area. (provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: There is no quantitative data on the spread of the species in relation to habitat material.

Habitat material can move freely within the single market in the EU enabling contaminated material to spread over long distances. Both seeds and rhizome material may be included as a contaminant. Because of the lack of quantitative data, confidence is low.

Pathway name: (3) Transport – stowaway: Machinery/ equipment

Qu. 3.3c. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: Stowaway on machinery and equipment is the unintentional spread of the species within the risk assessment area.

Qu. 3.4c. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: There is no information available on the volumes of movement along this pathway. However, in areas where the species is present, the amount of windblown seed can be high and thus there is a potential for spread as a high number of seeds can become incorporated into tread of tyres and other parts of machinery.

Qu. 3.5c. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely	CONFIDENCE	low
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	unlikely moderately likely likely very likely		medium high
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Response: The seeds of the species are small and therefore they could survive during transport along this spread pathway. However, there is no evidence if seeds are robust enough to survive movement along this pathway. The species is unlikely to increase along the pathway until it finds a suitable habitat.

Qu. 3.6c. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The seeds are relatively small and potentially can remain hidden in soil in cracks and crevices in machinery and equipment. Such machinery can be moved within the RA area transporting the seeds to new areas. ISPM 41 (IPPC 2017) ‘Intentional movement of machinery and equipment’ provides international measures for cleaning, which can be applied to local level movement also.

Qu. 3.7c. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The seeds and rhizome fragments are relatively small and potentially can remain hidden in soil in cracks and crevices in machinery and equipment. Such machinery can be moved within the RA area transporting the seed to new areas.

Qu. 3.8c. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: It would be likely that *C. selloana* can transfer to a suitable habitat if the species is a stowaway on machinery/ equipment. Landscaping machinery and construction machinery is used in suitable habitats of the species, especially in the Mediterranean region. The fact that the species is often recorded in urban development areas further supports the hypothesis that the species can be moved by used machinery and equipment.

Qu. 3.9c. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area. (provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: Used machinery and equipment can be moved within the RA area at a local and long distances scale. Any seed and rhizome material attached could be spread at a fast rate. However, it is only scored moderately likely as a number of measures can be adopted against this spread pathway to reduce the spread of the species. Inspections of used machinery and equipment coupled with cleaning will reduce the potential of spread of the species. A low uncertainty has been given as there is no quantitative data.

Pathway name: (4) People and their luggage/ equipment (in particular tourism)

Qu. 3.3d. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional unintentional	CONFIDENCE	Low medium high
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Response: The spread of *C. selloana* via people and their luggage/equipment, in particular tourism, is for this species, considered the unintentional spread of the species. In the sense of this pathway, spread of the species could be facilitated by seeds being incorporated into the tread of hiking boots or other equipment and moved accidentally to other areas within the risk assessment area.

Qu. 3.4d. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?
including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication

- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: There is no information available on the volumes of movement along this pathway. The seeds are hairy and extremely lightweight. They readily adhere to any rough surface such as clothing (John Lambrinos, pers. comm. 2020). However, the seed could adhere to Velcro or other material used by recreationalists.

Qu. 3.5d. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The seeds of the species are small and therefore they could survive during transport along this spread pathway. The substrate i.e. leisure equipment could be conducive to maintain survival. The species would not reproduce along this pathway or increase.

Qu. 3.6d. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Existing management practices occurring in the risk assessment area are unlikely to affect the spread of the species. Wind blown seeds, if anything are likely to benefit from management practices due to distance.

Qu. 3.7d. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The seeds are relatively small and potentially can remain undetected within the tread of hiking boots. Additionally, the seeds may not be easily identifiable to non-botanists and thus may be overlooked.

Qu. 3.8d. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: It would be unlikely that *C. selloana* can transfer to a suitable habitat if the seed of the species is attached to hiking boots, clothes or camping equipment for example. This type of equipment may be re-used outside in a habitat suitable for the establishment of the species.

Qu. 3.9d. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area.(please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: The overall potential rate of spread via this pathway is estimated as slowly with a low confidence. Recreationalists would also need to be in the natural environment when the seed are dispersed by wind (autumn months) and the seed would need to attach to clothes or equipment for propagules to spread.

Qu. 3.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?

RESPONSE	very easy easy	CONFIDENCE	low medium
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	with some difficulty difficult very difficult		high
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Response: Natural spread would be difficult to control within the risk assessment area. Seeds are wind dispersed and can be spread over long distances. The high number of populations where the species can spread from is also a limiting factor for controlling the spread of the species. The species is found throughout the risk assessment area and is frequently planted in gardens. As the species can spread as a contaminant of soil and habitat material, machinery and equipment and recreational equipment, biosecurity measures and inspections would need to be adopted where these spread pathways are active, and the species is present. This would involve multiple stakeholders and communication and awareness raising to reduce the spread pathways. A ban on sale could act to prevent further spread of the species within the risk assessment area.

Preventing the spread from already established populations, including those in private gardens will be difficult due to the wide planting of the species. Measures could include preventing the plant from flowering but this would be practically impossible to implement.

Qu. 3.11. Estimate the overall potential rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (indicate any key issues and provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in current conditions, providing insight in the risk of spread into (new areas in) the risk assessment area.

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: As a wind dispersed species that can spread up to 25 km by seed (Parsons and Cuthbertson 2001) and can be spread much further distance by human mediated activities (both deliberate: horticulture and unintentional: contaminant of habitat material and used machinery and equipment), the overall rate of spread can be estimated as very rapidly. Land use change in the Mediterranean region has promoted the spread of the species in recent years and if this trend continues further spread in this region and other suitable biogeographical regions (i.e. Atlantic and Continental biogeographical regions) is likely.

Qu. 3.12. Estimate the overall potential rate of spread in relevant biogeographical regions in foreseeable climate change conditions (provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if rates of spread are likely slowed down or accelerated.

RESPONSE	very slowly	CONFIDENCE	low
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	slowly moderately rapidly very rapidly		medium high
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Response: The SDM (see Annex VIII) shows that the area suitable for the establishment of *C. selloana* expands under climate change projections RCP 2.6 and 4.5 within areas of the Mediterranean, Atlantic and Continental biogeographical regions becoming more suitable. The potential distribution for establishment increases northwards into the Boreal biogeographical region. This can cause an increase of the use of the species as a garden ornamental species in areas where it had not previously occurred and this can increase the risk of spread. In addition, rate of urbanization and land development is likely to increase as the human population increases and this can act to facilitate the spread of the species. Biogeographical regions with established populations and high levels of urbanization may see high levels of spread as seen in the Mediterranean region.

Higher latitudes of the Boreal biogeographical region are likely to see lower spread rates as established populations will not be present.

4 MAGNITUDE OF IMPACT

Important instructions:

- Questions 5.1-5.5 relate to biodiversity and ecosystem impacts, 5.6-5.8 to impacts on ecosystem services, 5.9-5.13 to economic impact, 5.14-5.15 to social and human health impact, and 5.16-5.18 to other impacts. These impacts can be interlinked, for example, a disease may cause impacts on biodiversity and/or ecosystem functioning that leads to impacts on ecosystem services and finally economic impacts. In such cases the assessor should try to note the different impacts where most appropriate, cross-referencing between questions when needed.
- Each set of questions starts with the impact elsewhere in the world, then considers impacts in the risk assessment area (=EU excluding outermost regions) separating known impacts to date (i.e. past and current impacts) from potential future impacts (including foreseeable climate change).
- Only negative impacts are considered in this section (socio-economic benefits are considered in Qu. A.7)
- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

Biodiversity and ecosystem impacts

Qu. 4.1. How important is the impact of the organism on biodiversity at all levels of organisation caused by the organism in its non-native range excluding the risk assessment area?

including the following elements:

- Biodiversity means the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems
- impacted chemical, physical or structural characteristics and functioning of ecosystems

RESPONSE		CONFIDENCE	
	Minimal		low
	minor		medium
	moderate		high
	major		
	massive		

Response: There is considerable evidence that *C. selloana* has a detrimental impact on biodiversity in its invaded range excluding the risk assessment area. Although known for ruderal invasions *C. selloana* is recognized as an invasive pest in California where it has frequently occupied non-ruderal habitats over the past 50 years (Lambrinos 2001) and can easily invade waste areas, open and disturbed areas in Australia (Harradine 1991). Once established, mature plants along with the congeneric *C. jubata*, are very competitive. Large infestations of *C. selloana* and *C. jubata* threaten California's coastal ecosystems by crowding out native species, particularly in sensitive coastal dune areas (Di Tomaso 2010). The plant is reported to displace native plant communities in coastal habitats in California (Motooka et al. 2003). It becomes dense and can suppress the growth of other species, replacing ground cover, shrubs, and ferns in

New Zealand (MPI 2012). *Cortaderia selloana* competes with and replaces native plants in South Africa (Wells et al. 1986).

A US Fish and Wildlife Service report (2010a, 2010b) record it as a danger to a Threatened and Endangered species *Streptanthus glandulosus* subsp. *niger* in California “strangling the plant” as well as competing with *Verbesina dissita* (big-leaved crownbeard) by monopolising resources.

In New Zealand, *C. selloana* is recorded as a weed of concern, i.e. known to be affecting the structure, species composition or regeneration of native plants (Owen 1996) based on publications by Humphries et al. (1991) and Mooney et al. (1986). The database also provides a weediness score and *C. selloana* ranks 27th among 160 species. In a review, Gosling et al. (2000) carried out a survey of pampas grass control staff who reported the following habitats as being vulnerable to the effects of pampas grass: dunelands, off-shore islands, coastal cliffs, river flats, geothermal sites and wetlands. The perceived impacts were displacement of natural flora and fauna including one Giant Weta (Orthoptera: Anostomatidae) site.

Cortaderia selloana is recognised as one of the main priority invasive weeds in bushland reserves and native forestry in Tasmania (Young 1993). In Australia it replaces natural plant communities and excludes plants through shading and below ground competition since the root system of a single plant is estimated to occupy 103 cubic meters of soil (Parsons and Cuthbertson 2001).

Qu. 4.2. How important is the current known impact of the organism on biodiversity at all levels of organisation (e.g. decline in native species, changes in native species communities, hybridisation) in the risk assessment area (include any past impact in your response)?

Discuss impacts that are currently occurring or are likely occurring or have occurred in the past in the risk assessment area. Where there is no direct evidence of impact in the risk assessment area (for example no studies have been conducted), evidence from outside of the risk assessment area can be used to infer impacts within the risk assessment area.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: *Cortaderia selloana* has a major impact on biodiversity in Mediterranean countries in the risk assessment area. It is a common invader of both natural and ruderal habitats in Mediterranean coastal regions (Domènech and Vilà 2007). However, Pardo-Primoy and Fagúndez (2019) noted that the best predictor of *Cortaderia* invasion on industrial sites in Galicia was closeness to another invaded industrial site and low elevation. In Spain *C. selloana* invasion of old fields prevents their restoration to wetlands where some rare or endemic plant species⁷ occur (Pausas et al. 2006) such as *Spirodela polyrrhiza*, a floating plant thought to be extinct in the region until recently. *C. selloana* on the island of Corsica (France), seriously threatens the endemic species *Centranthus trinervis*, one of the endemic and critically endangered Mediterranean plants from the IUCN ‘Top 50 Mediterranean Island Plant’ list (de Montmollin and Strahm (2005); Brundu (2013)).

⁷ Pausas et al. (2006) do not specify specific species

Due to low decomposition of litter and standing dead leaves reduce the total soil N and increase C:N ratios due to low decomposition of litter, changes the relative frequencies of plant growth forms, reduces total plant cover, and alters soil nutrient properties (Domènech et al. 2006).

Cortaderia selloana increases its density and colonizes semi-natural areas in a short period of time, changing the vertical structure of the vegetation, altering ecosystem naturalness by competing with native plants (Sinnassamy 2001; Herrera Gallastegui and Campos Prieto 2006; Campos et al. 2004). Its invasion reduces plant species, life form richness and native species cover; the similarity of native species composition with non-invaded areas can decrease by more than 50% (Domènech et al. 2006). The plant is spreading along roads and within fragile dune ecosystems in Portugal (Marchante et al. 2005)

In France it is recognised that its rapid growth allows it to form dense colonies, accumulating significant above and below ground biomass thereby competing with native plants for access to resources including light, space, nutrients and water to the detriment of these species (AME 2003). It is believed to have a particular impact on low light-loving vegetation, especially on coastal sites where the vegetation is more likely to be endemic or of high conservation value (Petit 2011).

The authors and reviewers consider that the major score given to impact on biodiversity is justified even with the lack of scientific publications to quantify the negative impacts to biodiversity. In the invaded areas, stands of *C. selloana* occur with high levels of covering which exclude native plant species and in many cases these dense stands are present in sensitive areas which harbour rare and endangered species. Monospecific dense stands of any perennial species is a good proxy for negative impact on biodiversity. Additionally, the inclusion of the species in an EU LIFE biodiversity project supports the presence of negative impact on biodiversity that can be reversed on local control and eradication.

However, due to the limited amount of scientific evidence, a medium confidence has been given.

Qu. 4.3. How important is the potential future impact of the organism on biodiversity at all levels of organisation likely to be in the risk assessment area?
 See comment above. The potential future impact shall be assessed only for the risk assessment area.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: It is likely that *C. selloana* will continue to spread within the risk assessment area and is likely to establish in new areas. Therefore, in these areas, there will be additional impacts as seen in the current area of distribution. These are likely to initially be localised around urban development and human disturbed habitats but once established there is more likelihood of spread into more sensitive habitats.

Qu. 4.4. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism currently in the risk assessment area?
 including the following elements:

- native species impacted, including red list species, endemic species and species listed in the Birds and Habitats directives

- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: The Mediterranean is a biodiversity hotspot (e.g., Médail and Quézel 1999) and in this biogeographical region many coastal and sand dune ecosystems are home to endemic (and narrow endemic plants, also known as micro-endemic) and rare flora and associated fauna and their displacement by *C. selloana* would very likely be impacting on the current conservation status (e.g., on the Habitats directive priority habitat 2130*, i.e. fixed coastal dunes with herbaceous vegetation or "grey dunes"). For these reasons, the plant is the subject of an EU LIFE project, "StopCortaderia", which aims to stop the spread of *Cortaderia selloana* and reduce its presence in coastal Natura 2000 network sites, to safeguard sensitive habitats and native species (www.stopcortaderia.org). Targeted sites in Cantabria include the Special Conservation Areas of Dunas de Liencres y estuarios del río Pas (ES1300004); Río Agüera (ES1300012); Marismas de Santoña, Victoria y Joyel (ES1300007); Rías occidentales y dunas de Oyambre (ES1300003); Dunas del Puntal y Estuario del Miera (ES1300005); Costa Central y Ría de Ajo (ES1300006). In Spain, according to Asensi et al. (2014), in recent decades, the increase of *Cortaderia selloana* populations in coastal areas of Andalusia has been observed. Although generally occupying anthropic environments, its presence was detected near to *Retama monosperma* [*Retamion monospermae* Rivas-Mart. et Cantó in Rivas-Mart et al. 2002] and *Pinus pinea* wooded dunes communities in Huelva. Since 2007, the Ministry of Environment of the Junta de Andalucía has performed various programmes for the eradication of these species in ecosystems of interest (Asensi et al. 2014). Another LIFE project (Spain, LIFE Miera - Biodiversity conservation in river Miera - LIFE13 NAT/ES/000899) is dealing with *Baccharis halimifolia* and *Cortaderia selloana* as they are displacing indigenous riverbank species, affecting estuary habitats (1330 and 1410), dune ecosystems (2120 and 2130) and coastal heathland habitat (4040) (Scalera et al. 2017). As described by Herrera Gallastegui and Campos Prieto (2006), *C. selloana* is widespread in the Basque Country, where it invades large areas inside protected areas and environmentally sensitive areas, such as in the case of the Biosphere Reserve Urdaibai, the natural Park Armañon (Herrera Gallastegui and Campos Prieto 2006), and the estuarine area of río Butroe. In the Biosphere Reserve of Urdaibai (Bizkaia), *C. selloana* is very abundant in natural and semi-natural habitats: subalkaline zones of the marshes, scrubs, edges of holm oak, sands and meadows. It colonizes large areas in the upper estuary of the Oka River, mainly artificially drained lands, and expands into high marsh habitats, such as reed beds, and salt grasslands. Furthermore, it has spread to areas where removal work of another invasive species (*Baccharis halimifolia*) was carried out and no subsequent restoration work was carried out (Herrera Gallastegui and Campos Prieto 2006).

According to de Montmollin and Strahm (2005) and as reported in Brundu (2013) *Cortaderia selloana*, on the island of Corsica (France), seriously threatens the endemic species *Centranthus trinervis*, one of the endemic and critically endangered Mediterranean plants from the IUCN 'Top 50 Mediterranean Island Plant' list.

It is likely to displace ground nesting birds that favour dune ecosystems with low ground cover as the tall *C. selloana* would provide very different structure and resources though no published evidence has been found relating to individual species.

Qu. 4.5. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.4.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Future impacts are likely to be seen in the Mediterranean biogeographical region. Native species, habitats and protected sites at risk are detailed in 4.4. The climate modelling shows areas of the Mediterranean, Atlantic and Continental biogeographical regions becoming more suitable as well as the new inclusion of Boreal biogeographical region. Biogeographical regions with established populations and high levels of urbanization may see high levels of spread as seen in the Mediterranean region.

Ecosystem Services impacts

Qu. 4.6. How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?

- For a list of services use the CICES classification V5.1 provided in Annex V.
- Impacts on ecosystem services build on the observed impacts on biodiversity (habitat, species, genetic, functional) but focus exclusively on reflecting these changes in relation to their links with socio-economic well-being.
- Quantitative data should be provided whenever available and references duly reported.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: *C. selloana* has an impact on provisioning of biomass in the form of wild plants and associated specialist animals as it is known to form extensive monospecific stands in coastal sage scrub drainages and riparian areas in California (Vourlitis and Kroon 2013) which will also have an impact on provisioning of water. The reduction in biodiversity associated with *C. selloana* invasions that have been reported from USA, (Lambrinos 2001; Di Tomaso 2010) South Africa (Wells et al. 1986), New Zealand (Owen 1996) and Australia (Young 1993). Since the root system of a single plant is estimated to occupy 103 cubic meters

of soil (Parsons and Cuthbertson 2001) it will have an impact on regulation through altered soil quality. It is known to form extensive monospecific stands in coastal sage scrub drainages and riparian areas in California (Vourlitis and Kroon 2013) which will also have an impact on regulation through altered water quality.

It has an impact on cultural ecosystem services that are physical and experiential through reduction biodiversity, amenity values (by reducing access to and the value of natural habitats for recreation) as well as on the non-use value of wilderness areas that have an intrinsic value and has a small impact on human health.

Qu. 4.7. How important is the impact of the organism on provisioning, regulating, and cultural services currently in the different biogeographic regions or marine sub-regions where the species has established in the risk assessment area (include any past impact in your response)?

- See guidance to Qu. 4.6.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: *Cortaderia selloana* has impacts upon provisioning through the reduction in availability and diversity of wild plants. Its invasion reduces plant species, life form richness and native species cover; the similarity of native species composition with non-invaded areas can decrease by more than 50% (Domènech et al. 2006).

It also has an impact of regulation and maintenance of ecosystem services, impacting on baseline flows e.g. it is known to modify nutrient regimes (Domènech, et al. 2006) as well as extreme events such as fire (Starr et al. 2003; Harradine 1991). In the Mediterranean this potential for increased fire risk resulting from the dry leaves of tussocks is noted (Arianoutsou and Vilà 2012). Both of these impacts on regulation further affect succession patterns and thereby provisioning. Although there is no published data, there is the potential of impact on hydrology in riparian areas, in particular in the case of temporary (intermittent) Mediterranean rivers and temporary ponds through the large scale growth of a much larger transpiring plant than is usually found in the habitat. Impacts on cultural ecosystem services in the risk assessment area include reduced amenity value and biodiversity intrinsic value.

Qu. 4.8. How important is the impact of the organism on provisioning, regulating, and cultural services likely to be in the different biogeographic regions or marine sub-regions where the species can establish in the risk assessment area in the future?

- See guidance to Qu. 4.6.

RESPONSE	minimal minor moderate major	CONFIDENCE	low medium high
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	massive		
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Response: The impacts would be similar but on a larger scale. There is the potential of increased fire regimes (and increased intensity) in the risk assessment area as the area of establishment of the species expands with the climate change projection (Annex VIII). Further spread into natural habitats can have negative impacts on cultural ecosystem services through reduced amenity value and biodiversity intrinsic value. There will also be expanded impacts on regulation through altered water and soil quality.

Economic impacts

Qu. 4.9. How great is the overall economic cost caused by the organism within its current area of distribution (excluding the risk assessment area), including both costs of / loss due to damage and the cost of current management.

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the world these should be reported here. The assessment of the potential costs of / loss due to damage shall describe those costs quantitatively and/or qualitatively depending on what information is available. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage. As far as possible, it would be useful to separate costs of / loss due to the organism from costs of current management.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: In Australian forest plantations, pampas grass competes with seedling trees, especially softwood species, reducing establishment and retarding growth. In older forests, pampas grass increases the fire hazard (Harradine 1991 which increases expenditure on preventative measures and preparedness for outbreaks). Pampas grasses have been estimated to increase silvicultural tending costs by 144% in New Zealand and impedes access for silviculture (Gadcil et al, 1984). In 1983 these control costs, which were reported as “barely assuring tree survival”, were \$NZ350/ha (Gadcil et al. 1984) which accounting for inflation would be \$NZ 1,220/ha today, which equates to €675/ha. In 1987 the Tasmanian Inter-departmental Pampa Grass Committee estimated the cost of Pampas grass control in pine and eucalyptus plantations as being \$400 Aus/ha which would add \$1.4m to the cost of plantation establishment in the State, \$3.6m today (€2.15m) though this is not assumed to be an annual cost.

Pampas grass infestations on roadsides and waste areas are usually controlled by application of glyphosate. In the early 1990s herbicide cost varied from \$65 per hectare (\$128 today or €78) for seedlings to \$260 per hectare (\$512 today or €300) for established plants (Harradine 1991). Labour and equipment costs for the application may far exceed these herbicide costs in many areas where access is difficult.

In New Zealand, kiwifruit [*Actinidia deliciosa*] have been rejected for export as it was contaminated with *Cortaderia* seed which was attached to the fruit (Knowles and Tombleson 1987).

Qu. 4.10. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism currently in the risk assessment area (include any past costs in your response)?

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the EU these should be reported here. Assessment of the potential costs of damage on human health, safety, and the economy, including the cost of non-action. A full economic assessment at EU scale might not be possible, but qualitative data or different case studies from across the EU (or third countries if relevant) may provide useful information to inform decision making. In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: There is no published literature on incurred costs in the risk assessment area besides those relating to control efforts. The reduction in pasture quality and the injuries to humans will have a cost (AME-CBNPM 2003) but this has not been quantified. Nor has the financial and social cost of fires made more significant by the plant’s dry biomass. Given its common invasion on roadsides it is probable that the resultant lack of visibility for drivers may cause accidents which would have a significant economic and social impact but not evidence has been published. It is however, highly likely that any of the above costs incurred across the invaded range in the risk assessment area will be more than €10,000 making it more than minimal and probably more than the €100,000 per year threshold provided in the guidance for a score of “moderate” but a conservative score with a low confidence is given.

Qu. 4.11. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.10.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: The economic cost is likely to moderately increase in the future as there will be more areas available for the species to establish. Spread of the species may also increase as urbanisation and development increases. However, there is no evidence for this so no increase in score is given.

Qu. 4.12. How great are the economic costs / losses associated with managing this organism currently in the risk assessment area (include any past costs in your response)?

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Costs of control are significant in Spain (Andreu et al. 2009). The plant is the subject of a 4 year €3.6m EU Life project, Stop Cortaderia, (www.stopcortaderia.org). Other LIFE projects which included an element of Cortaderia management include EmysTer - Recovery of the habitat of amphibians and Emys orbicularis in the Baix Ter (LIFE04 NAT/ES/000059) Estuarios del Pais Vasco - Restoration of habitats of Community interest in the Basque Country's estuaries.(LIFE08 NAT/E/000055), BRIGHT - Bussacos Recovery from Invasions Generating Habitat Threats (BIODIV) (LIFE10 NAT/PT/000075) Life Anillo Verde - Green Belt of Bay of Santander: connecting nature and city (LIFE14 NAT/ES/000699) and LIFE SALLINA - Sustainable Actions on Loire Lagoons for Improvement aNd Assessment (LIFE17 NAT/FR/000519). Though the exact value of the Cortaderia actions is not presented here it is unlikely to take the control cost beyond €10m. Another financial impact is the fact that it reduces the aesthetic and recreational value of conservation areas (Bossard et al. 2000) though this has not been quantified.

Qu. 4.13. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.12.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Costs are likely to increase as the plant continues to spread and the need to manage it increases. Awareness for the need to manage the plant will be raised by the StopCortaderia LIFE project which in turn will be likely to drive demands for action from the public initially on a local scale but concerned citizens and land managers from other invaded areas are likely to become more aware and more demanding for action.

Social and human health impacts

Qu. 4.14. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism for the risk assessment area and for third countries, if relevant (e.g. with similar eco-climatic conditions).

The description of the known impact and the assessment of potential future impact on human health, safety and the economy, shall, if relevant, include information on

- illnesses, allergies or other affections to humans that may derive directly or indirectly from a species;
- damages provoked directly or indirectly by a species with consequences for the safety of people, property or infrastructure;
- direct or indirect disruption of, or other consequences for, an economic or social activity due to the presence of a species.

Social and human health impacts can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: *Cortaderia selloana* has been recognised as a clinically-significant allergen (Street et al. 1979) and is recorded as a common allergenic ornamental species in a selection of Spanish Cities (Velasco-Jiménez et al. 2014). *Cortaderia selloana* can cause superficial skin injuries that tend to cause inflammatory reactions but may also impact on animals that feed on the plant in pasturelands (Di-Tomaso et al. 2010; AME-CBNPM 2003; Peloté and Haury 2008). It can also have negative aesthetic impacts through changes to the appearance of the landscape, rendering the landscape uninteresting (Le Moigne and Magnanon 2007) and on lake margins in NZ it prevents public access and dominates the ecosystem (Gosling et al. 2000).

Qu. 4.15. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism in the future for the risk assessment area.

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No information has been found on the issue.

Other impacts

Qu. 4.16. How important is the organism in facilitating other damaging organisms (e.g. diseases) as food source, a host, a symbiont or a vector etc.?

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No information has been found on the issue. It is likely that *C. selloana* is not important in facilitating other damaging organisms and therefore a minimal score is given with a low level of confidence.

However, it should be noted that a survey and study in New Zealand on *C. selloana* and *C. jubata* revealed a wide range of native and introduced invertebrates associated with pampas grass in New Zealand. In particular, 62 fungal species were identified, based upon cultural morphology and diagnostic spore characters and confirmed by ITS sequencing. Of these, 33 are considered primary plant pathogens. The pathogens are a mix of grass- and crop-pathogens. Some have been known to cause a range of serious stem cankers and foliar blights on their target-host(s) – many of which are not grasses. The most frequently isolated grass-pathogen was *Nigrospora oryzae* – a seed-borne fungus causing minute leaf and grain spot in rice. *Leptosphaerulina chartarum*, a common pathogen from grassland vegetation, was also recovered. Other significant non-grass pathogens include *Neofusicoccum australe* – also identified as the cause of grapevine dieback in New Zealand (Bellgard et al. 2010).

Qu. 4.17. How important might other impacts not already covered by previous questions be resulting from introduction of the organism?

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No information has been found on other impacts not already covered in the risk assessment. Any additional impacts are however likely to be minimal as if not, they would be detailed in other regions of the world where the species is invasive.

Qu. 4.18. How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area?

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Since there are no known specialist natural enemies in the risk assessment area the expected impacts will not change. Only recently biocontrol agents have been sought in the native south American range and two smut fungi and some leafhopper species show some potential (Hayes 2015) so in future, were biocontrol agents to be introduced, then natural enemies may have an influence on the impact of *C. selloana* in the risk assessment area.

Qu. 4.19. Estimate the overall impact in the risk assessment area under current climate conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, in current conditions.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Impacts include above and below ground transformation of structure and nutrients with recorded negative impacts on fragile and protected ecosystems and displacement of species in the risk assessment area. This mirrors major impacts recorded in other invaded ranges. Since these are technically reversible (unless a narrow endemic plant species is lost) with enough resources a moderate score is given despite the vulnerable ecosystems at risk and the number of initiatives aimed at stopping its spread in such habitats. The impacts will be greatest where the species is currently established: Atlantic, Continental and Mediterranean biogeographical regions as these areas will have the greatest propagule pressure.

Qu. 4.20. Estimate the overall impact in the risk assessment area in foreseeable climate change conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, under future conditions.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: There is no evidence that future impacts will be any less reversible despite the likelihood of them being on a much larger scale so a moderate score is retained with medium confidence. The impacts will be greatest where the species is currently established: Atlantic, Continental and Mediterranean biogeographical regions as these areas will have the greatest propagule pressure.

RISK SUMMARIES			
	RESPONSE	CONFIDENCE	COMMENT
Summarise Introduction and Entry*	very unlikely unlikely moderately likely likely very likely	low medium high	The species has been introduced and entered the risk assessment area in the past. A number of active pathways exist (e.g. horticulture, ornamental purposes other than horticulture, contaminant of habitat material and contaminant of tourists). The plant is a very popular ornamental species in the risk assessment area. The very likely score is given by default as the species is already established in the risk assessment area.
Summarise Establishment*	very unlikely unlikely moderately likely likely very likely	low medium high	The species is established in the risk assessment area and further establishment is likely in both current and future climatic conditions. The species has a wide tolerance of habitats and other abiotic parameters which promotes its establishment.
Summarise Spread*	very slowly slowly moderately rapidly very rapidly	low medium high	Natural spread of wind dispersed seeds can distribute the species over long distances. Human mediated spread other than horticulture and contamination of habitat material and equipment contribute to the spread throughout the risk assessment area. Further spread is likely as there is a large suitable area for establishment for <i>C. selloana</i> in the risk assessment area. The overall rapidly score with medium confidence is based on the worst-case scenario detailed in section 3 for 'Ornamental purposes other than horticulture (escape from confinement)'
Summarise Impact*	minimal minor moderate major massive	low medium high	An overall major score has been given due to the major impacts reported on biodiversity and conservation value. Impacts include above and below ground transformation of structure and nutrients with recorded negative impacts on fragile and protected ecosystems and displacement of species in the risk assessment area, as well as significant socioeconomic impacts.

			In future climate scenarios the invaded area is expected to expand but again impacts tend to be most felt in ruderal habitats and none are irreversible.
Conclusion of the risk assessment (overall risk)	low moderate high	low medium high	Overall, <i>C. selloana</i> has a high risk to the EU with medium uncertainty. This score takes in account the major impact the species has in the risk assessment area with regard to impact on biodiversity.

*in current climate conditions and in foreseeable future climate conditions

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Distribution Summary

Please answer as follows:

- Yes if recorded, established or invasive
- if not recorded, established or invasive
- ? Unknown; data deficient

The columns refer to the answers to Questions A5 to A12 under Section A.

For data on marine species at the Member State level, delete Member States that have no marine borders.

In all other cases, provide answers for all columns.

Member States and the United Kingdom

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Austria	YES	NO	YES	YES	NO
Belgium	YES	NO	YES	YES	NO
Bulgaria	YES	NO	YES	YES	NO
Croatia	YES	NO	YES	YES	NO
Cyprus	YES	NO	YES	YES	NO
Czech Republic	YES	NO	YES	YES	NO
Denmark	YES	NO	YES	YES	NO
Estonia	NO	NO	YES	YES	NO
Finland	NO	NO	YES	YES	NO
France	YES	YES	YES	YES	YES
Germany	YES	NO	YES	YES	NO
Greece	YES	NO	YES	YES	NO
Hungary	NO	NO	YES	YES	NO
Ireland	YES	YES	YES	YES	YES
Italy	YES	YES	YES	YES	YES
Latvia	NO	NO	YES	YES	NO
Lithuania	NO	NO	YES	YES	NO
Luxembourg	NO	NO	YES	YES	NO
Malta	YES	NO	YES	YES	NO
Netherlands	YES	NO	YES	YES	NO
Poland	YES	NO	YES	YES	NO
Portugal	YES	YES	YES	YES	YES
Romania	NO	NO	YES	YES	NO
Slovakia	YES	NO	YES	YES	NO
Slovenia	YES	NO	YES	YES	NO
Spain	YES	YES	YES	YES	YES
Sweden	YES	NO	YES	YES	NO
United Kingdom	YES	YES	YES	YES	NO

Biogeographical regions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Alpine	YES		YES	YES	
Atlantic	YES	YES	YES	YES	
Black Sea	YES		YES	YES	
Boreal	YES		YES	YES	
Continental	YES	YES	YES	YES	YES
Mediterranean	YES	YES	YES	YES	YES
Pannonian			YES	YES	
Steppic	YES		YES	YES	

ANNEX I Scoring of Likelihoods of Events

(taken from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Description	Frequency
Very unlikely	This sort of event is theoretically possible, but is never known to have occurred and is not expected to occur	1 in 10,000 years
Unlikely	This sort of event has occurred somewhere at least once in the last millenium	1 in 1,000 years
Moderately likely	This sort of event has occurred somewhere at least once in the last century	1 in 100 years
Likely	This sort of event has happened on several occasions elsewhere, or on at least once in the last decade	1 in 10 years
Very likely	This sort of event happens continually and would be expected to occur	Once a year

ANNEX II Scoring of Magnitude of Impacts

(modified from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Biodiversity and ecosystem impact	Ecosystem Services impact	Economic impact (Monetary loss and response costs per year)	Social and human health impact, and other impacts
	<i>Question 5.1-5</i>	<i>Question 5.6-8</i>	<i>Question 5.9-13</i>	<i>Question 5.14-18</i>
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected ⁸	Up to 10,000 Euro	No social disruption. Local, mild, short-term reversible effects to individuals.
Minor	Some ecosystem impact, reversible changes, localised	Local and temporary, reversible effects to one or few services	10,000-100,000 Euro	Significant concern expressed at local level. Mild short-term reversible effects to identifiable groups, localised.
Moderate	Measureable long-term damage to populations and ecosystem, but reversible; little spread, no extinction	Measureable, temporary, local and reversible effects on one or several services	100,000-1,000,000 Euro	Temporary changes to normal activities at local level. Minor irreversible effects and/or larger numbers covered by reversible effects, localised.
Major	Long-term irreversible ecosystem change, spreading beyond local area	Local and irreversible or widespread and reversible effects on one / several services	1,000,000-10,000,000 Euro	Some permanent change of activity locally, concern expressed over wider area. Significant irreversible effects locally or reversible effects over large area.
Massive	Widespread, long-term population loss or extinction, affecting several species with serious ecosystem effects	Widespread and irreversible effects on one / several services	Above 10,000,000 Euro	Long-term social change, significant loss of employment, migration from affected area. Widespread, severe, long-term, irreversible health effects.

⁸ Not to be confused with "no impact".

ANNEX III Scoring of Confidence Levels

(modified from Bacher et al. 2017)

Each answer provided in the risk assessment must include an assessment of the level of confidence attached to that answer, reflecting the possibility that information needed for the answer is not available or is insufficient or available but conflicting.

The responses in the risk assessment should clearly support the choice of the confidence level.

Confidence level	Description
Low	There is no direct observational evidence to support the assessment, e.g. only inferred data have been used as supporting evidence <i>and/or</i> Impacts are recorded at a spatial scale which is unlikely to be relevant to the assessment area <i>and/or</i> Evidence is poor and difficult to interpret, e.g. because it is strongly ambiguous <i>and/or</i> The information sources are considered to be of low quality or contain information that is unreliable.
Medium	There is some direct observational evidence to support the assessment, but some information is inferred <i>and/or</i> Impacts are recorded at a small spatial scale, but rescaling of the data to relevant scales of the assessment area is considered reliable, or to embrace little uncertainty <i>and/or</i> The interpretation of the data is to some extent ambiguous or contradictory.
High	There is direct relevant observational evidence to support the assessment (including causality) <i>and</i> Impacts are recorded at a comparable scale <i>and/or</i> There are reliable/good quality data sources on impacts of the taxa <i>and</i> The interpretation of data/information is straightforward <i>and/or</i> Data/information are not controversial or contradictory.

ANNEX IV CBD pathway categorisation scheme

Overview of CBD pathway categorisation scheme showing how the 44 pathways relate to the six main pathway categories. All of the pathways can be broadly classified into 1) those that involve intentional transport (blue), 2) those in which the taxa are unintentionally transported (green) and 3) those where taxa moved between regions without direct transportation by humans and/or via artificial corridors (orange and yellow). **Note that the pathways in the category “Escape from confinement” can be considered intentional for the introduction into the risk assessment area and unintentional for the entry into the environment.**



ANNEX V Ecosystem services classification (CICES V5.1, simplified) and examples

For the purposes of this risk assessment, please feel free to use what seems as the most appropriate category / level / combination of impact (Section – Division – Group), reflecting information available.

Section	Division	Group	Examples (i.e. relevant CICES “classes”)
Provisioning	Biomass	Cultivated <i>terrestrial</i> plants	Cultivated terrestrial plants (including fungi, algae) grown for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from cultivated plants, fungi, algae and bacteria for direct use or processing (excluding genetic materials); Cultivated plants (including fungi, algae) grown as a <u>source of energy</u> <i>Example: negative impacts of non-native organisms to crops, orchards, timber etc.</i>
		Cultivated <i>aquatic</i> plants	Plants cultivated by in- situ aquaculture grown for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from in-situ aquaculture for direct use or processing (excluding genetic materials); Plants cultivated by in- situ aquaculture grown as an <u>energy source</u> . <i>Example: negative impacts of non-native organisms to aquatic plants cultivated for nutrition, gardening etc. purposes.</i>
		Reared animals	Animals reared for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from reared animals for direct use or processing (excluding genetic materials); Animals reared to provide <u>energy</u> (including mechanical) <i>Example: negative impacts of non-native organisms to livestock</i>
		Reared <i>aquatic</i> animals	Animals reared by in-situ aquaculture for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from animals grown by in-situ aquaculture for direct use or processing (excluding genetic materials); Animals reared by in-situ aquaculture as an <u>energy source</u> <i>Example: negative impacts of non-native organisms to fish farming</i>
		Wild plants (terrestrial and aquatic)	Wild plants (terrestrial and aquatic, including fungi, algae) used for <u>nutrition</u> ; <u>Fibres and other materials</u> from wild plants for direct use or processing (excluding genetic materials); Wild plants (terrestrial and aquatic, including fungi, algae) used as a <u>source of energy</u> <i>Example: reduction in the availability of wild plants (e.g. wild berries, ornamentals) due to non-native organisms (competition, spread of disease etc.)</i>
		Wild animals (terrestrial and aquatic)	Wild animals (terrestrial and aquatic) used for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from wild animals for direct use or processing (excluding genetic materials); Wild animals (terrestrial and aquatic) used as a <u>source of energy</u>

			<p><i>Example: reduction in the availability of wild animals (e.g. fish stocks, game) due to non-native organisms (competition, predations, spread of disease etc.)</i></p>
	<p>Genetic material from all biota</p>	<p>Genetic material from plants, algae or fungi</p>	<p><u>Seeds, spores and other plant materials</u> collected for maintaining or establishing a population; Higher and lower plants (whole organisms) used to <u>breed new strains or varieties</u>; Individual genes extracted from higher and lower plants for the <u>design and construction of new biological entities</u></p> <p><i>Example: negative impacts of non-native organisms due to interbreeding</i></p>
		<p>Genetic material from animals</p>	<p>Animal material collected for the purposes of maintaining or establishing a population; Wild animals (whole organisms) used to breed new strains or varieties; Individual genes extracted from organisms for the design and construction of new biological entities</p> <p><i>Example: negative impacts of non-native organisms due to interbreeding</i></p>
	<p>Water⁹</p>	<p>Surface water used for nutrition, materials or energy</p>	<p>Surface water for <u>drinking</u>; Surface water used as a material (<u>non-drinking purposes</u>); Freshwater surface water, coastal and marine water used as an <u>energy source</u></p> <p><i>Example: loss of access to surface water due to spread of non-native organisms</i></p>
		<p>Ground water for used for nutrition, materials or energy</p>	<p>Ground (and subsurface) water for <u>drinking</u>; Ground water (and subsurface) used as a material (<u>non-drinking purposes</u>); Ground water (and subsurface) used as an <u>energy source</u></p> <p><i>Example: reduced availability of ground water due to spread of non-native organisms and associated increase of ground water consumption by vegetation.</i></p>
<p>Regulation and Maintenance</p>	<p>Transformation of biochemical or physical inputs to ecosystems</p>	<p>Mediation of wastes or toxic substances of anthropogenic origin by living processes</p>	<p><u>Bio-remediation</u> by micro-organisms, algae, plants, and animals; <u>Filtration/sequestration/storage/accumulation</u> by micro-organisms, algae, plants, and animals</p> <p><i>Example: changes caused by non-native organisms to ecosystem functioning and ability to filtrate etc. waste or toxics</i></p>
		<p>Mediation of nuisances of anthropogenic origin</p>	<p>Smell reduction; noise attenuation; visual screening (e.g. by means of green infrastructure)</p> <p><i>Example: changes caused by non-native organisms to ecosystem structure, leading to reduced ability to mediate nuisances.</i></p>
	<p>Regulation of physical, chemical, biological conditions</p>	<p>Baseline flows and extreme event regulation</p>	<p>Control of <u>erosion</u> rates; Buffering and attenuation of <u>mass movement</u>; <u>Hydrological cycle and water flow regulation</u> (Including flood control, and coastal protection); <u>Wind</u> protection; <u>Fire</u> protection</p>

⁹ Note: in the CICES classification provisioning of water is considered as an abiotic service whereas the rest of ecosystem services listed here are considered biotic.

			<p><i>Example: changes caused by non-native organisms to ecosystem functioning or structure leading to, for example, destabilisation of soil, increased risk or intensity of wild fires etc.</i></p>
		<p>Lifecycle maintenance, habitat and gene pool protection</p>	<p><u>Pollination</u> (or 'gamete' dispersal in a marine context); <u>Seed dispersal</u>; Maintaining <u>nursery populations and habitats</u> (Including gene pool protection)</p> <p><i>Example: changes caused by non-native organisms to the abundance and/or distribution of wild pollinators; changes to the availability / quality of nursery habitats for fisheries</i></p>
		<p>Pest and disease control</p>	<p>Pest control; Disease control</p> <p><i>Example: changes caused by non-native organisms to the abundance and/or distribution of pests</i></p>
		<p>Soil quality regulation</p>	<p><u>Weathering processes</u> and their effect on soil quality; <u>Decomposition and fixing processes</u> and their effect on soil quality</p> <p><i>Example: changes caused by non-native organisms to vegetation structure and/or soil fauna leading to reduced soil quality</i></p>
		<p>Water conditions</p>	<p>Regulation of the <u>chemical condition</u> of freshwaters by living processes; Regulation of the chemical condition of salt waters by living processes</p> <p><i>Example: changes caused by non-native organisms to buffer strips along water courses that remove nutrients in runoff and/or fish communities that regulate the resilience and resistance of water bodies to eutrophication</i></p>
		<p>Atmospheric composition and conditions</p>	<p>Regulation of <u>chemical composition</u> of atmosphere and oceans; Regulation of <u>temperature and humidity</u>, including ventilation and transpiration</p> <p><i>Example: changes caused by non-native organisms to ecosystems' ability to sequester carbon and/or evaporative cooling (e.g. by urban trees)</i></p>
Cultural	Direct, in-situ and outdoor interactions with living systems that depend on presence in the environmental setting	<p>Physical and experiential interactions with natural environment</p>	<p>Characteristics of living systems that that enable activities promoting health, recuperation or enjoyment through <u>active or immersive interactions</u>; Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through <u>passive or observational interactions</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that make it attractive for recreation, wild life watching etc.</i></p>
		<p>Intellectual and representative interactions with natural environment</p>	<p>Characteristics of living systems that enable <u>scientific investigation</u> or the creation of traditional ecological knowledge; Characteristics of living systems that enable <u>education and training</u>; Characteristics of living systems that are resonant in terms of <u>culture or heritage</u>; Characteristics of living systems that enable <u>aesthetic experiences</u></p>

			<i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have cultural importance</i>
Indirect, remote, often indoor interactions with living systems that do not require presence in the environmental setting	Spiritual, symbolic and other interactions with natural environment		Elements of living systems that have <u>symbolic meaning</u> ; Elements of living systems that have <u>sacred or religious meaning</u> ; Elements of living systems used for <u>entertainment or representation</u>
			<i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have sacred or religious meaning</i>
	Other biotic characteristics that have a non-use value		Characteristics or features of living systems that have an <u>existence value</u> ; Characteristics or features of living systems that have an <u>option or bequest value</u> <i>Example: changes caused by non-native organisms to ecosystems designated as wilderness areas, habitats of endangered species etc.</i>

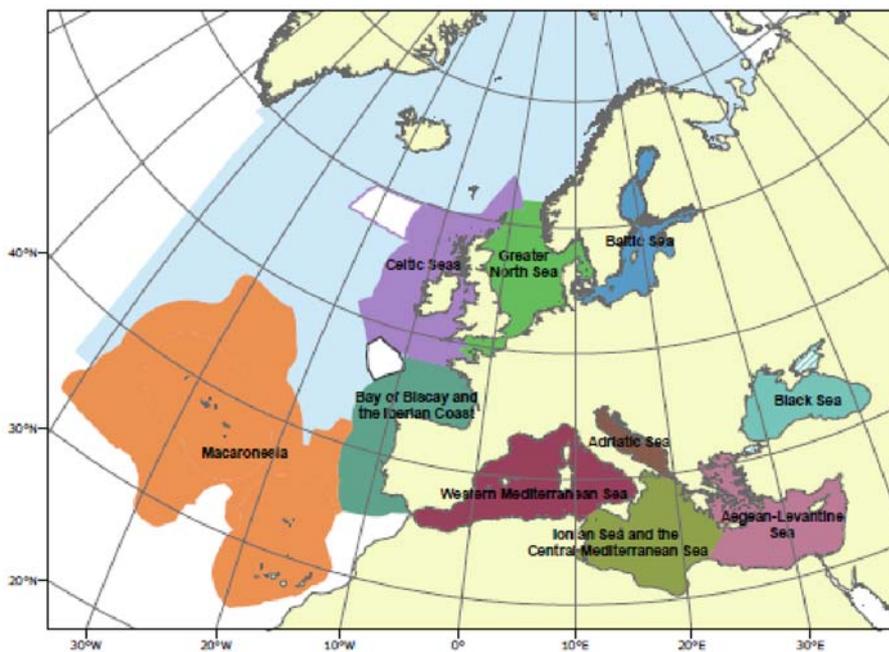
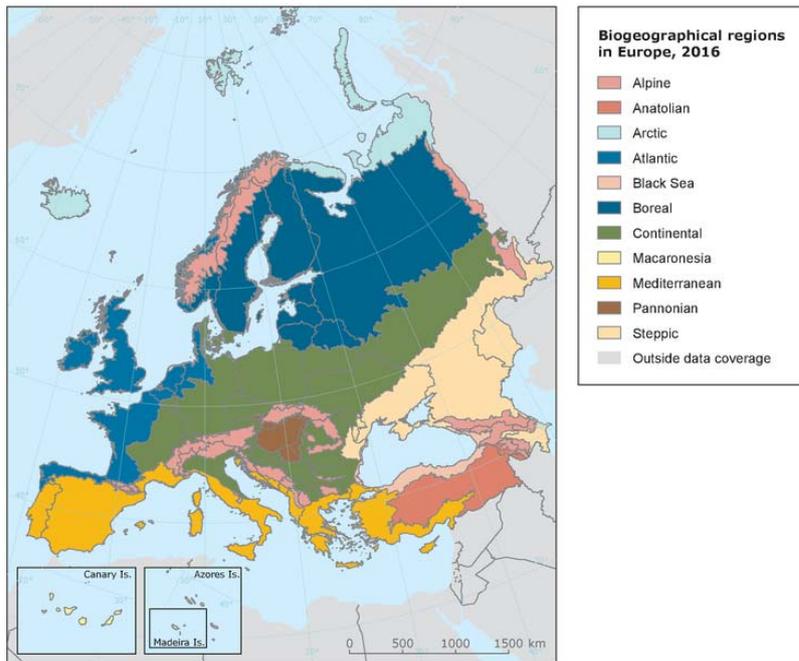
ANNEX VI EU Biogeographic Regions and MSFD Subregions

See <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2>

http://ec.europa.eu/environment/nature/natura2000/biogeog_regions/

and

<https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions-1/technical-document/pdf>



ANNEX VII Delegated Regulation (EU) 2018/968 of 30 April 2018

see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>

ANNEX VIII Species Distribution Model

Projection of climatic suitability for *Cortaderia selloana* establishment in Europe

Björn Beckmann, Rob Tanner, Giuseppe Brundu, Richard Shaw, Beth Purse and Dan Chapman

30 March 2020

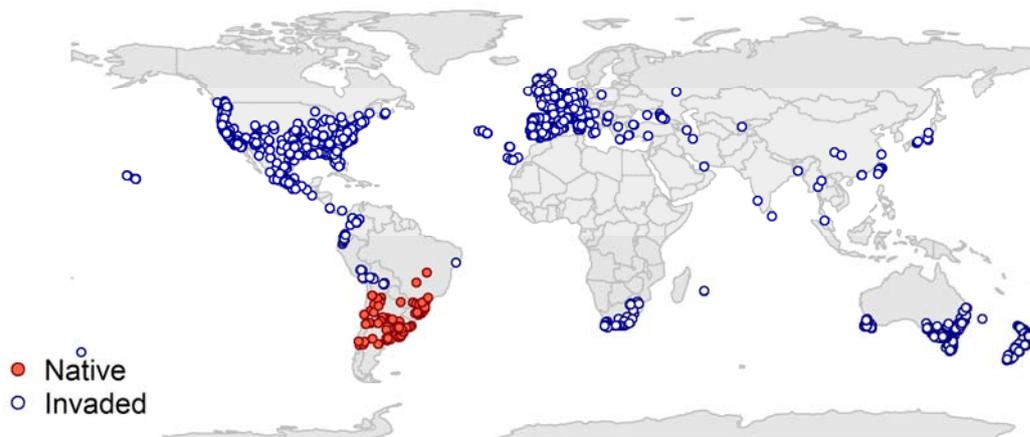
Aim

To project the suitability for potential establishment of *Cortaderia selloana* in Europe, under current and predicted future climatic conditions.

Data for modelling

Species occurrence data were obtained from the Global Biodiversity Information Facility (GBIF) (9771 records), iNaturalist (4206 records), the Atlas of Living Australia (1521 records), the Biodiversity Information Serving Our Nation database (BISON) (481 records), the Integrated Digitized Biocollections (iDigBio) (221 records), ITSAR (175 records), the Berkeley Ecoinformatics Engine database (98 records), and a small number of additional records from the risk assessment team. We scrutinised occurrence records from regions where the species is not known to be established and removed any dubious records (e.g. fossils) or where the georeferencing was too imprecise (e.g. records referenced to a country or island centroid) or outside of the coverage of the predictor layers (e.g. small island or coastal occurrences). The remaining records were gridded at a 0.25 x 0.25 degree resolution for modelling, yielding 1925 grid cells with occurrences (Figure 1a). As a proxy for recording effort, the density of Tracheophyta records held by GBIF was also compiled on the same grid (Figure 1b).

(a) Species distribution used in modelling



(b) Estimated recording effort (log10-scaled)

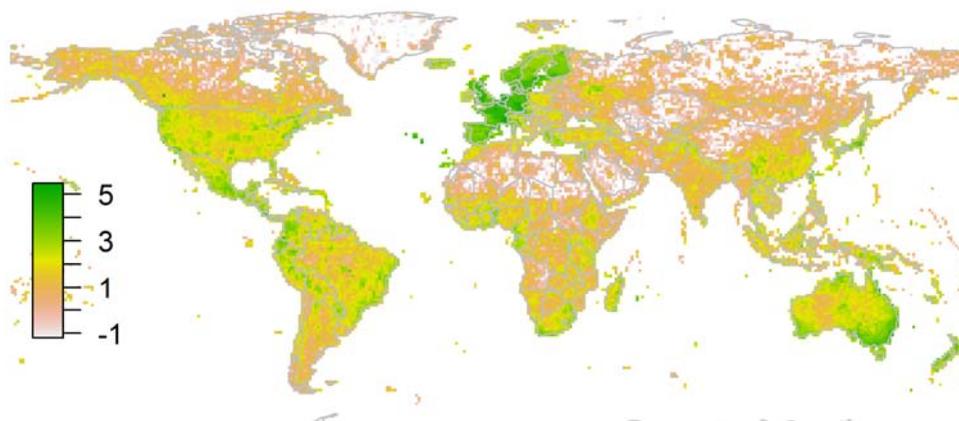


Figure 1. (a) Occurrence records obtained for *Cortaderia selloana* and used in the modelling, showing native and invaded distributions. (b) The recording density of Tracheophyta on GBIF, which was used as a proxy for recording effort.

Climate data were selected from the ‘Bioclim’ variables contained within the WorldClim database (Hijmans et al., 2005), originally at 5 arcminute resolution (0.083 x 0.083 degrees of longitude/latitude) and aggregated to a 0.25 x 0.25 degree grid for use in the model.

Based on the biology of *Cortaderia selloana*, the following climate variables were used in the modelling:

- Minimum temperature of the coldest month (Bio6)
- Mean temperature of the warmest quarter (Bio10)
- Climatic moisture index (CMI): ratio of mean annual precipitation to potential evapotranspiration, log+1 transformed. For its calculation, monthly potential evapotranspirations were estimated from the WorldClim monthly temperature data and solar radiation using the simple method of Zomer et al. (2008) which is based on the Hargreaves evapotranspiration equation (Hargreaves, 1994).

To estimate the effect of climate change on the potential distribution, equivalent modelled future climate conditions for the 2070s under the Representative Concentration Pathways (RCP) 2.6 and 4.5

were also obtained. There represent low and medium emissions scenarios, respectively. The above variables were obtained as averages of outputs of eight Global Climate Models (BCC-CSM1-1, CCSM4, GISS-E2-R, HadGEM2-AO, IPSL-CM5A-LR, MIROC-ESM, MRI-CGCM3, NorESM1-M), downscaled and calibrated against the WorldClim baseline (see http://www.worldclim.org/cmip5_5m).

The following habitat layers were also used:

- Human influence index (HII): As many non-native invasive species associate with anthropogenically disturbed habitats. We used the Global Human Influence Index Dataset of the Last of the Wild Project (Wildlife Conservation Society - WCS & Center for International Earth Science Information Network - CIESIN - Columbia University, 2005), which is developed from nine global data layers covering human population pressure (population density), human land use and infrastructure (built-up areas, nighttime lights, land use/land cover) and human access (coastlines, roads, railroads, navigable rivers). The index ranges between 0 and 1 and was ln+1 transformed for the modelling to improve normality.

Species distribution model

A presence-background (presence-only) ensemble modelling strategy was employed using the BIOMOD2 R package version 3.4.6 (Thuiller et al., 2020, Thuiller et al., 2009). These models contrast the environment at the species' occurrence locations against a random sample of the global background environmental conditions (often termed 'pseudo-absences') in order to characterise and project suitability for occurrence. This approach has been developed for distributions that are in equilibrium with the environment. Because invasive species' distributions are not at equilibrium and subject to dispersal constraints at a global scale, we took care to minimise the inclusion of locations suitable for the species but where it has not been able to disperse to (Chapman et al. 2019). Therefore the background sampling region included:

- The area accessible by native *Cortaderia selloana* populations, in which the species is likely to have had sufficient time to disperse to all locations. Based on presumed maximum dispersal distances, the accessible region was defined as a 400km buffer around the native range occurrences; AND
- A 30km buffer around the non-native occurrences, encompassing regions likely to have had high propagule pressure for introduction by humans and/or dispersal of the species; AND
- Regions where we have an *a priori* expectation of high unsuitability for the species so that absence is assumed irrespective of dispersal constraints (see Figure 2). The following rules were applied to define a region expected to be highly unsuitable for *Cortaderia selloana* at the spatial scale of the model:
 - Minimum temperature of the coldest month (Bio6) < -12
 - Mean temperature of the warmest quarter (Bio10) < 8.5
 - Climatic moisture index (CMI) < log1p(0.07)

Altogether, only 0.7% of occurrence grid cells were located in the unsuitable background region.

Within the background region, 3 samples of 5000 randomly sampled grid cells were obtained, weighting the sampling by recording effort (Figure 2).

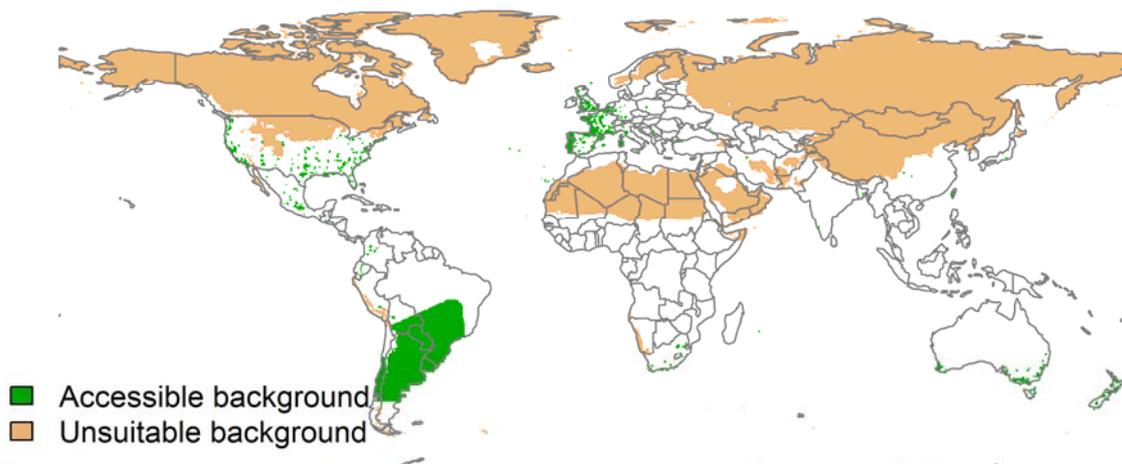


Figure 2. The background from which pseudo-absence samples were taken in the modelling of *Cortaderia selloana*. Samples were taken from a 400km buffer around the native range and a 30km buffer around non-native occurrences (together forming the accessible background), and from areas expected to be highly unsuitable for the species (the unsuitable background region). Samples were weighted by a proxy for recording effort (Figure 1(b)).

Each dataset (i.e. combination of the presences and the individual background samples) was randomly split into 80% for model training and 20% for model evaluation. With each training dataset, seven statistical algorithms were fitted with the default BIOMOD2 settings and rescaled using logistic regression, except where specified below:

- Generalised linear model (GLM)
- Generalised boosting model (GBM)
- Generalised additive model (GAM) with a maximum of four degrees of freedom per smoothing spline
- Random forest (RF)
- Maxent

Since the background sample was larger than the number of occurrences, prevalence fitting weights were applied to give equal overall importance to the occurrences and the background. Normalised variable importance was assessed and variable response functions were produced using BIOMOD2's default procedure.

Model predictive performance was assessed by the following three measures:

- AUC, the area under the receiver operating characteristic curve (Fielding & Bell 1997). Predictions of presence-absence models can be compared with a subset of records set aside for model evaluation (here 20%) by constructing a confusion matrix with the number of true positive, false positive, false negative and true negative cases. For models generating non-dichotomous scores (as here) a threshold can be applied to transform the scores into a dichotomous set of presence-absence predictions. Two measures that can be derived from the confusion matrix are sensitivity (the proportion of observed presences that are predicted as such, quantifying omission errors), and specificity (the proportion of observed absences that are predicted as such, quantifying commission errors). A receiver

operating characteristic (ROC) curve can be constructed by using all possible thresholds to classify the scores into confusion matrices, obtaining sensitivity and specificity for each matrix, and plotting sensitivity against the corresponding proportion of false positives (equal to $1 - \text{specificity}$). The use of all possible thresholds avoids the need for a selection of a single threshold, which is often arbitrary, and allows appreciation of the trade-off between sensitivity and specificity. The area under the ROC curve (AUC) is often used as a single threshold-independent measure for model performance (Manel, Williams & Ormerod 2001). AUC is the probability that a randomly selected presence has a higher model-predicted suitability than a randomly selected absence (Allouche et al. 2006).

- Cohen's Kappa (Cohen 1960). This measure corrects the overall accuracy of model predictions (ratio of the sum of true presences plus true absences to the total number of records) by the accuracy expected to occur by chance. The kappa statistic ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random. Advantages of kappa are its simplicity, the fact that both commission and omission errors are accounted for in one parameter, and its relative tolerance to zero values in the confusion matrix (Manel, Williams & Ormerod 2001). However, Kappa has been criticised for being sensitive to prevalence (the proportion of sites in which the species was recorded as present) and may therefore be inappropriate for comparisons of model accuracy between species or regions (McPherson, Jetz & Rogers 2004, Allouche et al. 2006).
- TSS, the true skill statistic (Allouche et al. 2006). TSS is defined as sensitivity + specificity - 1, and corrects for Kappa's dependency on prevalence. TSS compares the number of correct forecasts, minus those attributable to random guessing, to that of a hypothetical set of perfect forecasts. Like kappa, TSS takes into account both omission and commission errors, and success as a result of random guessing, and ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random (Allouche et al. 2006).

An ensemble model was created by first rejecting poorly performing algorithms with relatively extreme low AUC values and then averaging the predictions of the remaining algorithms, weighted by their AUC. To identify poorly performing algorithms, AUC values were converted into modified z-scores based on their difference to the median and the median absolute deviation across all algorithms (Iglewicz & Hoaglin, 1993). Algorithms with $z < -2$ were rejected. In this way, ensemble projections were made for each dataset and then averaged to give an overall suitability, as well as its standard deviation. The projections were then classified into suitable and unsuitable regions using the 'minROCDist' method, which minimizes the distance between the ROC plot and the upper left corner of the plot (point (0,1)).

We also produced limiting factor maps for Europe following Elith et al. (2010). For this, projections were made separately with each individual variable fixed at a near-optimal value. These were chosen as the median values at the occurrence grid cells. Then, the most strongly limiting factors were identified as the one resulting in the highest increase in suitability in each grid cell.

Results

The ensemble model suggested that suitability for *Cortaderia selloana* was most strongly determined by Minimum temperature of the coldest month (Bio6), accounting for 64% of variation explained, followed by Human influence index (HII) (18%), Mean temperature of the warmest quarter (Bio10) (11%) and Climatic moisture index (CMI) (7%) (Table 1, Figure 3).

Table 1. Summary of the cross-validation predictive performance (AUC, Kappa, TSS) and variable importance of the fitted model algorithms and the ensemble (AUC-weighted average of the best performing algorithms). Results are the average from models fitted to 3 different background samples of the data.

Algorithm	AUC	Kappa	TSS	Used in the ensemble	Variable importance (%)			
					Minimum temperature of the coldest month (Bio6)	Human influence index (HII)	Mean temperature of the warmest quarter (Bio10)	Climatic moisture index (CMI)
GLM	0.867	0.507	0.686	yes	56	28	14	2
GAM	0.870	0.509	0.696	yes	63	27	8	1
GBM	0.894	0.550	0.712	yes	77	4	4	15
RF	0.832	0.505	0.697	no	59	10	15	16
Maxent	0.890	0.540	0.704	yes	58	15	16	11
Ensemble	0.886	0.532	0.707		64	18	11	7

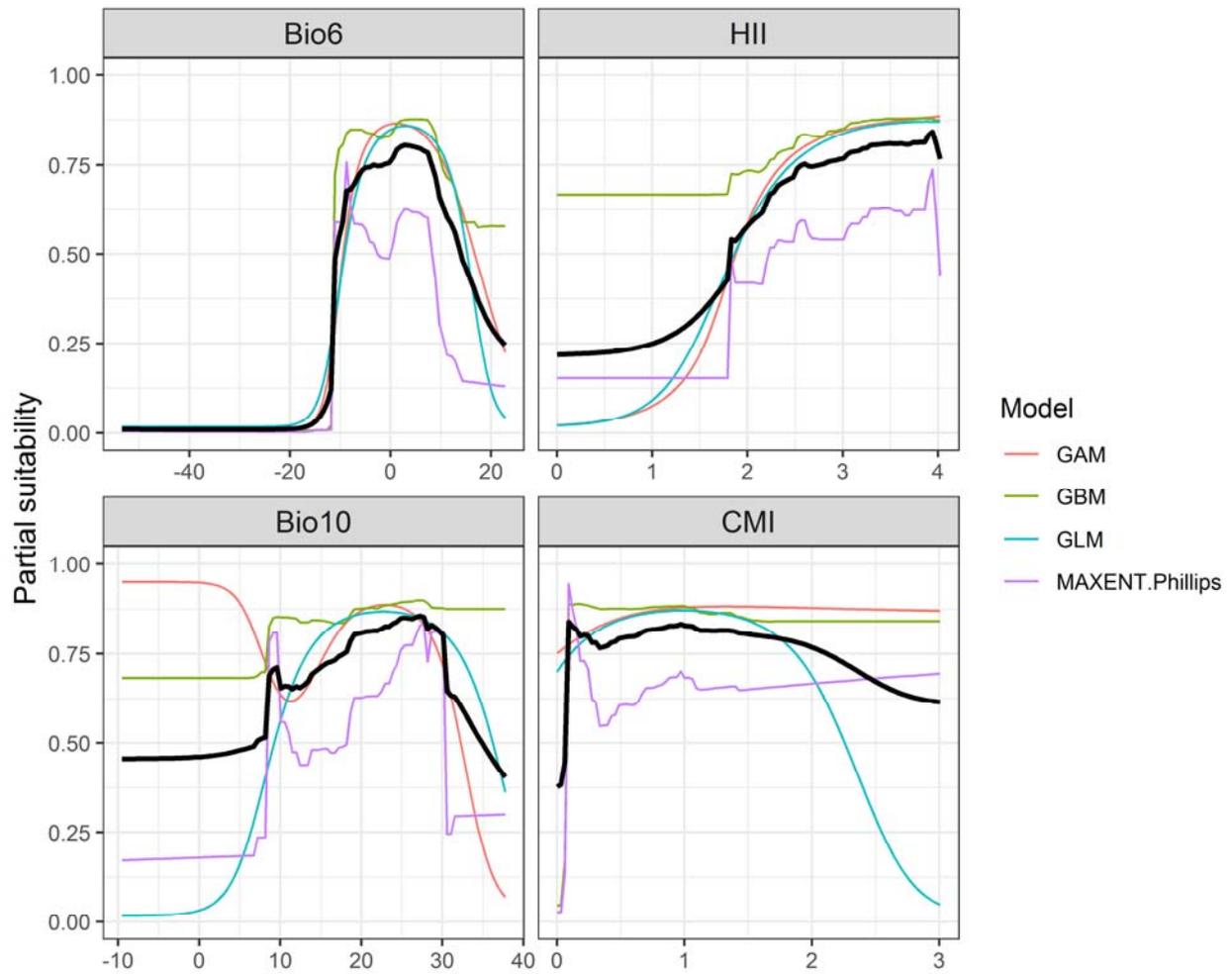


Figure 3. Partial response plots from the fitted models. Thin coloured lines show responses from the algorithms in the ensemble, while the thick black line is their ensemble. In each plot, other model variables are held at their median value in the training data. Some of the divergence among algorithms is because of their different treatment of interactions among variables.

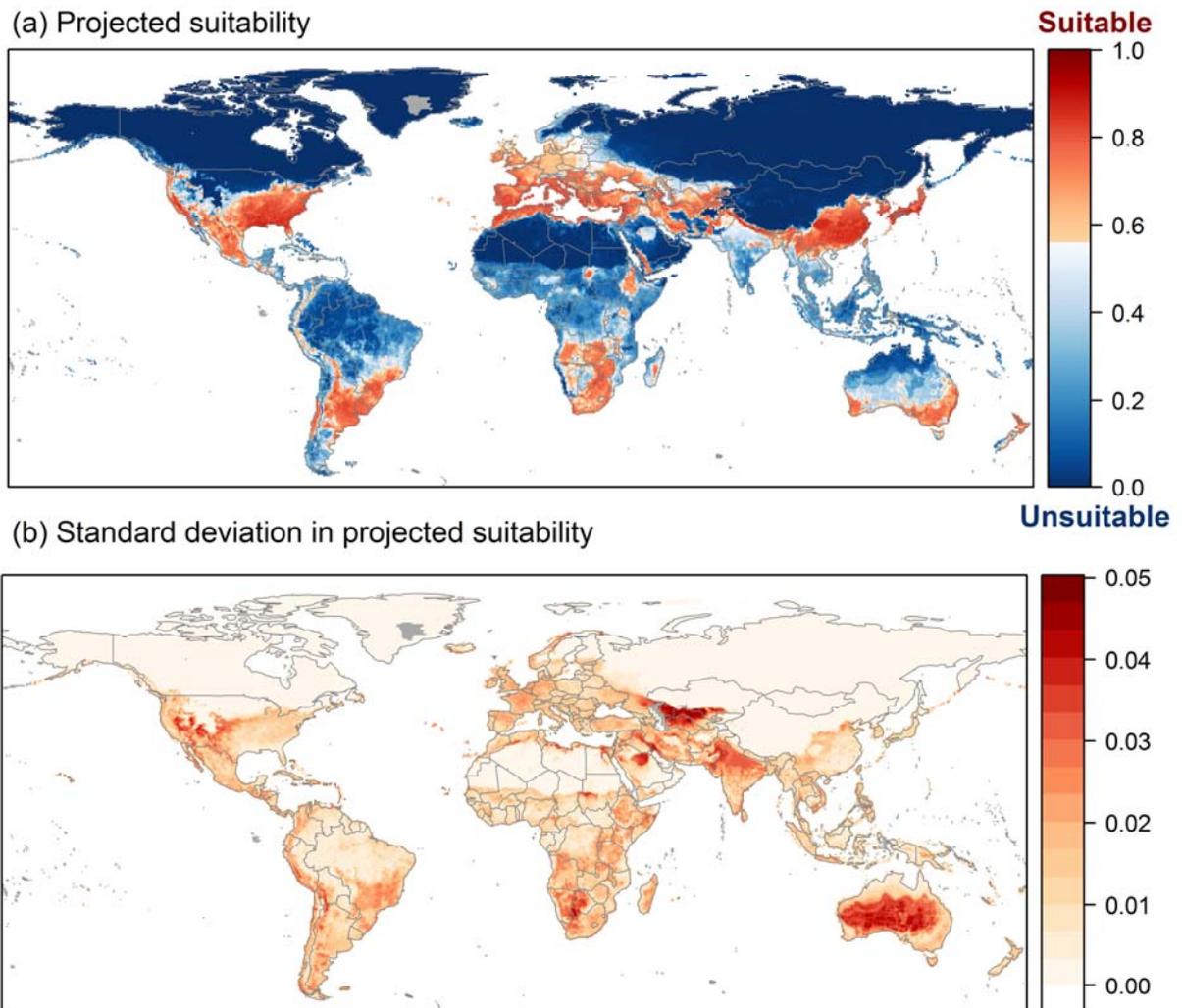


Figure 4. (a) Projected global suitability for *Cortaderia selloana* establishment in the current climate. For visualisation, the projection has been aggregated to a 0.5 x 0.5 degree resolution, by taking the maximum suitability of constituent higher resolution grid cells. Values > 0.56 may be suitable for the species. Grey areas have climatic conditions outside the range of the training data and were excluded from the projection. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 3 datasets.

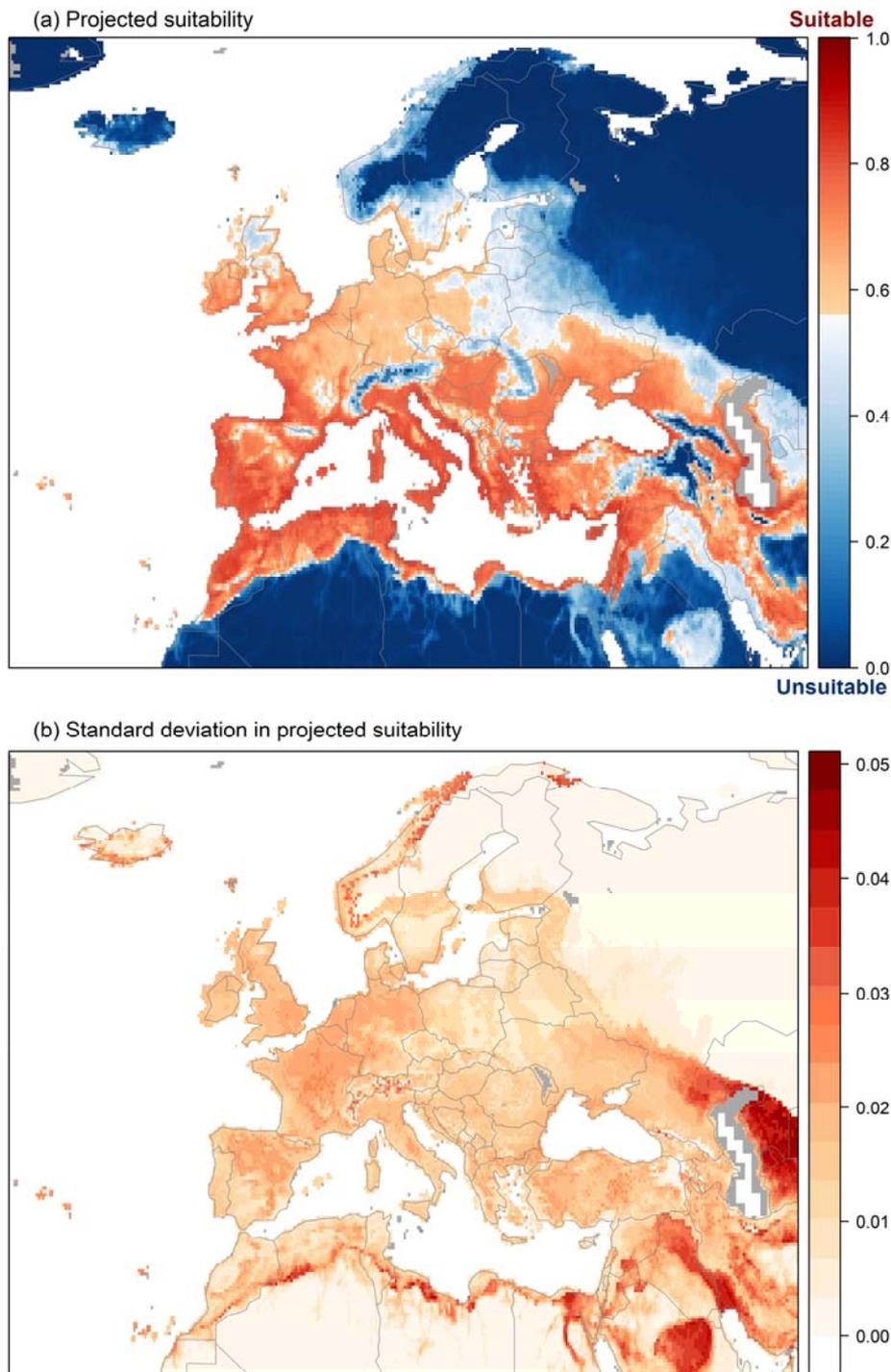


Figure 5. (a) Projected current suitability for *Cortaderia selloana* establishment in Europe and the Mediterranean region. Grey areas have climatic conditions outside the range of the training data and were excluded from the projection. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 3 datasets.

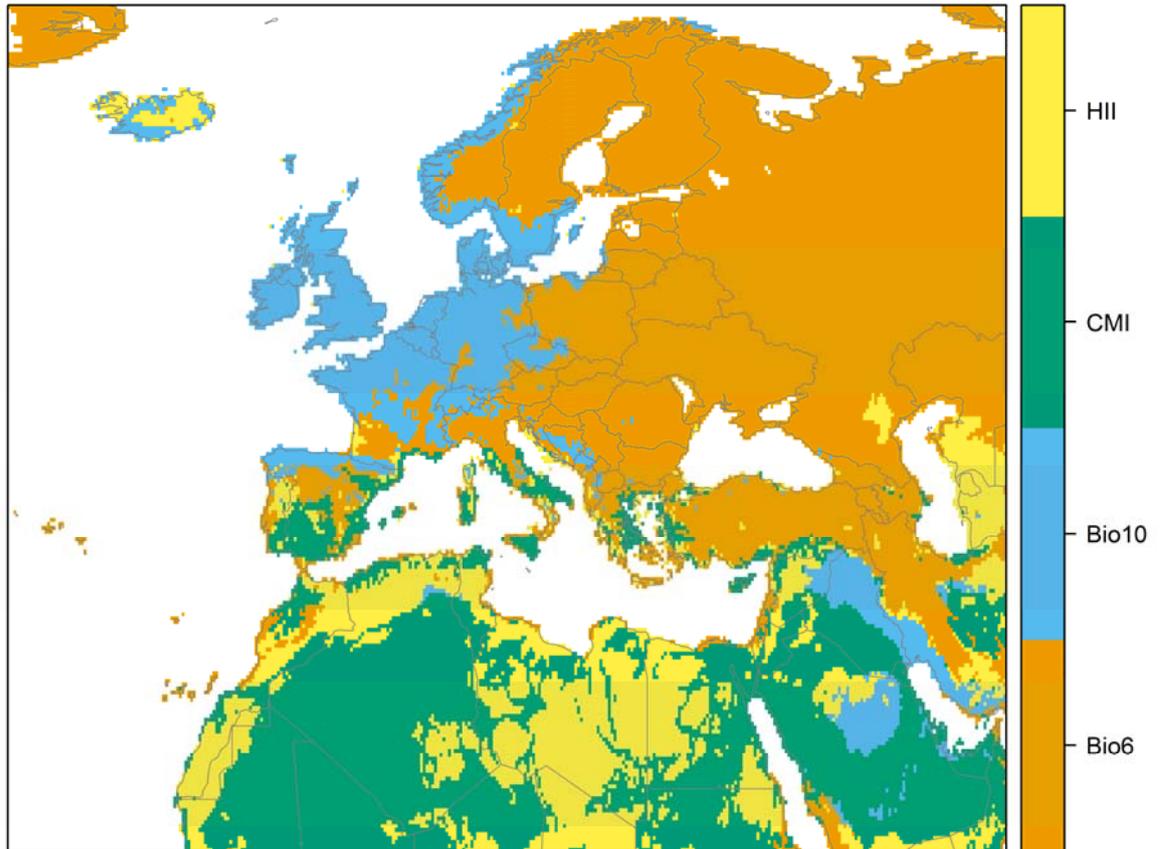


Figure 6. The most strongly limiting factors for *Cortaderia selloana* establishment estimated by the model in Europe and the Mediterranean region in current climatic conditions.

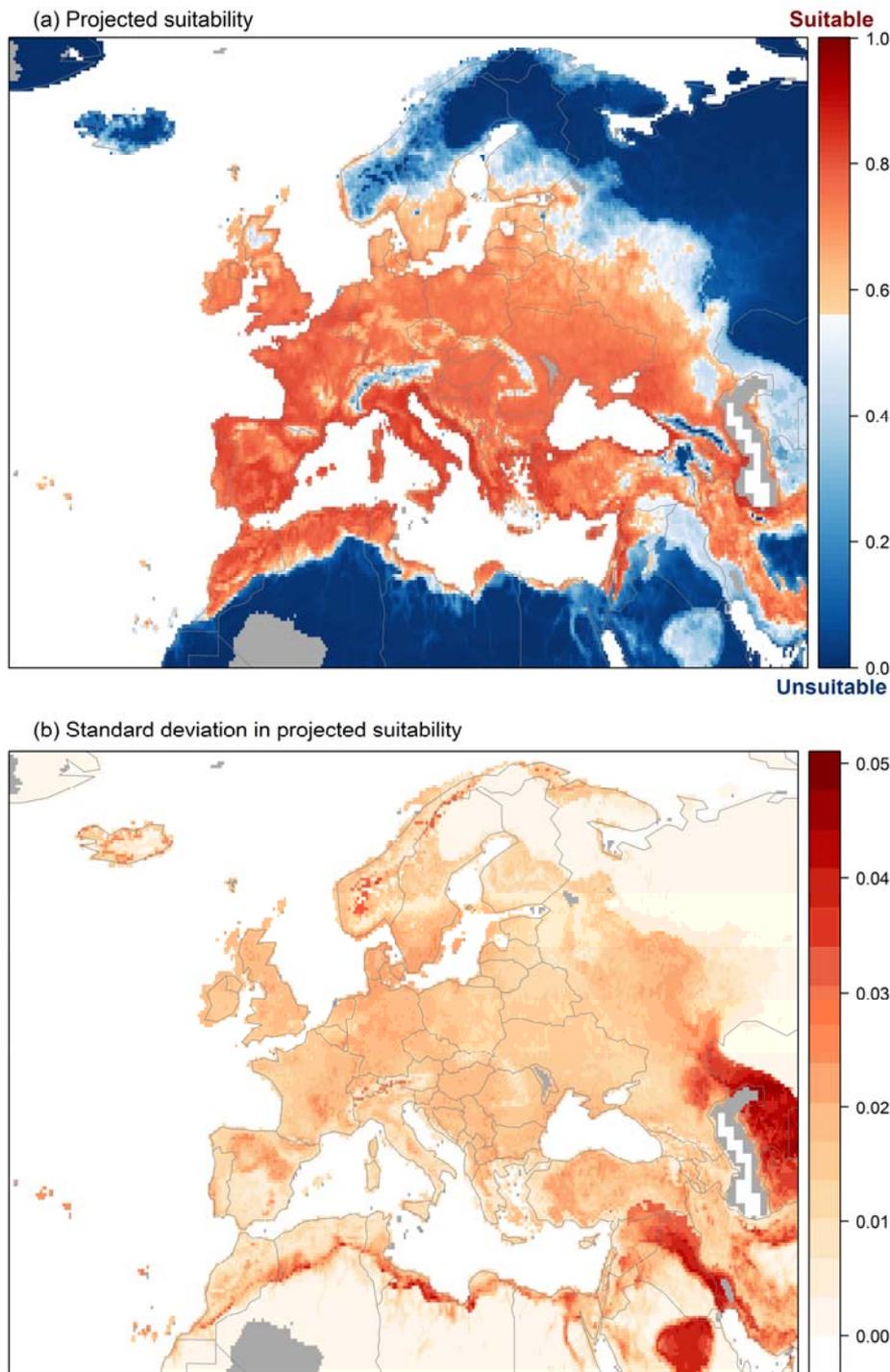


Figure 7. (a) Projected suitability for *Cortaderia selloana* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP2.6, equivalent to Figure 5. Grey areas have climatic conditions outside the range of the training data and were excluded from the projection. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 3 datasets.

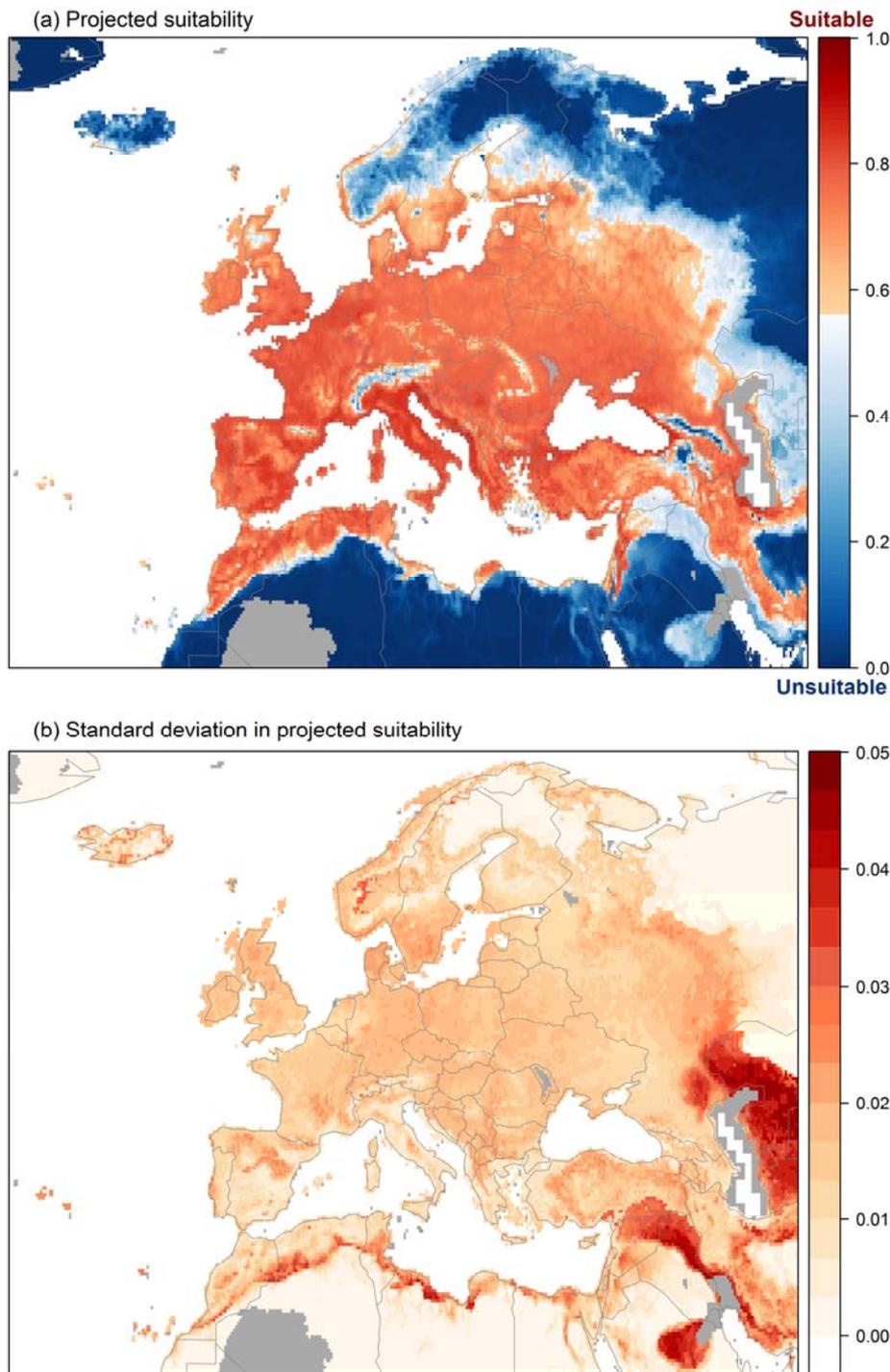


Figure 8. (a) Projected suitability for *Cortaderia selloana* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP4.5, equivalent to Figure 5. Grey areas have climatic conditions outside the range of the training data and were excluded from the projection. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 3 datasets.

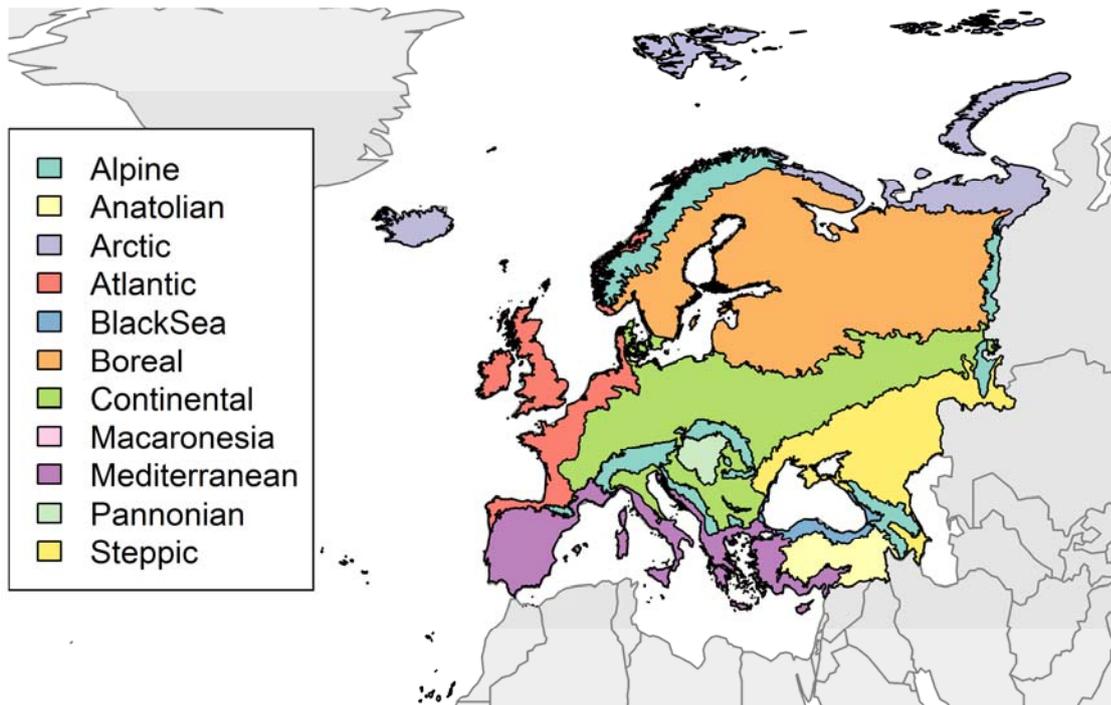
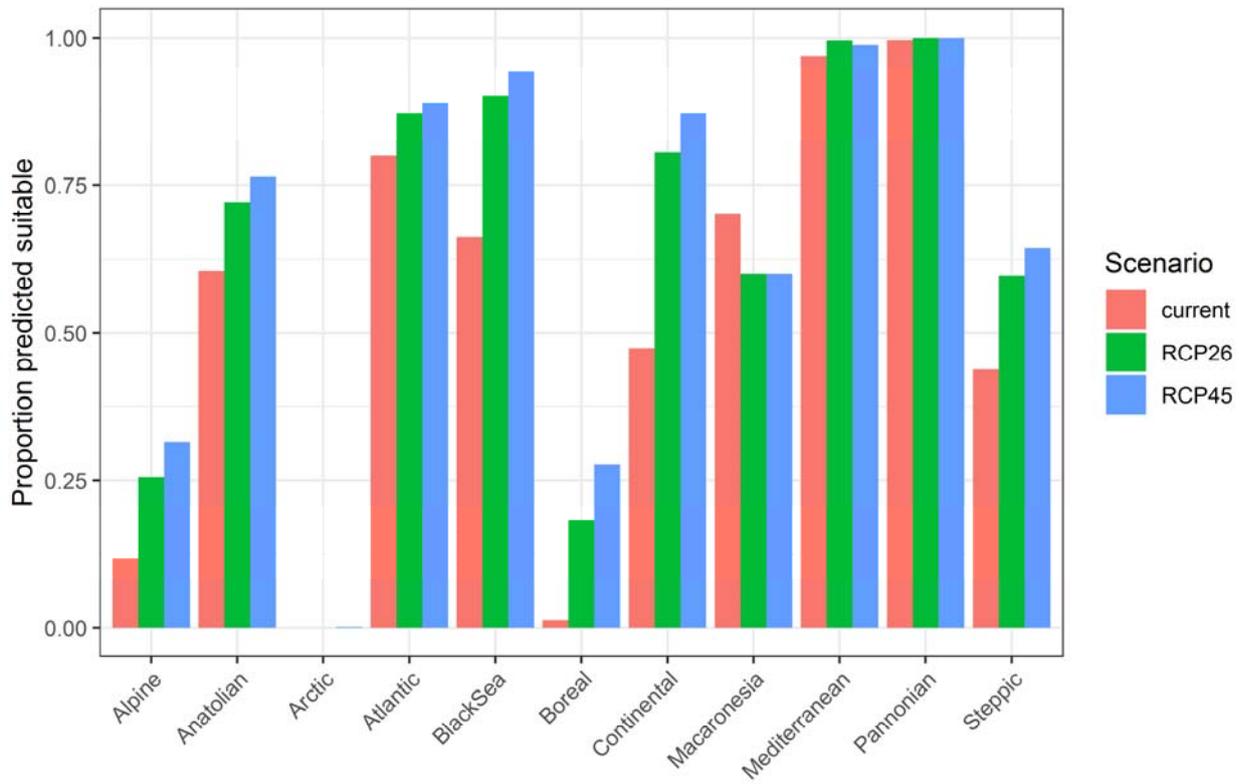


Figure 9. Variation in projected suitability for *Cortaderia selloana* establishment among Biogeographical regions of Europe (Bundesamt für Naturschutz (BfN), 2003). The bar plots show the proportion of grid cells in each region classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. The location of each region is also shown. The Arctic and Macaronesian biogeographical regions are not part of the study area, but are included for completeness.

Table 2. Variation in projected suitability for *Cortaderia selloana* establishment among Biogeographical regions of Europe (numerical values of Figure 9 above). The numbers are the proportion of grid cells in each region classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. The Arctic and Macaronesian biogeographical regions are not part of the study area, but are included for completeness.

Region	current	RCP26	RCP45
Alpine	0.12	0.26	0.31
Anatolian	0.61	0.72	0.76
Arctic	0.00	0.00	0.00
Atlantic	0.80	0.87	0.89
BlackSea	0.66	0.90	0.94
Boreal	0.01	0.18	0.28
Continental	0.47	0.80	0.87
Macaronesia	0.70	0.60	0.60
Mediterranean	0.97	1.00	0.99
Pannonian	1.00	1.00	1.00
Steppic	0.44	0.60	0.64

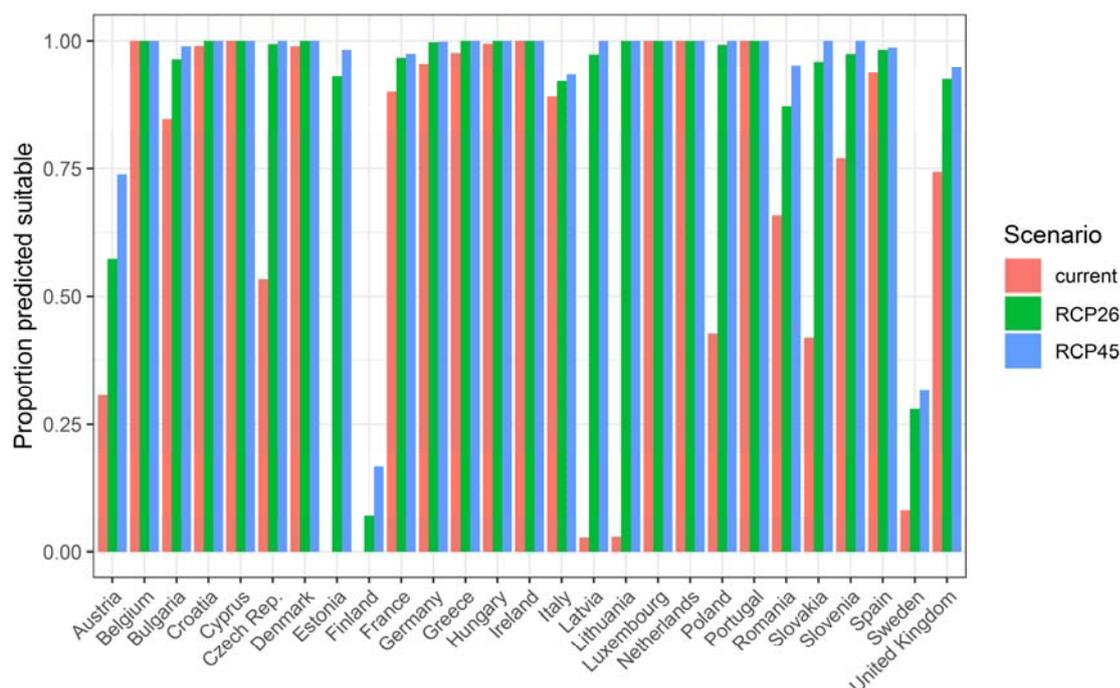


Figure 10. Variation in projected suitability for *Cortaderia selloana* establishment among European Union countries. The bar plots show the proportion of grid cells in each country classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. Malta has been excluded because the Human Influence Index dataset lacks coverage for Malta.

Table 3. Variation in projected suitability for *Cortaderia selloana* establishment among European Union countries and the UK (numerical values of Figure 10 above). The numbers are the proportion of grid cells in each country classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. Malta has been excluded because the Human Influence Index dataset lacks coverage for Malta.

Region	current	RCP26	RCP45
Austria	0.31	0.57	0.74
Belgium	1.00	1.00	1.00
Bulgaria	0.85	0.96	0.99
Croatia	0.99	1.00	1.00
Cyprus	1.00	1.00	1.00
Czech Rep.	0.53	0.99	1.00
Denmark	0.99	1.00	1.00
Estonia	0.00	0.93	0.98
Finland	0.00	0.07	0.17
France	0.90	0.97	0.97
Germany	0.96	1.00	1.00
Greece	0.98	1.00	1.00
Hungary	0.99	1.00	1.00
Ireland	1.00	1.00	1.00
Italy	0.89	0.92	0.93
Latvia	0.03	0.97	1.00
Lithuania	0.03	1.00	1.00
Luxembourg	1.00	1.00	1.00
Netherlands	1.00	1.00	1.00
Poland	0.43	0.99	1.00
Portugal	1.00	1.00	1.00
Romania	0.66	0.87	0.95
Slovakia	0.42	0.96	1.00
Slovenia	0.77	0.97	1.00
Spain	0.94	0.98	0.99
Sweden	0.08	0.28	0.31
United Kingdom	0.74	0.92	0.95

Caveats to the modelling

To remove spatial recording biases, the selection of the background sample was weighted by the density of Tracheophyta records on the Global Biodiversity Information Facility (GBIF). While this is preferable to not accounting for recording bias at all, it may not provide the perfect measure of recording bias.

There was substantial variation among modelling algorithms in the partial response plots (Figure 3). In part this will reflect their different treatment of interactions among variables. Since partial plots are made with other variables held at their median, there may be values of a particular variable at which this does not provide a realistic combination of variables to predict from.

Other variables potentially affecting the distribution of the species, such as land cover were not included in the model.

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**Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention"
Contract No 07.0202/2019/812602/ETU/ENV.D.2¹**

Name of organism: *Faxonius immunis* (Hagen, 1870)

Author(s) of the assessment: Elena Tricarico, Department of Biology, University of Florence, Italy
Frances Lucy, Institute of Technology Sligo, Ireland

Risk Assessment Area: The risk assessment area is the territory of the European Union 27 and the United Kingdom, excluding the EU-outermost regions.

Peer review 1: Christoph Chucholl, Fisheries Research Station Baden-Württemberg, Langenargen, Germany

Peer review 2: Antonín Kouba, University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Vodňany, Czech Republic

Date of completion: 15/10/2020

Date of revision: 15/10/2021

¹ This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA). A number of amendments have been introduced to ensure compliance with Regulation (EU) 1143/2014 on IAS and relevant legislation, including the Delegated Regulation (EU) 2018/968 of 30 April 2018, supplementing Regulation (EU) No 1143/2014 of the European Parliament and of the Council with regard to risk assessments in relation to invasive alien species (see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>).

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SECTION A – Organism Information and Screening

A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?

including the following elements:

- the taxonomic family, order and class to which the species belongs;
- the scientific name and author of the species, as well as a list of the most common synonym names;
- names used in commerce (if any)
- a list of the most common subspecies, lower taxa, varieties, breeds or hybrids

As a general rule, one risk assessment should be developed for a single species. However, there may be cases where it may be justified to develop one risk assessment covering more than one species (e.g. species belonging to the same genus with comparable or identical features and impact). It shall be clearly stated if the risk assessment covers more than one species, or if it excludes or only includes certain subspecies, lower taxa, hybrids, varieties or breeds (and if so, which subspecies, lower taxa, hybrids, varieties or breeds). Any such choice must be properly justified.

Response: The species is a single taxonomic entity, and it can be distinguished from other entities. The genus *Orconectes* (except cave-dwelling species) is now classified as *Faxonius* (Crandall & De Grave 2017).

Faxonius immunis (Hagen, 1870) (Malacostraca, Decapoda, Cambaridae)

Maiynomymys: *Orconectes immunis*, *Cambarus immunis*, *Cambarus signifer*

Common names: calico crayfish, papershell crayfish, mud crayfish (EN), Kalikokrebs (GE), Ecrevisse calicot, Écrevisse calico, Écrevisse à carapace fine (FR)

No hybrids with congeneric *Faxonius* species have been reported. No subspecies, lower taxa, varieties or breeds are currently known. Genetic studies using cytochrome c oxidase subunit I (COI) on European and North American specimens of *F. immunis* suggested that calico crayfish might represent a cryptic species complex (Filipová et al. 2011a).

A2. Provide information on the existence of other species that look very similar [that may be detected in the risk assessment area, either in the environment, in confinement or associated with a pathway of introduction]

Include both native and non-native species that could be confused with the species being assessed, including the following elements:

- other alien species with similar invasive characteristics, to be avoided as substitute species (in this case preparing a risk assessment for more than one species together may be considered);
- other alien species without similar invasive characteristics, potential substitute species;
- native species, potential misidentification and mis-targeting

Response: Native and non-native crayfish species present in the risk assessment area can be distinguished based on morphological and colour characteristics (Souty-Grosset et al. 2006; Kouba et al. 2014; IUCN 2018). The hair tufts on the ventral side of the chelae joints of the 1st and 2nd pereiopod are key features for recognizing the species together with a distinct tooth followed by a notch on the dactylus (movable finger) of the chelipeds. In addition, *F. immunis* features a very narrow areola (dorsal gap between the branchiocardiac grooves on the carapace), which – among other morphological characteristics of cambarid crayfish – clearly separates it from any native crayfish species, even if for non-experts sometimes difficulties can arise.

These are the other non-native *Faxonius* species present in Europe:

- *Faxonius juvenilis* (France) (Kouba et al. 2014);
- *Faxonius limosus*, present in Europe since the end of 19th Century, and established in Austria, Belarus, Belgium, Bulgaria, Croatia, Czech Republic, France, Germany, Hungary, Italy, Latvia, Lithuania, Luxembourg, Montenegro, Netherlands, Poland, Romania, Russia, Serbia, Slovakia, Slovenia, Spain, Switzerland, United Kingdom (Kouba et al. 2014; CABI 2020);
- *Faxonius virilis* (in the Netherlands and UK; Kouba et al. 2014);
- *Faxonius rusticus*, reported in 2019 in France (<http://especies-exotiques-envahissantes.fr/espece/faxonius-rusticus/?lang=en>)

Faxonius immunis has a brown or grey-green body with a less pronounced rostrum than *F. limosus*, no hepatic spines on lateral margins of carapace in front of the cervical groove, a typical pale zone in the middle of the carapace and pleon with four dark bands running along dorsal surface of pleon, and broad, flattened tuberculate chela with red/orange tips, with inner side of the dactylus straight at the distal part, followed by the distinct tooth and notch straight margin of movable finger and hair tufts on the ventral side of the chelae joints of the 1st and 2nd pereiopod (Souty-Grosset et al. 2006). Another key identification character of the species is the shape of the first pleopod of a Form I male: the corneous tip is short and sharply curved with an almost 90-degree bend. Form II pleopods retain this sharp curve (Schainost 2016).

A3. Does a relevant earlier risk assessment exist? Give details of any previous risk assessment, including the final scores and its validity in relation to the risk assessment area.

Response: For Europe, the species has been screened using FI-ISK, a tool for identifying potentially invasive freshwater invertebrates, with Italy used as the risk assessment area (Tricarico et al. 2010); the final outcome for *F. immunis* was high risk of invasiveness, and this is relevant to EU countries having similar conditions to Italy. For Germany, the species was assessed using a modified version of the GABLIS-protocol, with the outcome “Invasive species-Action List”, based on the transmission of crayfish plague and the localized distribution (Rabitsch & Nehring 2017). Species on the Action list should be eradicated immediately. For the Rhine-Meuse river district, in the Netherlands, the species has been screened using the internet-based Harmonia+ risk assessment protocol (D’hondt et al. 2015), with an outcome of high risk (Lemmers et al. 2021). For USA, the species has been screened by U.S. Fish and Wildlife Service (2015) with an uncertain outcome: “The biology and ecology of *O. immunis* have some documentation in the scientific literature. Controlled studies on the ecological impacts of *O. immunis* suggest that introduction of this species outside its native range could have detrimental

impacts to native flora and fauna, but the only documented impacts to date have been on another non-native species. Certainty of this assessment is low”.

A4. Where is the organism native?

including the following elements:

- an indication of the continent or part of a continent, climatic zone and habitat where the species is naturally occurring
- if applicable, indicate whether the species could naturally spread into the risk assessment area

Response: The species is native to North America, ranging from southern Quebec and New England westward across the upper Midwest to Wyoming and eastern Colorado and south to extreme northwestern Tennessee (Hobbs 1989; Hosabettu & Daniel 2020), and covering different climatic zones (following the classification of Köppen: oceanic; warm summer/humid continental; hot summer/humid continental; cold semi-arid; warm summer/mediterranean continental). It occurs in ponds, roadside ditches, flood plains, stagnant waters and sluggish streams with abundant aquatic plants and plant debris for cover; it has a preference for soft substrate and can tolerate high turbidity. The species usually avoids fast flowing, summer-cool streams (Souty-Grosset et al. 2006; Adams et al. 2010; but see Bovbjerg 1970). It is able to construct deep burrows and spread along the watercourses; in Germany the species has been found to actively disperse over land, moving from pond to pond (no dispersal rate has been reported in the study), over the entire year, especially between March and May, during the spawning season of this species, and between September and November, when *F. immunis* is mating (Hermann et al. 2018).

A5. What is the global non-native distribution of the organism outside the risk assessment area?

Response: The species has been probably introduced in the USA in New England, New Hampshire, Rhode Island, Vermont and Massachusetts. Its presence in Connecticut is uncertain (Taylor et al. 2007; Adam et al. 2010; <https://nas.er.usgs.gov/viewer/omap.aspx?speciesID=210>).

A6. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species been recorded and where is it established? The information needs be given separately for recorded (including casual or transient occurrences) and established occurrences. “Established” means the process of an alien species successfully producing viable offspring with the likelihood of continued survival².

A6a. Recorded: List regions

A6b. Established: List regions

² Convention on Biological Diversity, Decision VI/23

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

- Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea.

Comment on the sources of information on which the response is based and discuss any uncertainty in the response.

For delimitation of EU biogeographical regions please refer to <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2> (see also Annex VI).

For delimitation of EU marine regions and subregions consider the Marine Strategy Framework Directive areas; please refer to <https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions/technical-document/pdf> (see also Annex VI).

Response (6a): Continental (Gelmar et al. 2006; Collas et al. 2011) and Atlantic (Vermiert 2020). The response is based on publications reporting the presence of the species in these biogeographic regions, so the level of confidence is high.

Response (6b): Continental (Gelmar et al. 2006; Collas et al. 2011; Chucholl 2012) and Atlantic (Vermiert 2020). The response is based on publications reporting the presence and establishment of the species in these biogeographic regions, so the level of confidence is high.

A7. In which biogeographic region(s) or marine subregion(s) in the risk assessment area could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A7a. Current climate: List regions

A7b. Future climate: List regions

With regard to EU biogeographic and marine (sub)regions, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided.

However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (7a): Alpine (very few areas), Atlantic (very few areas), Black Sea, Boreal (very few areas), Continental, Mediterranean, Pannonian, Steppic

Response (7b): 2070 RCP 2.6/RCP 4.5 Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic. The differences between the two RCPs are, compared to the current situation, that under RCP 2.6 scenario there would be an increase of suitable areas in all the listed biogeographic regions (except a slight decrease in Pannonian), especially in Alpine, Atlantic, Black Sea, Boreal, Continental. On the contrary, under RCP 4.5, there would be an increase of suitable areas in Alpine, Atlantic, Boreal, Continental regions (more than under RCP 2.6), an increase similar to those under RCP 2.6 in Black Sea and Pannonian regions, but a decrease in Mediterranean and Steppic regions compared to RCP 2.6.

These responses are based on the SDM (Annex VIII). The model indicates that maximum temperature of the warmest month is the climatic variable currently limiting the establishment in the northern and coldest biogeographic regions. Temperature increase will favour the species establishment in the future, even if the increase forecasted under RCP 4.5 would decrease the percentage of suitable areas in Mediterranean region, that probably would become too warm for the species. The importance of temperature as predictor, followed by terrain slope, has been confirmed also by SDMs conducted on the species for southwestern Germany (Chucholl 2016).

A8. In which EU Member States has the species been recorded and in which EU Member States has it established? List them with an indication of the timeline of observations. The information needs be given separately for recorded and established occurrences.

A8a. Recorded: List Member States

A8b. Established: List Member States

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden

The description of the invasion history of the species shall include information on countries invaded and an indication of the timeline of the first observations, establishment and spread.

Response (8a): the first reliable record of the species was in Germany in 1993 in the catchment of the Upper Rhine Plain, followed by another record in 1997, always in the same region (Dehus et al. 1999; Gelmar et al. 2006); in France the first record was in 2010 in Alsace, in the Rothbach river, a tributary of the Rhine river (Collas et al. 2011). The species has been reported for the Netherlands in 2019 (Ottburg et al. 2019) but, after a check with German experts, it seemed to be a misidentification (Fran Oficialdegui, pers. comm.).

Response (8b): Germany, France (Gelmar et al. 2006; Collas et al. 2011; Chucholl 2012; Hermann et al. 2018a). In Germany, the species, after the two records in 1993 and 1997, established and started expanding along the Upper Rhine Plain. The species is reported also in the area of the Black Forest (Chucholl & Brinker 2017). In 2018, new records of the species were reported in the floodplain of the River Rhine between Wiesbaden and Ginsheim, being the first records for the federal state of Hesse, 53 kilometres from the next known locality (Herrmann et al. 2018b). In 2010, an established population was reported for the first time in France; the origin of French population is uncertain. Natural dispersal from Germany could have not occurred as this first French record was far from the areas where the species was reported for Germany. The hypotheses are two: some alive individuals were introduced from Germany or some individuals, kept at home for ornamental reasons, were dumped into the water (Collas et al. 2011). After this first record in France, the species started colonizing the French part of Rhine, some tributaries, and canals.

The species is currently colonizing a stretch of more than 150 km along the Upper Rhine plain in south-western Germany and eastern France (Filipová et al. 2011a; Ott 2017). In addition, there is an apparently disjunct occurrence downstream in the lower section of the Rhine (Düssel catchment) in western Germany (Vermiert 2020). Further isolated occurrences outside of the Upper Rhine Plain were recently reported from south-western Germany (i.e. near the city of Sindelfingen: Chucholl, unpubl. data, 2020).

In the Netherlands seven individuals were reported for three localities (Ottburg et al. 2019; Lemmers et al. 2021), but it was a misidentification.

A9. In which EU Member States could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A9a. Current climate: List Member States

A9b. Future climate: List Member States

With regard to EU Member States, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (9a): Austria, Bulgaria, Croatia, Czech Republic, France, Germany, Greece, Hungary, Italy, Poland, Portugal, Romania, Slovakia, Slovenia, Spain

Response (9b): 2070 RCP 2.6 Austria, Belgium, Bulgaria, Croatia, Czech Republic, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Lithuania, Luxembourg, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, and Sweden; 2070 RCP 4.5: Austria, Belgium, Bulgaria, Croatia, Czech Republic, Estonia, Finland, France, Germany, Greece, Hungary, Italy, Latvia, Lithuania, Luxembourg, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden; and in the United Kingdom. Under RCP 2.6, compared to the current situation, there will be an increase of suitable areas in all the listed countries (but slightly in Greece, Italy and Sweden), except in Austria, Croatia, Hungary, Portugal, Slovenia, Spain that will experience a decrease. Under RCP 4.5, compared to the current situation, there will be a much more marked increase of suitable areas in the listed countries (but very slightly in the United Kingdom), except Croatia, Greece, Hungary, Portugal, Spain, that will experience a decrease, and Austria and Slovenia that will remain the same.

These responses are based on the SDM (Annex VIII). Maximum temperature of the warmest month is resulted to be the climatic variable currently limiting the spread in the northern and coldest biogeographic regions. Temperature increase will favour the species spread in the future, but not Croatia, Greece, Hungary, Portugal, Spain, that probably would become too warm for the species.

A10. Is the organism known to be invasive (i.e. to threaten or adversely impact upon biodiversity and related ecosystem services) anywhere outside the risk assessment area?

Response: In USA, the species has been hypothesized to possibly cause impacts in its US introduced range (Jansen et al. 2009), being omnivorous and more aggressive than *F. limosus*, but up to now there are no published data of adverse impacts for this area.

A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea

Response: Continental and Atlantic: in Germany, the species has been proved to carry the crayfish plague lethal for European native crayfish (Filipová et al. 2013; Schrimpf et al. 2013) and to reduce native amphibians and macroinvertebrates, especially dragonfly larvae, other insects and molluscs (Ott 2016; Martens et al. 2018). Please see also Section 4 of the present Risk Assessment on impacts.

A12. In which EU Member States has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden

Response: Germany; the species has been proved to carry the crayfish plague lethal for European native crayfish (Filipová et al. 2013; Schrimpf et al. 2013) and to reduce native amphibians and macroinvertebrates, especially dragonfly larvae, other insects and molluscs (Ott 2016; Martens et al. 2018). In France, turbid waters are reported in invaded ponds due to the species burrowing activity (Francois et al. 2019). Moreover, it could outcompete the congeneric alien *F. limosus* (Chucholl et al. 2008).

A13. Describe any known socio-economic benefits of the organism.

including the following elements:

- Description of known uses for the species, including a list and description of known uses in the risk assessment area and third countries, if relevant.
- Description of social and economic benefits deriving from those uses, including a description of the environmental, social and economic relevance of each of those uses and an indication of associated beneficiaries, quantitatively and/or qualitatively depending on what information is available.

If the information available is not sufficient to provide a description of those benefits for the entire risk assessment area, qualitative data or different case studies from across the risk assessment area or third countries shall be used, if available.

Response: In North America, the species was grown and sold as bait (Huner 1994). Estimated 175 metric tonnes of crayfish were cultivated and another estimated 400-500 metric tonnes were harvested for this purpose (Huner 1997). It has been proposed that this species can be used to control submerged aquatic vegetation but the high numbers of required crayfish plus the possible impacts caused by its introduction has prevented promotion of its use (Letson & Makarewicz 1994).

No detailed economic data have been found on the use of the species in the ornamental pet trade, even though its presence in such trade has been reported (Chucholl 2013; Faulkes 2015).

SECTION B – Detailed assessment

Important instructions:

- In the case of lack of information the assessors are requested to use a standardized answer: “No information has been found.”
- With regard to the scoring of the likelihood of events or the magnitude of impacts see Annexes I and II.
- With regard to the confidence levels, see Annex III.
- Highlight the selected response score and confidence level in **bold** but keep the other scores in normal text (so that the selected score is evident in the final document).

1 PROBABILITY OF INTRODUCTION AND ENTRY

Important instructions:

- **Introduction** is the movement of the species into the risk assessment area (it may be either in captive conditions and/or in the environment, depending on the relevant pathways).
- **Entry** is the release/escape/arrival in the environment, i.e. occurrence in the wild
- Introduction and entry may coincide for species entering through pathways such as “corridor” or “unaided”, but it also may differ. If different, please consider all relevant pathways, both for the introduction into the risk assessment area and the entry in the environment.
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used (see Annex IV). For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document³ and the provided key to pathways⁴.
- For organisms which are already present (recorded or established) in the risk assessment area, the likelihood of introduction and entry should be scored as “very likely” by default.
- Repeated (independent) introductions and entries at separate locations in the risk assessment area should be considered here (see Qu. 1.7).

Qu. 1.1. List relevant pathways through which the organism could be introduced into the risk assessment area and/or enter into the environment. Where possible give details about the specific origins and end points of the pathways as well as a description of any associated commodities.

For each pathway answer questions 1.2 to 1.7 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 1.2a, 1.3a, etc. and then 1.2b, 1.3b etc. for the next pathway.

In this context a pathway is the route or mechanism of introduction and/or entry of the species.

³ <https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf>

⁴ <https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf>

The description of commodities with which the introduction of the species is generally associated shall include a list and description of commodities with an indication of associated risks (e.g. the volume of trade; the likelihood of a commodity being contaminated or acting as vector).

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 1.2-1.9.

Pathway name: Escape from confinement: Live food and live bait; Pet/aquarium/terrarium species; Contaminant on animals.

The introduction pathway to Europe is unknown; both pet trade and as live bait have been suggested (Dehus et al. 1999; Gelmar et al. 2006). As underlined by Gelmar et al. (2006) and Chucholl (2012), the species was not known in the German pet trade before its establishment in the Upper Rhine, it is however a common and popular species for fishing bait in the USA and Canada, therefore the introduction as live bait seems more likely. The “ornamental” trade became active only since its establishment in Germany (Chucholl 2013) and could well lead to repeated introductions, even if until now the species spread has mainly been reported as occurring naturally along the Rhine basin (Kouba et al. 2014; Hermann et al. 2018; but see France and recent findings in southwestern Germany: Collas et al. 2011; Vermiert 2020). The species seems to have been introduced in France as contaminant of fish stocks (Collas et al. 2015).

Escape from confinement: Live food and live bait

Qu. 1.2a. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional	CONFIDENCE	low
	unintentional		medium
			high

Response: The species probably was introduced as fishing bait, possibly by Canadian soldiers, who had been stationed at an airbase near the two localities where the species was first discovered (Chucholl 2013). The entry could have been intentional too, but unintentional escapes were also possible.

Qu. 1.3a. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication

- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Introduction of crayfish as food and bait in Europe has decreased in importance through the years, although still continues in some areas (Kouba et al. 2014). Considering the possibility of repeated and independent introductions via this pathway, the probability of introducing a large number of individuals is moderately likely. Source of these repeated introductions could be individuals from North America (even if there is a ban for using crayfish as a bait in numerous North American states) or from populations already established in Europe (Germany and France). No data are available of numbers of crayfish introduced as living bait into the risk assessment area. Likelihood of reinvasion is also moderately likely because of the possible repeated use as live bait.

Qu. 1.4a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species is established in the wild, proving that it can survive during the transport along this pathway (Dehus et al. 1999; Chucholl 2012). Reproduction and increase during the transport and storage, however, are unlikely, unless specimens are specifically kept and multiplied for use as baits. There is however no information that this has happened in the risk assessment area.

Qu. 1.5a. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely	CONFIDENCE	low medium high
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	very likely		
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Response: There are no biosecurity measures targeting the species before and during the transport; there could be border inspections for checking the introduction of crayfish already in the list of species of Union concern, and possibly during these checks also *F. immunis* could be intercepted. But up to now no interceptions have been reported for this species. Also for the entry there are no biosecurity measures, except for the code of conducts on IAS and recreational angling.

Qu. 1.6a. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species was previously detected in the wild only after its introduction and entry (Gelmar et al. 2006), so the probability of being introduced and released undetected is very likely. Even if the introduction would be carried out by anglers (intentionally or accidentally), it would not be reported, and thus the species would be undetected for the majority of people. Usually, the species would be detected only after establishing or when spreading.

Qu. 1.7a. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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Response: Repeated introductions and entry are linked to widespread release/escape by citizens/anglers, wherever they have access to rivers, lakes and any other suitable water bodies. The recent findings in southwestern Germany, and in the Netherlands, disjunct from the other records, confirms the likelihood of repeated introductions (Ottburg et al., 2019; Vermiert 2020).

Qu. 1.8a. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely	CONFIDENCE	low
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	unlikely moderately likely likely very likely		medium high
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Response: The species presumably entered the risk assessment area via this pathway; however, this pathway is decreasing in importance for crayfish, because their use as bait is currently forbidden in many U.S. states (the native area of many alien crayfish), so we can hypothesize that repeated and independent introductions as live bait are less frequent compared to the past (Kouba et al. 2014). However, we cannot completely discard the possibility that high abundance of the species in invaded European waterbodies means easy access to anglers who can use it as bait (C. Chucholl, pers. comm.)

Escape from confinement: Pet/aquarium/terrarium species

Qu. 1.2b. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: For this pathway, we are considering the possibility of repeated and independent introductions in the risk assessment area, as the species is already present in Europe. The species is deliberately introduced for ornamental reasons in aquaria and garden ponds (Holdich et al. 2009). The entry into the environment can be either intentional or unintentional, depending on whether it is the result of deliberate releases or accidental escapes.

Qu. 1.3b. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely	CONFIDENCE	low
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	unlikely moderately likely likely very likely		medium high
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Response: The species is already used as an ornamental species in Europe (Holdich et al. 2009; Chucholl 2013), even if numbers in trade may be lower compared to other more colorful alien crayfish (e.g. *Cambarellus* spp., *Procambarus* spp., *Cherax* spp.: Chucholl & Wendler 2017). Source of these repeated introductions could be specimens from populations established in Europe (Germany and France). For introductions and entry, we can hypothesize few crayfish, as they are bought by a single person and then eventually released by them as unwanted pets. Likelihood of reinvasion is likely because of the use as pet and the possibility of owners dumping unwanted pets into the wild. In addition, the species requires cold winter temperatures for reproduction, which may prompt deliberate releases into garden ponds, from where the species can easily escape (e.g., by over land migration).

Qu. 1.4b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: As testified by other alien crayfish introduced for this purpose (and established in the wild) and the use of the species in aquaria and ponds (Kouba et al. 2014), it can survive during the transport along this pathway. There is no reproduction or increase during transport, but the species could reproduce during storage in aquaria.

Qu. 1.5b. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: There are no biosecurity measures targeting the species before and during the transport; there could be border inspections for checking the introduction of crayfish already in the list of species of Union concern, and possibly during these checks also *F. immunis* could be intercepted. But up to

now no interceptions have been reported for this species. Also for the entry there are no biosecurity measures, except for the code of conducts on IAS and pets. The introduction of the species in France testified its survival to any possible management practices (Chucholl 2012).

Qu. 1.6b. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Pet owners can purchase the species online and can release individuals into the wild “secretly”, so the species is usually detected only once established. Calico crayfish could have been introduced and released into the wild in France as ornamental species (Chucholl 2013). This has happened also for many other invasive crayfish already present in Europe (Kouba et al. 2014).

Qu. 1.7b. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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Response: As there are no restrictions and it can be purchased online, its repeated introductions could be widespread; also entry could be widespread, being linked to release by private citizens potentially in all lakes, rivers and other suitable water bodies wherever they can have access. Urban waters are in general more prone to this phenomenon (Chucholl 2015; Patoka et al. 2016; Weiperth et al. 2017).

Qu. 1.8b. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species is used in aquaria and garden ponds (Holdich et al. 2009), so the probability of repeated introductions is likely.

Contaminant on animals

Qu. 1.2c. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: For this pathway, we are considering the possibility of repeated and independent introductions in the risk assessment area, as the species is already present in Europe. The species could be accidentally introduced through fish stocking (Collas et al. 2015).

Qu. 1.3c. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Source of these repeated introductions could be specimens from populations established in Europe. For introductions and entry, we can hypothesize few crayfish, as the species is introduced as contaminant. Likelihood of reinvasion could be moderately likely, it depends by the frequency of these stocking activities in the ponds, from where the species can easily escape (e.g., by over land migration).

Qu. 1.4c. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: As testified by the presence of the species in France (Collas et al. 2015), it can survive during the transport along this pathway. There is no reproduction or increase during transport.

Qu. 1.5c. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: It depends by the biosecurity measures targeting fish stocks, e.g. if stocks are inspected before the release.

Qu. 1.6c. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Its presence in France shows the possibility to be introduced and enter into the environments undetected (Colls et al. 2015). (

Qu. 1.7c. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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Response: Its repeated introductions could be widespread; also entry could be widespread, being linked to release into fish ponds that are numerous.

Qu. 1.8b. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Even if the other two previous listed pathways are more common, we cannot completely discard the probability to be introduced as contaminant as probably happened in France (Collas et al. 2015).

Qu. 1.9. Estimate the overall likelihood of introduction into the risk assessment area or entry into the environment based on all pathways and specify if different in relevant biogeographical regions in current conditions.

Provide a thorough assessment of the risk of introduction in relevant biogeographical regions in current conditions: providing insight into the risk of introduction into the risk assessment area.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species is already present in the Continental and Atlantic biogeographic regions in the EU (Kouba et al. 2014). Being a species present in Internet trade, it could be sold all across the risk assessment area, , in particular, according to the model developed on current climate, Black Sea, Continental. Mediterranean, Pannonian, and Steppic regions are (potentially) affected regarding introduction and entry.

Qu. 1.10. Estimate the overall likelihood of introduction into the risk assessment area or entry into the environment based on all pathways in foreseeable climate change conditions?

Thorough assessment of the risk of introduction in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of introduction (e.g. change in trade or user preferences)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely introduction within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: It is an adaptable species and, and we can expect that it could be still used in aquaria and urban ponds also in the future.

2 PROBABILITY OF ESTABLISHMENT

Important instructions:

- For organisms which are already established in parts of the risk assessment area or have previously been eradicated, the likelihood of establishment should be scored as “very likely” by default.
- Discuss the risk also for those parts of the risk assessment area, where the species is not yet established.

Qu. 2.1. How likely is it that the organism will be able to establish in the risk assessment area based on similarity of climatic and abiotic conditions in its distribution elsewhere in the world?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species is already established in the risk assessment area (Kouba et al. 2014). According to the model developed on current climate, the species could find many suitable areas and water bodies in Black Sea, Continental, Mediterranean, Pannonian, and Steppic regions, being a high adaptable species. Further suitable areas, even if few, are predicted also for Alpine, Atlantic and Boreal regions.

Qu. 2.2. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area? Consider if the organism specifically requires another species to complete its life cycle.

RESPONSE	very isolated isolated moderately widespread widespread ubiquitous	CONFIDENCE	low medium high
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Response: The species shows a high degree of plasticity in North America: it occurs in ponds, roadside ditches, flood plains, stagnant waters and sluggish streams with abundant aquatic plants and plant debris for cover; it has a preference for soft mud substrate (Maude & Williams 1983) and can tolerate high turbidity. It has a low potential to colonize highly fast flowing streams (Souty-Grosset et

al. 2006; Adams et al. 2010): when its ability to maintain itself in a current was tested, it started to slip downstream when flow exceeded 26 cm/sec (Maude & Williams 1983). It can tolerate water temperature up to 30 °C and low oxygen level (Tack 1941); egg development is faster at 25 °C than at 12 °C, but at 30 °C eggs die (Rach & Dawson 1991). It is able to construct deep burrows used during dry periods and in fall-winter when water temperatures are too low. As underlined by Chucholl (2012), this capability also allows it to inhabit shallow and temporary water bodies, a niche formerly not occupied by any native crayfish in Central Europe.

Qu. 2.3. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: In laboratory experiments, *F. immunis* was shown to be superior to the non-native *F. limosus* in direct aggressive interactions and competition for shelter (Chucholl et al. 2008). In the Upper Rhine, the abundance of *F. limosus* decreased after the colonization of *F. immunis* (Gelmar et al. 2006), probably because the latter is a strongly r-selected (i.e producing many eggs and offsprings) and burrowing species (Gelmar et al. 2006; Chucholl 2012). In the native range, *F. virilis* can outcompete *F. immunis* in streams because of greater aggressive behaviour (Bovbjerg 1970). This leads the latter to avoid the competition occupying temporary backwaters or pools. From laboratory experiments, there are contrasting results on interspecific pairs composed by the non-native marbled crayfish *Procambarus virginalis* and *F. immunis*: for Hossain et al. (2020), the former is competitively superior to *F. immunis*, while for Günter (2016) *F. immunis* is clearly superior in size-matched interspecific bouts. However, in natural conditions, the situation could be affected by other variables (e.g. predator pressure).

The possible competition with *Procambarus clarkii* and *Pacifastacus leniusculus*, the most widespread non-native species in Europe after *F. limosus*, has been experimentally tested only in the case of *P. leniusculus*. According to Wendler & Chucholl (2016). *F. immunis* is dominant over size- and sex-matched *P. leniusculus* in both direct interactions and competition for shelter. The inverse situation occurs, when *P. leniusculus* has a size advantage of 4 mm, which is closer to field conditions, since the latter grows considerably larger. The congeneric *F. limosus* outcompetes the native *Austropotamobius pallipes* (Kouba et al. 2014), so *F. immunis*, more aggressive than *F. limosus*, could outcompete the native one.

Qu. 2.4. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?

RESPONSE	N/A	CONFIDENCE	low
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	very unlikely unlikely moderately likely likely very likely		medium high
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Response: As with other crayfish, birds, fish and aquatic mammals can predate the species (Hamr 2002), but they do not have a major impact on the population, as they usually do not cause its extinction. *Faxonius immunis* can carry and transmit the crayfish plague (Schrimpf et al. 2013), but as other North American crayfish species *F. immunis* is not severely affected (Svoboda et al. 2017). Moreover, the already established populations of the species in Germany and France demonstrate that they can establish permanent populations despite resident fish and birds.

Qu. 2.5. How likely is the organism to establish despite existing management practices in the risk assessment area? Explain if existing management practices could facilitate establishment.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Management practices can be more effective at the early stage of invasion in a closed system, but not in an open system, such as a river. In USA calico crayfish has been demonstrated as highly sensitive to the insecticide permethrin (Thurston et al. 1985) but this cannot be used in every country and waterbodies. Early warning and rapid response processes for crayfish would not limit/prevent establishment in some cases (e.g. if the species results to be more diffused than expected). To prevent new introductions, a complete banning of the species from the trade plus an intensive educational campaign for citizens to avoid its further spread should be promoted. Management practices that promote the use of alien crayfish as a product, e.g. for bait or human consumption, may facilitate establishment (Kouba et al. 2014). Management practices in the environment, e.g. ecosystem restoration via creation of corridors and connections, can favour the spread and establishment of the species, as, for example, happened for the red swamp crayfish *Procambarus clarkii* in Italy (Mazza et al. 2018).

Qu. 2.6. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely	CONFIDENCE	low medium high
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	very likely		
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Response: It depends on the invaded habitat and the applied eradication methodology. As with all crayfish species, eradication is very challenging: this species is difficult to detect at low densities, and it cannot be easily removed by only physical means. Moreover, the species digs deep burrows that can allow it to survive some physical and chemical management actions, and has a high reproductive rate that facilitates the recovery of the population in case of incomplete eradication. In addition, it readily moves over land, allowing it to potentially avoid adverse conditions during eradication attempts. Eradication from complex natural habitats, in particular streams and rivers, is currently considered not feasible due to a lack of effective targeted methods and prohibitive collateral damage of non-selective methods (e.g., biocides) (Gherardi et al. 2011).

Qu. 2.7. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?

including the following elements:

- a list and description of the reproduction mechanisms of the species in relation to the environmental conditions in the risk assessment area
- an indication of the propagule pressure of the species (e.g. number of gametes, seeds, eggs or propagules, number of reproductive cycles per year) of each of those reproduction mechanisms in relation to the environmental conditions in the risk assessment area.
- If relevant, comment on the likelihood of establishment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in establishment whereas for others high propagule pressure (many thousands of individuals) may not.
- If relevant, comment on the adaptability of the organism to facilitate its establishment and if low genetic diversity in the founder population would have an influence on establishment.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species is omnivorous, with no ontogenetic shift in the diet observed in Europe (Chucholl 2012). It is a strongly r-selected species: it has a high growth rate, with early maturity occurring also at the end of first summer in young of the year; but its life span is short (estimated 2.5 years; Chucholl 2012). Laboratory experiments have showed that *F. immunis* juveniles were larger than *F. virilis* at the third instar and gained weight more rapidly (Wetzel & Brown 1993). In Europe, the species may reproduce two to three times during its life-time, depending on whether they attain sexual maturity at the end of their first summer, and a female can produce up to 500 eggs (Chucholl 2012). Contrary to other cambarids, mating occurs in autumn and female incubate the eggs during the winter in the burrows, releasing the offspring in early spring (Chucholl 2012). As in other *Faxonius* species, storage of spermatophores in the *annulus ventralis* could enable females to mate with a suitable mate even at low population densities (and this is very important when establishing in a new

environment; Buřič et al. 2013). Moreover, extended maternal care (especially present in cambarids and parastacids) allow a good survival of offspring (Mathews 2011). Low genetic diversity is not a problem for successful invasion in crayfish as reported for the congeneric *F. limosus* (Filipová et al. 2011b).

In the USA (but the species could follow the same patterns in Europe: C. Chucholl, pers. comm.), it can tolerate low oxygen levels and water temperature up to 30 °C (even 36 °C: Wiens & Armitage 1961) and low oxygen levels (Tack 1941); egg development is faster at 25°C than at 12°C, but at 30°C eggs die (Rach & Dawson 1991). When temperature preferences were tested, calico crayfish avoided temperature extremes (6 °C and 36 °C): they wandered freely through the intermediate, being most active at night when they tended to select a temperature around 22 °C while, during the day, they were inactive and selected areas with a temperature around 4 degrees cooler (Crawshaw 1974). The species has been reported also to have a high dispersal capability overland (Hermann et al. 2018).

Qu. 2.8. If the organism does not establish, then how likely is it that casual populations will continue to occur?

Consider, for example, a species which cannot reproduce in the risk assessment area, because of unsuitable climatic conditions or host plants, but is present because of recurring introduction, entry and release events. This may also apply for long-living organisms.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Continuous release of the species as unwanted pet (or bait) would likely occur.

Qu. 2.9. Estimate the overall likelihood of establishment in the risk assessment area under current climatic conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under current climatic conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in current conditions: providing insight in the risk of establishment in (new areas in) the risk assessment area.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species is already established in the Continental and Atlantic biogeographic regions of Europe (Kouba et al. 2014; Vermiert 2020). According to the model developed on current climate, the

species could find many suitable areas to establish permanent populations in Black Sea, Continental, Mediterranean, Pannonian, and Steppic regions, while very few suitable areas are present in Alpine, Atlantic and Boreal regions.

Qu. 2.10. Estimate the overall likelihood of establishment in the risk assessment area under foreseeable climate change conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under foreseeable climate change conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of establishment (e.g. increase in average winter temperature, increase in drought periods)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely establishment within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Based on the SDM (Annex VIII), in the 2070s under climate change scenario RCP2.6/RCP 4.5, the areas suitable for the species will increase (except for a slight decrease in the Mediterranean region under RCP 4.5) in the future, particularly in the Alpine, Atlantic, Boreal and Continental regions.

3 PROBABILITY OF SPREAD

Important instructions:

- Spread is defined as the expansion of the geographical distribution of an alien species within the risk assessment area.
- Repeated releases at separate locations do not represent continuous spread and should be considered in the probability of introduction and entry section (Qu. 1.7).

Qu. 3.1. How important is the expected spread of this organism within the risk assessment area by natural means? (List and comment on each of the mechanisms for natural spread.)

including the following elements:

- a list and description of the natural spread mechanisms of the species in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of spread discussed in relation to the species biology and the environmental conditions in the risk assessment area.

The description of spread patterns here refers to the CBD pathway category “Unaided (Natural Spread)”. It should include elements of the species life history and behavioural traits able to explain its ability to spread, including: reproduction or growth strategy, dispersal capacity, longevity, dietary requirements, environmental and climatic requirements, specialist or generalist characteristics.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Currently, the species is colonizing new areas upstream and downstream by natural spread. Since its first report, it is currently reported in a stretch of more than 150 km, spreading through canals, ditches and streams, colonizing a variety of aquatic systems from the floodplain to the slope of the Black Forest and occupying different habitat types (Chucholl 2012; Ott 2017). In Germany, the species has been found to actively disperse overland, moving from pond to pond (no dispersal rate has been reported in the study), over the entire year, especially between March and May, during the spawning season of this species, and between September and November, when *F. immunis* is mating (Hermann et al. 2018).

Qu. 3.2a. List and describe relevant pathways of spread other than "unaided". For each pathway answer questions 3.3 to 3.9 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 3.3a, 3.4a, etc. and then 3.3b, 3.4b etc. for the next pathway.

including the following elements:

- a list and description of pathways of spread with an indication of their importance and associated risks (e.g. the likelihood of spread in the risk assessment area, based on these pathways; likelihood of survival, or reproduction, or increase during transport and storage;

ability and likelihood of transfer from the pathway to a suitable habitat or host) in relation to the environmental conditions in the risk assessment area.

- an indication of the rate of spread for each pathway discussed in relation to the species biology and the environmental conditions in the risk assessment area.
- All relevant pathways of spread (except “Unaided (Natural Spread)”, which is assessed in Qu. 4.1) should be considered. The classification of pathways developed by the Convention of Biological Diversity shall be used (see Annex IV).

Pathway name: Corridor (interconnected waterways/basins/seas)

Qu. 3.3a. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional	CONFIDENCE	low
	unintentional		medium
			high

Response: The Rhine river is connected with other rivers by canals, so the species could spread using this pathway, as happened for other aquatic alien species (Leuven et al. 2009).

Qu. 3.4a. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely	CONFIDENCE	low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response: There are no data on the propagule pressure, but, as shown by the natural spread of the species along the Rhine, the species could spread also through this pathway. Some traits mentioned above- successful reproduction, low genetic diversity, possible maternal care- could facilitate the spread. Reinvasion could happen, as the species actively disperses, even overland (Chucholl 2012;

Hermann et al. 2018). As rate, we can consider a similar rate to the reported natural spread: in around ten years it colonized a stretch of almost 100 km along the Rhine (Chucholl 2012).

Qu. 3.5a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely	CONFIDENCE	low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response: The species could survive along this pathway, as also showed by its active dispersal along the Rhine. It could even reproduce while spreading. This happened for other macroinvertebrates (Leuven et al. 2009).

Qu. 3.6a. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely	CONFIDENCE	low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response: Some barriers along the interconnected water bodies could slow the spread of species, but they would not affect its survival.

Qu. 3.7a. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely	CONFIDENCE	low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response: Interconnected canals and rivers allowed the undetected spread of many invasive species in Europe (Panov et al. 2007, 2009); this could happen also for *F. immunis*.

Qu. 3.8a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: During natural dispersal, the species usually arrives and settles in suitable habitats or moves on; the same could happen using this pathway.

Qu. 3.9a. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area. (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: Considering that the Rhine is connected with other rivers and that the species could rapidly disperse unaided (almost 100 km within 10 years: Chucholl 2012), we could hypothesize a similar rapid spread along this pathway.

Qu. 3.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?

RESPONSE	very easy easy with some difficulty difficult very difficult	CONFIDENCE	low medium high
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Response: Once in an aquatic system, particularly a well-connected one such as the Rhine basin, it is difficult to contain the species. Physical barriers have been tested on other alien crayfish, but they can also stop native species movement (Gherardi et al. 2011). Containment could be hypothesized for closed (or almost confined systems: e.g. ponds or areas that could be fenced).

Qu. 3.11. Estimate the overall potential rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (indicate any key issues and provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in current conditions, providing insight in the risk of spread into (new areas in) the risk assessment area.

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: The species has colonized almost 100 km of river area within approx. 10 years (Chucholl 2012). We can expect that the species will continue to spread (unaided or using corridors) in the Continental and Atlantic region. The lower Rhine drainage is probably very prone to invasion by *F. immunis* (C. Chucholl, pers. comm.), as evidenced by the recent reports in the Netherlands (Ottburg et al. 2019).

Qu. 3.12. Estimate the overall potential rate of spread in relevant biogeographical regions in foreseeable climate change conditions (provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if rates of spread are likely slowed down or accelerated.

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: It is likely that the species will be able to spread also in the future, as the availability of suitable areas will increase with climate change (Annex VIII). As climate change will favour the species, we can hypothesize that in the future at least a similar rate of spread to the current one could occur, considering that all the regions will almost become suitable.

4 MAGNITUDE OF IMPACT

Important instructions:

- Questions 4.1-4.5 relate to biodiversity and ecosystem impacts, 4.6-4.8 to impacts on ecosystem services, 4.9-4.13 to economic impact, 4.14-4.15 to social and human health impact, and 4.16-4.18 to other impacts. These impacts can be interlinked, for example, a disease may cause impacts on biodiversity and/or ecosystem functioning that leads to impacts on ecosystem services and finally economic impacts. In such cases the assessor should try to note the different impacts where most appropriate, cross-referencing between questions when needed.
- Each set of questions starts with the impact elsewhere in the world, then considers impacts in the risk assessment area (=EU27+UK excluding outermost regions) separating known impacts to date (i.e. past and current impacts) from potential future impacts (including foreseeable climate change).
- Only negative impacts are considered in this section (socio-economic benefits are considered in Qu. A.7)

Biodiversity and ecosystem impacts

Qu. 4.1. How important is the impact of the organism on biodiversity at all levels of organisation caused by the organism in its non-native range excluding the risk assessment area?

including the following elements:

- Biodiversity means the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems
- impacted chemical, physical or structural characteristics and functioning of ecosystems

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: In the USA, the species has been hypothesized to have the potential to cause impacts in the introduced range (Jansen et al. 2009), being omnivorous and more aggressive than the congeneric *F. limosus*, but up to now there are no published data and details of impacts for this area.

Qu. 4.2. How important is the current known impact of the organism on biodiversity at all levels of organisation (e.g. decline in native species, changes in native species communities, hybridisation) in the risk assessment area (include any past impact in your response)?

Discuss impacts that are currently occurring or are likely occurring or have occurred in the past in the risk assessment area. Where there is no direct evidence of impact in the risk assessment area (for

example no studies have been conducted), evidence from outside of the risk assessment area can be used to infer impacts within the risk assessment area.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: In Europe, the species has been found to carry crayfish plague, lethal for European native crayfish (Schrimpf et al. 2013). The species is omnivorous: from the stomach content analyses, Chucholl (2012) showed that in Germany the main food item is detritus, followed by macroinvertebrates and macrophytes, with no ontogenetic shift in the diet clearly reported up to now. In the same study, the species was found to prey on a wide spectrum of macroinvertebrates, mainly Chironomidae larvae, Cladocera and Ephemeroptera larvae. Moreover, “The relatively high importance of Cladocera in its diet, along with the positive selection of *Dreissena*, indicates that *O. immunis* is able to access the plankton pool as an energy resource, in addition to benthic macroinvertebrates, macrophytes and detritus. The relatively high importance of energy-rich macroinvertebrate prey in its diet probably supports the sustained high growth rate of *O. immunis*” (Chucholl 2012). Chucholl (2012) noted that “in Lake Bärensee (the study lake in Germany), the decline of macrophyte species coincided with the first observations of *O. immunis* in the lake, suggesting that *O. immunis* might negatively affect macrophyte biomass, as was experimentally shown by Letson and Makarewicz (1994)”.

From studies carried out in USA, it results that plant material is often the dominant food item found in the stomachs, followed by zooplankton (*Daphnia*), insect remains, isopods (*Asellus*), midge larvae (*Chironomus*), rotifers and diatoms (Tack 1941), and that calico crayfish juveniles were found to filter feed whereas adults may do so opportunistically (Budd et al. 1978).

In Germany, Hermann et al. (2016) from laboratory experiments on juvenile growth in Europe inferred that juveniles could eat mainly macrozoobenthos, in accordance with findings in other crayfish species (juveniles usually are more carnivores), even if no other evidence on this issue has been reported. Ott (2016) reports negative impacts on amphibians, molluscs, caddisflies, and dragonflies in the river Rhine. Ott (2018) reports that “In the floodplain of the river Rhine in the two German federal states of Baden-Württemberg and Rhineland-Palatinate the calico crayfish (*Orconectes immunis*) increased its distribution area remarkably, which has a strong influence on the dragonfly fauna of the floodplain, also affecting several dragonfly species of the EC habitats directive (e.g. *Leucorrhinia caudalis*, *Ophiogomphus cecilia*).” In Germany, Herrmann et al. (2018c) and Martens et al. (2018) found a significant reduction in Coleoptera, Odonata, Mollusca and Trichoptera between the years 2015 and 2017 in a pond created for amphibian conservation, comparing the populations of these invertebrates before and after *F. immunis* has built up high population densities. They also found that that *F. immunis* can grow up to densities > 15 crayfish/m² in small conservation ponds leading to minimum levels of presence of odonates. In agreement with the major impact of *F. immunis* on macroinvertebrates in small ponds, Wendler (2018) demonstrated *F. immunis* to exhibit the highest *per capita* effect on gammarid prey among four invasive and one native crayfish species using comparative function response analysis. Finally, it is a burrowing species, so together with damages to

riverbank, increase in turbidity with cascade effects on native species (less oxygen and light for the species living under the water surface with their consequent reduction) can be expected, as recorded for other invasive alien crayfish intensively burrowing in Europe (e.g. *Procambarus clarkii*; Souty-Grosset et al. 2016).

A recent laboratory experiment on functional response of different alien crayfish present in Europe, *F. immunis* included, showed that this species, together with the signal crayfish *Pacifastacus leniusculus*, is one of the most impactful invaders, with the highest effects on macrobenthos and detritus (Chucholl & Chucholl 2021).

Qu. 4.3. How important is the potential future impact of the organism on biodiversity at all levels of organisation likely to be in the risk assessment area?
 See comment above. The potential future impact shall be assessed only for the risk assessment area.

RESPONSE	minimal	CONFIDENCE	low
	minor		medium
	moderate		high
	major		
	massive		

Response: According to the SDM (Annex VIII), the species will find more suitable climatic areas in Europe in the future, starting from the Continental and Atlantic regions already invaded. Being an omnivorous species, it can cause a decrease in macrophyte cover, macroinvertebrates abundance and diversity, changing trophic interaction and community composition.

Qu. 4.4. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism currently in the risk assessment area?
 including the following elements:

- native species impacted, including red list species, endemic species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	minimal	CONFIDENCE	low
	minor		medium
	moderate		high
	major		
	massive		

Response: Impact on native crayfish species listed in the Habitat Directive, such as the white-clawed crayfish, *Austropotamobius pallipes*, the stone crayfish *A. torrentium* and the noble crayfish *Astacus astacus*, could be significant through disease (i.e. crayfish plague lethally affecting native species; Schrimpf et al. 2013). Impact could be significant also on native macroinvertebrates (e.g. odonates *Leucorrhinia caudalis*, *Ophiogomphus cecilia*, both species in the European Red List of Dragonflies and in the Habitat Directive) and amphibians (e.g. *Rana* spp: Ott 2016, 2017). All invaded habitats could be affected as a result of the species consumption of macroinvertebrates and macrophytes, in addition to burrowing and sediment mobilization (“milky water”). In this way, it can alter the ecological status of water bodies according to the Water Framework Directive. It has negative effects in protected areas (Francois et al. 2019) and has the potential to affect habitats and species listed in the Habitat Directive (e.g. 3150 Natural eutrophic lakes with *Magnopotamion* or *Hydrocharition*; 3270 Rivers with muddy banks with *Chenopodion rubri* pp and *Bidention* pp vegetation; abovementioned dragonflies and crayfish).

Qu. 4.5. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism likely to be in the future in the risk assessment area?

including the following elements:

- native species impacted, including red list species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	minimal	CONFIDENCE	low
	minor		medium
	moderate		high
	major		
	massive		

Response: According to the SDM (Annex VIII), the species will find more suitable climatic areas in Europe in the future, so we can expect a major impact on species and habitats of conservation concerns listed in the answer of Qu. 4.4. areas.

Ecosystem Services impacts

Qu. 4.6. How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?

- For a list of services use the CICES classification V5.1 provided in Annex V.
- Impacts on ecosystem services build on the observed impacts on biodiversity (habitat, species, genetic, functional) but focus exclusively on reflecting these changes in relation to their links with socio-economic well-being.

- Quantitative data should be provided whenever available and references duly reported.
- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: In the USA introduced range, no information has been found. We cannot discard, however, that, being a burrowing species, together with damage to riverbanks, the increase in turbidity can have cascading effects on native species and ecosystem services (please see the response to the subsequent Qu 4.7 for details) (e.g. *Procambarus clarkii*; Souty-Grosset et al. 2016).

Qu. 4.7. How important is the impact of the organism on provisioning, regulating, and cultural services currently in the different biogeographic regions or marine sub-regions where the species has established in the risk assessment area (include any past impact in your response)?

- See guidance to Qu. 4.6.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Increase in turbidity is reported in some ponds (Francois et al. 2019). Being a burrowing species, it can damage riverbanks leading to soil erosion and changes in sediments (regulating services: baseline flows and extreme event regulation; soil quality regulation), while the increase in turbidity can have a cascade effect on native species, primary production and trophic chains (provisioning services: wild plants and wild animals) and water quality (regulating services: water conditions, and provisioning services: water quality), as recorded for other invasive alien crayfish intensively burrowing in Europe (e.g. *Procambarus clarkii*; Souty-Grosset et al. 2016). Also cultural services can be impacted, because “milky water” ponds do not attract people (physical and experiential interactions with natural environment), and the species alters the pristine characteristics of the habitat (intellectual and representative interactions with natural environment).

Qu. 4.8. How important is the impact of the organism on provisioning, regulating, and cultural services likely to be in the different biogeographic regions or marine sub-regions where the species can establish in the risk assessment area in the future?

- See guidance to Qu. 4.6.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: According to the predictive climatic models, the species will find more suitable climatic areas in Europe in the future (see Annex VIII), so we can expect still major impacts on ecosystem services (provisioning: wild plants, wild animals, water quality; regulating services: baseline flows and extreme event regulation, soil quality regulation, water conditions; cultural services: physical and experiential interactions with natural environment, intellectual and representative interactions with natural environment).

Economic impacts

Qu. 4.9. How great is the overall economic cost caused by the organism within its current area of distribution (excluding the risk assessment area), including both costs of / loss due to damage and the cost of current management.

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the world these should be reported here. The assessment of the potential costs of / loss due to damage shall describe those costs quantitatively and/or qualitatively depending on what information is available. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage. As far as possible, it would be useful to separate costs of / loss due to the organism from costs of current management.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Souty-Grosset et al. (2006) report that the species in the USA is considered a pest in rice paddies, where it burrows extensively and causes damage to young rice plants. However, there is no quantification of this damage available. No information has been found for costs of damaged banks and their management.

Qu. 4.10. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism currently in the risk assessment area (include any past costs in your response)?

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the EU these should be reported here. Assessment of the potential costs of damage on human health, safety, and the economy, including the cost of non-action. A full economic assessment at EU scale might not be possible, but qualitative data or different case studies from across the EU (or third countries if relevant) may provide useful information to inform decision making. In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No information has been found on the issue in the risk assessment area. We can only hypothesize the response by considering the estimated costs caused by *Procambarus clarkii* for damaging banks (e.g. 1000 euro/m², info provided by an Italian Land Reclamation Authority reported in Souty-Grosset et al. 2016), because also *F. immunis* is considered a highly burrowing species as *P. clarkii*.

Qu. 4.11. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.10.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: This very much depends on the time elapsed between first introduction and when action is taken to control the species. If action is taken immediately to mitigate its spread, then costs could be relatively low (for example damage to banks would be limited), but if left to spread the costs would potentially be major (the costs linked to riverbanks could increase; increase of turbidity could also affect fish populations with a potential consequence on fishing activities).

Qu. 4.12. How great are the economic costs / losses associated with managing this organism currently in the risk assessment area (include any past costs in your response)?

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No published information has been found on the issue. Management actions are ongoing in some ponds in Germany, but no detailed information is available on costs. Considering the current extent of the species, we can only hypothesize the response, because the species could be managed only in certain areas (closed and/or confined water bodies).

Qu. 4.13. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.12.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: This very much depends on when action is taken to control the species if it is introduced. If action is taken immediately to mitigate its spread, then costs could be relatively low, but if left to spread the costs would potentially be major. Intensive trapping an area of 10,000 m² with 120 traps for 171 trap days could cost approximately 30,000 euro to reduce species abundance by at least 70%. But intensive trapping should be permanently maintained through time. Eradication of crayfish from a water body of 19,000 m² could cost around 46,500 euro of biocides. Drainage at least once of a pond of approximately 400,000 m³ could cost around 40-50,000 euro (E. Tricarico, pers. comm.). Costs are estimated on the basis of the experience with measures targeting other invasive crayfish present in the risk assessment area.

Social and human health impacts

Qu. 4.14. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism for the risk assessment area and for third countries, if relevant (e.g. with similar eco-climatic conditions).

The description of the known impact and the assessment of potential future impact on human health, safety and the economy, shall, if relevant, include information on

- illnesses, allergies or other affections to humans that may derive directly or indirectly from a species;
- damages provoked directly or indirectly by a species with consequences for the safety of people, property or infrastructure;
- direct or indirect disruption of, or other consequences for, an economic or social activity due to the presence of a species.

Social and human health impacts can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No human health impacts have been reported up to now. Risk of flooding might increase if dykes are destabilized by crayfish burrowing.

Qu. 4.15. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism in the future for the risk assessment area.

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: As in the future, the suitable areas for the species will increase we can hypothesize that risk of flooding might increase for crayfish burrowing, because they actively dig and this could lead to instability/collapse of banks, as already happened for a similar burrowing species *Procambarus clarkii* (Haubrock et al. 2019).

Other impacts

Qu. 4.16. How important is the organism in facilitating other damaging organisms (e.g. diseases) as food source, a host, a symbiont or a vector etc.?

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: The species carries the crayfish plague, lethal for European native crayfish (Filipová et al. 2013; Schrimpf et al. 2013). We cannot discard the possibility that the species could carry other pathogens and diseases as found for the congeneric *F. limosus*.

Qu. 4.17. How important might other impacts not already covered by previous questions be resulting from introduction of the organism?

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: NA

Qu. 4.18. How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area?

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Even if fish and birds could predate on the species, they regulate the population to a low level of density but do not cause extinction. A recent study showed that the species, together with the signal crayfish *Pacifastacus leniusculus*, is one of the most impactful invaders (Chucholl & Chucholl 2021), thus overcoming also other notable invasive crayfish as *Faxonius limosus* and *Procambarus fallax* forma *virginalis*.

Qu. 4.19. Estimate the overall impact in the risk assessment area under current climate conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, in current conditions.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Some studies already demonstrated relevant ecological impacts (reduction of macroinvertebrate abundance; decline of macrophytes; vector of crayfish plague, lethal for native crayfish; highly burrowing activity with damages to banks) in the Continental region where the species is present (Chucholl 2012; Ott 2016, 2018; Herrmann et al., 2018c; Martens et al. 2018), but further studies are needed to better assess them, particularly in different habitats and through time, and to deeply investigate the effects of its intense burrowing activity. Studies to understand the ecosystem services impacts and quantify the economic impacts are also necessary.

Qu. 4.20. Estimate the overall impact in the risk assessment area in foreseeable climate change conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, under future conditions.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: As the species will find more suitable areas for introduction, entry and establishment in the future (see Annex VIII), an increase of its impacts has to be expected. According to the SDM (Annex VIII), impacts might increase in the Alpine, Atlantic, Black Sea, Boreal, and Continental biogeographical regions (under both RCP 2.6 and RCP 4.5), but decrease in the Mediterranean and Steppic regions (under RCP 4.5).

RISK SUMMARIES			
	RESPONSE	CONFIDENCE	COMMENT
Summarise Introduction and Entry*	very unlikely unlikely moderately likely likely very likely	low medium high	The species is already present in Europe (France and Germany) since 1993.
Summarise Establishment*	very unlikely unlikely moderately likely likely very likely	low medium high	The species is already established in Europe (Germany from 1997, France from 2010).
Summarise Spread*	very slowly slowly moderately rapidly very rapidly	low medium high	After its first detection in 1993, the species is currently colonizing a riverine stretch of more than 150 km, and it is still spreading. Considering the favourable current and future climatic conditions and its capability to disperse also overland, the species will continue to spread rapidly.
Summarise Impact*	minimal minor moderate major massive	low medium high	The studies on the impacts are recent and still ongoing, but they have already demonstrated ecological impacts (reduction of macroinvertebrate abundance; decline of macrophytes; vector of crayfish plague; highly burrowing activity with damages to banks). Considering that the species is omnivorous, colonizes a wide range of habitats and extensively burrows, with favorable current and future climate conditions we can expect major impacts.
Conclusion of the risk assessment (overall risk)	low moderate high	low medium high	Based on the evidence from the recent literature, species and habitats protected under the EU Nature Directives are under threat and there is risk that this will increase in the future if the species spreads. The species can cause also moderate economic damages in the future.

*in current climate conditions and in foreseeable future climate conditions

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Distribution Summary

Please answer as follows:

- Yes if recorded, established or invasive
- if not recorded, established or invasive
- ? Unknown; data deficient and/or?

The columns refer to the answers to Questions A5 to A12 under Section A.

For data on marine species at the Member State level, delete Member States that have no marine borders.

In all other cases, provide answers for all columns.

Member States and the United Kingdom

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Austria	-	-	X	X	-
Belgium	-	-		X	-
Bulgaria	-	-	X	X	-
Croatia	-	-	X	X	-
Cyprus	-	-			-
Czech Republic	-	-	X	X	-
Denmark	-	-			-
Estonia	-	-		X	-
Finland	-	-		X	-
France	YES	YES	X	X	YES
Germany	YES	YES	X	X	YES
Greece	-	-	X	X	-
Hungary	-	-	X	X	-
Ireland	-	-			-
Italy	-	-	X	X	-
Latvia	-	-		X	-
Lithuania	-	-		X	-
Luxembourg	-	-		X	-
Malta	-	-			-
Netherlands	-	-		X	-
Poland	-	-	X	X	-
Portugal	-	-	X	X	-
Romania	-	-	X	X	-
Slovakia	-	-	X	X	-
Slovenia	-	-	X	X	-
Spain	-	-	X	X	-
Sweden	-	-		X	-
United Kingdom	-	-	-	X	-

Biogeographical regions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Alpine	-	-	X	X	-
Atlantic	YES	YES	X	X	?
Black Sea	-	-	X	X	-
Boreal	-	-	X	X	-
Continental	YES	YES	X	X	YES
Mediterranean	-	-	X	X	-
Pannonian	-	-	X	X	-
Steppic	-	-	X	X	-

Marine regions and subregions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Baltic Sea					
Black Sea					
North-east Atlantic Ocean					
Bay of Biscay and the Iberian Coast					
Celtic Sea					
Greater North Sea					
Mediterranean Sea					
Adriatic Sea					
Aegean-Levantine Sea					
Ionian Sea and the Central Mediterranean Sea					
Western Mediterranean Sea					

ANNEX I Scoring of Likelihoods of Events

(taken from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Description	Frequency
Very unlikely	This sort of event is theoretically possible, but is never known to have occurred and is not expected to occur	1 in 10,000 years
Unlikely	This sort of event has occurred somewhere at least once in the last millenium	1 in 1,000 years
Moderately likely	This sort of event has occurred somewhere at least once in the last century	1 in 100 years
Likely	This sort of event has happened on several occasions elsewhere, or on at least once in the last decade	1 in 10 years
Very likely	This sort of event happens continually and would be expected to occur	Once a year

ANNEX II Scoring of Magnitude of Impacts

(modified from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Biodiversity and ecosystem impact	Ecosystem Services impact	Economic impact (Monetary loss and response costs per year)	Social and human health impact, and other impacts
	<i>Question 5.1-5</i>	<i>Question 5.6-8</i>	<i>Question 5.9-13</i>	<i>Question 5.14-18</i>
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected ⁵	Up to 10,000 Euro	No social disruption. Local, mild, short-term reversible effects to individuals.
Minor	Some ecosystem impact, reversible changes, localised	Local and temporary, reversible effects to one or few services	10,000-100,000 Euro	Significant concern expressed at local level. Mild short-term reversible effects to identifiable groups, localised.
Moderate	Measureable long-term damage to populations and ecosystem, but reversible; little spread, no extinction	Measureable, temporary, local and reversible effects on one or several services	100,000-1,000,000 Euro	Temporary changes to normal activities at local level. Minor irreversible effects and/or larger numbers covered by reversible effects, localised.
Major	Long-term irreversible ecosystem change, spreading beyond local area	Local and irreversible or widespread and reversible effects on one / several services	1,000,000-10,000,000 Euro	Some permanent change of activity locally, concern expressed over wider area. Significant irreversible effects locally or reversible effects over large area.
Massive	Widespread, long-term population loss or extinction, affecting several species with serious ecosystem effects	Widespread and irreversible effects on one / several services	Above 10,000,000 Euro	Long-term social change, significant loss of employment, migration from affected area. Widespread, severe, long-term, irreversible health effects.

⁵ Not to be confused with “no impact”.

ANNEX III Scoring of Confidence Levels

(modified from Bacher et al. 2017)

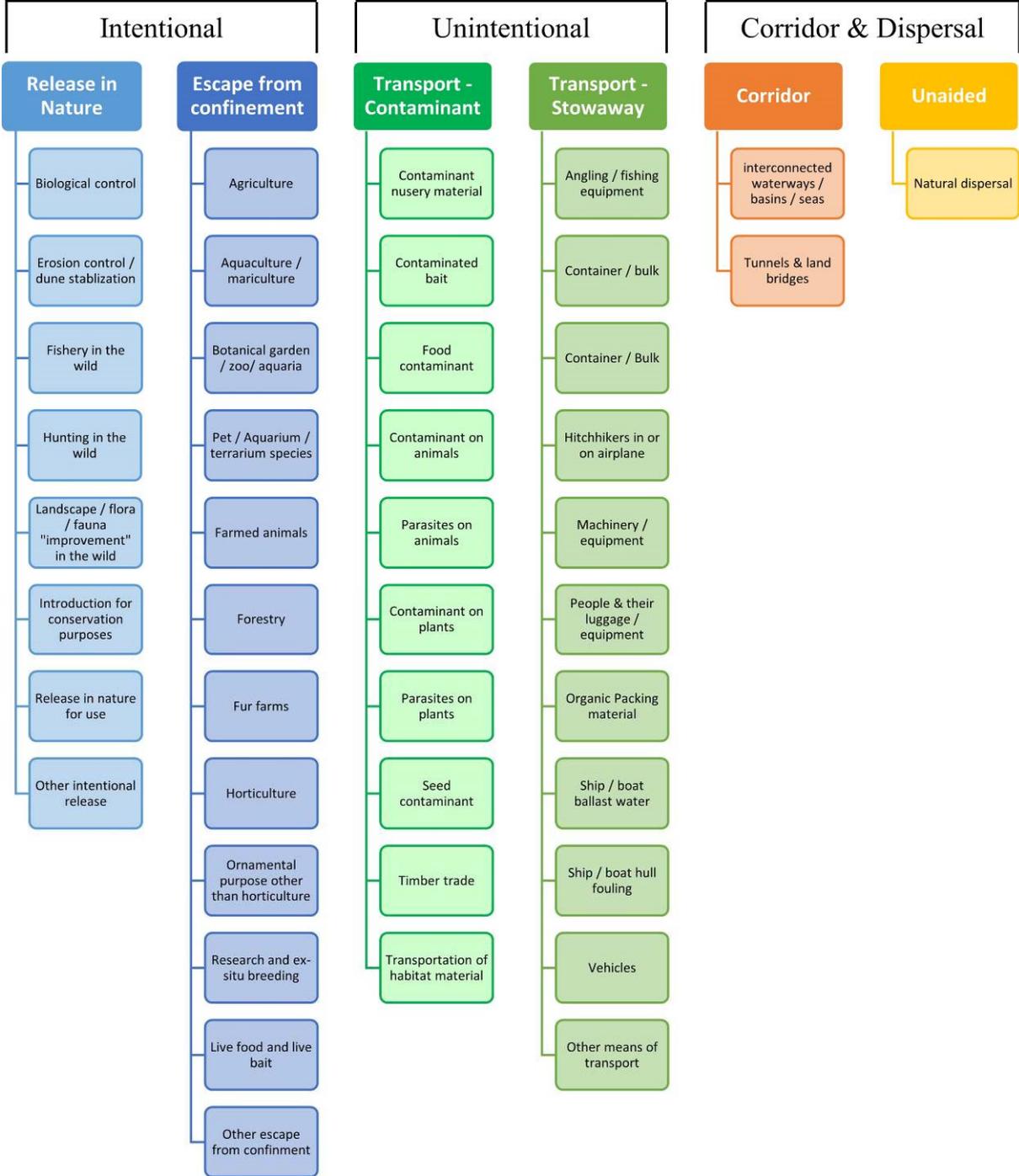
Each answer provided in the risk assessment must include an assessment of the level of confidence attached to that answer, reflecting the possibility that information needed for the answer is not available or is insufficient or available but conflicting.

The responses in the risk assessment should clearly support the choice of the confidence level.

Confidence level	Description
Low	There is no direct observational evidence to support the assessment, e.g. only inferred data have been used as supporting evidence <i>and/or</i> Impacts are recorded at a spatial scale which is unlikely to be relevant to the assessment area <i>and/or</i> Evidence is poor and difficult to interpret, e.g. because it is strongly ambiguous <i>and/or</i> The information sources are considered to be of low quality or contain information that is unreliable.
Medium	There is some direct observational evidence to support the assessment, but some information is inferred <i>and/or</i> Impacts are recorded at a small spatial scale, but rescaling of the data to relevant scales of the assessment area is considered reliable, or to embrace little uncertainty <i>and/or</i> The interpretation of the data is to some extent ambiguous or contradictory.
High	There is direct relevant observational evidence to support the assessment (including causality) <i>and</i> Impacts are recorded at a comparable scale <i>and/or</i> There are reliable/good quality data sources on impacts of the taxa <i>and</i> The interpretation of data/information is straightforward <i>and/or</i> Data/information are not controversial or contradictory.

ANNEX IV CBD pathway categorisation scheme

Overview of CBD pathway categorisation scheme showing how the 44 pathways relate to the six main pathway categories. All of the pathways can be broadly classified into 1) those that involve intentional transport (blue), 2) those in which the taxa are unintentionally transported (green) and 3) those where taxa moved between regions without direct transportation by humans and/or via artificial corridors (orange and yellow). **Note that the pathways in the category “Escape from confinement” can be considered intentional for the introduction into the risk assessment area and unintentional for the entry into the environment.**



ANNEX V Ecosystem services classification (CICES V5.1, simplified) and examples

For the purposes of this risk assessment, please feel free to use what seems as the most appropriate category / level / combination of impact (Section – Division – Group), reflecting information available.

Section	Division	Group	Examples (i.e. relevant CICES “classes”)
Provisioning	Biomass	Cultivated <i>terrestrial</i> plants	<p>Cultivated terrestrial plants (including fungi, algae) grown for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from cultivated plants, fungi, algae and bacteria for direct use or processing (excluding genetic materials); Cultivated plants (including fungi, algae) grown as a <u>source of energy</u></p> <p><i>Example: negative impacts of non-native organisms to crops, orchards, timber etc.</i></p>
		Cultivated <i>aquatic</i> plants	<p>Plants cultivated by in- situ aquaculture grown for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from in-situ aquaculture for direct use or processing (excluding genetic materials); Plants cultivated by in- situ aquaculture grown as an <u>energy source</u>.</p> <p><i>Example: negative impacts of non-native organisms to aquatic plants cultivated for nutrition, gardening etc. purposes.</i></p>
		Reared animals	<p>Animals reared for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from reared animals for direct use or processing (excluding genetic materials); Animals reared to provide <u>energy</u> (including mechanical)</p> <p><i>Example: negative impacts of non-native organisms to livestock</i></p>
		Reared <i>aquatic</i> animals	<p>Animals reared by in-situ aquaculture for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from animals grown by in-situ aquaculture for direct use or processing (excluding genetic materials); Animals reared by in-situ aquaculture as an <u>energy source</u></p> <p><i>Example: negative impacts of non-native organisms to fish farming</i></p>
		Wild plants (terrestrial and aquatic)	<p>Wild plants (terrestrial and aquatic, including fungi, algae) used for <u>nutrition</u>; <u>Fibres and other materials</u> from wild plants for direct use or processing (excluding genetic materials); Wild plants (terrestrial and aquatic, including fungi, algae) used as a <u>source of energy</u></p> <p><i>Example: reduction in the availability of wild plants (e.g. wild berries, ornamentals) due to non-native organisms (competition, spread of disease etc.)</i></p>
		Wild animals (terrestrial and aquatic)	<p>Wild animals (terrestrial and aquatic) used for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from wild animals for direct use or processing (excluding genetic materials); Wild animals (terrestrial and aquatic) used as a <u>source of energy</u></p>

			<i>Example: reduction in the availability of wild animals (e.g. fish stocks, game) due to non-native organisms (competition, predations, spread of disease etc.)</i>
	Genetic material from all biota	Genetic material from plants, algae or fungi	<u>Seeds, spores and other plant materials</u> collected for maintaining or establishing a population; Higher and lower plants (whole organisms) used to <u>breed new strains or varieties</u> ; Individual genes extracted from higher and lower plants for the <u>design and construction of new biological entities</u> <i>Example: negative impacts of non-native organisms due to interbreeding</i>
		Genetic material from animals	Animal material collected for the purposes of maintaining or establishing a population; Wild animals (whole organisms) used to breed new strains or varieties; Individual genes extracted from organisms for the design and construction of new biological entities <i>Example: negative impacts of non-native organisms due to interbreeding</i>
	Water ⁶	Surface water used for nutrition, materials or energy	Surface water for <u>drinking</u> ; Surface water used as a material (<u>non-drinking purposes</u>); Freshwater surface water, coastal and marine water used as an <u>energy source</u> <i>Example: loss of access to surface water due to spread of non-native organisms</i>
		Ground water for used for nutrition, materials or energy	Ground (and subsurface) water for <u>drinking</u> ; Ground water (and subsurface) used as a material (<u>non-drinking purposes</u>); Ground water (and subsurface) used as an <u>energy source</u> <i>Example: reduced availability of ground water due to spread of non-native organisms and associated increase of ground water consumption by vegetation.</i>
Regulation & Maintenance	Transformation of biochemical or physical inputs to ecosystems	Mediation of wastes or toxic substances of anthropogenic origin by living processes	<u>Bio-remediation</u> by micro-organisms, algae, plants, and animals; <u>Filtration/sequestration/storage/accumulation</u> by micro-organisms, algae, plants, and animals <i>Example: changes caused by non-native organisms to ecosystem functioning and ability to filtrate etc. waste or toxics</i>
		Mediation of nuisances of anthropogenic origin	<u>Smell reduction</u> ; <u>noise attenuation</u> ; <u>visual screening</u> (e.g. by means of green infrastructure) <i>Example: changes caused by non-native organisms to ecosystem structure, leading to reduced ability to mediate nuisances.</i>
	Regulation of physical, chemical, biological conditions	Baseline flows and extreme event regulation	Control of <u>erosion</u> rates; Buffering and attenuation of <u>mass movement</u> ; <u>Hydrological cycle and water flow regulation</u> (Including flood control, and coastal protection); <u>Wind</u> protection; <u>Fire</u> protection

⁶ Note: in the CICES classification provisioning of water is considered as an abiotic service whereas the rest of ecosystem services listed here are considered biotic.

			<p><i>Example: changes caused by non-native organisms to ecosystem functioning or structure leading to, for example, destabilisation of soil, increased risk or intensity of wild fires etc.</i></p>
		<p>Lifecycle maintenance, habitat and gene pool protection</p>	<p>Pollination (or 'gamete' dispersal in a marine context); <u>Seed dispersal</u>; Maintaining <u>nursery populations and habitats</u> (Including gene pool protection)</p> <p><i>Example: changes caused by non-native organisms to the abundance and/or distribution of wild pollinators; changes to the availability / quality of nursery habitats for fisheries</i></p>
		<p>Pest and disease control</p>	<p>Pest control; Disease control</p> <p><i>Example: changes caused by non-native organisms to the abundance and/or distribution of pests</i></p>
		<p>Soil quality regulation</p>	<p><u>Weathering processes</u> and their effect on soil quality; <u>Decomposition and fixing processes</u> and their effect on soil quality</p> <p><i>Example: changes caused by non-native organisms to vegetation structure and/or soil fauna leading to reduced soil quality</i></p>
		<p>Water conditions</p>	<p>Regulation of the <u>chemical condition</u> of freshwaters by living processes; Regulation of the chemical condition of salt waters by living processes</p> <p><i>Example: changes caused by non-native organisms to buffer strips along water courses that remove nutrients in runoff and/or fish communities that regulate the resilience and resistance of water bodies to eutrophication</i></p>
		<p>Atmospheric composition and conditions</p>	<p>Regulation of <u>chemical composition</u> of atmosphere and oceans; Regulation of <u>temperature and humidity</u>, including ventilation and transpiration</p> <p><i>Example: changes caused by non-native organisms to ecosystems' ability to sequester carbon and/or evaporative cooling (e.g. by urban trees)</i></p>
Cultural	Direct, in-situ and outdoor interactions with living systems that depend on presence in the environmental setting	<p>Physical and experiential interactions with natural environment</p>	<p>Characteristics of living systems that that enable activities promoting health, recuperation or enjoyment through <u>active or immersive interactions</u>;</p> <p>Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through <u>passive or observational interactions</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that make it attractive for recreation, wild life watching etc.</i></p>
		<p>Intellectual and representative interactions with natural environment</p>	<p>Characteristics of living systems that enable <u>scientific investigation</u> or the creation of traditional ecological knowledge;</p> <p>Characteristics of living systems that enable <u>education and training</u>;</p> <p>Characteristics of living systems that are resonant in terms of <u>culture or heritage</u>;</p> <p>Characteristics of living systems that enable <u>aesthetic experiences</u></p>

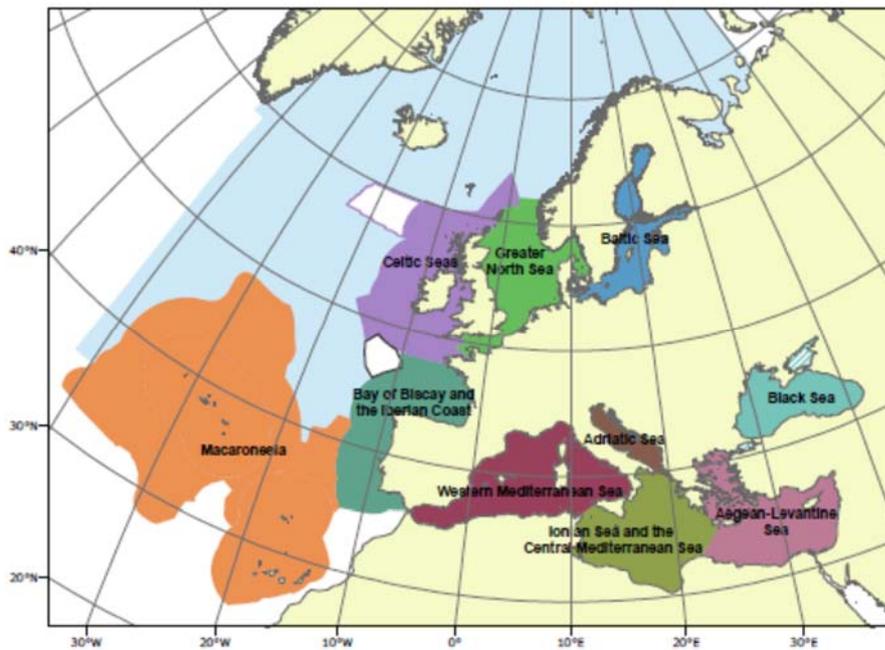
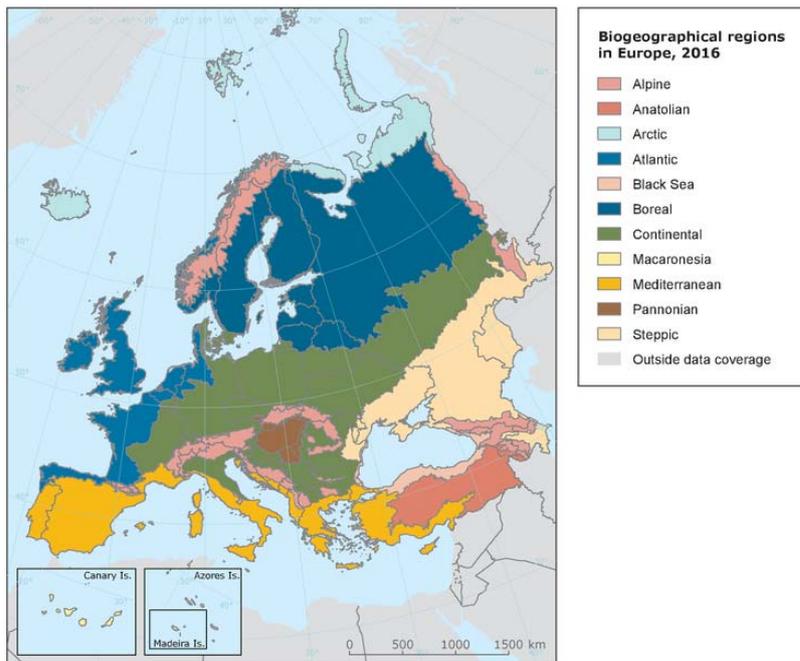
			<i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have cultural importance</i>
	Indirect, remote, often indoor interactions with living systems that do not require presence in the environmental setting	Spiritual, symbolic and other interactions with natural environment	<p>Elements of living systems that have <u>symbolic meaning</u>;</p> <p>Elements of living systems that have <u>sacred or religious meaning</u>;</p> <p>Elements of living systems used for <u>entertainment or representation</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have sacred or religious meaning</i></p>
		Other biotic characteristics that have a non-use value	<p>Characteristics or features of living systems that have an <u>existence value</u>;</p> <p>Characteristics or features of living systems that have an <u>option or bequest value</u></p> <p><i>Example: changes caused by non-native organisms to ecosystems designated as wilderness areas, habitats of endangered species etc.</i></p>

ANNEX VI EU Biogeographic Regions and MSFD Subregions

See <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2> ,
http://ec.europa.eu/environment/nature/natura2000/biogeog_regions/

and

<https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions-1/technical-document/pdf>



ANNEX VII Delegated Regulation (EU) 2018/968 of 30 April 2018

see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>

ANNEX VIII Species Distribution Model

Projection of environmental suitability for *Faxonius immunis* establishment in Europe

Björn Beckmann, Elena Tricarico, Frances Lucy and Dan Chapman

10 October 2020

Aim

To project the suitability for potential establishment of *Faxonius immunis* in Europe, under current and predicted future climatic conditions.

Data for modelling

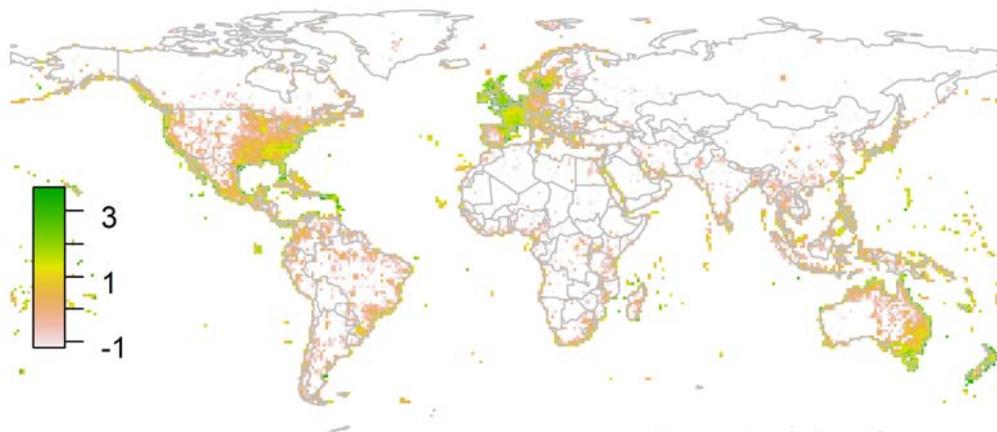
Species occurrence data were obtained from the Integrated Digitized Biocollections (iDigBio) (1038 records), the Global Biodiversity Information Facility (GBIF) (371 records), the Biodiversity Information Serving Our Nation database (BISON) (247 records), iNaturalist (82 records), and additional records from the risk assessment team. We scrutinised occurrence records from regions where the species is not known to be established and removed any dubious records (e.g. fossils) or where the georeferencing was too imprecise (e.g. records referenced to a country or island centroid) or outside of the coverage of the predictor layers (e.g. small island or coastal occurrences). The remaining records were gridded at a 0.25 x 0.25 degree resolution for modelling, yielding 550 grid cells with occurrences (Figure 1a). As a proxy for recording effort, the density of Decapoda records held by GBIF was also compiled on the same grid (Figure 1b).

Figure 1. (a) Occurrence records obtained for *Faxonius immunis* and used in the modelling, showing native and invaded distributions. (b) The recording density of Decapoda on GBIF, which was used as a proxy for recording effort.

(a) Species distribution used in modelling



(b) Estimated recording effort (log10-scaled)



Climate data were selected from the 'Bioclim' variables contained within the WorldClim database (Hijmans et al. 2005), originally at 5 arcminute resolution (0.083 x 0.083 degrees of longitude/latitude) and aggregated to a 0.25 x 0.25 degree grid for use in the model.

Based on the biology of *Faxonius immunis*, the following climate variables were used in the modelling:

- Temperature seasonality (Bio4)
- Maximum temperature of the warmest month (Bio5)
- Minimum temperature of the coldest month (Bio6)
- Mean temperature of the wettest quarter (Bio8)
- Annual precipitation (Bio12)
- Precipitation of the wettest month (Bio13)

- Precipitation seasonality (Bio15)

To estimate the effect of climate change on the potential distribution, equivalent modelled future climate conditions for the 2070s under the Representative Concentration Pathways (RCP) 2.6 and 4.5 were also obtained. These represent low and medium emissions scenarios, respectively. The above variables were obtained as averages of outputs of eight Global Climate Models (BCC-CSM1-1, CCSM4, GISS-E2-R, HadGEM2-AO, IPSL-CM5A-LR, MIROC-ESM, MRI-CGCM3, NorESM1-M), downscaled and calibrated against the WorldClim baseline (see http://www.worldclim.org/cmip5_5m).

Species distribution model

A presence-background (presence-only) ensemble modelling strategy was employed using the BIOMOD2 R package version 3.4.6 (Thuiller et al. 2009; Thuiller et al. 2020). These models contrast the environment at the species' occurrence locations against a random sample of the global background environmental conditions (often termed 'pseudo-absences') in order to characterise and project suitability for occurrence. This approach has been developed for distributions that are in equilibrium with the environment. Because invasive species' distributions are not at equilibrium and subject to dispersal constraints at a global scale, we took care to minimise the inclusion of locations suitable for the species but where it has not been able to disperse to (Chapman et al. 2019). Therefore the background sampling region included:

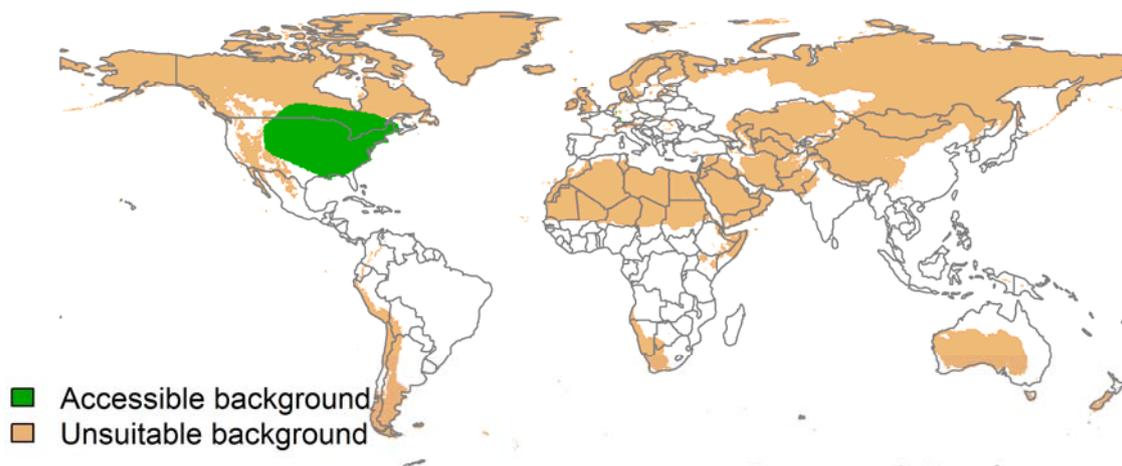
- The area accessible by native *Faxonius immunis* populations, in which the species is likely to have had sufficient time to disperse to all locations. Based on presumed maximum dispersal distances, the accessible region was defined as a 400km buffer around the native range occurrences; AND
- A 30km buffer around the non-native occurrences, encompassing regions likely to have had high propagule pressure for introduction by humans and/or dispersal of the species; AND
- Regions where we have an *a priori* expectation of high unsuitability for the species so that absence is assumed irrespective of dispersal constraints (see Figure 2). The following rules were applied to define a region expected to be highly unsuitable for *Faxonius immunis* at the spatial scale of the model:
 - Minimum temperature of the coldest month (Bio6) < -24°C
 - Maximum temperature of the warmest month (Bio5) < 21°C
 - Annual precipitation (Bio12) < 350mm

Altogether, only 0.7% of occurrence grid cells were located in the unsuitable background region.

Within the unsuitable background region, 10 samples of 5000 randomly sampled grid cells were obtained. In the accessible background (comprising the accessible areas around native and non-native occurrences as detailed above), the same number of pseudo-absence samples were drawn as there were presence records (550), weighting the sampling by a proxy for recording effort (Figure 2).

Figure 2. The background from which pseudo-absence samples were taken in the modelling of *Faxonius immunis*. Samples were taken from a 400km buffer around the native range and a 30km buffer around non-native occurrences (together forming the accessible background), and from areas

expected to be highly unsuitable for the species (the unsuitable background region). Samples from the accessible background were weighted by a proxy for recording effort (Figure 1(b)).



Each dataset (i.e. combination of the presences and the individual background samples) was randomly split into 80% for model training and 20% for model evaluation. With each training dataset, five statistical algorithms were fitted with the default BIOMOD2 settings and rescaled using logistic regression, except where specified below:

- Generalised linear model (GLM)
- Generalised boosting model (GBM)
- Generalised additive model (GAM) with a maximum of four degrees of freedom per smoothing spline
- Random forest (RF)
- Maxent

Since the total background sample was larger than the number of occurrences, prevalence fitting weights were applied to give equal overall importance to the occurrences and the background. Normalised variable importance was assessed and variable response functions were produced using BIOMOD2's default procedure.

Model predictive performance was assessed by the following three measures:

- AUC, the area under the receiver operating characteristic curve (Fielding & Bell 1997). Predictions of presence-absence models can be compared with a subset of records set aside for model evaluation (here 20%) by constructing a confusion matrix with the number of true positive, false positive, false negative and true negative cases. For models generating non-dichotomous scores (as here) a threshold can be applied to transform the scores into a dichotomous set of presence-absence predictions. Two measures that can be derived from the confusion matrix are sensitivity (the proportion of observed presences that are predicted as such, quantifying omission errors), and specificity (the proportion of observed absences that are predicted as such, quantifying commission errors). A receiver operating characteristic (ROC) curve can be constructed by using

all possible thresholds to classify the scores into confusion matrices, obtaining sensitivity and specificity for each matrix, and plotting sensitivity against the corresponding proportion of false positives (equal to $1 - \text{specificity}$). The use of all possible thresholds avoids the need for a selection of a single threshold, which is often arbitrary, and allows appreciation of the trade-off between sensitivity and specificity. The area under the ROC curve (AUC) is often used as a single threshold-independent measure for model performance (Manel et al. 2001). AUC is the probability that a randomly selected presence has a higher model-predicted suitability than a randomly selected absence (Allouche et al. 2006).

- Cohen's Kappa (Cohen 1960). This measure corrects the overall accuracy of model predictions (ratio of the sum of true presences plus true absences to the total number of records) by the accuracy expected to occur by chance. The kappa statistic ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random. Advantages of kappa are its simplicity, the fact that both commission and omission errors are accounted for in one parameter, and its relative tolerance to zero values in the confusion matrix (Manel et al. 2001). However, Kappa has been criticised for being sensitive to prevalence (the proportion of sites in which the species was recorded as present) and may therefore be inappropriate for comparisons of model accuracy between species or regions (McPherson et al. 2004; Allouche et al. 2006).
- TSS, the true skill statistic (Allouche et al. 2006). TSS is defined as $\text{sensitivity} + \text{specificity} - 1$, and corrects for Kappa's dependency on prevalence. TSS compares the number of correct forecasts, minus those attributable to random guessing, to that of a hypothetical set of perfect forecasts. Like kappa, TSS takes into account both omission and commission errors, and success as a result of random guessing, and ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random (Allouche et al. 2006).

An ensemble model was created by first rejecting poorly performing algorithms with relatively extreme low AUC values and then averaging the predictions of the remaining algorithms, weighted by their AUC. To identify poorly performing algorithms, AUC values were converted into modified z-scores based on their difference to the median and the median absolute deviation across all algorithms (Iglewicz & Hoaglin 1993). Algorithms with $z < -2$ were rejected. In this way, ensemble projections were made for each dataset and then averaged to give an overall suitability, as well as its standard deviation. The projections were then classified into suitable and unsuitable regions using the 'minROCDist' method, which minimizes the distance between the ROC plot and the upper left corner of the plot (point (0,1)).

We also produced limiting factor maps for Europe following Elith et al. (2010). For this, projections were made separately with each individual variable fixed at a near-optimal value. These were chosen as the median values at the occurrence grid cells. Then, the most strongly limiting factors were identified as the one resulting in the highest increase in suitability in each grid cell.

Results

The ensemble model suggested that suitability for *Faxonius immunis* was most strongly determined by Maximum temperature of the warmest month (Bio5), accounting for 30.6% of variation explained, followed by Annual precipitation (Bio12) (28.5%), Temperature seasonality (Bio4) (18.5%), Minimum temperature of the coldest month (Bio6) (7.9%), Precipitation of the wettest month (Bio13) (7.5%), Mean temperature of the wettest quarter (Bio8) (4.3%) and Precipitation seasonality (Bio15) (2.5%) (Table 1, Figure 3).

Table 1. Summary of the cross-validation predictive performance (AUC, Kappa, TSS) and variable importance of the fitted model algorithms and the ensemble (AUC-weighted average of the best performing algorithms). Results are the average from models fitted to 10 different background samples of the data.

Algorithm	AUC	Kappa	TSS	Used in the ensemble	Variable importance (%)						
					Maximum temperature of the warmest month (Bio5)	Annual precipitation (Bio12)	Temperature seasonality (Bio4)	Minimum temperature of the coldest month (Bio6)	Precipitation of the wettest month (Bio13)	Mean temperature of the wettest quarter (Bio8)	Precipitation seasonality (Bio15)
GLM	0.986	0.820	0.936	yes	28	33	7	11	10	6	5
GAM	0.987	0.835	0.945	yes	26	24	13	16	11	6	3
GBM	0.988	0.831	0.948	yes	40	35	19	1	3	1	0
RF	0.990	0.862	0.950	yes	28	22	35	4	6	4	1
Maxent	0.945	0.800	0.857	no	35	20	15	12	9	4	5
Ensemble	0.990	0.856	0.946		31	29	19	8	7	4	3

Figure 3. Partial response plots from the fitted models. Thin coloured lines show responses from the algorithms in the ensemble, while the thick black line is their ensemble. In each plot, other model variables are held at their median value in the training data. Some of the divergence among algorithms is because of their different treatment of interactions among variables.

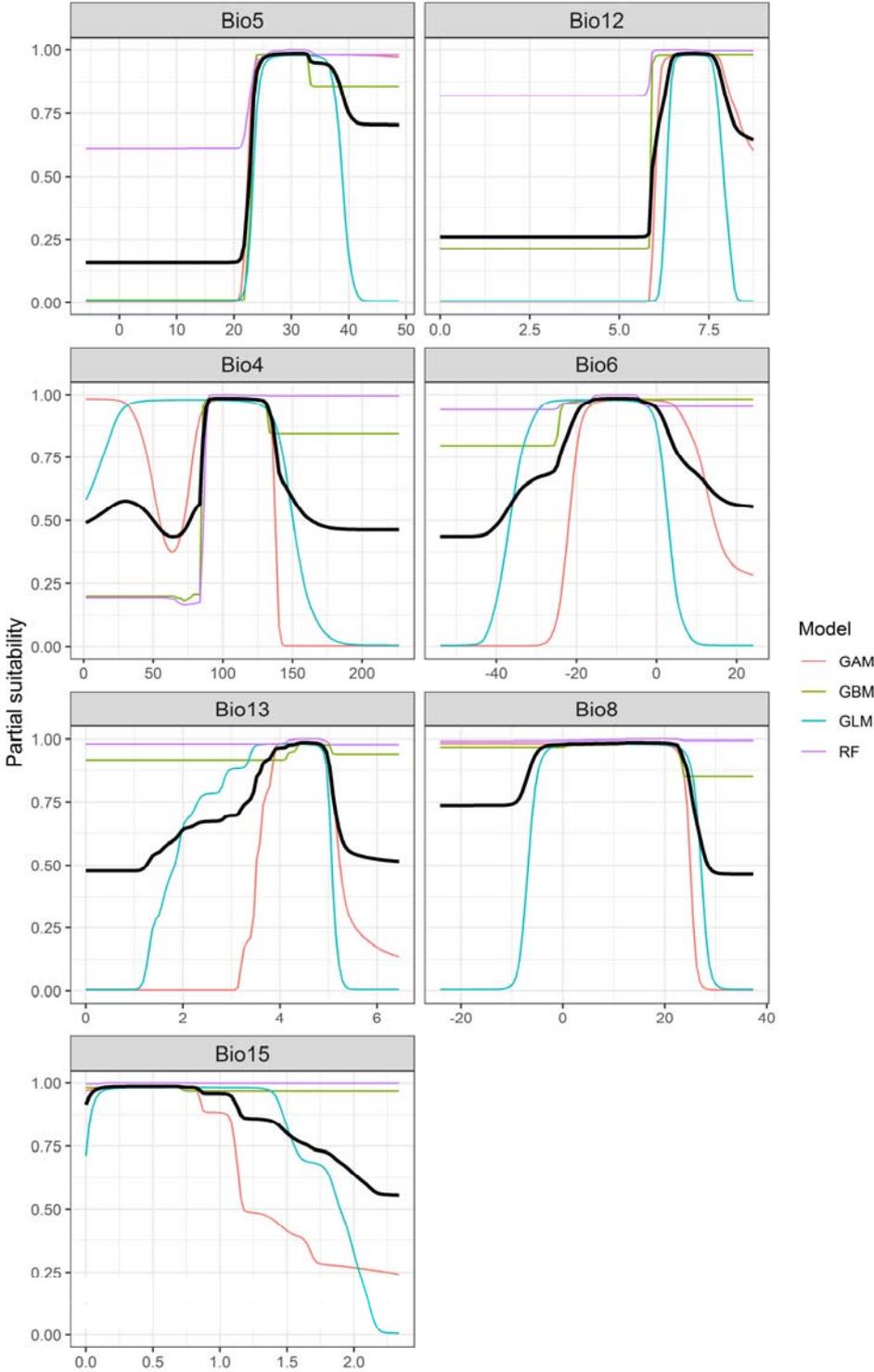


Figure 4. (a) Projected global suitability for *Faxonius immunis* establishment in the current climate. For visualisation, the projection has been aggregated to a 0.5 x 0.5 degree resolution, by taking the maximum suitability of constituent higher resolution grid cells. Values > 0.47 may be suitable for the species. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

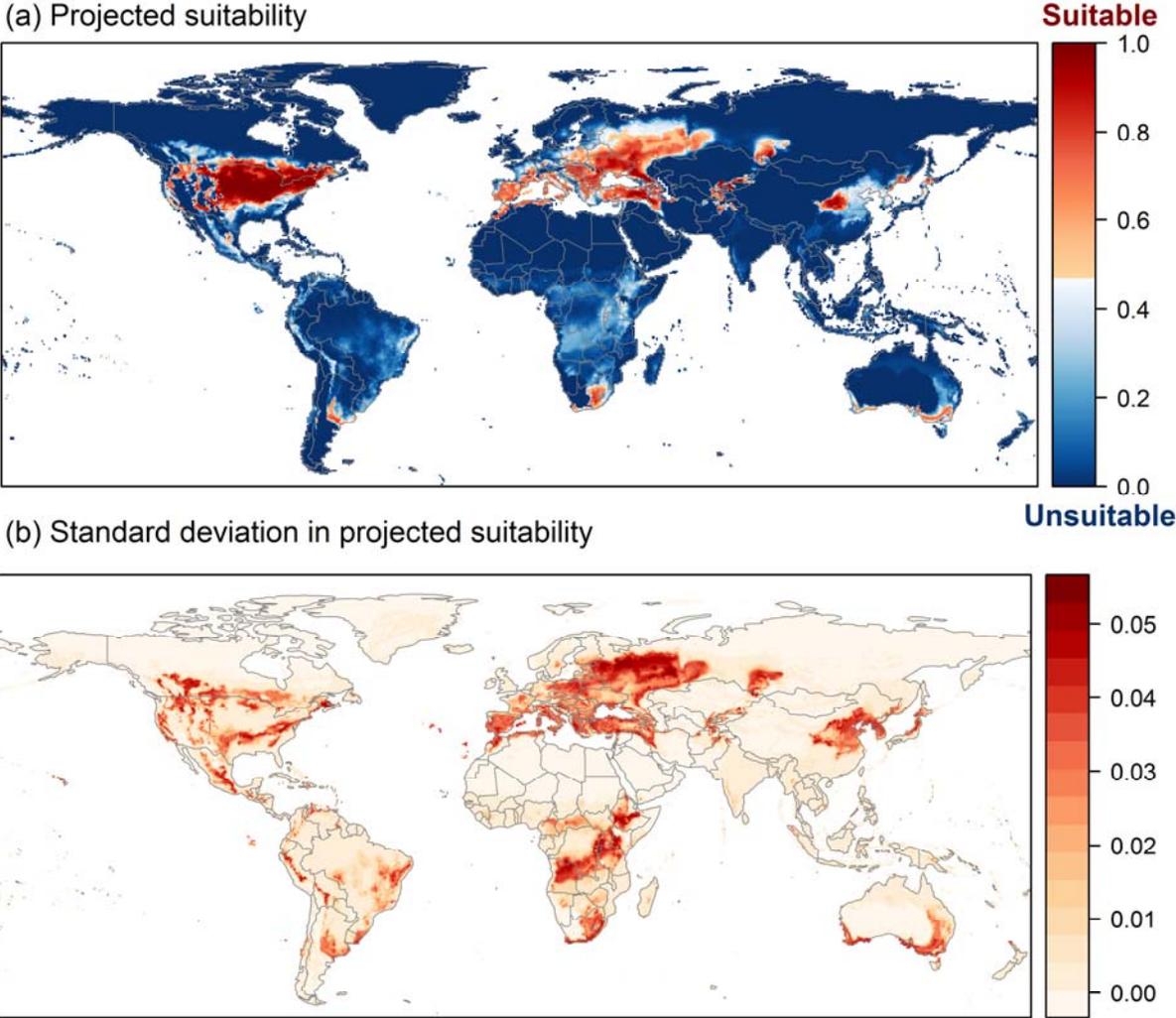


Figure 5. (a) Projected current suitability for *Faxonius immunis* establishment in Europe and the Mediterranean region. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

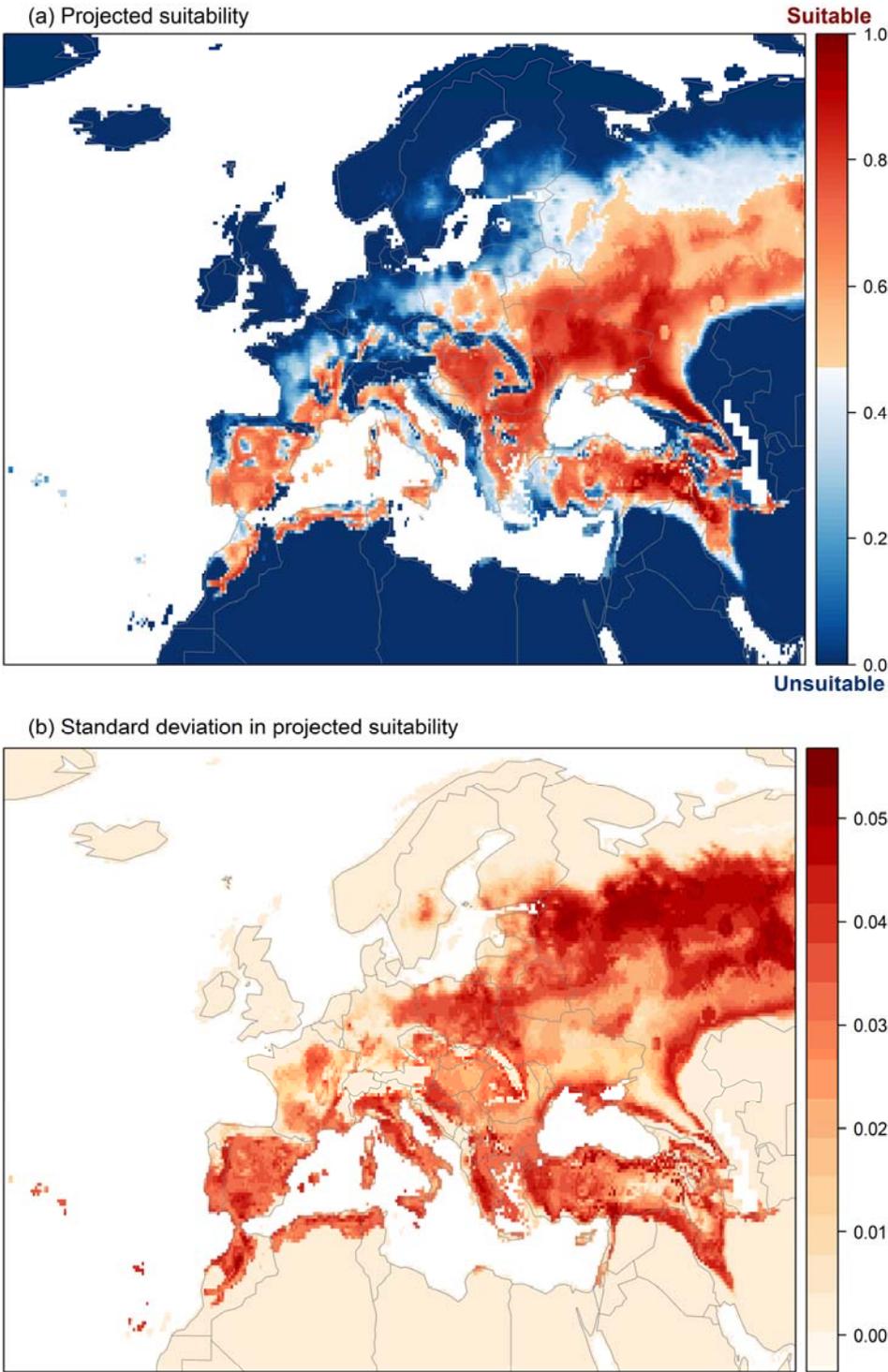


Figure 6. The most strongly limiting factors for *Faxonius immunis* establishment estimated by the model in Europe and the Mediterranean region in current climatic conditions.

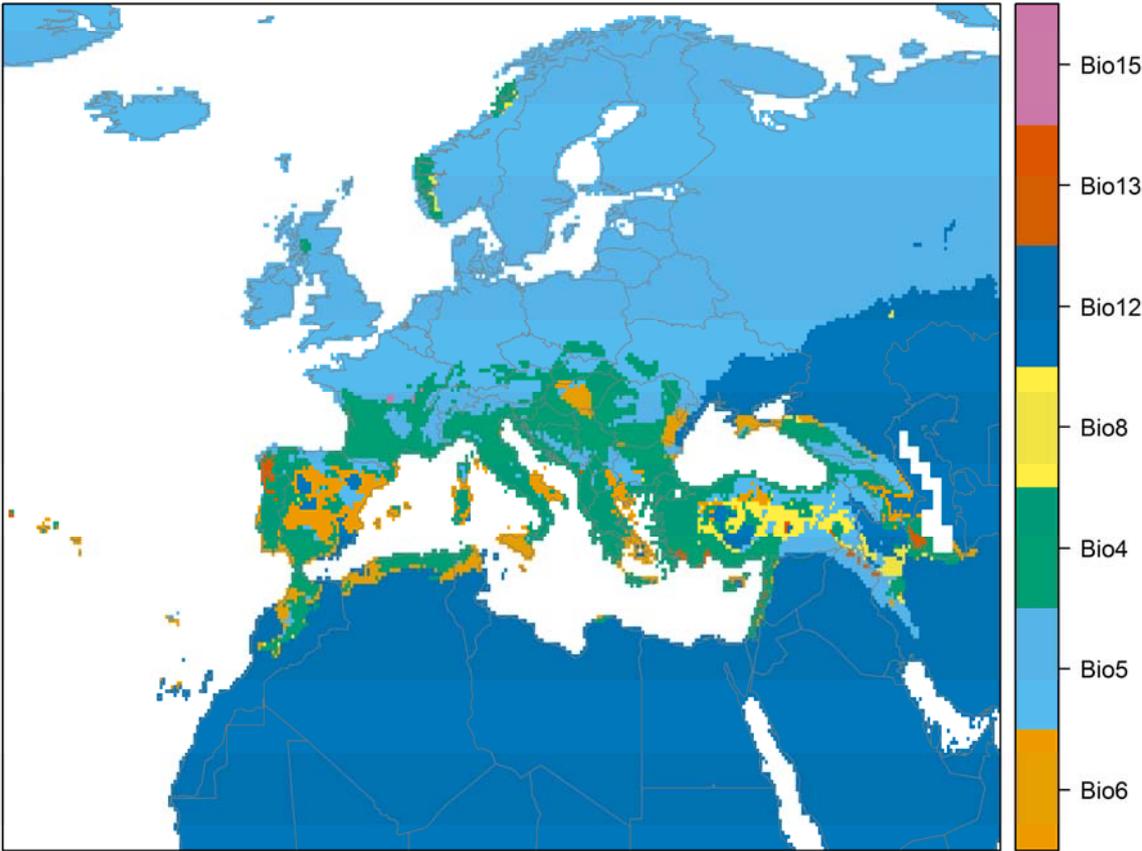


Figure 7. (a) Projected suitability for *Faxonius immunis* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP2.6, equivalent to Figure 5. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

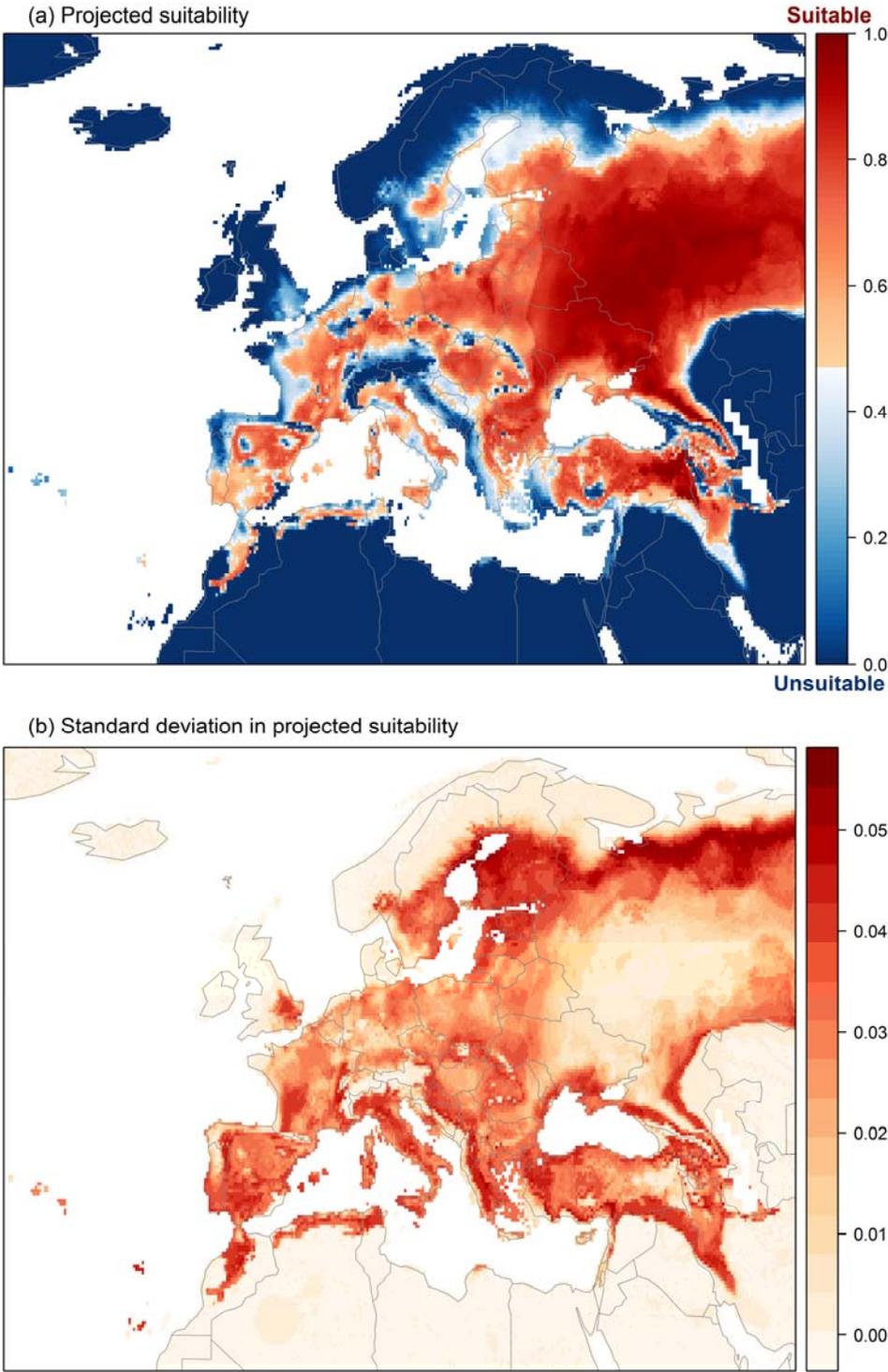


Figure 8. (a) Projected suitability for *Faxonius immunis* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP4.5, equivalent to Figure 5. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

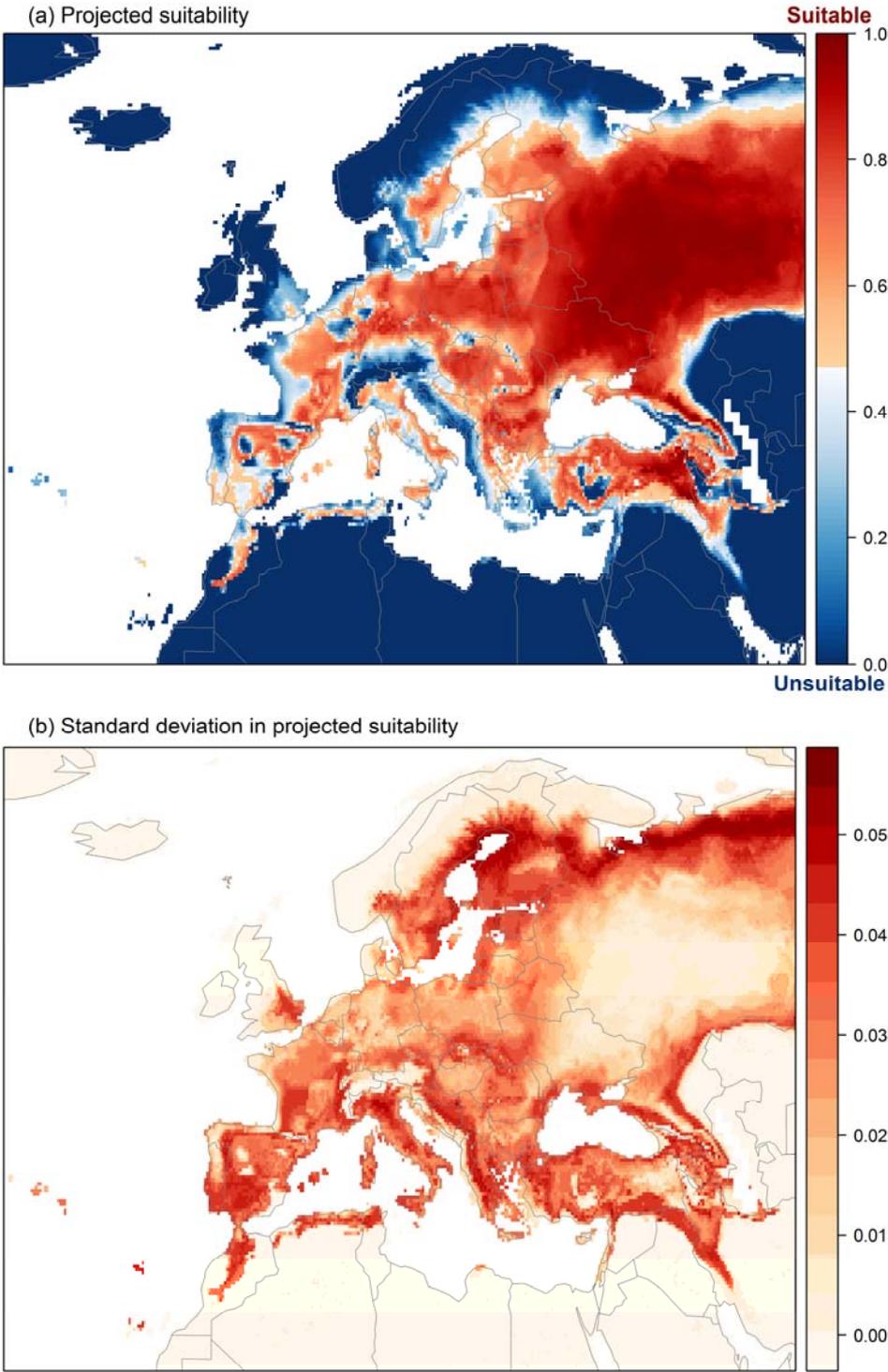


Figure 9. Variation in projected suitability for *Faxonius immunis* establishment among Biogeographical Regions of Europe (<https://www.eea.europa.eu/data-and-maps/data/biogeographical-regions-europe-3>). The bar plots show the proportion of grid cells in each region classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. The location of each region is also shown. The Arctic and Macaronesian biogeographical regions are not part of the study area, but are included for completeness.

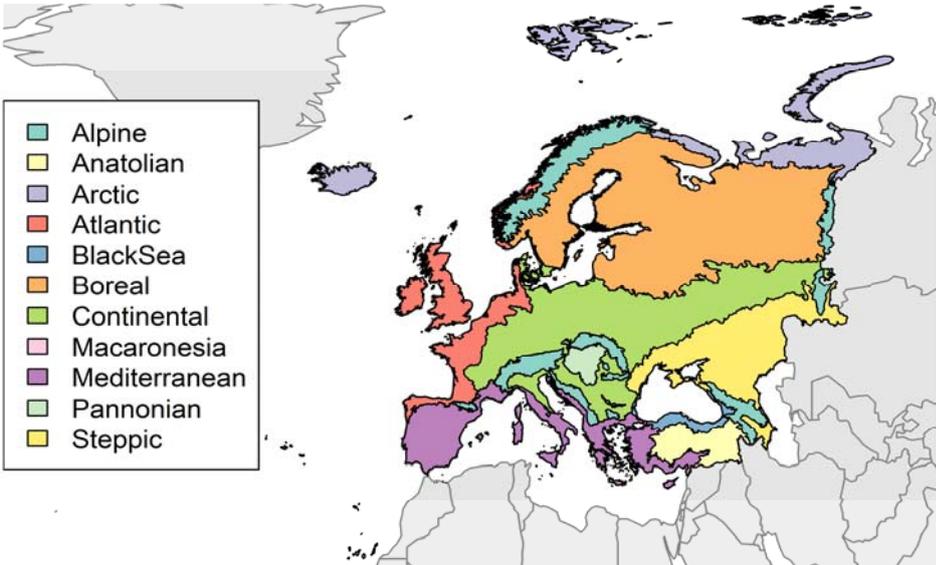
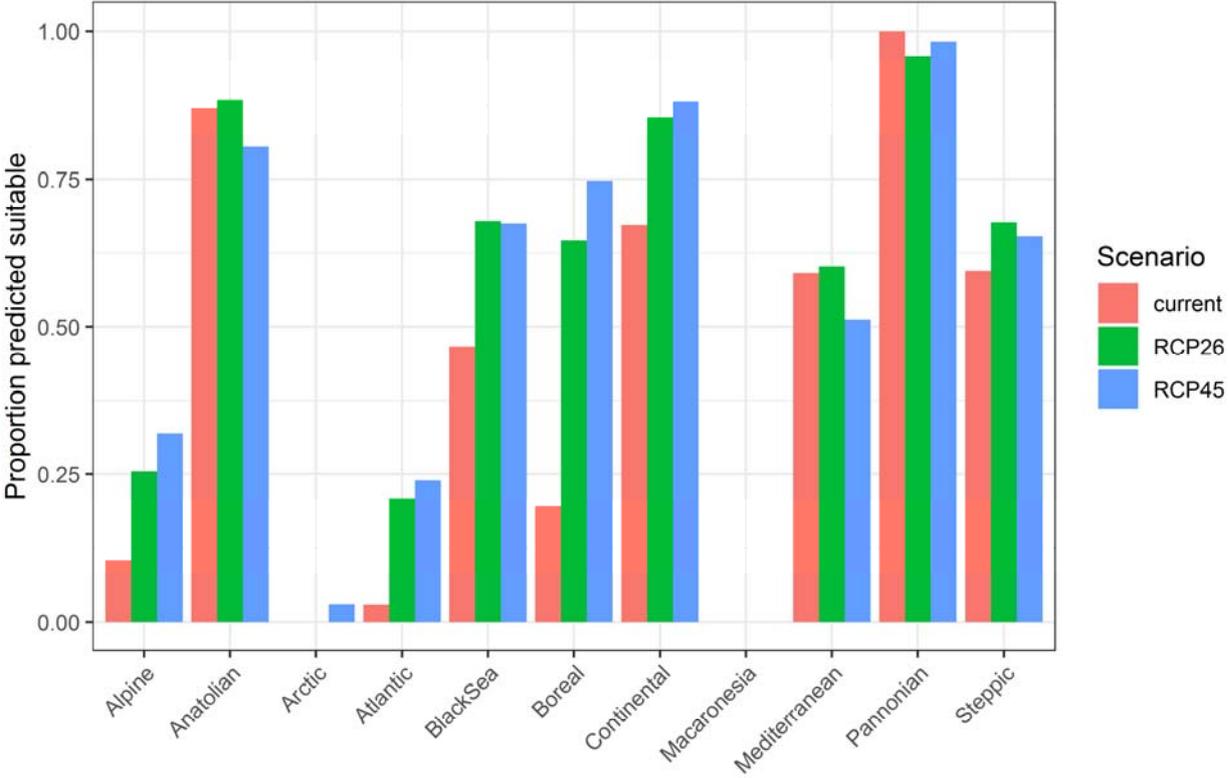


Table 2. Variation in projected suitability for *Faxonius immunis* establishment among Biogeographical regions of Europe (numerical values of Figure 9 above). The numbers are the proportion of grid cells in each region classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. The Arctic and Macaronesian biogeographical regions are not part of the study area, but are included for completeness.

Biogeographical region	Current climate	RCP26	RCP45
Alpine	0.10	0.25	0.32
Anatolian	0.87	0.88	0.80
Arctic	0.00	0.00	0.03
Atlantic	0.03	0.21	0.24
BlackSea	0.47	0.68	0.67
Boreal	0.19	0.64	0.75
Continental	0.67	0.85	0.88
Macaronesia	0.00	0.00	0.00
Mediterranean	0.59	0.60	0.51
Pannonian	1.00	0.96	0.98
Steppic	0.59	0.68	0.65

Figure 10. Variation in projected suitability for *Faxonius immunis* establishment among European Union countries and the UK. The bar plots show the proportion of grid cells in each country classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios.

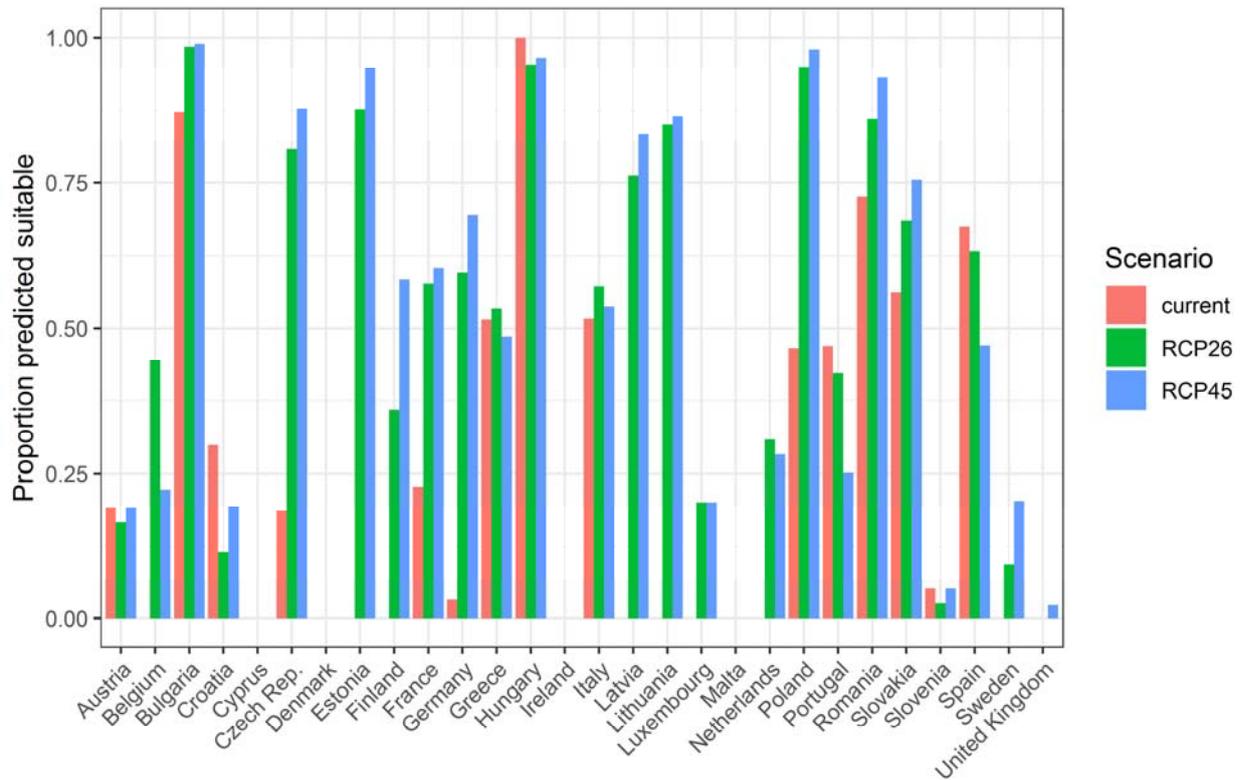


Table 3. Variation in projected suitability for *Faxonius immunis* establishment among European Union countries and the UK (numerical values of Figure 10 above). The numbers are the proportion of grid cells in each country classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios.

Country	Current climate	RCP26	RCP45
Austria	0.19	0.17	0.19
Belgium	0.00	0.44	0.22
Bulgaria	0.87	0.98	0.99
Croatia	0.30	0.12	0.19
Cyprus	0.00	0.00	0.00
Czech Rep.	0.19	0.81	0.88
Denmark	0.00	0.00	0.00
Estonia	0.00	0.88	0.95
Finland	0.00	0.36	0.58
France	0.23	0.58	0.60
Germany	0.03	0.60	0.69
Greece	0.51	0.53	0.49
Hungary	1.00	0.95	0.97
Ireland	0.00	0.00	0.00
Italy	0.52	0.57	0.54
Latvia	0.00	0.76	0.83
Lithuania	0.00	0.85	0.87
Luxembourg	0.00	0.20	0.20
Malta	0.00	0.00	0.00
Netherlands	0.00	0.31	0.28
Poland	0.47	0.95	0.98
Portugal	0.47	0.42	0.25
Romania	0.73	0.86	0.93
Slovakia	0.56	0.68	0.76
Slovenia	0.05	0.03	0.05
Spain	0.67	0.63	0.47
Sweden	0.00	0.09	0.20
United Kingdom	0.00	0.00	0.02

Caveats to the modelling

To remove spatial recording biases, the selection of the background sample from the accessible background was weighted by the density of terrestrial Decapoda records on the Global Biodiversity Information Facility (GBIF). While this is preferable to not accounting for recording bias at all, it may not provide the perfect measure of recording bias.

There was substantial variation among modelling algorithms in the partial response plots (Figure 3). In part this will reflect their different treatment of interactions among variables. Since partial plots are made with other variables held at their median, there may be values of a particular variable at which this does not provide a realistic combination of variables to predict from.

Other variables potentially affecting the distribution of the species, such as types of land cover were not included in the model.

Finally, Filipová et al. (2011) suggested that calico crayfish might represent a cryptic species complex, “making the forecasting of ecological properties of European *F. immunis* populations based on data from populations in its indigenous North American range difficult because the latter might comprise different cryptic species” (Chucholl 2012).

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**Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention"
Contract No 07.0202/2019/812602/ETU/ENV.D.2¹**

Name of organism: *Misgurnus anguillicaudatus* (Cantor, 1842)

Author(s) of the assessment: Hugo Verreycken, INBO, Brussels, Belgium

Risk Assessment Area: The risk assessment area is the territory of the European Union 27 and the United Kingdom, excluding the EU-outermost regions.

Peer review 1: Elena Tricarico, Department of Biology, University of Florence, Italy

Peer review 2: Frances Lucy, Centre for Environmental Research Innovation and Sustainability CERIS, Dept of Environmental Science, Institute of Technology Sligo, Ireland

Date of completion: 15/10/2020

Date of revision: 15/10/2021

¹ This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA). A number of amendments have been introduced to ensure compliance with Regulation (EU) 1143/2014 on IAS and relevant legislation, including the Delegated Regulation (EU) 2018/968 of 30 April 2018, supplementing Regulation (EU) No 1143/2014 of the European Parliament and of the Council with regard to risk assessments in relation to invasive alien species (see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>).

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SECTION A – Organism Information and Screening

A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?

including the following elements:

- the taxonomic family, order and class to which the species belongs;
- the scientific name and author of the species, as well as a list of the most common synonym names;
- names used in commerce (if any)
- a list of the most common subspecies, lower taxa, varieties, breeds or hybrids

As a general rule, one risk assessment should be developed for a single species. However, there may be cases where it may be justified to develop one risk assessment covering more than one species (e.g. species belonging to the same genus with comparable or identical features and impact). It shall be clearly stated if the risk assessment covers more than one species, or if it excludes or only includes certain subspecies, lower taxa, hybrids, varieties or breeds (and if so, which subspecies, lower taxa, hybrids, varieties or breeds). Any such choice must be properly justified.

Response: The present risk assessment covers one species (no lower taxa or hybrids): *Misgurnus anguillicaudatus* (Cantor, 1842). It belongs to the genus *Misgurnus* Lacepède 1803, which is part of the Cobitidae (loaches or weatherfishes) (Froese and Pauly 2019).

Actinopterygii > Cypriniformes (Carps) > Cobitidae (Loaches or weatherfishes) > Cobitinae > *Misgurnus* Lacepède 1803 > *Misgurnus anguillicaudatus* (Cantor, 1842)

Kingdom: Animalia

Phylum: Chordata

Class: Actinopterygii (ray-finned fishes)

Order: Cypriniformes (Carps)

Family: Cobitidae (Loaches)

Genus: *Misgurnus* Lacepède 1803

Cobitidae consists of 28 genera and 262 species (Froese and Pauly 2019); in the closely related genera *Misgurnus* and *Paramisgurnus* the taxonomy is not clear and the number of recognised species differs from study to study and database to database.

According to the Integrated Taxonomic Information System (www.itis.org) *Misgurnus* spp. comprises of the following species:

Misgurnus anguillicaudatus (Cantor, 1842) – valid – oriental weatherfish, dojo, Japanese weatherfish

(*Misgurnus bipartitus* (Sauvage and Dabry de Thiersant, 1874) – **invalid**)

Misgurnus buphoensis Kim and Park, 1995 – valid

(*Misgurnus erikssoni* Rendahl, 1922 – **invalid**)

Misgurnus fossilis (Linnaeus, 1758) – valid – weatherfish

Misgurnus mizolepis Günther, 1888 – valid

Misgurnus mohoity (Dybowski, 1869) – valid

Misgurnus nikolskyi Vasil'eva, 2001 – valid

Misgurnus tonkinensis Rendahl, 1937 – valid

For the genus *Paramisgurnus*, only one valid species *Paramisgurnus dabryanus* Dabry de Thiersant, 1872 is recognised.

Other views on the taxonomy of the genera *Misgurnus* and *Paramisgurnus* can be found (e.g. Eschmeyer's Catalog of Fishes) but recently it has become clear that the taxonomic understanding of the weatherfish genera is still poor and there are many challenges associated with accurate species assignment in this group (Belle et al. 2021).

Also FishBase (Froese and Pauly 2019) mentions *M. bipartitus* as an invalid name and considers it as a synonym for *M. mohoity*; this was based on a study by Bogutskaya and Naseka (2004). A recent study by Zangl et al. (2020), however, keeps *M. bipartitus* and *M. mohoity* as separate species. They consider eight valid species in the genus *Misgurnus* and one in *Paramisgurnus*. Furthermore, this last study also states that the specimens of *M. anguillicaudatus* in Austria (and parts of Germany) were misidentified and should be *M. bipartitus* (based on morphology and genetics) instead. Genetic data from INBO (Brys et al. 2020) point in the same direction and conclude that the non-native weatherfish occurring in Belgium (and the Netherlands) is not *M. anguillicaudatus* but indeed *M. bipartitus*.

According to Kottelat and Freyhof (2007), only two non-native 'oriental' weatherfish were recorded in the risk assessment (RA) area (*M. anguillicaudatus* and *Paramisgurnus dabryanus*). However, Zięba et al. (2010) report that a fish imported under the name *Misgurnus anguillicaudatus* in the UK was found to be *M. mizolepis*. This reproducing loach population in a pond in Essex is since eradicated (Zięba et al. 2010).

The preferred common name in English is oriental weatherfish, but also dojo, Japanese weatherfish, pond loach and Asian weather loach are used as common names. Scientific (non-valid) synonyms for *M. anguillicaudatus* following FishBase (Froese and Pauly 2019) are *Cobitis anguillicaudata*, *Misgurnus anguillicaudatus anguillicaudatus*, *Misgurnus fossilis anguillicaudatus*, *Misgurnus aguillcadatus*, *Nemachilus lividus*, *Misgurnus lividus*, *Nemacheilus lividus*, *Misgurnus crossochilus*, *Ussuria leptcephala*, *Misgurnus mizolepis grangeri*, *Misgurnus mohoity leopardus*, *Misgurnus mohoity yunnan*, *Misgurnus punctatus*, *Misgurnus mizolepis punctatus*, *Misgurnus mizolepis unicolor*, *Misgurnus elongatus*, *Misgurnus mizolepis elongatus*, *Cobitis fossilis*, *Cobitis fossilis mohoity*, *Misgurnus mohoity*, *Misgurnus mizolepis*, *Misgurnus mizolepis multimaculatus*.

In the RA area, *M. anguillicaudatus* (oriental weatherfish) is currently established in four countries. Populations are reported from The Netherlands (van Kessel et al. 2013a; Niemeijer and van Kessel 2016), Germany (Freyhof and Korte 2005), Spain (Franch et al. 2008), and Italy (Razzetti et al. 2001). Possibly some of these populations are misidentified and are in fact *M. bipartitus* (see Zangl et al. 2020); confirmed populations of *M. bipartitus* exist in Austria and Germany (Zangl et al. 2020), Belgium and The Netherlands (Brys et al. 2020). In France, two records of *M. anguillicaudatus* are known from the Seine basin (N. Poulet, pers. com.) but the species is not established in this Member State. Other, non-established Asian weatherfish species are reported from Switzerland and Germany, namely *P. dabryanus* (large-scale loach) (Freyhof and Brooks 2011; Stoeckle et al. 2019) and from the UK, *M. mizolepis* (Zięba et al. 2010).

Misgurnus anguillicaudatus is known to hybridise naturally with *P. dabryanus* (You et al. 2009). In aquaculture, Fujimoto et al. (2008a) were able to produce hybrids of *M. anguillicaudatus* and *M. mizolepis*. Artificial reproduction was also used to successfully crossbreed *M. anguillicaudatus* with the native *M. fossilis* (J. Wanzenböck, pers. comm.).

The known common names of *M. anguillicaudatus* in European languages other than English are the following: FR: loche asiatique, IT: cobite di stagno orientale, DK: kinesisk smerling, DE: Ostasiatischer Schlammpeitzger, NL: Aziatische modderkruiper, SLO: azijska činklja.

A2. Provide information on the existence of other species that look very similar [that may be detected in the risk assessment area, either in the environment, in confinement or associated with a pathway of introduction]

Include both native and non-native species that could be confused with the species being assessed, including the following elements:

- other alien species with similar invasive characteristics, to be avoided as substitute species (in this case preparing a risk assessment for more than one species together may be considered);
- other alien species without similar invasive characteristics, potential substitute species;
- native species, potential misidentification and mis-targeting

Response: Loaches and weatherfishes from the genus *Misgurnus* have a long eel-like body and ten chin barbels. The Global Invasive Species Database (GISD; <http://www.iucngisd.org/gisd/species.php?sc=1537>) describes *Misgurnus anguillicaudatus* as “brown to yellow with greenish, gray-brown to black marbling, with a paler ventral. Its eel-like body is long, laterally compressed, commonly measuring around 15 cm, but reaching a maximum size near 30 cm. It has a small, narrow mouth and subinferior with 10 barbels, 4 of them, clearly smaller than the other, placed below the lower lip. Its lips are thick and fleshy. Its lateral line is short and does not exceed the length of the pectoral fin. Its pectoral fins are triangular with a stout spine. The dorsal fin originates above the pelvic fin origin and is single and short-based. It bears conspicuous adipose crests along the ventral and dorsal mid-lines of the caudal peduncle. It also has a characteristic dark spot in the upper half of the base of the caudal fin. It has 9 dorsal rays, 6-7 pelvic rays, and 7-8 anal rays. Generally males have larger pectoral fins and females fuller abdomens”. Males can be distinguished from females (sexual dimorphism) by the enlarged second ray of the pectoral fin and the presence (vs. absence in females) of the lamina circularis (Urquhart and Koetsier 2011).

Kottelat and Freyhof (2007) state the *M. anguillicaudatus* can be distinguished from other species of *Misgurnus* in Europe by (1) dark, irregular spots on the body and no stripes, (2) the caudal peduncle has low adipose crests, (3) lamina circularis (i.e. ventral base of the second pectoral ray broadens into a rounded plate and covers the bases of the other pectoral rays) is present in males and (4) there is a dark spot on the upper caudal base.

In the RA area, only one species of the genus *Misgurnus* is native (*Misgurnus fossilis*). Its native range encompasses Europe north of the Alps, from the Meuse eastward to the Neva drainages and Lake Ladoga, the northern Black Sea basin from the Danube eastward to Kuban and the Caspian basin in the Volga and Ural drainages (Kottelat and Freyhof 2007). It is not native in Great Britain, Scandinavia and Iberia. The European weatherfish *M. fossilis* also has an anguilliform body shape

and ten barbels but has a broad midlateral line from eye to caudal base and a narrow stripe from opercle at least to pelvic origin instead of irregular spots on the body for *M. anguillicaudatus*. A lamina circularis is absent in males while it is present in *M. anguillicaudatus* (Kottelat and Freyhof 2007). Other native loaches belong to separate genera (*Cobitis* and *Sabanejewia*) and can be readily distinguished from *Misgurnus* because the suborbital spine is externally visible (*versus* hidden under muscles in *Misgurnus*) and the lower lip has 0-2 short, barbel-like mental lobes (*vs.* 4 long, barbel-like mental lobes in *Misgurnus*) (Kottelat and Freyhof, 2007).

Discussed below are other non-native *Misgurnus* and *Paramisgurnus* species that can be potential substitute species.

Misgurnus bipartitus is morphologically very similar to *M. anguillicaudatus*, but good identification keys are not available (Frable 2008). Nichols (1943) provides a dichotomous key for identifying *M. anguillicaudatus* from other *Misgurnus* species, but does not mention *M. bipartitus* and separates *M. mohoity* from other Chinese *Misgurnus* species by the fact that the skin is not thickened and the scales of *M. mohoity* are regular and fully exposed where for other *Misgurnus* species the skin is more or less thickened and the scales are more or less embedded. Yi et al. (2017) provide a morphological comparison of four Chinese *Misgurnus* species (*P. dabryanus*, *M. anguillicaudatus*, *M. bipartitus*, *M. mohoity*) and conclude that morphometric measurements can be used to distinguish between these species but also warn that “efficient delimitation of these four closely species based on one or several morphological characters seems to be difficult and limited”.

Zangl et al. (2020) based their morphological identification of *M. bipartitus* on characters given in the key by Vasil’eva (2001 and the translated Chinese key therein) namely the caudal peduncle depth 2.4–2.5 times in caudal peduncle length *vs.* 1.3–1.8 times in *M. anguillicaudatus* and the maximum body depth 8.2–8.6 times in standard length (SL) *vs.* < 7.5 times in *M. anguillicaudatus*. The latter species is more elongated (Nichols 1943).

The Chinese fine-scaled loach *M. mizolepis* is closely related to *M. anguillicaudatus*. This species can be readily distinguished from the oriental weatherfish because it has a greater number of fine surface scales covering its body rather than irregularly embedded scales (Frable 2008). Moreover the peduncular keels (adipose crests near caudal peduncle) are greatly developed in *M. mizolepis* while they are low in *M. anguillicaudatus* (Nichols 1943; Kottelat and Freyhof 2007). This is contrary to what the CABI datasheet on this species states “It has conspicuous adipose crests along the ventral and dorsal mid-lines of the caudal peduncle” (CABI 2020).

As with *M. mizolepis* the adipose crests near the caudal peduncle are greatly developed in the large-scale loach *P. dabryanus*. This species can be distinguished from *M. anguillicaudatus* because the dark dot on the upper caudal base is missing and from *M. fossilis* by the absence of a broad midlateral stripe from eye to caudal base, and the absence of the narrow stripe from opercle to the pelvic origin (Stoeckle et al. 2019).

Correct identification of *Misgurnus* and *Paramisgurnus* species remains difficult, morphologically as well as genetically, even when DNA sequence data are available, as systematics and taxonomy are still not fully resolved (Zangl et al. 2020). Belle et al. (2021) highlight this problem and conclude that their analysis of genetic weatherfish identification using mtDNA barcodes confirms the still incomplete and unresolved classical and molecular taxonomy of *Misgurnus* and *Paramisgurnus* genera. Nonetheless, they found distinct existing phylogenetic clusters for the genera *Misgurnus* and *Paramisgurnus* and they suggest DNA barcoding and genotyping of detected non-native or traded weatherfish specimens to assign a specimen to the right species.

A3. Does a relevant earlier risk assessment exist? Give details of any previous risk assessment, including the final scores and its validity in relation to the risk assessment area.

Response: A risk assessment was performed for South Australia in 2014 (Fredberg et al. 2014). The authors used FISK and NAPRA tools to assess, rank and score the environmental, economic and social impacts and costs of *M. anguillicaudatus* and found that the species has a high risk of establishment and several potential impacts were suggested, including competition for spawning sites and for food and shelter, disturbance and/or predation of fish and frog eggs, alteration of habitat and decreased water quality. Fredberg et al. (2014), however, concluded that these impacts were largely speculative and further research is needed to fill several knowledge gaps.

For the USA, an Ecological Risk Screening Summary was written by The U.S. Fish and Wildlife Service in 2012. *Misgurnus anguillicaudatus* is established in the US and they concluded that there is a high risk of additional introductions, establishment, and impacts in other areas because this species is highly adaptable, quick to reproduce, and is extremely popular in the aquaculture and aquarium industry. The climate match with the native range is high and multiple impacts are expected (reductions in macroinvertebrate populations, altered aquatic habitats, and the fish are vectors for certain fish parasites). Their overall risk was estimated as high but with a medium certainty of the assessment.

Interesova et al. (2020) assessed a number of non-native fishes in the River Ob Basin (Russia) and rated *M. anguillicaudatus* as having a high risk of becoming invasive. The species was also assessed for state of New York and the New York Invasiveness Rank was High (http://nyis.info/wp-content/uploads/2017/10/1ae76_Misgurnus-anguillicaudatus-Ecological.pdf).

In the Netherlands, Spikmans et al. (2010) made a pest risk analysis for ten non-native fish species based on literature and climate match. For oriental weatherfish they found a high current climate match with most of the Dutch rivers suitable for establishment of this fish. They do not assess the invasiveness of this species but only summarise the literature on possible impacts (predation, competition and disease transmission).

Almeida et al. (2013), using FISK for the Iberian Peninsula, and Radočaj et al. (2021), using AS-ISK for Croatia and Slovenia, both report high scores for the invasiveness of *M. anguillicaudatus*.

All the above risk assessments are valid for the RA area because of a reasonable to good climate match and the high adaptability of the oriental weatherloach.

A4. Where is the organism native?

including the following elements:

- an indication of the continent or part of a continent, climatic zone and habitat where the species is naturally occurring
- if applicable, indicate whether the species could naturally spread into the risk assessment area

Response: The oriental weatherfish is native to the East Asian temperate regions, including Cambodia, China, India, Japan, Korea, Laos, Taiwan, Thailand, Vietnam and the Tugur and Amur River catchments in Siberia (Kottelat and Freyhof 2007). In its native habitat, *M. anguillicaudatus* is found in mangrove swamps along rivers and lakes. In addition, the species is found in ponds, marshes and rice fields (Spikmans et al. 2010). In general, the species prefers stagnant or weakly flowing water (Froese and Pauly 2019). The oriental weatherfish is mainly found in waters with a muddy bottom. The species burrows in sediment or leaf litter, with only its head protruding (Simon et al. 2006, Froese and Pauly 2019). Natural spread from its native area into the risk assessment area is not possible.

A5. What is the global non-native distribution of the organism outside the risk assessment area?

Response: Outside the RA area, oriental weatherfish was introduced to Australia (Koster et al. 2002 (extensive overview of Australian populations), Keller and Lake 2007, Fredberg et al. 2014), North America (several authors, e.g. Tabor et al. 2001, Simon et al. 2006), South America (Abilhoa et al. 2013) and Asia outside of their natural range (Belle et al. 2017).

A6. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species been recorded and where is it established? The information needs to be given separately for recorded (including casual or transient occurrences) and established occurrences. “Established” means the process of an alien species successfully producing viable offspring with the likelihood of continued survival².

A6a. Recorded: List regions

A6b. Established: List regions

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

- Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea.

Comment on the sources of information on which the response is based and discuss any uncertainty in the response.

For delimitation of EU biogeographical regions please refer to <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2> (see also Annex VI).

For delimitation of EU marine regions and subregions consider the Marine Strategy Framework Directive areas; please refer to <https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and->

² Convention on Biological Diversity, Decision VI/23

subregions/technical-document/pdf (see also Annex VI).

Response (6a): **Continental** (Freyhof and Korte 2005; Belle et al. 2017), **Mediterranean** (Franch et al. 2008; Razzetti et al. 2001). All references are very trustworthy and report the first records first hand. Although van Kessel et al. (2013a) report *M. anguillicaudatus* from the **Atlantic** region, this species turned out to be *M. bipartitus* after a genetic screening (Brys et al. 2020).

Response (6b): **Continental** (Freyhof and Korte (2005) report adults and juveniles and suggest that a successfully reproducing population has been established in Germany), **Mediterranean** (Franch et al. (2008) state that oriental weatherfish has an established population in the Ebro, northeastern Iberian Peninsula). All references are very trustworthy and report the first records first hand. Natural reproduction and presence of young fishes was reported for The Netherlands (Atlantic region) by Binnendijk et al. (2017), however, this species turned out to be *M. bipartitus* after a genetic screening (Brys et al. 2020).

A7. In which biogeographic region(s) or marine subregion(s) in the risk assessment area could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A7a. Current climate: List regions

A7b. Future climate: List regions

With regard to EU biogeographic and marine (sub)regions, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (7a): The species could currently establish in all EU biogeographic regions (Annex VIII). The highest suitability is in the Black Sea, Continental, Mediterranean and Pannonian regions followed from high to low by the Steppic, Boreal, Atlantic and Alpine regions (see figure 1).

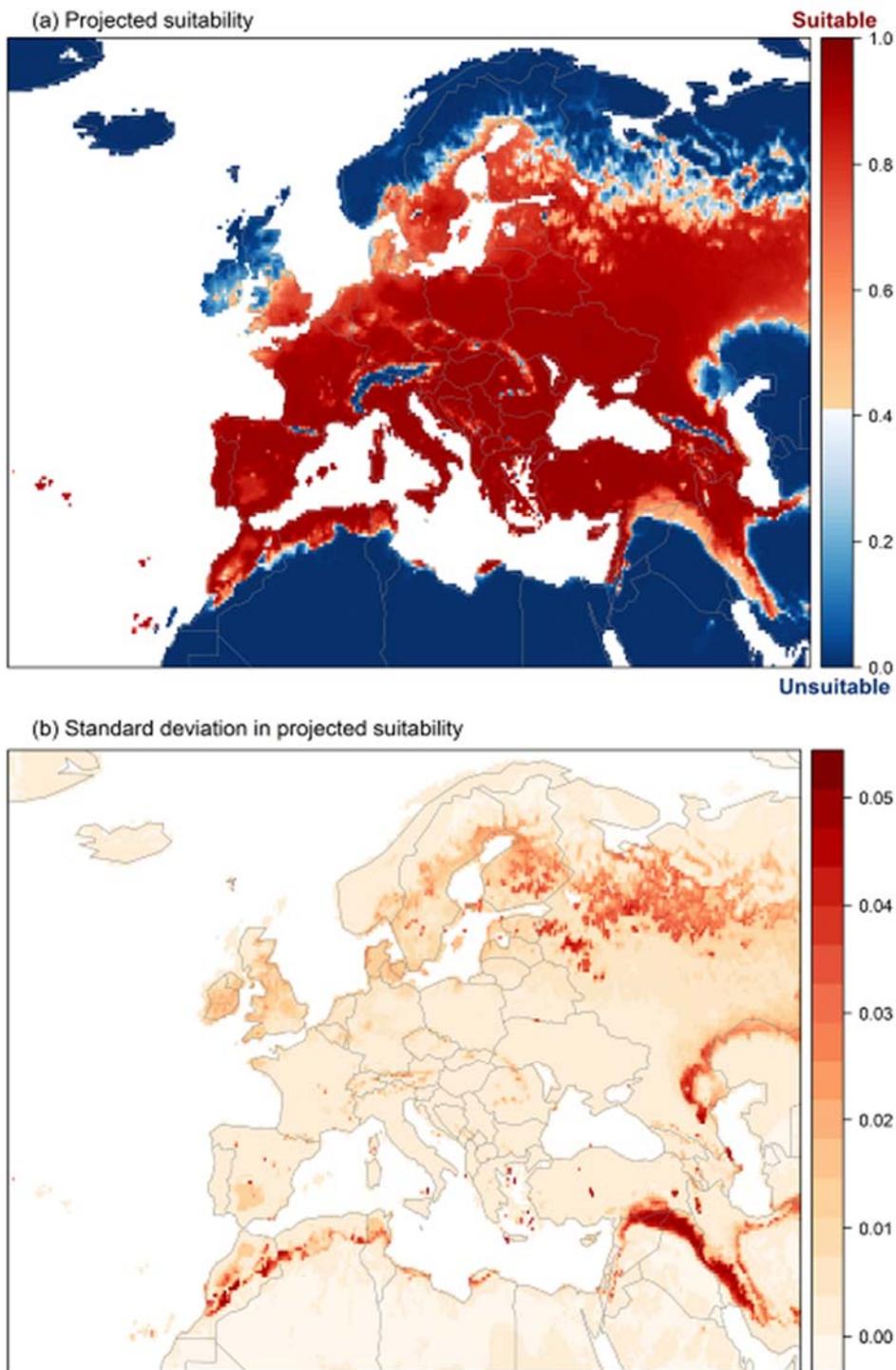


Figure 1: (a) Projected current suitability for *Misgurnus anguillicaudatus* establishment in Europe and the Mediterranean region. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

Response (7b): The species could establish in the 2070s under climate change scenario RCP2.6 and scenario RCP4.5 in all EU biogeographic regions (Annex VIII). The highest suitability is in the Black

Sea, Continental, Mediterranean and Pannonian regions followed from high to low by the Steppic, Atlantic, Boreal and Alpine regions (see figure 2 for scenario RCP2.6).

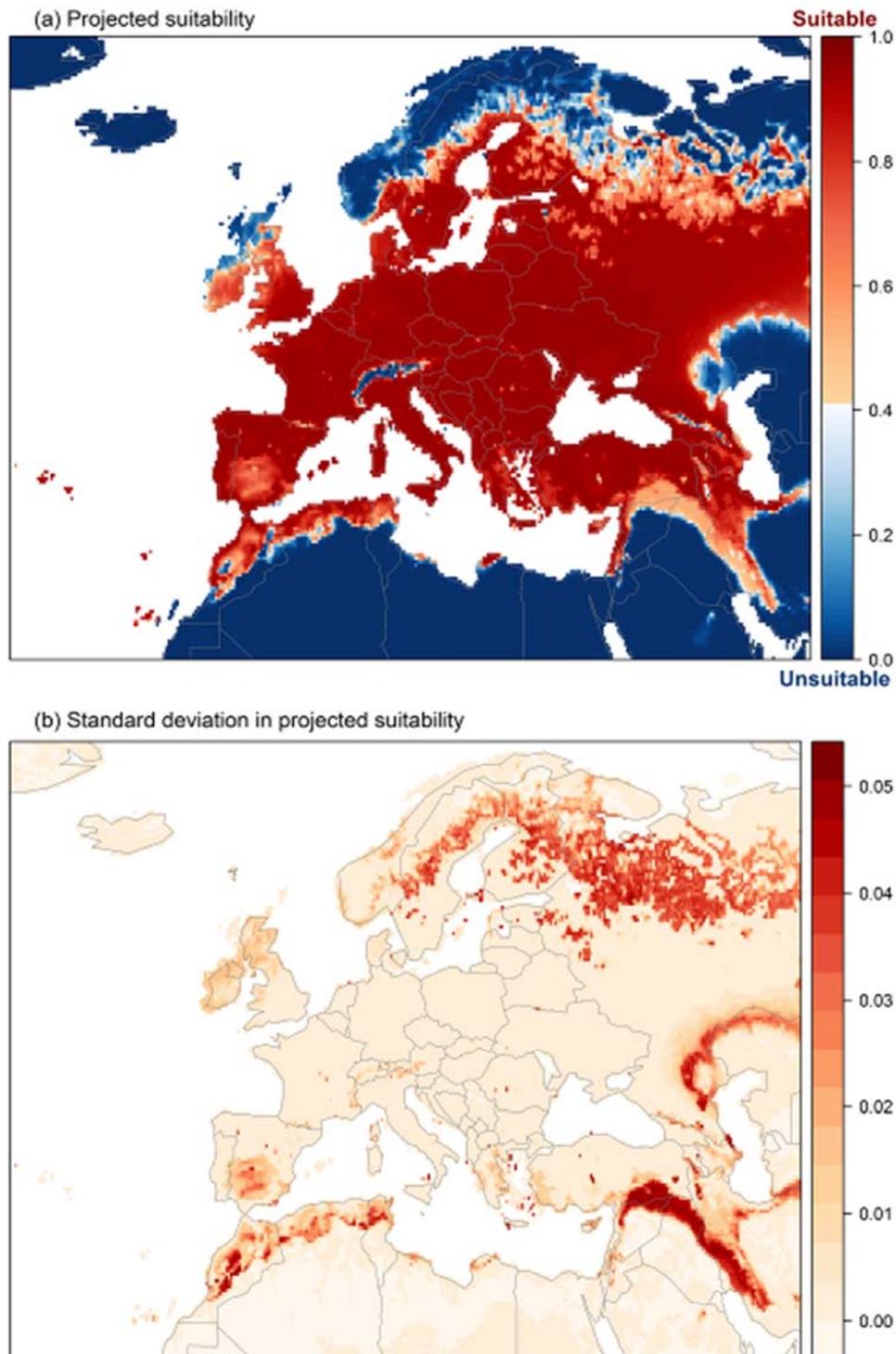


Figure 2: (a) Projected suitability for *Misgurnus anguillicaudatus* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP2.6, equivalent to Figure 1. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

It is not totally clear which aspects of climate change are most likely to affect the risk assessment but the SDM (Annex VIII) suggested that suitability for *Misgurnus anguillicaudatus* was strongly determined by the maximum temperature of the warmest month (27.1%) and by annual precipitation (23.8%). In both climate change scenarios, the suitable area will increase in the colder parts of the RA area e.g. UK, Ireland, Finland and Sweden.

A8. In which EU Member States has the species been recorded and in which EU Member States has it established? List them with an indication of the timeline of observations. The information needs be given separately for recorded and established occurrences.

A8a. Recorded: List Member States

A8b. Established: List Member States

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden

The description of the invasion history of the species shall include information on countries invaded and an indication of the timeline of the first observations, establishment and spread.

Response (8a): The oriental weatherfish was first recorded in Germany in 1990 (Freyhof and Korte 2005). Subsequently first records came from Italy in 1997 (Razzetti et al. 2001), Spain in 2001 (Franch et al. 2008) and the Netherlands in 2012 (van Kessel et al. 2013a).

It must be noted that possibly some of these populations are misidentified and in fact are other *Misgurnus* species. Recently it became clear, after genetic screening, that confirmed populations of *M. bipartitus* exist in Austria and Germany (Zangl et al. 2020), and Belgium and the Netherlands (Brys et al. 2020). It is, however, impossible to check all the reported *M. anguillicaudatus* specimens on their correct identification.

Response (8b): After the first record in 1990 in Germany (Freyhof and Korte 2005), in 2002, 43 specimens were caught ranging from 5.5 to 20.0 cm standard length; these specimens included adults and juveniles, suggesting a successfully reproducing, established population. Some specimens were caught in 2016 in the River Inn (South Germany), but establishment is not confirmed (Belle et al. 2017).

Razzetti et al. (2001) reported the first record for Italy in 1997, more individuals were caught in 1998 and the presence of dozens of adults and young fish of both sexes (total length between 9 and 21 cm) in 2000 confirmed the establishment of this species there.

Since the first Spanish record in 2001, the population of oriental weatherfish is expanding (Franch et al. 2008). The capture of over 1,000 weatherfish shows that its population is composed of both juvenile and adult individuals suggesting an established population.

A9. In which EU Member States could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current

climate and under foreseeable climate change conditions.

A9a. Current climate: List Member States

A9b. Future climate: List Member States

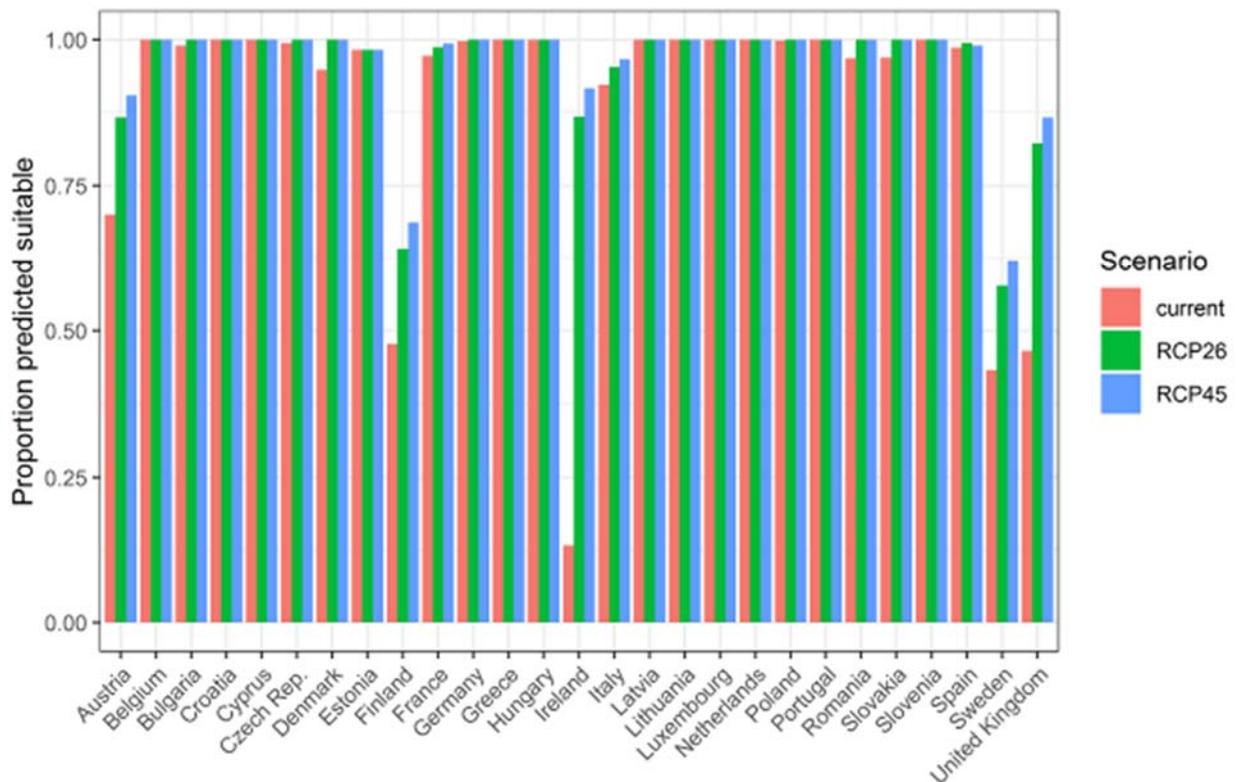
With regard to EU Member States, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (9a): Under current climate conditions *M. anguillicaudatus* could establish in all EU MS and the UK; there seems to be a high establishment potential for most MS but less so for Ireland, UK, Sweden, Finland and Austria (see figure 3).



Response (9b): In the 2070s, under climate change scenario RCP2.6 and scenario RCP4.5 *M. anguillicaudatus* could establish in all EU MS and the UK: there seems to be a high establishment

potential for most MS with increased potential (compared to current conditions) for Ireland, UK, Sweden, Finland and Austria (see Figure 3).

It is not totally clear which aspects of climate change are most likely to affect the risk assessment but the SDM (Annex VIII) suggested that suitability for *Misgurnus anguillicaudatus* was strongly determined by the maximum temperature of the warmest month (27.1%) and by annual precipitation (23.8%). In both climate change scenarios, the suitable area for establishment will increase in the colder parts of the RA area e.g. UK, Ireland, Finland and Sweden.

A10. Is the organism known to be invasive (i.e. to threaten or adversely impact upon biodiversity and related ecosystem services) anywhere outside the risk assessment area?

Response: Several authors reported adverse impacts but most of these impacts are anecdotal. For instance, Nobile et al. (2017) sum up possible impacts of established oriental weatherfishes for Brazil as follows: niche overlap, competition for resources and space, introduction of parasites, predation on eggs of fishes and restructuring of the aquatic ecosystem.

Keller and Lake (2007) report the potential impacts for Australia. In a mesocosm experiment, they found that oriental weatherfish caused significant reductions in macroinvertebrate numbers and biomass and that this fish was also associated with elevated ammonia, nitrate/nitrite (NO_x), and turbidity levels. However, the authors conclude: “We are not able to conclusively determine that oriental weatherfish are having large impacts in natural systems”.

In Asia, the species is known to hybridize with other species of *Misgurnus* and *Paramisgurnus* (You et al. 2009; Fujimoto et al. 2008a), but it is not clear what the impact of this phenomenon will be.

Schmidt and Schmidt (2014) conclude that *M. anguillicaudatus* does not seem to affect other vertebrates in the Hudson River Valley (New York) but that attention should be paid on the potential negative interactions (competition for food) with the native eastern mudminnow *Umbra pygmaea*. Similarly, López et al. (2012) suggest potential competition for food resources with benthic species such as river blenny (*Salaria fluviatilis*), European eel (*Anguilla anguilla*) and Iberian loach (*Cobitis paludica*). Also, Radočaj et al. (2021) state that in Croatia and Slovenia, *M. anguillicaudatus* can produce hybrids with congeneric the native *M. fossilis* with impacts on their genetic diversity

Misgurnus anguillicaudatus has been responsible for the establishment of the parasitic flatworm *Gyrodactylus macracanthus* (Dove and Ernst 1998) in Australia. Ogawa (1994) names this loach as host of the gill parasite *Ancyrocephalus cruciatus*, fin parasite *Gyrodactylus micracanthus*, and other unidentified fin and gill parasites of the genus *Gyrodactylus*. *Misgurnus anguillicaudatus* can also host the parasite *Acanthocephalus opsariichthydis* (Amin et al. 2007). In addition, the gill parasites *Trichodina lechriodontata* and *Trichodina modesta* were found in oriental weatherfish (Zhao and Tang 2007). Also Reyda et al. (2019) report the introduction of at least three *Gyrodactylus* species in the USA through oriental weatherfish. These parasites can potentially infect indigenous fish species and can cause mortalities in aquaculture as was the case with another *Gyrodactylus* parasite (*G. salaris*) in Norway (Salte et al. 2010).

A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness? Indicate the area endangered by the organism as

detailed as possible.

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea

Response: Established populations in the RA area are reported in the Continental (Freyhof and Korte 2005) and Mediterranean biogeographic regions (Ebro delta (Franch et al. 2008) and the province of Pavia (Razzetti et al. 2001)). López et al. (2012) state densities of up to 100 individuals m⁻² of sampled area. Although it is not always explicitly stated in the papers that report the establishment of self-sustaining populations, most authors mention potential negative impacts that imply invasiveness (because of growing numbers and expanding populations).

A12. In which EU Member States has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden

Response: Invasiveness is not explicitly stated in the papers that report established, self-sustaining populations in the RA area. Most impacts mentioned are anecdotal but imply invasiveness. In the future, invasiveness may be expected (because of growing numbers and expanding populations) in Germany (central east near Frankfurt am Main), Italy (Po river basin), and Spain (northeast, Ebro delta). Regarding, the Ebro population (northeastern Iberia) this population is highly abundant and its control is no longer possible. According with López et al. (2012) this species has already spread to other drainages within the northeastern part of Iberia. In the Netherlands (southeastern part near Weert) and Belgium (northeastern part near Bocholt), the congener *M. bipartitus* is showing an explosive population growth in just a few years (author's own observation).

A13. Describe any known socio-economic benefits of the organism.

including the following elements:

- Description of known uses for the species, including a list and description of known uses in the risk assessment area and third countries, if relevant.
- Description of social and economic benefits deriving from those uses, including a description of the environmental, social and economic relevance of each of those uses and an indication of

associated beneficiaries, quantitatively and/or qualitatively depending on what information is available.

If the information available is not sufficient to provide a description of those benefits for the entire risk assessment area, qualitative data or different case studies from across the risk assessment area or third countries shall be used, if available.

Response: *Misgurnus anguillicaudatus* is sold as an ornamental fish in the aquarium trade in the RA area as well as in the rest of the world (Strecker et al. 2011; Chang et al. 2009; Franch et al. 2008; Tabor et al. 2001), as a food source (Park et al. 2006) and as live bait (Franch et al. 2008). However, no published figures on quantities and costs were found.

In China (and other Asian countries), oriental weatherfish is a highly valued food fish and also used in traditional Chinese medicine. Since the 1990s the entire life cycle of this fish can be produced in aquaculture facilities and as of 2010 more than 204.552 tons were produced in aquaculture in China (Milton et al. 2018).

SECTION B – Detailed assessment

Important instructions:

- In the case of lack of information the assessors are requested to use a standardized answer: “No information has been found.”
- With regard to the scoring of the likelihood of events or the magnitude of impacts see Annexes I and II.
- With regard to the confidence levels, see Annex III.
- Highlight the selected response score and confidence level in **bold** but keep the other scores in normal text (so that the selected score is evident in the final document).

1 PROBABILITY OF INTRODUCTION AND ENTRY

Important instructions:

- **Introduction** is the movement of the species into the risk assessment area (it may be either in captive conditions and/or in the environment, depending on the relevant pathways).
- **Entry** is the release/escape/arrival in the environment, i.e. occurrence in the wild
- Introduction and entry may coincide for species entering through pathways such as “corridor” or “unaided”, but it also may differ. If different, please consider all relevant pathways, both for the introduction into the risk assessment area and the entry in the environment.
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used (see Annex IV). For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document³ and the provided key to pathways⁴.
- For organisms which are already present (recorded or established) in the risk assessment area, the likelihood of introduction and entry should be scored as “very likely” by default.
- Repeated (independent) introductions and entries at separate locations in the risk assessment area should be considered here (see Qu. 1.7).

Qu. 1.1. List relevant pathways through which the organism could be introduced into the risk assessment area and/or enter into the environment. Where possible give details about the specific origins and end points of the pathways as well as a description of any associated commodities.

For each pathway answer questions 1.2 to 1.7 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 1.2a, 1.3a, etc. and then 1.2b, 1.3b etc. for the next pathway.

In this context a pathway is the route or mechanism of introduction and/or entry of the species.

The description of commodities with which the introduction of the species is generally associated shall include a list and description of commodities with an indication of associated risks (e.g. the

³ <https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf>

⁴ <https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf>

volume of trade; the likelihood of a commodity being contaminated or acting as vector).

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 1.2-1.9.

Two main pathways are identified:

Pathway name: Escape from confinement – Pet/Aquarium/Terrarium species

This pathway is estimated to be the most important for introduction and entry of the oriental weatherfish in the RA area, with the species being available for sale on the internet⁵ and in shops. Disposal of aquarium fishes and escape into the wild from ponds happens frequently (Chan et al. 2019).

Pathway name: Escape from confinement – Live food and live bait

Although no data are available on the use of oriental weatherfish as live food or live bait in the RA area; it is an important food source in its native range, especially in China. So it can be assumed that this fish is intentionally imported to e.g. Chinatowns in Europe. The use of this species as live bait in the RA area is unclear and only mentioned as a possible introduction route by Franch et al. (2008). In Australia as well as in the USA and Japan, Koster et al. (2002) confirmed the use of this species as live bait.

Pathway: Escape from confinement – Pet/Aquarium/Terrarium species

Qu. 1.2a. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: *Misgurnus anguillicaudatus* has been imported into the RA area as an aquarium and garden pond species, it still is sold in many fish shops and on the internet. Entry into the wild was probably both intentional (release of unwanted specimens) and unintentional (flooding of garden ponds).

Qu. 1.3a. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.

⁵ <https://www.justcichlids.com/index.php/pages/Dojo-loach-for-sale.html>;
<https://verduijncichlids.com/product/misgurnus-anguillicaudatus/>

- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The aquarium trade is responsible for the worldwide movement of over 2 billion live freshwater fish per year (Chan et al. 2019). In Australia ornamental trade has become the leading vector for non-native fish introductions since the 1970s with species established in Australian drainages including oriental weatherfish. Oriental weatherfish were imported to Australia by the aquarium industry at a rate of up to 50,000 per year until the sale was banned in 1986 (Keller and Lake 2007). For the RA area, no exact data are available but several authors (e.g. van Kessel et al. 2013a, Zangl et al. 2020) mention this pathway (“aquarium trade”) as the most likely for some Member States. Although the propagule pressure of introductions into the RA area may be high, the entry of the introduced fishes into the wild will be much lower. Still, releases of aquarium fish occur frequently (Chan et al. 2019). No information has been found on the percentage of introduced fishes for ornamental purposes in the risk assessment area or entering the natural environment.

Qu. 1.4a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Live transport of aquarium species is well organised, the survival during the passage would be high as with other fish transports. Moreover, oriental weatherfish is a hardy fish that can withstand reduced dissolved oxygen conditions (Koetsien and Urquhart 2012) and low temperatures (Urquhart and Koetsier 2014). Reproduction during transport is unlikely but not impossible because reproduction as well as fertilization of the eggs happens in the water column. Eggs hatch within 30 hours and larvae remain in the water column for a short period before settling on the bottom (Frable 2008). Survival of specimens that get released in the wild (dumped from aquaria or escaped after flooding) is bound to be high because of the hardiness of oriental weatherfish and its ability to survive several days out of the water (Koster et al. 2002).

Qu. 1.5a. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Intentional fish transport is meant to keep the fish alive, so no management practices to imperil the survival would be in place. Aquarium holders will do their best to keep their fish alive under the best conditions and no management practices would be in place to prevent entry and survival in the wild.

Qu. 1.6a. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Misgurnus* species can be difficult to distinguish from each other for non-trained people, e.g. *M. anguillicaudatus* imported for the aquarium trade in Great Britain turned out to be *M. mizolepis* instead (Zięba et al. 2010), the same can happen the other way round. Escape into the wild of aquarium and pond fishes happens frequently and may be noticed only years later (Chan et al. 2019). *M. anguillicaudatus* can be detected during regular monitoring for e.g. the Water Framework Directive but there may be several years between first occurrence and first detection. Also, this species has a benthic lifestyle and often lives in marshlike habitats which are difficult to sample. New detection methods, e.g. environmental DNA (eDNA) may be used for better and quicker detection of this cryptic species (Brys et al. 2020).

Qu. 1.7a. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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Response: Introductions via the aquarium trade can happen in any place, especially since *M. anguillicaudatus* is also for sale through the internet⁶. Intentional as well as unintentional entry into the wild can also occur in most places but propagule pressure is bound to be higher in a neighbourhood with dense human populations (Copp et al. 2005).

Qu. 1.8a. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The oriental weatherfish is already established in several Member States (MS) of the RA area as a consequence of releases/escapes connected to the aquarium trade (Zangl et al. 2020). Additional introductions are still possible and probably ongoing since this species is readily available via the aquarium trade and the internet. Entry into the wild has already been encountered for several decades now, with natural populations in at least six MS (NL, BE, DE, AT, ES, IT).

Pathway: Escape from confinement – Live food and live bait

Qu. 1.2b. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: As this fish is highly valued as a food source (especially in China), intentional import of this species to e.g. Chinatowns in the RA area cannot be excluded. The import of oriental weatherfish for use as live bait is not documented for the RA area but was for Australia, the USA and Japan (Koster et al. 2002).

Qu. 1.3b. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one

⁶ <https://verduijncichlids.com/product/misgurnus-anguillicaudatus/>
<https://www.gdaquarium.nl/a-28161018/tropische-vissen-database/misgurnus-anguillicaudatus-gold-weeraal-goud/#description>

year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Although no information has been found on organised imports of live weatherfish for food or as baits into the RA area, it is still possible that this species, farmed in large numbers in Asia, is intentionally imported to e.g. Chinatowns in Europe. Several authors (Franch et al. 2008, Milton et al. 2018) report that *M. anguillicaudatus* is important as a food source, especially in China (and some other East Asian countries). The use of this species in the RA area as live bait is unclear and only mentioned as a possible introduction route by Franch et al. (2008) but was confirmed for Australia, the USA and Japan (Koster et al. 2002).

Qu. 1.4b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Live transport of food species is well organised, the survival during the passage would be high as with other fish transports. Moreover, oriental weatherfish is a hardy fish that can withstand reduced dissolved oxygen conditions (Koetsien and Urquhart 2012) and low temperatures (Urquhart and Koetsier 2014). Reproduction during the transport is unlikely but not impossible because reproduction as well as fertilization of the eggs happens in the water column. Eggs hatch within 30 hours and larvae remain in water column for a short period before settling on the bottom (Frale 2008). Survival of specimens that get released in the wild (e.g. release of excess live bait) is expected

to be high because of the hardiness of oriental weatherfish and its ability to survive several days out of the water (Koster et al. 2002).

Qu. 1.5b. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Intentional fish transport is meant to keep the fish alive, so no management practices to imperil the survival would be in place. Neither would management practices be in place to prevent survival of escaped fish in the wild.

Qu. 1.6b. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Misgurnus* species can be difficult to distinguish from each other for non-trained people, e.g. *M. anguillicaudatus* imported for the aquarium trade in Great Britain turned out to be *M. mizolepis* instead (Zięba et al. 2010), the same can happen the other way round. Escape into the wild of live food fishes happens frequently and may be noticed only years later (Rixon et al. 2005).

Qu. 1.7b. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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Response: Introductions for the live food trade or as live bait can happen in any place, especially since *M. anguillicaudatus* is also for sale through the internet. Although no information has been found on

organised import of live weatherfish for food into the RA area, it is possible that this species is imported in Asian neighbourhoods of cities in Europe. Intentional as well as unintentional entry into wild can occur in these places where propagule pressure is higher near the neighbourhood of live food markets, but these live food markets are probably not widespread in the RA area.

Qu. 1.8b. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Importation of live fishes is known as an important pathway for the introduction of invasive, non-native fishes in some regions in the world (Rixon et al. 2005), but probably less so in the RA area. However no specific information has been found on this subject.

Qu. 1.9. Estimate the overall likelihood of introduction into the risk assessment area or entry into the environment based on all pathways and specify if different in relevant biogeographical regions in current conditions.

Provide a thorough assessment of the risk of introduction in relevant biogeographical regions in current conditions: providing insight in to the risk of introduction into the risk assessment area.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The oriental weatherfish is already established in several Member States of the RA area. Additional introductions are still possible and probably ongoing since this species is readily available via the aquarium trade and the internet. It is also farmed in high numbers in China for the food industry. Several MS have already reported established populations in the wild as a consequence of deliberate introductions from aquaria or unintentional escapes from garden ponds.

Qu. 1.10. Estimate the overall likelihood of introduction into the risk assessment area or entry into the environment based on all pathways in foreseeable climate change conditions?

Thorough assessment of the risk of introduction in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this

risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of introduction (e.g. change in trade or user preferences)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely introduction within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species is established from NW Europe, over Central Europe to the south in Spain and Italy. The risk of introduction and/or entry is very likely and will probably not change much in foreseeable climate change conditions. Possibly northern MS will be at higher risk of invasion in the future because the temperature range of *M. anguillicaudatus* is very broad, this can be assumed from its broad native habitat. It is not clear which aspects of climate change are most likely to affect the risk assessment but an increase in average winter temperature will increase the suitable area in the colder parts of the RA area e.g. Great Britain, Ireland, Finland and Sweden (Annex VIII).

2 PROBABILITY OF ESTABLISHMENT

Important instructions:

- For organisms which are already established in parts of the risk assessment area or have previously been eradicated, the likelihood of establishment should be scored as “very likely” by default.
- Discuss the risk also for those parts of the risk assessment area, where the species is not yet established.

Qu. 2.1. How likely is it that the organism will be able to establish in the risk assessment area based on similarity of climatic and abiotic conditions in its distribution elsewhere in the world?

RESPONSE		CONFIDENCE	
	very unlikely		low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response: Oriental weatherfish is a fish native mainly to the temperate climate region of east Asia (Köppen-Geiger climate classification) (Kottelat and Freyhof 2007). All temperate regions in the world (e.g. USA, Australia, Europe) have reported established populations of this species. In the RA area, the species is established from Central Europe (Germany) to the south in Spain and Italy. Probably all MS can contain established populations of *M. anguillicaudatus*, because it is a very hardy species that can tolerate harsh conditions like reduced dissolved oxygen conditions (Koetsien and Urquhart 2012) and low temperatures (Urquhart and Koetsier 2014). Specimens of this fish or its congener *M. bipartitus* have already been found in a cold alpine stretch of the River Inn in Germany and Austria (Zangl et al. 2020) but it is not known whether they are established there. Figure 1 (at page 11) shows a high (projected) suitability for establishment in most of the EU MS.

Qu. 2.2. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area? Consider if the organism specifically requires another species to complete its life cycle.

RESPONSE		CONFIDENCE	
	very isolated		low
	isolated		medium
	moderately widespread		high
	widespread		
	ubiquitous		

Response: *M. anguillicaudatus* occurs in rivers, lakes and ponds (Froese and Pauly 2019) and also in swamps and ricefields (You et al. 2009). It prefers muddy bottoms, where they hide in the muck and

leaf litter with only their heads sticking out. This species is very hardy and can survive a wide range of temperatures and environmental conditions. It is typically found in slow or still waters with muddy or silty bottoms abundant with aquatic plants (Froese and Pauly 2019). However, Schmidt and Schmidt (2014) noticed that their sample sites in the Hudson Valley (NY, USA) were mostly devoid of aquatic plants. Several authors (e.g. Franch et al. 2008; Zangl et al. 2020) mention also rivers as suitable habitat for the oriental weatherfish. The oriental weatherfish can breathe atmospheric oxygen by using its intestine as an accessory respiratory organ, allowing it to live in oxygen-poor waters and to bury itself in soft substrates to survive long droughts (Koetsier and Urquhart 2012). All these suitable habitats are widely available in the RA area.

Qu. 2.3. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Misgurnus anguillicaudatus* is established in the RA area in a wide range of habitats and climate conditions (see Q 2.1) where also other species occur. The congener species, *M. bipartitus*, for instance co-exists, in very high densities, with *M. fossilis* and *Cobitis taenia* (spined loach), two species with a similar habitat use and food items in its Dutch and Belgian non-native range (Brys et al. 2020). and *M. bipartitus* occurs there. In the Dutch population, top predators like pike *Esox lucius* and perch *Perca fluviatilis* were present in the fish community (Binnendijk et al. 2017) and not limiting establishment of *M. bipartitus*. Similar situations can be expected for the close relative *M. anguillicaudatus*.

Qu. 2.4. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?

RESPONSE	N/A very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: As in other parts of the world (USA, Australia, Brazil), *M. anguillicaudatus* became established in several regions in the RA area despite the presence of native predators (e.g. northern pike *Esox lucius* and Eurasian perch *Perca fluviatilis*), parasites or pathogens (Koster et al. 2002, Binnendijk et al. 2017).

Qu. 2.5. How likely is the organism to establish despite existing management practices in the risk assessment area? Explain if existing management practices could facilitate establishment.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Currently, no management practices seem to be ongoing in the RA area, no information has been found on this subject. Previous efforts to eradicate (or at least manage) the Dutch population (Binnendijk et al. 2017) failed. Over several years and multiple times per year Binnendijk et al. (2017) tried to catch (with dipnets and electrofishing) as many loaches as possible and removed them from the infested site. Although many specimens were captured (n= 763), eradication efforts were stopped because the numbers kept increasing with new and smaller (i.e. natural reproduction) caught each year. Other eradication methods were deemed unfeasible on the basis of financial, practical, ecological or legal reasons (Binnendijk et al. 2017). The Dutch management effort is the only management practice reported for the RA area.

In the context of European, national and regional legislations (e.g. Decision on the free migration of fish species in the hydrographic catchment areas of the Benelux countries⁷) several actions are undertaken to remove fish migration barriers from rivers and streams. These management practices to restore the natural connections may actually facilitate spread and enhance establishment of non-native species to new areas (Krieg and Zenker 2020). However, in the case of the oriental weatherfish, weirs and dams may have been less of a barrier for dispersal because Lintermans et al. (1990) reported observations of this species moving overland.

Qu. 2.6. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The oriental weatherfish can breathe atmospheric oxygen by using its intestine as an accessory respiratory organ, allowing it to live in oxygen-poor waters and to bury itself in soft substrates to survive long droughts (Koetsier and Urquhart 2012). Eradication campaigns for fish often use the principle of water removal (e.g. drying of ponds) or oxygen depletion (e.g. use of piscicides) to

⁷ https://www.vmm.be/wetgeving/M_2009_1_NL.pdf

kill unwanted fish species. Because of its unique feature to breathe atmospheric oxygen, oriental weatherfish is able to survive oxygen deprived conditions for many days. In experimental circumstances in the USA, some individuals survived for over 81 days in desiccated conditions with soil moisture content less than 3% (Koetsier and Urquhart 2014). An effort to eradicate (or at least manage) the Dutch *M. bipartitus* population, by using multiple catches and removals of individuals with electrofishing apparatus, fykes and dipnets failed (Binnendijk et al. 2017). On the other hand, a reproducing population of *M. mizolepis* in a small, plastic-lined garden pond in Essex has been eradicated (Zięba et al. 2010).

Qu. 2.7. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?

including the following elements:

- a list and description of the reproduction mechanisms of the species in relation to the environmental conditions in the risk assessment area
- an indication of the propagule pressure of the species (e.g. number of gametes, seeds, eggs or propagules, number of reproductive cycles per year) of each of those reproduction mechanisms in relation to the environmental conditions in the risk assessment area.
- If relevant, comment on the likelihood of establishment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in establishment whereas for others high propagule pressure (many thousands of individuals) may not.
- If relevant, comment on the adaptability of the organism to facilitate its establishment and if low genetic diversity in the founder population would have an influence on establishment.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *Misgurnus anguillicaudatus* matures quickly, can reproduce multiple times in a season, and produces a great number of offspring (Nico et al. 2020). The male wraps his body around the female and stimulates her to release a cloud of eggs, which he simultaneously fertilizes. These fish are nonguarders and release their eggs in open water as well as on substratum (Breder and Rosen 1966). This indicates broad environmental conditions for reproduction. Yamamoto and Tagawa (2000) reported 2000 eggs per female for introduced weatherfish in Hawaii. However, Urquhart (2013) found an estimated number of mature ova in female fish ranged from zero up to 46,18 for a self-sustaining population in Idaho (USA). Within this study area, oriental weatherfish lived 5–7 years, reaching sexual maturity within the first year of life. Females remained fecund throughout their lifetime, and spawned twice per year, with young produced in the late spring (May) and early autumn (October) seasons.

The oriental weatherfish is also a very hardy species with broad temperature tolerances (Urquhart and Koetsier 2014) and can withstand low dissolved oxygen conditions and dessication (Koetsier and Urquhart 2012).

No data are available about the genetic diversity of the oriental weatherfish populations in the RA area. Belcik (2017) investigated the population genetics of introduced oriental weatherfish populations in the Chicago Area Waterways and concluded that the results from his study suggest a single introduction to these waterways before 1987, with a subsequent range expansion. This author also suggests that the population is genetically identical to the weatherfish introduced into Australia before 1984, and that these populations were derived from the same native population in Asia. Therefore, it seems fair to conclude that low genetic diversity of this species probably will not inhibit its ability to establish in new areas. Binnendijk et al. (2017) also assume a single introduction in the southeast of the Netherlands was responsible for the establishment for a large population of *M. bipartitus*.

Qu. 2.8. If the organism does not establish, then how likely is it that casual populations will continue to occur?

Consider, for example, a species which cannot reproduce in the risk assessment area, because of unsuitable climatic conditions or host plants, but is present because of recurring introduction, entry and release events. This may also apply for long-living organisms.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Most MS contain suitable habitat for oriental weatherfish to survive and inhabit, even if establishment would not be possible, there is a continued chance of e.g. aquarium specimens being dumped (Chan et al. 2019) as there is a continued sale of this species for the aquarium trade throughout the RA area and they will survive because of their broad tolerance for physiological variables, a flexible diet and a high reproductive potential (Koetsier and Urquhart 2012).

Qu. 2.9. Estimate the overall likelihood of establishment in the risk assessment area under current climatic conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under current climatic conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in current conditions: providing insight in the risk of establishment in (new areas in) the risk assessment area.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: *M. anguillicaudatus* is established in several MS in the RA area (the Netherlands, Belgium, Germany, Spain, Italy). The SDM (Annex VIII) pointed out that most MS and most biogeographical regions have favourable conditions for establishment. Suitable habitats are available all over the RA area and because of its flexibility and broad tolerance for physiological variables, new introductions of oriental weatherfish will almost certainly lead to new established populations. Moreover, indications exist that oriental weatherfish can reproduce by gynogenesis (Morishima et al. 2002), a form of asexual reproduction in which growth and development of embryos occur without fertilization and where the egg are stimulated to develop simply by the presence of sperm: in the case of *M. anguillicaudatus* the sperm of a cyprinid species e.g. gibel carp *Carassius gibelio* is sufficient. This means that even the escape into the wild of one mature female can be enough to start a population. Also, there may be several years between first occurrence and first detection as *M. anguillicaudatus* has a benthic lifestyle and often lives in marshlike habitats which are difficult to sample with traditional methods like electrofishing and fykes. New detection methods, e.g. environmental DNA (eDNA) may be used for better and quicker detection of this cryptic species (Brys et al. 2020).

Qu. 2.10. Estimate the overall likelihood of establishment in the risk assessment area under foreseeable climate change conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under foreseeable climate change conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of establishment (e.g. increase in average winter temperature, increase in drought periods)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely establishment within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: The species could establish in the 2070s under climate change scenario RCP2.6 in all EU biogeographic regions (Annex VIII). The highest calculated suitability is in the Black Sea, Continental, Mediterranean and Pannonian regions followed from high to low by the Steppic, Atlantic,

Boreal and Alpine regions (see figure 2). Under scenario RCP4.5, conditions get better for most ecoregions in the RA area (figure 4). The SDM (Annex VIII) suggested that suitability for *Misgurnus anguillicaudatus* was strongly determined by the maximum temperature of the warmest month (27.1%), followed by annual precipitation (23.8%) while minimum temperature of the coldest month only accounted for 8.8% of the variation.

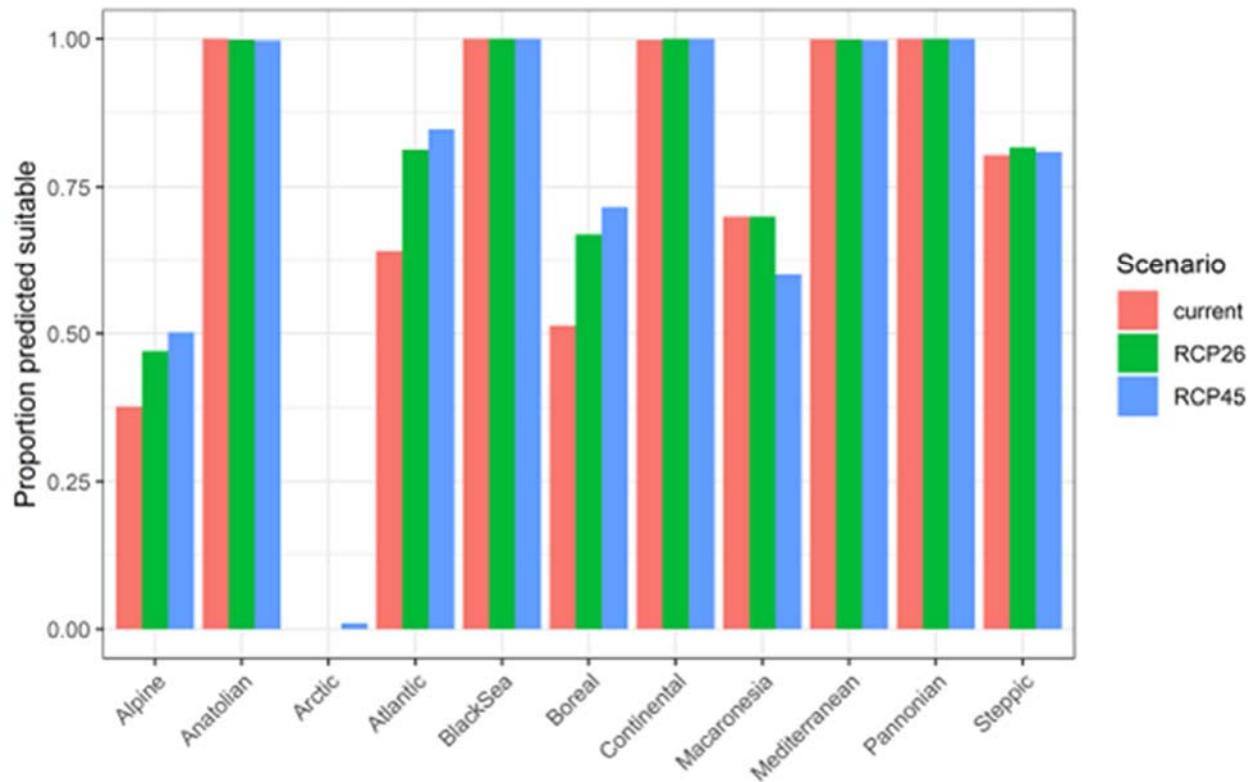


Figure 4: Variation in projected suitability for *Misgurnus anguillicaudatus* establishment among biogeographical regions of Europe. The bar plots show the proportion of grid cells in each region classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. The Anatolian, Arctic and Macaronesian biogeographical regions are not part of the study area, but are included for completeness.

3 PROBABILITY OF SPREAD

Important instructions:

- Spread is defined as the expansion of the geographical distribution of an alien species within the risk assessment area.
- Repeated releases at separate locations do not represent continuous spread and should be considered in the probability of introduction and entry section (Qu. 1.7).

Qu. 3.1. How important is the expected spread of this organism within the risk assessment area by natural means? (List and comment on each of the mechanisms for natural spread.)

including the following elements:

- a list and description of the natural spread mechanisms of the species in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of spread discussed in relation to the species biology and the environmental conditions in the risk assessment area.

The description of spread patterns here refers to the CBD pathway category “Unaided (Natural Spread)”. It should include elements of the species life history and behavioural traits able to explain its ability to spread, including: reproduction or growth strategy, dispersal capacity, longevity, dietary requirements, environmental and climatic requirements, specialist or generalist characteristics.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Natural spread can occur between connecting waters. Although overland movements are theoretically possible because of its air breathing capacity, Koetsier and Urquhart (2012) concluded that even in worsening environmental conditions (dessication experiments) oriental weatherfish rather bury themselves in the mud than trying to move away. However, Koster et al. (2002) reported the find of a specimen of oriental weatherfish moving overland during a wet day a short distance outside of a garden pond in Australia. Schmidt and Schmidt (2014) report that oriental weatherfish had colonized at least 20 km of the Dwaar Kill mainstem (plus several tributaries) and it is present in at least 8.5 km of the Wallkill River; this spread was probably realised over the last 20-30 years.

In the RA area, in the Ebro delta, oriental weatherfish was first detected in 2001 and by 2007 the species had spread over 31 1 x 1 km UTM quadrates (Franch et al. 2008). López et al. (2012) state that this species has large capacity to disperse from the established populations, finally colonizing all the Ebro delta.

In the Netherlands, natural spread of the congener *M. bipartitus* was also relatively high; from the discovery in 2012 to the end of 2016, this oriental weatherfish had moved twelve kilometers downstream and one kilometer (and possibly more) upstream (Binnendijk et al. 2017). In the downstream direction it looks like the spread was stopped by a (temporary) barrier. The Belgian population originated from natural spread from the neighbouring Dutch population, through a natural connection, with seven years between first records in the Netherlands and Belgium (Brys et al. 2020).

Qu. 3.2a. List and describe relevant pathways of spread other than "unaided". For each pathway answer questions 3.3 to 3.9 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 3.3a, 3.4a, etc. and then 3.3b, 3.4b etc. for the next pathway.

including the following elements:

- a list and description of pathways of spread with an indication of their importance and associated risks (e.g. the likelihood of spread in the risk assessment area, based on these pathways; likelihood of survival, or reproduction, or increase during transport and storage; ability and likelihood of transfer from the pathway to a suitable habitat or host) in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of spread for each pathway discussed in relation to the species biology and the environmental conditions in the risk assessment area.
- All relevant pathways of spread (except “Unaided (Natural Spread)”, which is assessed in Qu. 3.1) should be considered. The classification of pathways developed by the Convention of Biological Diversity shall be used (see Annex IV).

Only one pathway for spread was identified: Corridor – Interconnected waterways / basins / seas. All over the introduced range, secondary spread was noticed from initial infection sources. In the RA area most river catchments are connected by man-made canals, which act as corridors for the spread of invasive aquatic species (Leuven et al. 2009), however, it is unclear how important this pathway is for *M. anguillicaudatus* in the RA area.

Pathway name: Corridor – Interconnected waterways / basins / seas

Qu. 3.3a. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response: In the RA area most river catchments are connected by man-made canals, which act as corridors for the spread of invasive aquatic species (Leuven et al. 2009). Although the canals are intentionally built to connect water basins to allow boat traffic between them, the spread through these systems is unintentional.

Qu. 3.4a. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Range expansion of the oriental weatherfish through man-made channels and canals was not investigated in the RA area. However, in the USA, oriental weatherfish used the North Shore Channel and the Chicago Sanitary and Ship Canal to move from the Lake Michigan Basin side toward the rivers and tributaries of the Mississippi River Basin (Belcik 2017). In Australia, these fish have also been collected from concrete flood control channels, and rock-filled wire cages used as soil conservation structures (Koster et al. 2002). In the native range, oriental weatherfish are known to inhabit irrigation channels for rice fields (Fujimoto et al. 2008b). The above indicates that channels and canals are valid corridors for the dispersal of the oriental weatherfish.

No numbers are available to give an indication of the propagule pressure through this pathway. However, a few specimens are enough to start a new population (e.g. Dutch and German population: van Kessel et al. 2013a, Freyhof and Korte 2005). On top of this, indications exist that oriental weatherfish can reproduce by gynogenesis (Morishima et al. 2002).

Qu. 3.5a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: NA

Qu. 3.6a. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Currently, no management practices seem to be ongoing in the RA area, no information has been found on this subject. Previous efforts to eradicate (or at least manage) the Dutch population (Binnendijk et al. 2017) failed. Over several years and multiple times per year Binnendijk et al. (2017) tried to catch (with dipnets and electrofishing) as many loaches as possible and removed them from the infested site. Although many specimens were captured (n= 763), eradication efforts were stopped because the numbers kept increasing with new and smaller young-of-the-year individuals (i.e. natural reproduction) caught each year. Other eradication methods were deemed unfeasible on the basis of financial, practical, ecological or legal reasons (Binnendijk et al. 2017). Chemicals (e.g. rotenone) can be used to eradicate fish in small, closed waters but are very difficult to apply in large, open water systems like canals. Also are these chemicals not species-specific and cause high collateral damage among non-target species. The use of these chemicals is also legally restricted (Koster et al. 2002).

Qu. 3.7a. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: No information has been found on dedicated monitoring of oriental weatherfish in the RA area. *M. anguillicaudatus* can be detected during regular monitoring for e.g. the Water Framework Directive but there may be several years between first occurrence and first detection. Also, this species has a benthic lifestyle and often lives in marshlike habitats which are difficult to sample. New detection methods, e.g. environmental DNA (eDNA) may be used for better and quicker detection of this cryptic species (Brys et al. 2020).

Qu. 3.8a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response: Much of the RA area offers suitable habitat for oriental weatherfish. The corridors provide a perfect pathway between the suitable habitats in different river catchments. There are no data about the presence of oriental weatherfish in canals in the RA area. In other introduced areas e.g. Belcik (2017) reported that the oriental weatherfish used the North Shore Channel and the Chicago Sanitary and Ship Canal to move from the Lake Michigan Basin side toward the rivers and tributaries of the Mississippi River Basin.

Qu. 3.9a. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area. (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: No information has been found on the spread potential through this pathway in the RA area, but considering the moderate expected spread by natural means and the estimated relatively low number of existing populations of oriental weatherfish in the RA area, the rate of spread based on this pathway is thought to be moderate even though environmental conditions are favourable in most MS of the RA area.

Qu. 3.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?

RESPONSE	very easy easy with some difficulty difficult very difficult	CONFIDENCE	low medium high
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Response: Natural spread is very difficult to contain. In the RA area most river catchments are connected by artificial canals. Once oriental weatherfish is introduced, it will spread almost unnoticed, until one or a few specimens are coincidentally detected. Containment of this species in large river and other natural water systems is almost impossible, in common with most fish species. In smaller systems oriental weatherfish may be contained using piscicides (e.g. rotenone) but with high collateral damage. Also, piscicides may have legal restrictions of its use in several Member States of the RA area.

Qu. 3.11. Estimate the overall potential rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (indicate any key issues and provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in current conditions, providing insight in the risk of spread into (new areas in) the risk assessment area.

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: Oriental weatherloach do not appear to undertake migrations over long distances as part of their life-cycle (Lucas and Barras 2001). However, little appears to be known about their general movement patterns (Koster et al. 2002). As indicated in Q 3.1, the rate of spread in most countries and continents is not very fast (probably less than 10 km per year). A study by Schultz (1960) determined that in the Shiawassee River in Michigan, their expansion was slow, around 0.8 km per year but a study conducted in Australia concluded that oriental weatherfish can expand their range more quickly at about 7.2 km per year (Lintermans et al. 1990). Frable (2008) states that within just two years, oriental weatherfish populations had spread more than 35km in Washington. Koster et al. (2002) report that oriental weatherfish may be restricted in their ability to colonise in an upstream direction.

Currently there are only a few known established populations of oriental weatherfish in the RA area and, despite the high availability of suitable habitat, it is estimated that the overall potential rate of spread under current conditions is rather slow. The spread via corridors (e.g. canals) and through natural waterways is probably only of importance for Member States/regions with neighbouring existing populations in the RA area.

Qu. 3.12. Estimate the overall potential rate of spread in relevant biogeographical regions in foreseeable climate change conditions (provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if rates of spread are likely slowed down or accelerated.

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response: Although the establishment potential is expected to increase in the foreseeable climate change conditions (see Qs A7 and A9), the rate of spread rate is not expected to change compared to current conditions.

4 MAGNITUDE OF IMPACT

Important instructions:

- Questions 4.1-4.5 relate to biodiversity and ecosystem impacts, 4.6-4.8 to impacts on ecosystem services, 4.9-4.13 to economic impact, 4.14-4.15 to social and human health impact, and 4.16-4.18 to other impacts. These impacts can be interlinked, for example, a disease may cause impacts on biodiversity and/or ecosystem functioning that leads to impacts on ecosystem services and finally economic impacts. In such cases the assessor should try to note the different impacts where most appropriate, cross-referencing between questions when needed.
- Each set of questions starts with the impact elsewhere in the world, then considers impacts in the risk assessment area (=EU+UK, excluding outermost regions) separating known impacts to date (i.e. past and current impacts) from potential future impacts (including foreseeable climate change).
- Only negative impacts are considered in this section (socio-economic benefits are considered in Qu. A.7)
- ##

Biodiversity and ecosystem impacts

Qu. 4.1. How important is the impact of the organism on biodiversity at all levels of organisation caused by the organism in its non-native range excluding the risk assessment area?

including the following elements:

- Biodiversity means the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems
- impacted chemical, physical or structural characteristics and functioning of ecosystems

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Fredberg et al. (2014) suggest several potential impacts of oriental weatherfish in Australia including competition for spawning sites and for food and shelter, disturbance and/or predation of fish and frog eggs, alteration of habitat and decreased water quality. They, however, concluded that these impacts were largely speculative and further research is needed to fill several knowledge gaps.

For the USA, the U.S. Fish and Wildlife Service (2012) reported that there is a high risk of impacts because this species is highly adaptable, quick to reproduce, and is extremely popular in the aquaculture and aquarium industry. The climate match with the native range is high and multiple impacts are expected (reductions in macroinvertebrate populations, altered aquatic habitats, and the fish are vectors for certain fish parasites).

Several authors reported adverse impacts but most of these impacts are anecdotal. For instance, Nobile et al. (2017) sum up possible impacts of established oriental weatherfishes for Brazil as follows: niche overlap, competition for resources and space, introduction of parasites, predation on eggs of fishes and restructuration of the aquatic ecosystem.

Keller and Lake (2007) report the potential impacts for Australia. In a mesocosm experiment, they found that oriental weatherfish caused significant reductions in macroinvertebrate numbers and biomass and that this fish was also associated with elevated ammonia, nitrate/nitrite (NOx), and turbidity levels. However, the authors conclude: “We are not able to conclusively determine that oriental weatherfish are having large impacts in natural systems”.

In Asia, the species is known to hybridize with other species of *Misgurnus* and *Paramisgurnus* (You et al. 2009; Fujimoto et al. 2008a) but it is not clear what the impact of this phenomenon will be. *Misgurnus anguillicaudatus* is known to hybridise naturally with *P. dabryanus* (You et al. 2009). In aquaculture, Fujimoto et al. (2008a) were able to produce hybrids of *M. anguillicaudatus* and *M. mizolepis*.

Schmidt and Schmidt (2014) conclude that *M. anguillicaudatus* does not seem to affect other vertebrates in the Hudson River Valley (New York) but that attention should be paid on the potential negative interactions with the native eastern mudminnow *Umbra pygmaea*.

M. anguillicaudatus has been responsible for the establishment of the parasitic flatworm *Gyrodactylus macracanthus* (Dove and Ernst 1998) in Australia. Ogawa (1994) names this loach as host of the gill parasite *Ancyrocephalus cruciatus*, fin parasite *Gyrodactylus micracanthus*, and other unidentified fin and gill parasites of the genus *Gyrodactylus*. *Gyrodactylus* species can potentially infect indigenous fish species and can cause mortalities in aquaculture as was the case with another *Gyrodactylus* parasite (*G. salaris*) in Norway (Salte et al. 2010). *M. anguillicaudatus* can also host the parasite *Acanthocephalus opsariichthydis* (Amin et al. 2007). In addition, the gill parasites *Trichodina lechriodontata* and *Trichodina modesta* were found in oriental weatherfish (Zhao and Tang 2007). Also Reyda et al. (2019) report the introduction of at least three *Gyrodactylus* species in the USA through oriental weatherfish.

Qu. 4.2. How important is the current known impact of the organism on biodiversity at all levels of organisation (e.g. decline in native species, changes in native species communities, hybridisation) in the risk assessment area (include any past impact in your response)?

Discuss impacts that are currently occurring or are likely occurring or have occurred in the past in the risk assessment area. Where there is no direct evidence of impact in the risk assessment area (for example no studies have been conducted), evidence from outside of the risk assessment area can be used to infer impacts within the risk assessment area.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No studies were found that explicitly investigate the impact in the RA area. Within the RA area, the same biodiversity impacts as in Qu 4.1., however, can be assumed. Of major concern is the fact that *M. anguillicaudatus* is known to hybridise naturally with other weatherfishes e.g. with *P. dabryanus* (You et al. 2009). Artificial reproduction was used to successfully crossbreed *M. anguillicaudatus* with the native *M. fossilis* (J. Wanzenböck, pers. comm.). The European weatherfish *M. fossilis* is highly endangered in parts of its native territory, e.g. Belgium (Verreycken et al. 2014) with populations declining everywhere in the EU (Freyhof and Brooks 2011). Co-occurrence of a close relative of the oriental weatherfish, *M. bipartitus*, with European weatherfish was found in sites in the Netherlands and in Belgium and because *M. bipartitus* occurs in much larger numbers than the rare European weatherfish, the latter is automatically exposed to a much greater hybridization pressure (Brys et al. 2020).

Qu. 4.3. How important is the potential future impact of the organism on biodiversity at all levels of organisation likely to be in the risk assessment area?

See comment above. The potential future impact shall be assessed only for the risk assessment area.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Probably the current potential impact will not change much in the near future. However, if *M. anguillicaudatus* spreads further and more oriental weatherfish populations would establish then the potential impact might increase. Especially in areas where *M. fossilis* populations are declining, the relative pressure of *M. anguillicaudatus* on this native species will be higher in the future. Also when higher densities of oriental weatherfish occur, there could be other impacts, for example alteration of macroinvertebrate communities and decline of water quality in lakes and streams (Keller and Lake 2007).

Qu. 4.4. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism currently in the risk assessment area?

including the following elements:

- native species impacted, including red list species, endemic species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	minimal minor	CONFIDENCE	low medium
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	moderate major massive		high
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Response: The European weatherfish is protected by EU law (Habitats Directive Annex II and Bern Convention Annex III). It is listed as a species of least concern but as declining everywhere in the native range on the IUCN Red List of Threatened Species (Freyhof and Brooks 2011). Hartvich et al. (2010) report the conservation status of *M. fossilis* as follows: Endangered on the Red List in the Czech Republic; Danger of extinction, category 1 in Austria; Vulnerable in Croatia, Near Threatened in Slovakia and Vulnerable in Poland. The species is also listed as Endangered on the Red List of Flanders (Belgium) (Verreycken et al. 2014) and as Vulnerable on the Red List of The Netherlands (Spikmans and Kranenbarg 2016). The goal to preserve and improve populations of *M. fossilis* in the EU can be seriously hampered by the presence of oriental weatherfish through possible competition and hybridization. Probably no other native species are impacted directly by the oriental weatherfish as there are no closely related species present in the RA area.

The impact of oriental weatherfish on protected sites in the RA area (e.g. Natura 2000 habitats or particular habitats listed in the Habitats Directive) is not clear. However, as oriental weatherfish was associated with elevated ammonia, nitrate/nitrite (NO_x), and turbidity levels in mesocosm experiments in Australia (Keller and Lake 2007), the impact on these protected habitats may become moderate to major should high densities of oriental weatherfish establish there.

Also the ecological status of water bodies according to the Water Framework Directive (WFD) can be lower due to the presence of non-native species. Some groups (e.g. habitat-sensitive) fish species are of great importance for the WFD assessment of certain water types. Water managers assess the ecological status of waters, among other things, on the relative share of "habitat sensitive" species in the WFD assessment. The negative effects on these species as a result of competition with non-native species may have a negative effect on the results of WFD assessments (van Kessel et al. 2013b).

Qu. 4.5. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.4.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No major changes are expected for the future. However, should *M. anguillicaudatus* further expand its spread and establish in more sites and at higher densities, then slightly higher impacts as mentioned in Qu. 4.4. can be expected.

Ecosystem Services impacts

Qu. 4.6. How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?

- For a list of services use the CICES classification V5.1 provided in Annex V.
- Impacts on ecosystem services build on the observed impacts on biodiversity (habitat, species, genetic, functional) but focus exclusively on reflecting these changes in relation to their links with socio-economic well-being.
- Quantitative data should be provided whenever available and references duly reported.
- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No information has been found on the issue but two ecosystem services impacts can be possible in a minor form (1) Provisioning/Genetic material/ Genetic material from animals i.e. possible loss of local genetic material of *M. fossilis* as a consequence of hybridisation with oriental weatherfish (Brys et al. 2020) and (2) Regulations and Maintenance/Regulation of physical/chemical/biological condition/Water condition through elevated ammonia, nitrate/nitrite (NOx), and turbidity levels (Keller and Lake 2007).

Qu. 4.7. How important is the impact of the organism on provisioning, regulating, and cultural services currently in the different biogeographic regions or marine sub-regions where the species has established in the risk assessment area (include any past impact in your response)?

- See guidance to Qu. 4.6.

RESPONSE	N/A minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No information has been found on the issue.

Qu. 4.8. How important is the impact of the organism on provisioning, regulating, and cultural services likely to be in the different biogeographic regions or marine sub-regions where the

species can establish in the risk assessment area in the future?

- See guidance to Qu. 4.6.

RESPONSE	N/A minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No information has been found on the issue.

Economic impacts

Qu. 4.9. How great is the overall economic cost caused by the organism within its current area of distribution (excluding the risk assessment area), including both costs of / loss due to damage and the cost of current management.

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the world these should be reported here. The assessment of the potential costs of / loss due to damage shall describe those costs quantitatively and/or qualitatively depending on what information is available. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage. As far as possible, it would be useful to separate costs of / loss due to the organism from costs of current management.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No economic costs (e.g. management) of this species are mentioned. Established populations of oriental weatherfish are not actively managed in any state or territory in Australia, nor at a national level (Koster et al. 2002) but eradication with rotenone was trialled in the Wingecarribee River system and was unsuccessful. Eradication campaigns with rotenone are known to be costly. In the USA, rotenone treatments of large areas to remove (but not completely eradicate) common carps and ictalurid catfishes were reported to cost between US\$25,000 for 31 ha (\approx €730/ha) and US\$40,000 for 492 ha (\approx €74/ha) (U.S. Fish and Wildlife Service - Environmental Conservation Online System, 2020). In Hawaii, the control of tilapia with rotenone (CFT Legumine) was estimated to cost US\$5000 for 81 ha (€56/ha) (only product was counted, not personnel and equipment) (Tavares 2009).

Qu. 4.10. How great is the economic cost of / loss due to damage (excluding costs of

management) of the organism currently in the risk assessment area (include any past costs in your response)?

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the EU these should be reported here. Assessment of the potential costs of damage on human health, safety, and the economy, including the cost of non-action. A full economic assessment at EU scale might not be possible, but qualitative data or different case studies from across the EU (or third countries if relevant) may provide useful information to inform decision making. In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No economic costs of this species are known.

Qu. 4.11. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.10.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No economic costs of this species are known for the current nor future situation.

Qu. 4.12. How great are the economic costs / losses associated with managing this organism currently in the risk assessment area (include any past costs in your response)?

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	minimal minor	CONFIDENCE	low medium
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	moderate major massive		high
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Response: The only reported management programme of oriental weatherfish in the RA area is for the Netherlands. Efforts to eradicate (or at least manage) the Dutch population (Binnendijk et al. 2017) failed. Over several years and multiple times per year Binnendijk et al. (2017) tried to catch (with dipnets and electrofishing) as many loaches as possible and removed them from the infested site. Although many specimens were captured (n= 763), eradication efforts were stopped because the numbers kept increasing with new and smaller young-of-the-year individuals (i.e. natural reproduction) caught each year. The costs of these management efforts were not reported. Other eradication methods were deemed unfeasible on the basis of financial, practical, ecological or legal reasons (Binnendijk et al. 2017).

Qu. 4.13. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.12.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: Currently no targeted management schemes exist for oriental weatherfish in the RA area. In the past, only one management programme of oriental weatherfish in the RA area was reported (i.e. for the Netherlands (Binnendijk et al. 2017)). However, if *M. anguillicaudatus* were to further spread and establish large populations, then management costs would increase with expanding populations.

Social and human health impacts

Qu. 4.14. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism for the risk assessment area and for third countries, if relevant (e.g. with similar eco-climatic conditions).

The description of the known impact and the assessment of potential future impact on human health, safety and the economy, shall, if relevant, include information on

- illnesses, allergies or other affections to humans that may derive directly or indirectly from a species;
- damages provoked directly or indirectly by a species with consequences for the safety of people, property or infrastructure;
- direct or indirect disruption of, or other consequences for, an economic or social activity due to the presence of a species.

Social and human health impacts can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No information has been found on the issue and although no information on social and human health impacts is available for the RA area, it can be assumed that this is only minimal since reports from other (longer) invaded areas (e.g. Australia and the USA) do not explicitly mention these impacts either (Frable 2008, Koster et al. 2002).

Qu. 4.15. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism in the future for the risk assessment area.

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No information has been found on the issue but see previous answer.

Other impacts

Qu. 4.16. How important is the organism in facilitating other damaging organisms (e.g. diseases) as food source, a host, a symbiont or a vector etc.?

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: *Misgurnus anguillicaudatus* has been responsible for the establishment of the parasitic flatworm *Gyrodactylus macracanthus* (Dove and Ernst 1998) in Australia. Ogawa (1994) names this

loach as host of the gill parasite *Ancyrocephalus cruciatus*, fin parasite *Gyrodactylus micracanthus*, and other unidentified fin and gill parasites of the genus *Gyrodactylus*. *Misgurnus anguillicaudatus* can also host the parasite *Acanthocephalus opsariichthydis* (Amin et al. 2007). In addition, also the gill parasites *Trichodina lechriodontata* and *Trichodina modesta* were found in oriental weatherfish (Zhao and Tang 2007). Reyda et al. (2019) report the introduction of at least three *Gyrodactylus* species in the USA through oriental weatherfish. These parasites can potentially infect indigenous fish species and can cause mortalities in aquaculture as was the case with another *Gyrodactylus* parasite (*G. salaris*) in Norway (Salte et al. 2010).

Qu. 4.17. How important might other impacts not already covered by previous questions be resulting from introduction of the organism?

RESPONSE	N/A	CONFIDENCE	low
	minimal		medium
	minor		high
	moderate		
	major		
	massive		

Response: No information has been found on the issue.

Qu. 4.18. How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area?

RESPONSE	minimal	CONFIDENCE	low
	minor		medium
	moderate		high
	major		
	massive		

Response: The main impact of oriental weatherfish could be the possible hybridisation with the native, endangered European weatherfish *M. fossilis* (Brys et al. 2020). However, it can also indirectly affect native species through competition (e.g. for food (Schmidt and Schmidt 2014) and may affect the aquatic environment by modifying conditions such as altering water quality (Keller and Lake 2007). The natural control of the populations of oriental weatherfish by other organisms may be limited but their role is mostly unknown. Though there have been occasional reports of oriental weatherfish being eaten by redfin perch and brown trout, they often occur in shallow degraded habitats which large native predators may avoid (Koster et al. 2002). In the Dutch population of *M. bipartitus*, top predators like pike *Esox lucius* and perch *Perca fluviatilis* were present in the fish community (Binnendijk et al. 2017) and not limiting the establishment of this invasive species.

Qu. 4.19. Estimate the overall impact in the risk assessment area under current climate conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, in current conditions.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: The major concern of *M. anguillicaudatus* is the potential hybridisation with the native *M. fossilis*. The European weatherfish is highly endangered in parts of its native territory with populations declining everywhere in the EU (Freyhof and Brooks 2013). Co-occurrence of oriental weatherfish with European weatherfish can expose a great hybridization pressure on the latter (Brys et al. 2020). Oriental weatherfish can also indirectly affect native species through competition and may affect the aquatic environment by modifying conditions such as altering water quality and uprooting plants (Keller and Lake 2007). *Misgurnus anguillicaudatus* is also identified as carrier of several parasites and some of these (e.g. *Gyrodactylus* species) may be harmful to wild populations of native fish and to the aquaculture industry.

Qu. 4.20. Estimate the overall impact in the risk assessment area in foreseeable climate change conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, under future conditions.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response: No large changes in impact are to be expected in future climate conditions although some biogeographical regions (see Qu. A7) may have a higher establishment suitability. Little is known about the overall impact in the risk assessment area in the current situation (see Qu. 4.19), however, in the future, the overall impact may increase if more oriental weatherfish populations become established.

RISK SUMMARIES			
	RESPONSE	CONFIDENCE	COMMENT
Summarise Introduction and Entry*	very unlikely unlikely moderately likely likely very likely	low medium high	The oriental weatherfish is present in several member states (MS) of the RA area. Additional introductions are possible and probably ongoing since this species is available via the aquarium trade and the internet. It is also farmed in high numbers in China for the food industry.
Summarise Establishment*	very unlikely unlikely moderately likely likely very likely	low medium high	Several MS have established populations in the wild as a consequence of deliberate introductions and unintentional escapes. The species could currently establish in all EU biogeographic regions and conditions improve for most regions under climate change.
Summarise Spread*	very slowly slowly moderately rapidly very rapidly	low medium high	The rate of spread is not very fast (probably less than 10 km per year). Currently there are only a few known established populations in the RA area and despite the high availability of suitable habitat it is estimated that the overall potential rate of spread under current and future conditions is only moderate.
Summarise Impact*	minimal minor moderate major massive	low medium high	The major concern of <i>M. anguillicaudatus</i> is the potential hybridisation with the endangered native <i>M. fossilis</i> . Oriental weatherfish can also indirectly affect native species through competition and may affect the aquatic environment by modifying conditions such as altering water quality and uprooting plants and carry several parasites which may be harmful to wild populations of native fish and to the aquaculture industry. Future impacts are mostly unknown but may increase if more populations of oriental weatherfish were to establish.
Conclusion of the risk assessment (overall risk)	low moderate high	low medium high	Since the risks of introduction and entry and of establishment are high (with high confidence) and impacts are moderate (with

			medium confidence), the overall risk is high. Specifically, the threat to the endangered native European weatherfish <i>M. fossilis</i> is of great concern.
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*in current climate conditions and in foreseeable future climate conditions

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Distribution Summary

Please answer as follows:

- Yes if recorded, established or invasive
- if not recorded, established or invasive
- ? Unknown; data deficient

The columns refer to the answers to Questions A5 to A12 under Section A.

For data on marine species at the Member State level, delete Member States that have no marine borders. In all other cases, provide answers for all columns.

Member States and the United Kingdom

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Austria	?	?	Yes	Yes	?
Belgium	?	?	Yes	Yes	?
Bulgaria	–	–	Yes	Yes	–
Croatia	–	–	Yes	Yes	–
Cyprus	–	–	Yes	Yes	–
Czech Republic	–	–	Yes	Yes	–
Denmark	–	–	Yes	Yes	–
Estonia	–	–	Yes	Yes	–
Finland	–	–	?	Yes	–
France	–	–	Yes	Yes	–
Germany	Yes	Yes	Yes	Yes	Yes
Greece	–	–	Yes	Yes	–
Hungary	–	–	Yes	Yes	–
Ireland	–	–	?	Yes	–
Italy	Yes	Yes	Yes	Yes	Yes
Latvia	–	–	Yes	Yes	–
Lithuania	–	–	Yes	Yes	–
Luxembourg	–	–	Yes	Yes	–
Malta	–	–	Yes	Yes	–
Netherlands	?	?	Yes	Yes	Yes
Poland	–	–	Yes	Yes	–
Portugal	–	–	Yes	Yes	–
Romania	–	–	Yes	Yes	–
Slovakia	–	–	Yes	Yes	–
Slovenia	–	–	Yes	Yes	–
Spain	Yes	Yes	Yes	Yes	Yes
Sweden	–	–	?	Yes	–
United Kingdom	–	–	Yes	Yes	–

Biogeographical regions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Alpine	–	–	?	?	–
Atlantic	?	?	Yes	Yes	?
Black Sea	–	–	Yes	Yes	–
Boreal	–	–	?	Yes	–
Continental	Yes	Yes	Yes	Yes	Yes
Mediterranean	Yes	Yes	Yes	Yes	Yes
Pannonian	–	–	Yes	Yes	–
Steppic	–	–	Yes	Yes	–

ANNEX I Scoring of Likelihoods of Events

(taken from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Description	Frequency
Very unlikely	This sort of event is theoretically possible, but is never known to have occurred and is not expected to occur	1 in 10,000 years
Unlikely	This sort of event has occurred somewhere at least once in the last millenium	1 in 1,000 years
Moderately likely	This sort of event has occurred somewhere at least once in the last century	1 in 100 years
Likely	This sort of event has happened on several occasions elsewhere, or on at least once in the last decade	1 in 10 years
Very likely	This sort of event happens continually and would be expected to occur	Once a year

ANNEX II Scoring of Magnitude of Impacts

(modified from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Biodiversity and ecosystem impact	Ecosystem Services impact	Economic impact (Monetary loss and response costs per year)	Social and human health impact, and other impacts
	<i>Question 5.1-5</i>	<i>Question 5.6-8</i>	<i>Question 5.9-13</i>	<i>Question 5.14-18</i>
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected ⁸	Up to 10,000 Euro	No social disruption. Local, mild, short-term reversible effects to individuals.
Minor	Some ecosystem impact, reversible changes, localised	Local and temporary, reversible effects to one or few services	10,000-100,000 Euro	Significant concern expressed at local level. Mild short-term reversible effects to identifiable groups, localised.
Moderate	Measureable long-term damage to populations and ecosystem, but reversible; little spread, no extinction	Measureable, temporary, local and reversible effects on one or several services	100,000-1,000,000 Euro	Temporary changes to normal activities at local level. Minor irreversible effects and/or larger numbers covered by reversible effects, localised.
Major	Long-term irreversible ecosystem change, spreading beyond local area	Local and irreversible or widespread and reversible effects on one / several services	1,000,000-10,000,000 Euro	Some permanent change of activity locally, concern expressed over wider area. Significant irreversible effects locally or reversible effects over large area.
Massive	Widespread, long-term population loss or extinction, affecting several species with serious ecosystem effects	Widespread and irreversible effects on one / several services	Above 10,000,000 Euro	Long-term social change, significant loss of employment, migration from affected area. Widespread, severe, long-term, irreversible health effects.

⁸ Not to be confused with “no impact”.

ANNEX III Scoring of Confidence Levels

(modified from Bacher et al. 2017)

Each answer provided in the risk assessment must include an assessment of the level of confidence attached to that answer, reflecting the possibility that information needed for the answer is not available or is insufficient or available but conflicting.

The responses in the risk assessment should clearly support the choice of the confidence level.

Confidence level	Description
Low	There is no direct observational evidence to support the assessment, e.g. only inferred data have been used as supporting evidence <i>and/or</i> Impacts are recorded at a spatial scale which is unlikely to be relevant to the assessment area <i>and/or</i> Evidence is poor and difficult to interpret, e.g. because it is strongly ambiguous <i>and/or</i> The information sources are considered to be of low quality or contain information that is unreliable.
Medium	There is some direct observational evidence to support the assessment, but some information is inferred <i>and/or</i> Impacts are recorded at a small spatial scale, but rescaling of the data to relevant scales of the assessment area is considered reliable, or to embrace little uncertainty <i>and/or</i> The interpretation of the data is to some extent ambiguous or contradictory.
High	There is direct relevant observational evidence to support the assessment (including causality) <i>and</i> Impacts are recorded at a comparable scale <i>and/or</i> There are reliable/good quality data sources on impacts of the taxa <i>and</i> The interpretation of data/information is straightforward <i>and/or</i> Data/information are not controversial or contradictory.

ANNEX IV CBD pathway categorisation scheme

Overview of CBD pathway categorisation scheme showing how the 44 pathways relate to the six main pathway categories. All of the pathways can be broadly classified into 1) those that involve intentional transport (blue), 2) those in which the taxa are unintentionally transported (green) and 3) those where taxa moved between regions without direct transportation by humans and/or via artificial corridors (orange and yellow). **Note that the pathways in the category “Escape from confinement” can be considered intentional for the introduction into the risk assessment area and unintentional for the entry into the environment.**



ANNEX V Ecosystem services classification (CICES V5.1, simplified) and examples

For the purposes of this risk assessment, please feel free to use what seems as the most appropriate category / level / combination of impact (Section – Division – Group), reflecting information available.

Section	Division	Group	Examples (i.e. relevant CICES “classes”)
Provisioning	Biomass	Cultivated <i>terrestrial</i> plants	<p>Cultivated terrestrial plants (including fungi, algae) grown for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from cultivated plants, fungi, algae and bacteria for direct use or processing (excluding genetic materials); Cultivated plants (including fungi, algae) grown as a <u>source of energy</u></p> <p><i>Example: negative impacts of non-native organisms to crops, orchards, timber etc.</i></p>
		Cultivated <i>aquatic</i> plants	<p>Plants cultivated by in- situ aquaculture grown for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from in-situ aquaculture for direct use or processing (excluding genetic materials); Plants cultivated by in- situ aquaculture grown as an <u>energy source</u>.</p> <p><i>Example: negative impacts of non-native organisms to aquatic plants cultivated for nutrition, gardening etc. purposes.</i></p>
		Reared animals	<p>Animals reared for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from reared animals for direct use or processing (excluding genetic materials); Animals reared to provide <u>energy</u> (including mechanical)</p> <p><i>Example: negative impacts of non-native organisms to livestock</i></p>
		Reared <i>aquatic</i> animals	<p>Animals reared by in-situ aquaculture for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from animals grown by in-situ aquaculture for direct use or processing (excluding genetic materials); Animals reared by in-situ aquaculture as an <u>energy source</u></p> <p><i>Example: negative impacts of non-native organisms to fish farming</i></p>
		Wild plants (terrestrial and aquatic)	<p>Wild plants (terrestrial and aquatic, including fungi, algae) used for <u>nutrition</u>; <u>Fibres and other materials</u> from wild plants for direct use or processing (excluding genetic materials); Wild plants (terrestrial and aquatic, including fungi, algae) used as a <u>source of energy</u></p> <p><i>Example: reduction in the availability of wild plants (e.g. wild berries, ornamentals) due to non-native organisms (competition, spread of disease etc.)</i></p>
		Wild animals (terrestrial and aquatic)	<p>Wild animals (terrestrial and aquatic) used for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from wild animals for direct use or processing (excluding genetic materials); Wild animals (terrestrial and aquatic) used as a <u>source of energy</u></p> <p><i>Example: reduction in the availability of wild animals (e.g. fish stocks, game) due to non-native organisms (competition,</i></p>

			<i>predations, spread of disease etc.)</i>
	Genetic material from all biota	Genetic material from plants, algae or fungi	<p><u>Seeds, spores and other plant materials</u> collected for maintaining or establishing a population; Higher and lower plants (whole organisms) used to <u>breed new strains or varieties</u>; Individual genes extracted from higher and lower plants for the <u>design and construction of new biological entities</u></p> <p><i>Example: negative impacts of non-native organisms due to interbreeding</i></p>
		Genetic material from animals	<p>Animal material collected for the purposes of maintaining or establishing a population; Wild animals (whole organisms) used to breed new strains or varieties; Individual genes extracted from organisms for the design and construction of new biological entities</p> <p><i>Example: negative impacts of non-native organisms due to interbreeding</i></p>
	Water ⁹	Surface water used for nutrition, materials or energy	<p>Surface water for <u>drinking</u>; Surface water used as a material (<u>non-drinking purposes</u>); Freshwater surface water, coastal and marine water used as an <u>energy source</u></p> <p><i>Example: loss of access to surface water due to spread of non-native organisms</i></p>
		Ground water for used for nutrition, materials or energy	<p>Ground (and subsurface) water for <u>drinking</u>; Ground water (and subsurface) used as a material (<u>non-drinking purposes</u>); Ground water (and subsurface) used as an <u>energy source</u></p> <p><i>Example: reduced availability of ground water due to spread of non-native organisms and associated increase of ground water consumption by vegetation.</i></p>
Regulation & Maintenance	Transformation of biochemical or physical inputs to ecosystems	Mediation of wastes or toxic substances of anthropogenic origin by living processes	<p><u>Bio-remediation</u> by micro-organisms, algae, plants, and animals; <u>Filtration/sequestration/storage/accumulation</u> by micro-organisms, algae, plants, and animals</p> <p><i>Example: changes caused by non-native organisms to ecosystem functioning and ability to filtrate etc. waste or toxics</i></p>
		Mediation of nuisances of anthropogenic origin	<p><u>Smell reduction</u>; <u>noise attenuation</u>; <u>visual screening</u> (e.g. by means of green infrastructure)</p> <p><i>Example: changes caused by non-native organisms to ecosystem structure, leading to reduced ability to mediate nuisances.</i></p>
	Regulation of physical, chemical, biological conditions	Baseline flows and extreme event regulation	<p>Control of <u>erosion</u> rates; Buffering and attenuation of <u>mass movement</u>; <u>Hydrological cycle and water flow regulation</u> (Including flood control, and coastal protection); <u>Wind</u> protection; <u>Fire</u> protection</p> <p><i>Example: changes caused by non-native organisms to ecosystem functioning or structure leading to, for example, destabilisation of soil, increased risk or intensity of wild fires etc.</i></p>

⁹ Note: in the CICES classification provisioning of water is considered as an abiotic service whereas the rest of ecosystem services listed here are considered biotic.

		<p>Lifecycle maintenance, habitat and gene pool protection</p>	<p><u>Pollination</u> (or 'gamete' dispersal in a marine context); <u>Seed dispersal</u>; Maintaining <u>nursery populations and habitats</u> (Including gene pool protection)</p> <p><i>Example: changes caused by non-native organisms to the abundance and/or distribution of wild pollinators; changes to the availability / quality of nursery habitats for fisheries</i></p>
		<p>Pest and disease control</p>	<p>Pest control; Disease control</p> <p><i>Example: changes caused by non-native organisms to the abundance and/or distribution of pests</i></p>
		<p>Soil quality regulation</p>	<p><u>Weathering processes</u> and their effect on soil quality; <u>Decomposition and fixing processes</u> and their effect on soil quality</p> <p><i>Example: changes caused by non-native organisms to vegetation structure and/or soil fauna leading to reduced soil quality</i></p>
		<p>Water conditions</p>	<p>Regulation of the <u>chemical condition</u> of freshwaters by living processes; Regulation of the chemical condition of salt waters by living processes</p> <p><i>Example: changes caused by non-native organisms to buffer strips along water courses that remove nutrients in runoff and/or fish communities that regulate the resilience and resistance of water bodies to eutrophication</i></p>
		<p>Atmospheric composition and conditions</p>	<p>Regulation of <u>chemical composition</u> of atmosphere and oceans; Regulation of <u>temperature and humidity</u>, including ventilation and transpiration</p> <p><i>Example: changes caused by non-native organisms to ecosystems' ability to sequester carbon and/or evaporative cooling (e.g. by urban trees)</i></p>
Cultural	Direct, in-situ and outdoor interactions with living systems that depend on presence in the environmental setting	<p>Physical and experiential interactions with natural environment</p>	<p>Characteristics of living systems that that enable activities promoting health, recuperation or enjoyment through <u>active or immersive interactions</u>;</p> <p>Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through <u>passive or observational interactions</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that make it attractive for recreation, wild life watching etc.</i></p>
		<p>Intellectual and representative interactions with natural environment</p>	<p>Characteristics of living systems that enable <u>scientific investigation</u> or the creation of traditional ecological knowledge;</p> <p>Characteristics of living systems that enable <u>education and training</u>;</p> <p>Characteristics of living systems that are resonant in terms of <u>culture or heritage</u>;</p> <p>Characteristics of living systems that enable <u>aesthetic experiences</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have cultural importance</i></p>

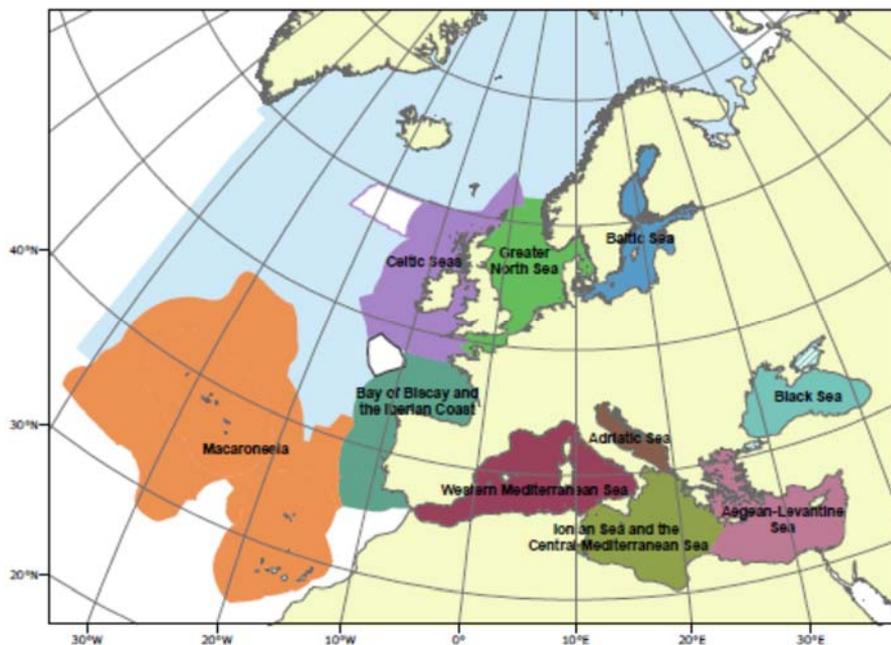
<p>Indirect, remote, often indoor interactions with living systems that do not require presence in the environmental setting</p>	<p>Spiritual, symbolic and other interactions with natural environment</p>	<p>Elements of living systems that have <u>symbolic meaning</u>; Elements of living systems that have <u>sacred or religious meaning</u>; Elements of living systems used for <u>entertainment or representation</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have sacred or religious meaning</i></p>
	<p>Other biotic characteristics that have a non-use value</p>	<p>Characteristics or features of living systems that have an <u>existence value</u>; Characteristics or features of living systems that have an <u>option or bequest value</u></p> <p><i>Example: changes caused by non-native organisms to ecosystems designated as wilderness areas, habitats of endangered species etc.</i></p>

ANNEX VI EU Biogeographic Regions and MSFD Subregions

See <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2>,
http://ec.europa.eu/environment/nature/natura2000/biogeog_regions/

and

<https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions-1/technical-document/pdf>



ANNEX VII Delegated Regulation (EU) 2018/968 of 30 April 2018

see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>

ANNEX VIII Species Distribution Model

Projection of environmental suitability for *Misgurnus anguillicaudatus* establishment in Europe

Björn Beckmann, Hugo Verreycken and Dan Chapman

08 October 2020

Aim

To project the suitability for potential establishment of *Misgurnus anguillicaudatus* in Europe, under current and predicted future climatic conditions.

Data for modelling

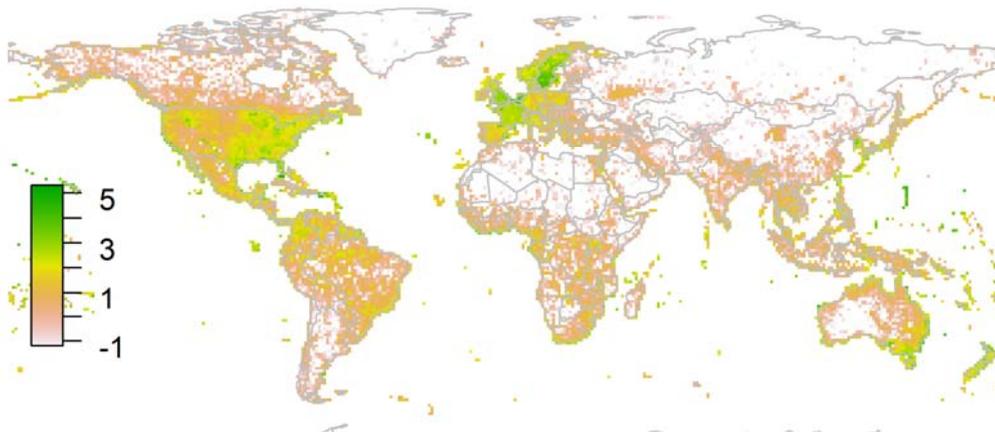
Species occurrence data were obtained from the Global Biodiversity Information Facility (GBIF) (8448 records), the Atlas of Living Australia (979 records), the Integrated Digitized Biocollections (iDigBio) (219 records), iNaturalist (181 records), the Biodiversity Information Serving Our Nation database (BISON) (109 records), the Berkeley Ecoinformatics Engine database (1 records), and 18 additional records from the risk assessment team which were not included in the above and taken from individual publications. We scrutinised occurrence records from regions where the species is not known to be established and removed any dubious records (e.g. fossils) or where the georeferencing was too imprecise (e.g. records referenced to a country or island centroid) or outside of the coverage of the predictor layers (e.g. small island or coastal occurrences). The remaining records were gridded at a 0.25 x 0.25 degree resolution for modelling, yielding 494 grid cells with occurrences (Figure 1a). As a proxy for recording effort, the density of Actinopterygii records held by GBIF was also compiled on the same grid (Figure 1b).

Figure 1. (a) Occurrence records obtained for *Misgurnus anguillicaudatus* and used in the modelling, showing native and invaded distributions. (b) The recording density of Actinopterygii on GBIF, which was used as a proxy for recording effort.

(a) Species distribution used in modelling



(b) Estimated recording effort (log10-scaled)



Climate data were selected from the 'Bioclim' variables contained within the WorldClim database (Hijmans et al., 2005), originally at 5 arcminute resolution (0.083 x 0.083 degrees of longitude/latitude) and aggregated to a 0.25 x 0.25 degree grid for use in the model.

Based on the biology of *Misgurnus anguillicaudatus*, the following climate variables were used in the modelling:

- Temperature seasonality (Bio4)
- Maximum temperature of the warmest month (Bio5)
- Minimum temperature of the coldest month (Bio6)
- Annual precipitation (Bio12)
- Precipitation seasonality (Bio15)

To estimate the effect of climate change on the potential distribution, equivalent modelled future climate conditions for the 2070s under the Representative Concentration Pathways (RCP) 2.6 and 4.5 were also obtained. These represent low and medium emissions scenarios, respectively. The above variables were obtained as averages of outputs of eight Global Climate Models (BCC-CSM1-1, CCSM4, GISS-E2-R, HadGEM2-AO, IPSL-CM5A-LR, MIROC-ESM, MRI-CGCM3, NorESM1-M), downscaled and calibrated against the WorldClim baseline (see http://www.worldclim.org/cmip5_5m).

The following habitat layers were also used:

- Human influence index (HII): As many non-native invasive species associate with anthropogenically disturbed habitats. We used the Global Human Influence Index Dataset of the Last of the Wild Project (Wildlife Conservation Society - WCS & Center for International Earth Science Information Network - CIESIN - Columbia University, 2005), which is developed from nine global data layers covering human population pressure (population density), human land use and infrastructure (built-up areas, nighttime lights, land use/land cover) and human access (coastlines, roads, railroads, navigable rivers). The index ranges between 0 and 1 and was $\ln+1$ transformed for the modelling to improve normality.

Species distribution model

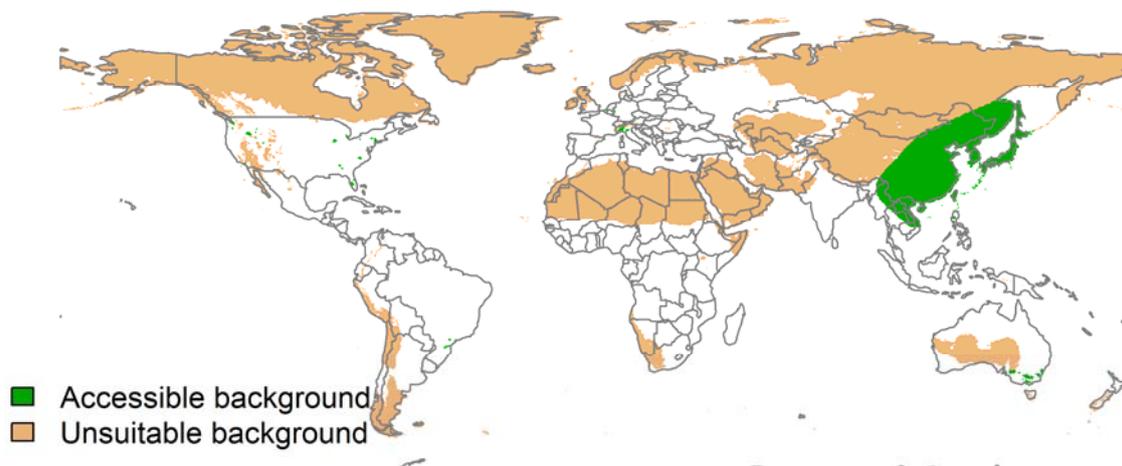
A presence-background (presence-only) ensemble modelling strategy was employed using the BIOMOD2 R package version 3.4.6 (Thuiller et al., 2020, Thuiller et al., 2009). These models contrast the environment at the species' occurrence locations against a random sample of the global background environmental conditions (often termed 'pseudo-absences') in order to characterise and project suitability for occurrence. This approach has been developed for distributions that are in equilibrium with the environment. Because invasive species' distributions are not at equilibrium and subject to dispersal constraints at a global scale, we took care to minimise the inclusion of locations suitable for the species but where it has not been able to disperse to (Chapman et al. 2019). Therefore the background sampling region included:

- The area accessible by native *Misgurnus anguillicaudatus* populations, in which the species is likely to have had sufficient time to disperse to all locations. Based on presumed maximum dispersal distances, the accessible region was defined as a 400km buffer around the native range occurrences; AND
- A 30km buffer around the non-native occurrences, encompassing regions likely to have had high propagule pressure for introduction by humans and/or dispersal of the species; AND
- Regions where we have an *a priori* expectation of high unsuitability for the species so that absence is assumed irrespective of dispersal constraints (see Figure 2). The following rules were applied to define a region expected to be highly unsuitable for *Misgurnus anguillicaudatus* at the spatial scale of the model:
 - Minimum temperature of the coldest month (Bio6) < -23°C
 - Maximum temperature of the warmest month (Bio5) < 19°C
 - Annual precipitation (Bio12) < 250mm

Altogether, only 1% of occurrence grid cells were located in the unsuitable background region.

Within the unsuitable background region, 10 samples of 5000 randomly sampled grid cells were obtained. In the accessible background (comprising the accessible areas around native and non-native occurrences as detailed above), the same number of pseudo-absence samples were drawn as there were presence records (494), weighting the sampling by a proxy for recording effort (Figure 2).

Figure 2. The background from which pseudo-absence samples were taken in the modelling of *Misgurnus anguillicaudatus*. Samples were taken from a 400km buffer around the native range and a 30km buffer around non-native occurrences (together forming the accessible background), and from areas expected to be highly unsuitable for the species (the unsuitable background region). Samples from the accessible background were weighted by a proxy for recording effort (Figure 1(b)).



Each dataset (i.e. combination of the presences and the individual background samples) was randomly split into 80% for model training and 20% for model evaluation. With each training dataset, five statistical algorithms were fitted with the default BIOMOD2 settings and rescaled using logistic regression, except where specified below:

- Generalised linear model (GLM)
- Generalised boosting model (GBM)
- Generalised additive model (GAM) with a maximum of four degrees of freedom per smoothing spline
- Random forest (RF)
- Maxent

Since the total background sample was larger than the number of occurrences, prevalence fitting weights were applied to give equal overall importance to the occurrences and the background. Normalised variable importance was assessed and variable response functions were produced using BIOMOD2's default procedure.

Model predictive performance was assessed by the following three measures:

- AUC, the area under the receiver operating characteristic curve (Fielding & Bell 1997). Predictions of presence-absence models can be compared with a subset of records set aside for model evaluation (here 20%) by constructing a confusion matrix with the number of true positive, false positive, false negative and true negative cases. For models generating non-dichotomous scores (as here) a threshold can be applied to transform the scores into a dichotomous set of presence-absence predictions. Two measures that can be derived from the confusion matrix are sensitivity (the proportion of observed presences that are predicted as such, quantifying omission errors), and specificity (the proportion of observed absences that are predicted as such, quantifying commission errors). A receiver operating characteristic (ROC) curve can be constructed by using all possible thresholds to classify the scores into confusion matrices, obtaining sensitivity and specificity for each matrix, and plotting sensitivity against the corresponding proportion of false positives (equal to $1 - \text{specificity}$). The use of all possible thresholds avoids the need for a selection of a single threshold, which is often arbitrary, and allows appreciation of the trade-off between sensitivity and specificity. The area under the ROC curve (AUC) is often used as a single threshold-independent measure for model performance (Manel, Williams & Ormerod 2001). AUC is the probability that a randomly selected presence has a higher model-predicted suitability than a randomly selected absence (Allouche et al. 2006).
- Cohen's Kappa (Cohen 1960). This measure corrects the overall accuracy of model predictions (ratio of the sum of true presences plus true absences to the total number of records) by the accuracy expected to occur by chance. The kappa statistic ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random. Advantages of kappa are its simplicity, the fact that both commission and omission errors are accounted for in one parameter, and its relative tolerance to zero values in the confusion matrix (Manel, Williams & Ormerod 2001). However, Kappa has been criticised for being sensitive to prevalence (the proportion of sites in which the species was recorded as present) and may therefore be inappropriate for comparisons of model accuracy between species or regions (McPherson, Jetz & Rogers 2004, Allouche et al. 2006).
- TSS, the true skill statistic (Allouche et al. 2006). TSS is defined as sensitivity + specificity - 1, and corrects for Kappa's dependency on prevalence. TSS compares the number of correct forecasts, minus those attributable to random guessing, to that of a hypothetical set of perfect forecasts. Like kappa, TSS takes into account both omission and commission errors, and success as a result of random guessing, and ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random (Allouche et al. 2006).

An ensemble model was created by first rejecting poorly performing algorithms with relatively extreme low AUC values and then averaging the predictions of the remaining algorithms, weighted by their AUC. To identify poorly performing algorithms, AUC values were converted into modified z-scores based on their difference to the median and the median absolute deviation across all algorithms (Iglewicz & Hoaglin, 1993). Algorithms with $z < -2$ were rejected. In this way, ensemble projections were made for each dataset and then averaged to give an overall suitability, as well as its standard deviation. The projections were then classified into suitable and unsuitable regions using the 'minROCDist' method, which minimizes the distance between the ROC plot and the upper left corner of the plot (point (0,1)).

We also produced limiting factor maps for Europe following Elith et al. (2010). For this, projections were made separately with each individual variable fixed at a near-optimal value. These were chosen as the median values at the occurrence grid cells. Then, the most strongly limiting factors were identified as the one resulting in the highest increase in suitability in each grid cell.

Results

The ensemble model suggested that suitability for *Misgurnus anguillicaudatus* was most strongly determined by Human influence index (HII), accounting for 34.3% of variation explained, followed by Maximum temperature of the warmest month (Bio5) (27.4%), Annual precipitation (Bio12) (23.7%), Temperature seasonality (Bio4) (7.5%), Minimum temperature of the coldest month (Bio6) (6.9%) and Precipitation seasonality (Bio15) (0.1%) (Table 1, Figure 3).

Table 1. Summary of the cross-validation predictive performance (AUC, Kappa, TSS) and variable importance of the fitted model algorithms and the ensemble (AUC-weighted average of the best performing algorithms). Results are the average from models fitted to 10 different background samples of the data.

Algorithm	AUC	Kappa	TSS	Used in the ensemble	Variable importance (%)					
					Human influence index (HII)	Maximum temperature of the warmest month (Bio5)	Annual precipitation (Bio12)	Temperature seasonality (Bio4)	Minimum temperature of the coldest month (Bio6)	Precipitation seasonality (Bio15)
GLM	0.962	0.632	0.892	yes	31	32	20	14	2	0
GAM	0.963	0.630	0.894	yes	30	24	27	7	13	0
GBM	0.962	0.636	0.890	yes	42	26	24	1	6	0
RF	0.944	0.607	0.883	no	24	22	35	8	7	5
Maxent	0.957	0.634	0.891	no	18	22	27	5	24	4
Ensemble	0.964	0.633	0.893		34	27	24	8	7	0

Figure 3. Partial response plots from the fitted models. Thin coloured lines show responses from the algorithms in the ensemble, while the thick black line is their ensemble. In each plot, other model variables are held at their median value in the training data. Some of the divergence among algorithms is because of their different treatment of interactions among variables.

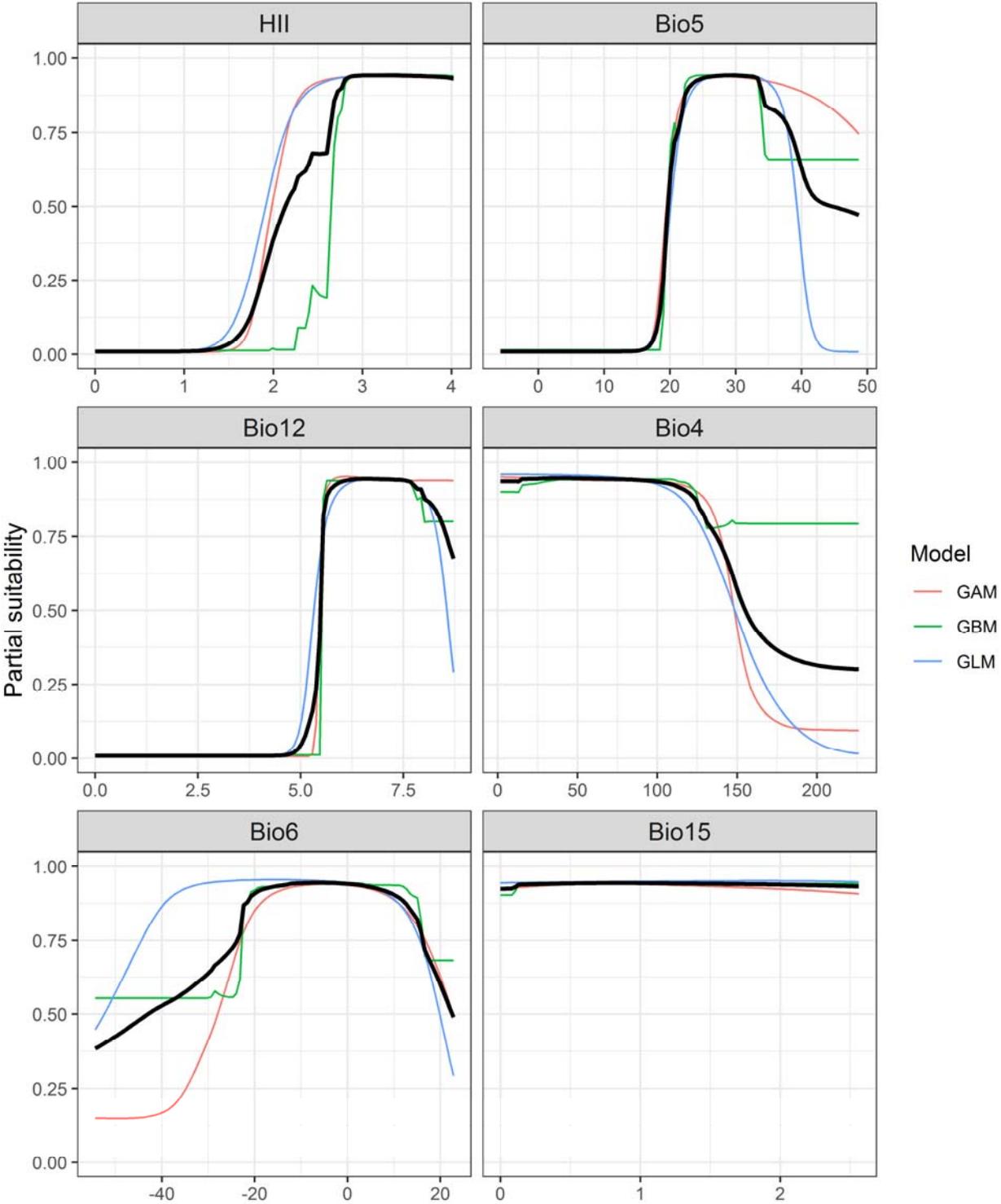


Figure 4. (a) Projected global suitability for *Misgurnus anguillicaudatus* establishment in the current climate. For visualisation, the projection has been aggregated to a 0.5 x 0.5 degree resolution, by taking the maximum suitability of constituent higher resolution grid cells. Values > 0.43 may be suitable for the species. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

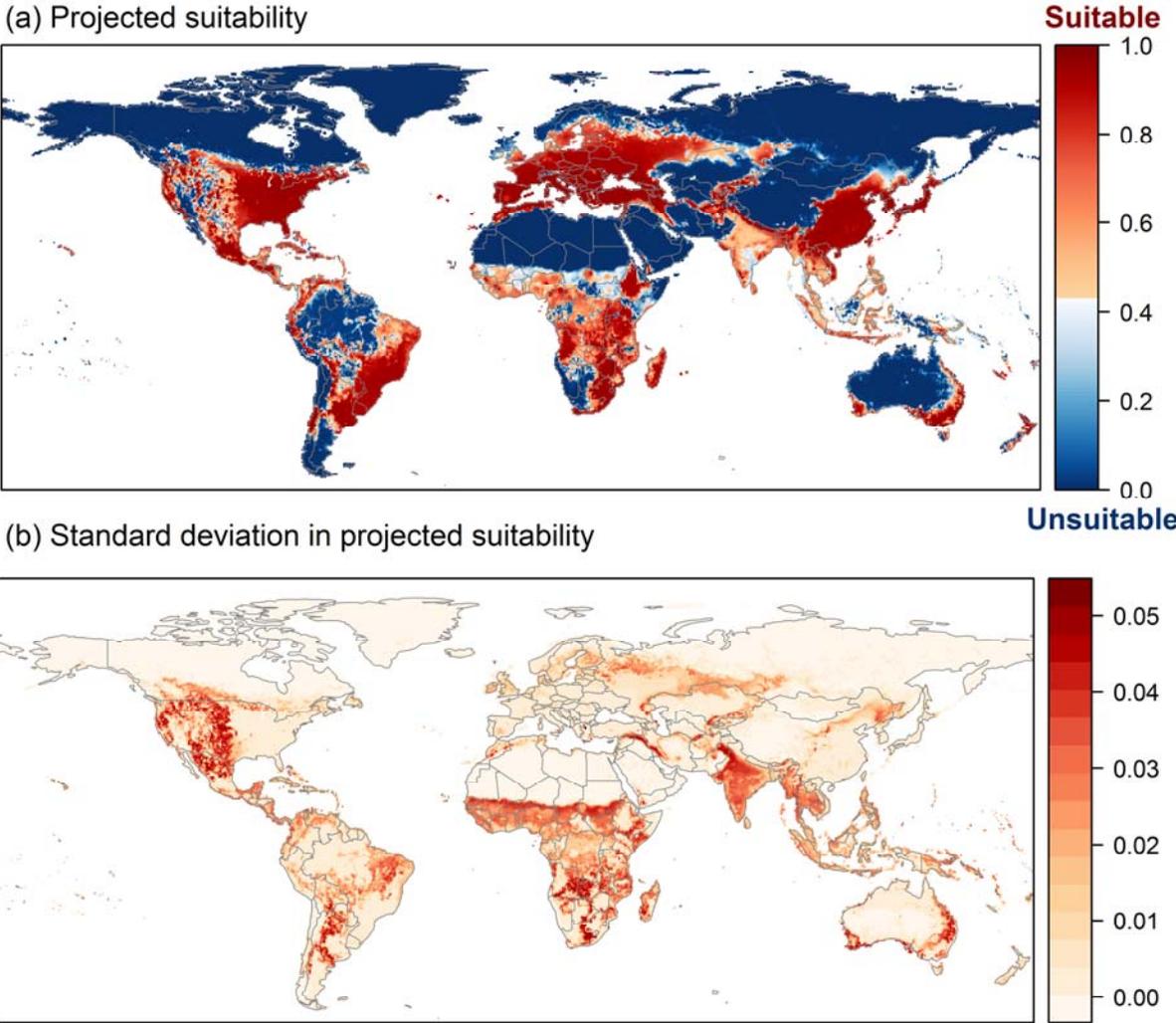


Figure 5. (a) Projected current suitability for *Misgurnus anguillicaudatus* establishment in Europe and the Mediterranean region. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

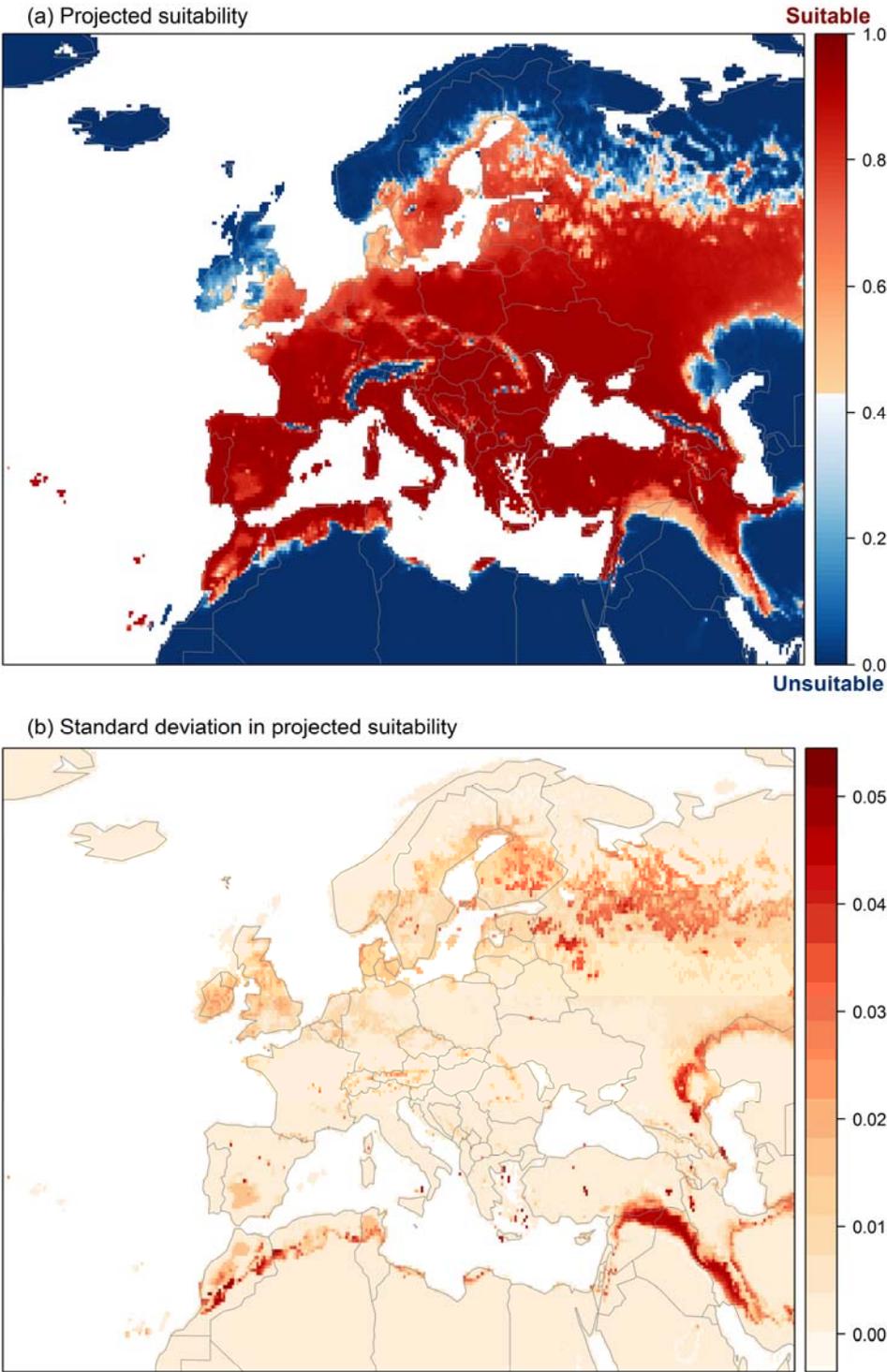


Figure 6. The most strongly limiting factors for *Misgurnus anguillicaudatus* establishment estimated by the model in Europe and the Mediterranean region in current climatic conditions.



Figure 7. (a) Projected suitability for *Misgurnus anguillicaudatus* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP2.6, equivalent to Figure 5. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

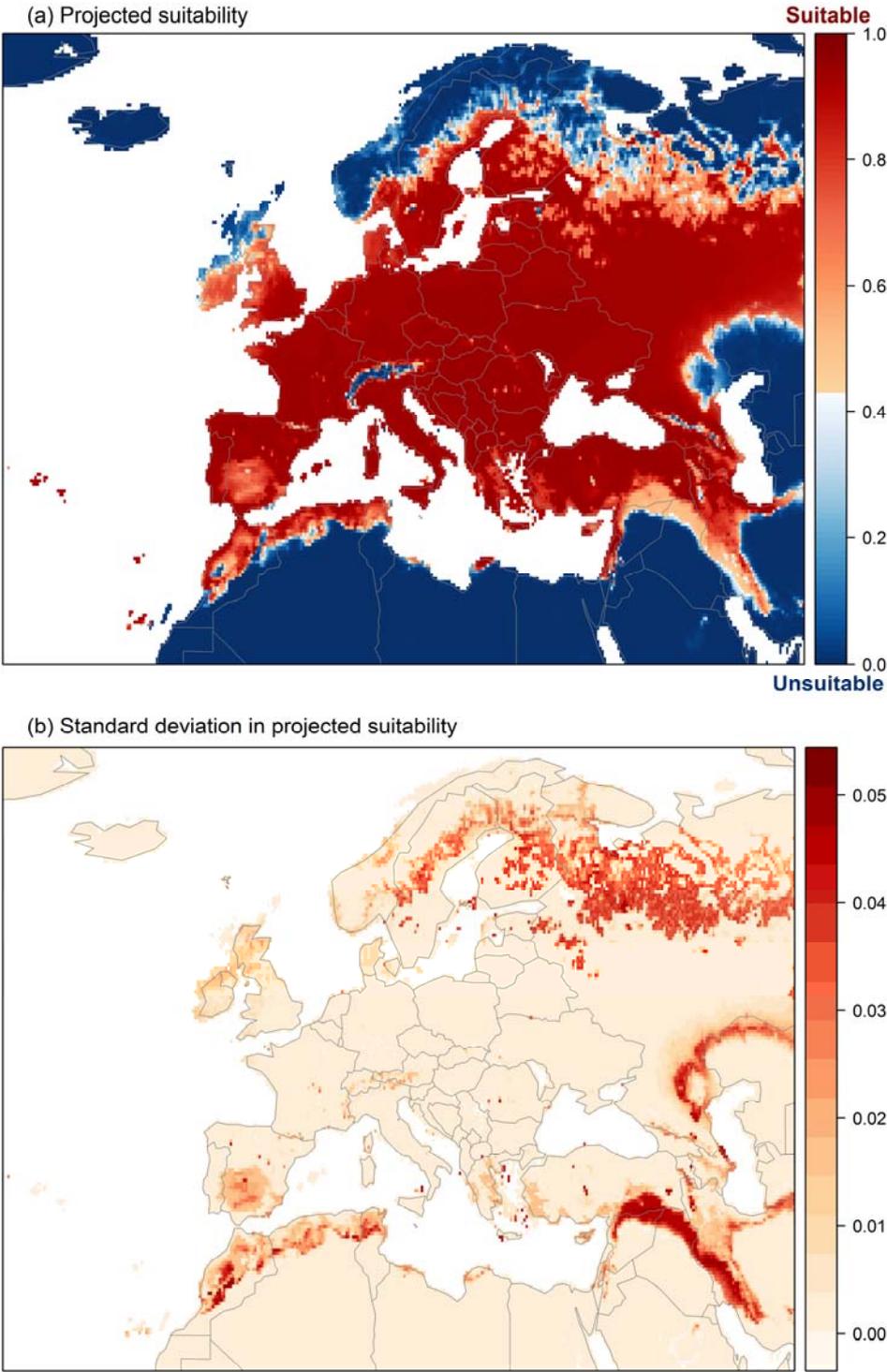


Figure 8. (a) Projected suitability for *Misgurnus anguillicaudatus* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP4.5, equivalent to Figure 5. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

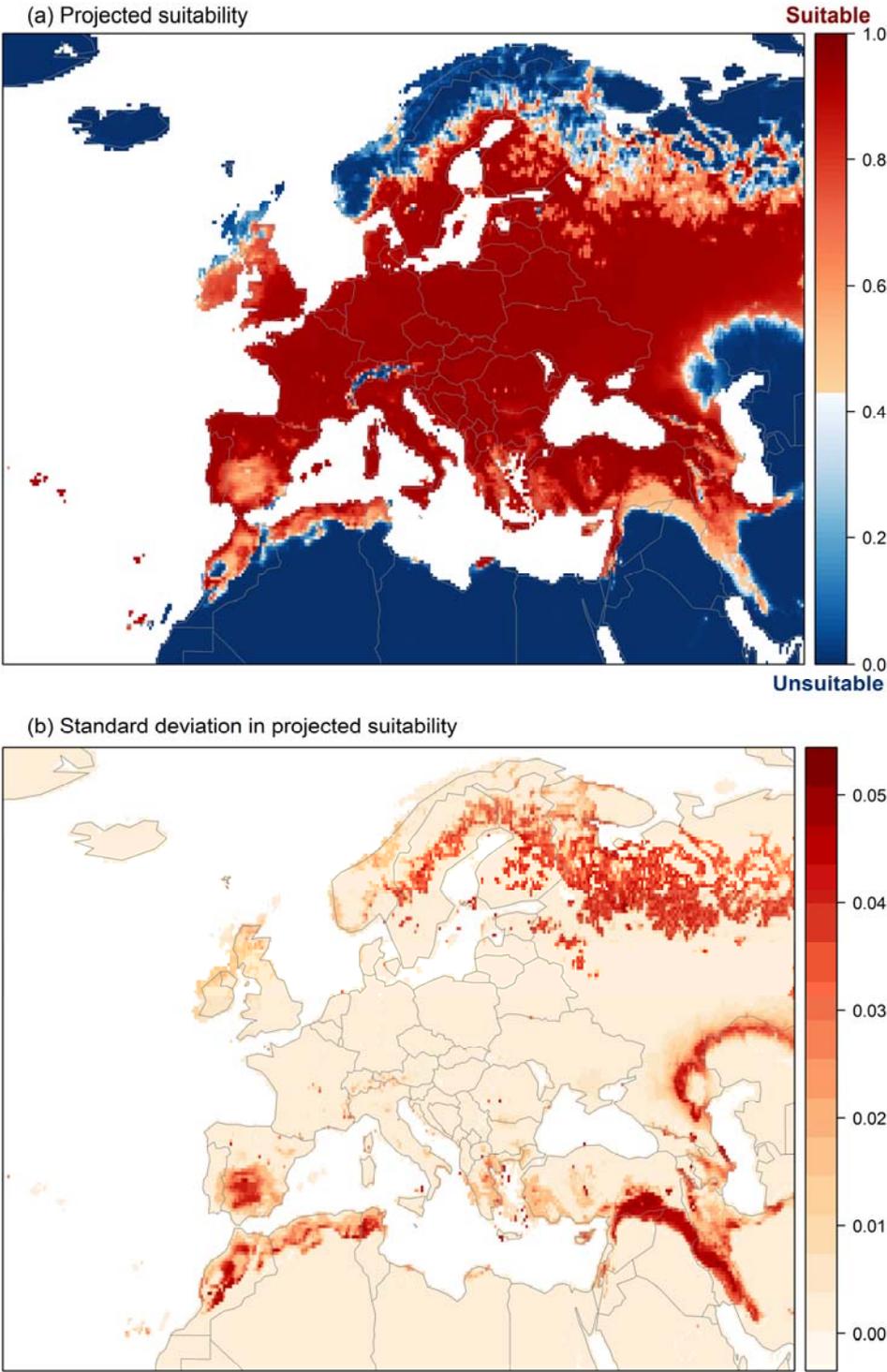


Figure 9. Variation in projected suitability for *Misgurnus anguillicaudatus* establishment among Biogeographical Regions of Europe (<https://www.eea.europa.eu/data-and-maps/data/biogeographical-regions-europe-3>). The bar plots show the proportion of grid cells in each region classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. The location of each region is also shown. The Arctic and Macaronesian biogeographical regions are not part of the study area, but are included for completeness.

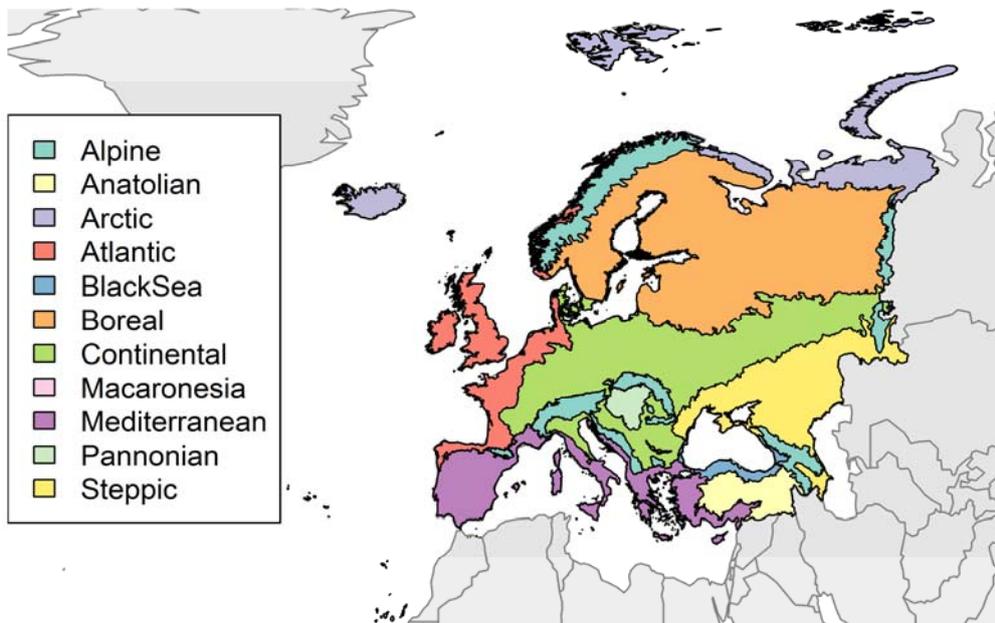
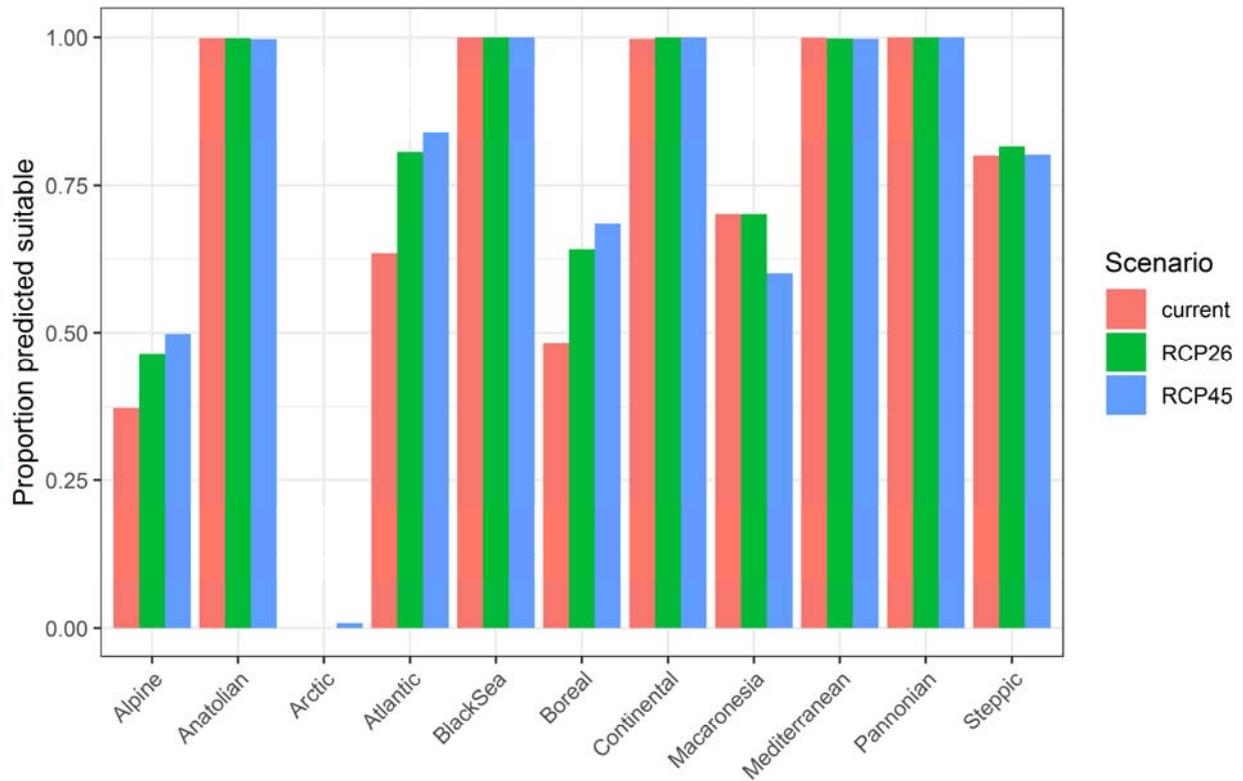


Table 2. Variation in projected suitability for *Misgurnus anguillicaudatus* establishment among Biogeographical regions of Europe (numerical values of Figure 9 above). The numbers are the proportion of grid cells in each region classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. The Arctic and Macaronesian biogeographical regions are not part of the study area, but are included for completeness.

Region	current climate	RCP26	RCP45
Alpine	0.37	0.46	0.50
Anatolian	1.00	1.00	1.00
Arctic	0.00	0.00	0.01
Atlantic	0.63	0.81	0.84
BlackSea	1.00	1.00	1.00
Boreal	0.48	0.64	0.68
Continental	1.00	1.00	1.00
Macaronesia	0.70	0.70	0.60
Mediterranean	1.00	1.00	1.00
Pannonian	1.00	1.00	1.00
Steppic	0.80	0.82	0.80

Figure 10. Variation in projected suitability for *Misgurnus anguillicaudatus* establishment among European Union countries and the UK. The bar plots show the proportion of grid cells in each country classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. Malta has been excluded because the Human Influence Index dataset lacks coverage for Malta.

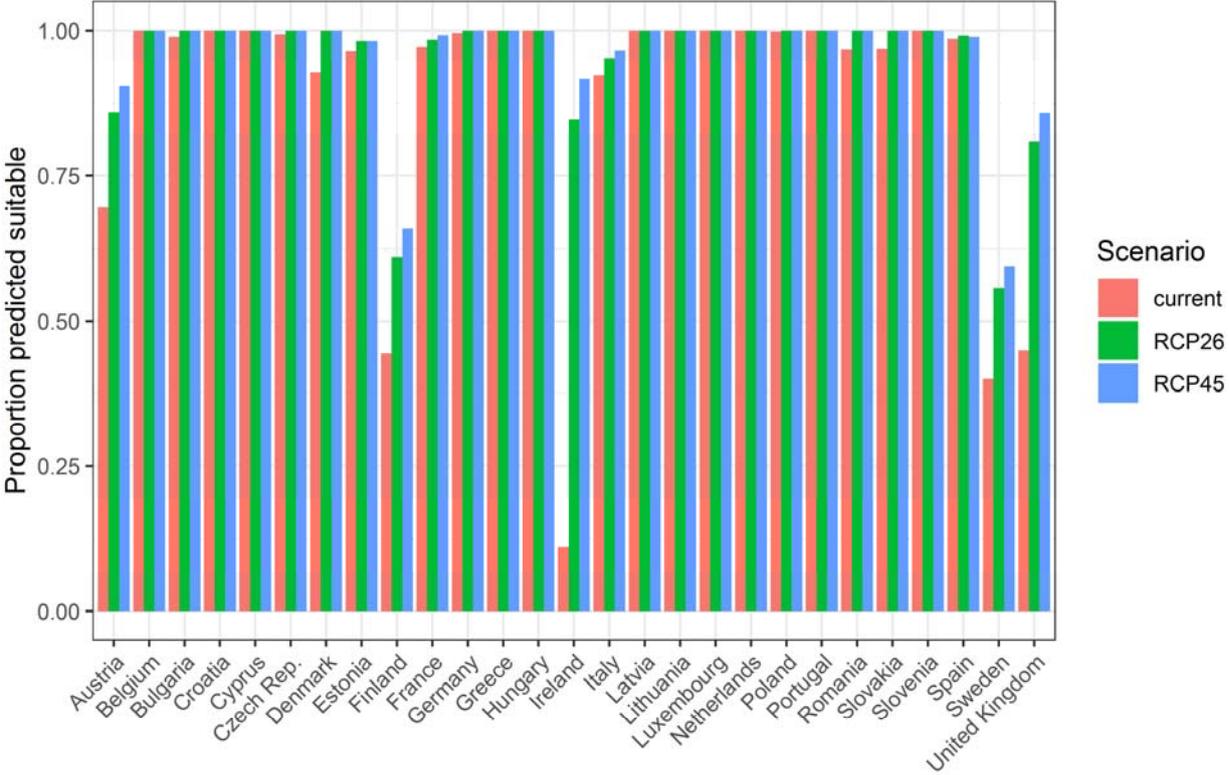


Table 3. Variation in projected suitability for *Misgurnus anguillicaudatus* establishment among European Union countries and the UK (numerical values of Figure 10 above). The numbers are the proportion of grid cells in each country classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. Malta has been excluded because the Human Influence Index dataset lacks coverage for Malta.

Region	current climate	RCP26	RCP45
Austria	0.69	0.86	0.90
Belgium	1.00	1.00	1.00
Bulgaria	0.99	1.00	1.00
Croatia	1.00	1.00	1.00
Cyprus	1.00	1.00	1.00
Czech Rep.	0.99	1.00	1.00
Denmark	0.93	1.00	1.00
Estonia	0.96	0.98	0.98
Finland	0.44	0.61	0.66
France	0.97	0.98	0.99
Germany	1.00	1.00	1.00
Greece	1.00	1.00	1.00
Hungary	1.00	1.00	1.00
Ireland	0.11	0.85	0.92
Italy	0.92	0.95	0.97
Latvia	1.00	1.00	1.00
Lithuania	1.00	1.00	1.00
Luxembourg	1.00	1.00	1.00
Netherlands	1.00	1.00	1.00
Poland	1.00	1.00	1.00
Portugal	1.00	1.00	1.00
Romania	0.97	1.00	1.00
Slovakia	0.97	1.00	1.00
Slovenia	1.00	1.00	1.00
Spain	0.99	0.99	0.99
Sweden	0.40	0.56	0.59
United Kingdom	0.45	0.81	0.86

Caveats to the modelling

To remove spatial recording biases, the selection of the background sample from the accessible background was weighted by the density of Actinopterygii records on the Global Biodiversity Information Facility (GBIF). While this is preferable to not accounting for recording bias at all, it may not provide the perfect measure of recording bias.

There was substantial variation among modelling algorithms in the partial response plots (Figure 3). In part this will reflect their different treatment of interactions among variables. Since partial plots are made with other variables held at their median, there may be values of a particular variable at which this does not provide a realistic combination of variables to predict from.

Other variables potentially affecting the distribution of the species, such as types of land cover were not included in the model.

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Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention"
Contract No 07.0202/2018/788519/ETU/ENV.D.2¹

Name of organism: *Pycnonotus cafer* (Linnaeus, 1766), red-vented bulbul

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Risk Assessment Area: The risk assessment area is the territory of the European Union 27 and the United Kingdom, excluding the outermost regions of the EU.

Peer review 1: *Tom Evans, Institute of Biology, Free University of Berlin & Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Berlin, Germany*

Peer review 2: *Jørgen Eilenberg, University of Copenhagen, Plant and Environmental Sciences, Denmark*

Acknowledgments: we thank Giuseppe Brundu and Rob Tanner (EPPO) for information on alien and invasive plants in the Mediterranean. We are grateful to Keith Bensusan (Gibraltar Botanic Gardens) and Carlos Gutiérrez-Expósito (Estación Biológica de Doñana) for information on the status of the species in southern Spain. We thank Iolanda Rocha (CIBIO-InBIO) for providing information on the status of reptiles on Mediterranean islands.

Date of completion: 31 October 2019

Date of revision: 4 March 2021

¹ This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA). A number of amendments have been introduced to ensure compliance with Regulation (EU) 1143/2014 on IAS and relevant legislation, including the Delegated Regulation (EU) 2018/968 of 30 April 2018, supplementing Regulation (EU) No 1143/2014 of the European Parliament and of the Council with regard to risk assessments in relation to invasive alien species (see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>).

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SECTION A – Organism Information and Screening

A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?

including the following elements:

- the taxonomic family, order and class to which the species belongs;
- the scientific name and author of the species, as well as a list of the most common synonym names;
- names used in commerce (if any)
- a list of the most common subspecies, lower taxa, varieties, breeds or hybrids

As a general rule, one risk assessment should be developed for a single species. However, there may be cases where it may be justified to develop one risk assessment covering more than one species (e.g. species belonging to the same genus with comparable or identical features and impact). It shall be clearly stated if the risk assessment covers more than one species, or if it excludes or only includes certain subspecies, lower taxa, hybrids, varieties or breeds (and if so, which subspecies, lower taxa, hybrids, varieties or breeds). Any such choice must be properly justified.

Response:

The red-vented bulbul belongs to the class Aves, order Passeriformes, family Pycnonotidae. The scientific name for the red-vented bulbul is *Pycnonotus cafer*, and the author is Linnaeus, 1766. Earlier names include *Turdus cafer* (Linnaeus, 1766), *Molpastes haemorrhous* (J.F. Gmelin, 1789), *Pycnonotus pygaeus* (Sharpe, 1881) and *Molpastes cafer* (Baker, 1930). Other names for the species in English and in other languages include common bulbul or sooty-headed bulbul (Thibault, 2018a), bulbul ventirrojo (ES), Bulbul à ventre rouge (FR), Rußbülbül (DE), Kala buulbuul (NL), roodbuikbuulbuul (NL) and Bilbil czerwonoplamy (PL).

The genus *Pycnonotus* comprises 49 species (Delacour, 1943; Dickinson and Dekker, 2002; Gill et al., 2021).

Pycnonotus cafer comprises eight subspecies (Dickinson et al., 2002):

- Central Indian red-vented bulbul (*P. c. humayuni* Deignan, 1951), found in south-eastern Pakistan, north-western and north-central India;
- Punjab red-vented bulbul (*P. c. intermedius* Blyth, 1846), in Kashmir, Kohat down to the Salt Range and along the western Himalayas to Kumaon, originally described as a separate species;
- *P. c. bengalensis* (Blyth, 1845), in central and eastern Himalayas from Nepal to Assam, north-eastern India and Bangladesh, originally described as a separate species;
- *P. c. stanfordi* (Deignan, 1949), in northern Burma and south-western China;
- *P. c. melanchimus* (Deignan, 1949), in south-central Burma and northern Thailand;
- *P. c. wetmorei* (Deignan, 1960), in eastern India;
- *P. c. cafer* (Linnaeus, 1766), in southern India;
- *P. c. haemorrhousus* (Gmelin, 1789), in Sri Lanka (Dickinson et al., 2002).

In its native range, *P. c. humayuni* is known to hybridize with *Pycnonotus leucogenys* (Gray, 1835). These hybrids were once described as subspecies *magrathi* (Sibley & Short, 1959). Hybridisation with

Pycnonotus leucogenys has also been observed in the United Arab Emirates (Khan, 1993) and Bahrain (Khamis, 2010). The offspring of hybrids in Bahrain is believed to be sterile (Khamis, 2010). In Myanmar, there is some natural hybridization with *Pycnonotus aurigaster* (Sharpe, 1909; Rasmussen & Anderton, 2005), with hybrids formerly considered as the subspecies *P. c. burmanicus* and *P. c. nigropileus* (Dickinson et al. 2002; Dickinson & Dekker 2002). Hybridisation with *Pycnonotus leucotis* (Gould, 1836) has been observed in Kuwait (Gregory, 2005), Qatar (Nation et al., 1997) and Iran (Azin et al., 2008). Hybridisation with *Pycnonotus xanthopygos* (Hemprich & Ehrenberg, 1833) has been observed in the United Arab Emirates (Khan, 1993).

A2. Provide information on the existence of other species that look very similar [that may be detected in the risk assessment area, either in the environment, in confinement or associated with a pathway of introduction]

Include both native and non-native species that could be confused with the species being assessed, including the following elements:

- other alien species with similar invasive characteristics, to be avoided as substitute species (in this case preparing a risk assessment for more than one species together may be considered);
- other alien species without similar invasive characteristics, potential substitute species;
- native species, potential misidentification and mis-targeting

Response:

Pycnonotus cafer has brown feathering and a black crest on its head, neck and throat (Thibault, 2018a; Pratt et al., 1987). Its sub-caudal feathers have a bright crimson colour, hence the species' name (Berger, 1981; Zia et al., 2014). It has a pale greyish white lower belly and rump which is highly visible in flight, and a long tail with a white tip (Zia et al., 2014). The tips of the back and breast feathers are white and this light edging gives them a scaly appearance (Thibault, 2018a). The tail is black with a narrow white tip (Thibault, 2018a). The red-vented bulbul measures about 21 cm in length (Berger, 1972) and its weight can vary from 26 to 45 grams (Long, 1981). Males can measure up to 23 cm in length, slightly larger than females, which is the only sexual dimorphism in the red-vented bulbul (Stuart & Stuart, 1999). Juveniles look like adults but with paler feathering and brownish edging on the feathers (Thibault, 2018a). It may live for up to 11 years in captivity (Walker, 2008).

The red-whiskered bulbul (*P. jocosus*) or crested bulbul, has been introduced to many regions of the world, including in Europe (Spain). The red-whiskered bulbul has an erect black/dark brown crest, a dark brown/black head with prominent white cheek patches and red whiskers below each eye. It is widely kept as a cage bird, escaped and established in many places, including Australia, Madagascar, Hawaii, Japan, the Seychelles, the USA and Spain. It is a pest of agriculture and gardens, feeding on fruits, vegetables, flower buds and insects. Furthermore, it is known to have environmental impacts through the dispersal of seeds of invasive plants, interspecific competition and predation on geckos and invertebrates (Hawaii Invasive Species Council, 2017; Cottrell, 2017).

In Europe, both the red-vented bulbul and the red-whiskered bulbul are established sympatrically in Valencia, Spain (Lever, 2015; Dyer et al., 2017), so it is possible that one species could be confused for the other in this region. Another alien species physically resembling the red-vented bulbul is the sooty-headed bulbul (*P. aurigaster*), mostly because of its red vent and black head. However, the breast and belly of *P. aurigaster* is lighter and only birds of the *chrysorrhoides* group have a red vent (Fishpool & Tobias, 2019). The sooty-headed bulbul is one of the most abundant and widespread native bulbuls on Java and Bali. It has been introduced to Sumatra, Singapore and Borneo where its alien populations have been expanding since the 1980s. It is suggested that this spread can be attributed to escaped birds from captivity (Phillipps & Phillipps, 2011). However, the species currently has no known alien populations in Europe. Hybrids between red-vented and sooty-headed bulbul do occur and can be difficult to identify (see A1). The only other bulbul with a known alien population is the yellow-vented bulbul (*P. goiavier*), which is native to the Malay Peninsula, Borneo, Thailand and the Philippines, and has an alien population on Sulawesi (Lever, 2005). However, the yellow-vented bulbul does not possess similar physical characteristics to the red-vented bulbul.

The only native species that somewhat resembles the red-vented bulbul and that therefore could be misidentified as such is the common bulbul (*P. barbatus*). This species is one of the commonest birds in Africa but is very rare in the wild in the risk assessment area. In recent years, common bulbul has bred in Tarifa (Cádiz, Andalusia, Spain) in an area that is potentially suitable for the red-vented bulbul as well. Birds are still present in the area, but only one pair has been known to breed and its status in Europe is currently tenuous (personal communication K. Bensusan, 22/10/2019). In 2013, two adult common bulbuls were observed feeding a young, which represented the first breeding record for the species in Europe (van den Berg & Haas, 2013).

A3. Does a relevant earlier risk assessment exist? Give details of any previous risk assessment, including the final scores and its validity in relation to the risk assessment area.

Response:

No other risk assessments for the species were found. However, as a well-known invader listed on the IUCN/ISSG list of 100th of the worst invasive species (Lowe et al., 2004), the species was included in several scoring exercises of invasive bird impacts globally. The results of these exercises are discussed in Qu. 5.1. An assessment of the impacts of the red-vented bulbul, undertaken using the Environmental Impact Classification for Alien Taxa (EICAT) classified the species as having Moderate (MO) impacts² through competition with native birds and by spreading the seeds of alien plants (Evans et al., 2016). The red-vented bulbul is also listed in the DAISIE database, however without any specific assessment.

A4. Where is the organism native?

including the following elements:

² EICAT categories are, from low to high impact: Minimal Concern (MC), Minor (MN), Moderate (MO), Major (MR), Massive (MV). MO impacts cause declines in populations of native species but do not cause native species extinctions.

- an indication of the continent or part of a continent, climatic zone and habitat where the species is naturally occurring
- if applicable, indicate whether the species could naturally spread into the risk assessment area

Response:

Pycnonotus cafer is native to the Indian Subcontinent, Southeast Asia, and Malay Peninsula (Long, 1981). It occurs naturally from Eastern Pakistan to southern China and Vietnam, and from Northern India to Sri Lanka. The species is linked to an equatorial climate according to the Kopper-Geigen classification (Kottek et al., 2006) and can live in diverse habitat types. The species is found in open areas, dry scrub, plains, cropland, natural forests, forest edges as well as plantations and prefers anthropogenic environments (urban areas, gardens, parks, farms) (Vander Velde, 2002). In India, the species is used for bulbul fighting, a traditional yet recently prohibited custom at harvest festivals (Ratnagar, 2015).

A5. What is the global non-native distribution of the organism outside the risk assessment area?

Response:

According to Thibault et al. (2018c), *Pycnonotus cafer* was introduced into 18 countries and established in 17 of them. The species is now present on at least 37 islands and seven continental locations (Thibault et al., 2018c). In order of first detection the countries where red-vented bulbul was introduced are: Fiji, Australia (extinct), Tonga, the Independent State of Samoa, New Zealand (eradicated), American Samoa, USA (Hawaii, Texas), Qatar, United Arab Emirates, French Polynesia, Saudi Arabia, Kuwait, New Caledonia, Bahrain, Oman, Spain (mainland Spain and Fuerteventura - Canary Islands), the Marshall Islands and Iran (Thibault et al., 2018c).

The first record of the red-vented bulbul outside of its native range was in Fiji in 1903 (Parham, 1955) where the species established and colonised several islands (Thibault et al., 2018c). It was probably brought there by Indian immigrants in the early 1900s (Watling, 1978), as it was widely used in bird fights in India because of its aggressive behaviour (Ali & Ripley, 1971). The bird has been recorded 6100 times in eight Pacific archipelagos since this first detection (Thibault 2018a).

The red-vented bulbul was detected in Melbourne and Sydney, Australia in 1918 and again in Melbourne in 1942 (Lendon, 1952; Watling, 1978; Dyer et al., 2017). Although a small population persisted for some time in the suburbs of Melbourne since 1918 (Watling 1978), it did however not successfully establish in Australia (Thibault et al., 2018c). The subspecies *P. c. bengalensis* was found in Melbourne in 1982 (Dyer et al., 2017). There are no records of the species since then. It is considered to be eradicated in Australia (<http://www.issg.org/database>).

The red-vented bulbul was deliberately introduced in the 1940s in Tonga to control unwanted insects (Watling, 1978). The red-vented bulbul probably reached the Independent State of Samoa in the early 1950's, and has since spread to several islands in the archipelago (Dhondt, 1977). The first observation of red-vented bulbul in Auckland, New Zealand was in 1952, but the species was eradicated in 1955

(Turbott, 1956; Watling, 1978). The red-vented bulbul was introduced in American Samoa in the late 1950s and quickly became established (Clapp & Sibley 1966; Freifeld, 1999). First observations for Hawaii (USA) date from 1966. In French Polynesia, the red-vented bulbul was first noticed in the residential area of Papeete in 1979. They are now common on Tahiti on elevations of up to 1000 m, possibly having negative interactions with the Tahiti monarch (*Pomarea nigra*) (Blanvillain et al., 2003). The species was intentionally released in Nouméa (New Caledonia) around 1983 by bird dealers to avoid prosecution (Thibault et al., 2018c). The red-vented bulbul was first sighted on the Marshall Islands in 2000, near the major commercial dock of Majuro, and was known to hitchhike on ships to other areas of the archipelago. In 2002, there were already several breeding populations (Vander Velde, 2002). Several individuals were seen by Christina Sylvester on the Kwajalein Atoll in November 2018 (<http://www.underwaterkwaj.com/land/bird-kwaj/bird-kwaj.htm>, visited on 07/06/2019).

In the Middle East, the red-vented bulbul was first detected in Qatar in 1971 (Nation et al., 1997). It has been reported 3080 times in five countries around the Persian Gulf since this first detection. The red-vented bulbul was first detected in the United Arab Emirates in 1974 where it is now common and expanding (Pedersen & Aspinall, 2010), and was first detected in Saudi Arabia in the 1980s (personal communication J. Babington). In Kuwait, the red-vented bulbul was first observed in 1981, and it is currently scarce, with a declining range (Gregory, 2005). According to Khamis (2010), the red-vented bulbul was first recorded in Bahrain in 1986, likely following an escape. At present, the bird maintains a self-sustaining population here. The first observation in Oman dates from 1987 and the red-vented bulbul is now a common bird there (Thibault et al., 2018a). In Iran, the red-vented bulbul was first recorded in 2007 when 10-12 individuals were observed in the east of Kish Island, Hormozgan Province (Azin et al., 2008).

The red-vented bulbul was first observed in Houston (Texas, USA) near the end of the 1990's. At least 14 sightings were reported between May 1999 and March 2004. These sightings have been estimated to represent 32 birds at 10 sites in Houston (Eubanks et al., 2006).

A population is also established on Fuerteventura (Canary Islands, Spain) with the first sightings in the late 1990s. By 2017, the species had spread over the entire island (SEO/Birdlife 2017; Nowakowski and Dulisz 2019).

A6. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species been recorded and where is it established? The information needs to be given separately for recorded and established occurrences.

A6a. Recorded: List regions

A6b. Established: List regions

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

- Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea.

Comment on the sources of information on which the response is based and discuss any uncertainty in the response.

For delimitation of EU biogeographical regions please refer to <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2> (see also Annex V).

For delimitation of EU marine regions and subregions consider the Marine Strategy Framework Directive areas; please refer to <https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions/technical-document/pdf> (see also Annex V).

Response (6a): Mediterranean, Atlantic

In the Netherlands, there have been multiple sightings of individual birds in the wild since 1999 (Avifauna Groningen, 2000; waarneming.nl). A number of these birds were sighted in the middle of urban areas suggesting an escaped origin. In Belgium, there was one observation of an escape in a natural area in a military Domain in 2005 (Brecht, Antwerp province). There are probably many more unreported incidental records of escaped birds across the risk assessment area. In Spain, there are some observations in Málaga and Torremolinos (personal communication A. Paterson).

Response (6b): Mediterranean

In Valencia (Spain), the species seems to be established, but no research has targeted this species yet. Breeding was observed in 2017 in gardens of urbanized areas surrounding the city. The range is not big, but they seem to be fairly common. There, the species is sympatric with the well-established red-whiskered bulbul (*P. jocosus*). (<https://www.miteco.gob.es/es/biodiversidad/temas/inventarios-nacionales/>; personal communication C. Gutiérrez-Expósito, 12/12/2018).

A7. In which biogeographic region(s) or marine subregion(s) in the risk assessment area could the species establish in the future under current climate and under foreseeable climate change? The information needs to be given separately for current climate and under foreseeable climate change conditions.

A7a. Current climate: List regions

A7b. Future climate: List regions

With regard to EU biogeographic and marine (sub)regions, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (7a): Mediterranean

According to the species distribution model (SDM, Annex VII), the red-vented bulbul could potentially establish in the Mediterranean biogeographical region. However, only around 2% of this bioregion is predicted to be suitable under current climatic conditions.

Response (7b): Mediterranean.

Under climate change scenarios RCP 2.6 and RCP 4.5, only the Mediterranean biogeographical region is deemed suitable for the establishment of *P. cafer*. The proportion of the region predicted to be suitable increases under both RCP scenarios, to 4% under RCP 2.6 and 6% under RCP 4.5.

A8. In which EU Member States has the species been recorded and in which EU Member States has it established? List them with an indication of the timeline of observations. The information needs be given separately for recorded and established occurrences.

A8a. Recorded: List Member States

A8b. Established: List Member States

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, the Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden

The description of the invasion history of the species shall include information on countries invaded and an indication of the timeline of the first observations, establishment and spread.

Response (8a): Spain, Belgium, The Netherlands

In the province of Málaga (Spain), breeding was observed in 2001 and 2002, however, it seems that the red-vented bulbul is not established there anymore. The last documented sighting of red-vented bulbul in Málaga province dates back to 2007. In Torremolinos (Málaga), a group of birds was observed in 1998, 2001 and 2002 (personal communication A. Paterson). On November 9, 2000, three individuals were observed singing and on May 27, 2001, a couple copulating. On 7 July 2002, four specimens were observed, of which two were juveniles. On September 9, 2002, a second clutch was confirmed, and the pair was observed with a chick, indicating the species successfully established in this area. However, there are no indications red-vented bulbul is currently still established around Torremolinos (<https://www.miteco.gob.es/es/biodiversidad/temas/inventarios-nacionales/>).

In Belgium, the red-vented bulbul was observed in August 2005 in the province of Antwerp (<http://waarnemingen.be/waarneming/view/41885488>). This is the only known observation for Belgium.

In the Netherlands, the red-vented bulbul was observed on five occasions, in September and October of 2006, and in July 2009 (<https://data.biodiversitydata.nl/obsint/observation/OBS.44081179>).

Response (8b): Spain

In Valencia (Valencia, Spain), the species seems to be established, as breeding was observed in 2017 when a family group of bulbuls were seen in urban gardens surrounding the city (personal communication C. Gutiérrez-Expósito, 12/12/2018). Their range is small, but they are not uncommon. The species is sympatric with a well-established red-whiskered bulbul (*P. jocosus*) population. Successful breeding of red-vented bulbul was reported in 2002 around Torremolinos but there, the last sighting was performed in 2007 (see above).

A9. In which EU Member States could the species establish in the future under current climate and under foreseeable climate change? The information needs to be given separately for current climate and under foreseeable climate change conditions.

A9a. Current climate: List Member States

A9b. Future climate: List Member States

With regard to EU Member States, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (9a): Cyprus, Spain, Greece, France and potentially other Mediterranean member states with islands (Italy, Portugal, Malta) for which the confidence on the SDM is lower. Under current climate, these countries have suitable areas for the establishment of the red-vented bulbul. Yet the suitable area represents only 2% of the Mediterranean bioregion (see 7a, Annex VII, figure 9).

Overall, the main limiting factor in most of the risk assessment area is annual mean temperature (Bio1). Since *P. cafer* is a species of subtropical climate, it is reasonable to assume that most of Europe will be too cold for successful reproduction. Second, precipitation of the wettest month

(Bio13) is the main limiting factor in some parts of southern Europe. The red-vented bulbul indeed avoids deserts and needs trees/shrubs for nesting (Zia et al., 2014), and a low precipitation of the wettest month indicates this. The ensemble model suggested that suitability for red-vented bulbul was most strongly determined by Annual mean temperature (Bio1), accounting for 43.5% of variation explained, followed by Mean temperature of the warmest quarter (Bio10) (24.7%), Precipitation of the wettest month (Bio13) (8.8%), Minimum temperature of the coldest month (Bio6) (8.3%), Annual precipitation (Bio12) (5.9%), Human influence index (HII) (5.6%), Precipitation of the driest month (Bio14) (2.3%) and Global tree cover (Tree) (0.9%). For more details, see Annex VII.

Response (9b):

RCP 2.6: Cyprus, France, Greece, Spain and potentially other Mediterranean member states with islands (Italy, Portugal, Malta) for which the confidence on the SDM is lower.

RCP 4.5: Cyprus, France, Greece, Spain and potentially other Mediterranean member states with islands (Italy, Portugal, Malta) for which the confidence on the SDM is lower.

For more details, see Annex VII.

A10. Is the organism known to be invasive (i.e. to threaten or adversely impact upon biodiversity and related ecosystem services) anywhere outside the risk assessment area?

Response:

Yes. The red-vented bulbul impacts native biodiversity outside of the risk assessment area in three ways:

1/ Frugivory: most damage relates to its diverse diet that comprises fruits and berries (Islam & Williams, 2000; Brooks, 2013), flowers, buds, insects and small reptiles (Vander Velde, 2002). It feeds on cultivated (food and ornamental) plants and is considered a problematic seed disperser of invasive alien plants, such as *Lantana camara* (Spotswood et al, 2013).

2/ Competition: its aggressive behaviour towards other birds has been reported to lead to niche contraction of some native birds (e.g. Tahiti flycatcher (*Pomarea nigra*)) in the Pacific (Blanvillain et al., 2003). Its numerical abundance was inversely correlated with the abundance of nine bird species in New Caledonia (Thibault et al., 2018d).

3/ Hybridisation: in the Middle East, crossbreeding with native bulbul species (*P. leucogenys*, *P. leucotis*, *P. xanthopygos*) threatens the genetic integrity of these native bird populations (Khan, 1993; Nation et al., 1997; Gregory, 2005; Azin et al., 2008; Khamis, 2010).

On the semi-arid island of Fuerteventura (Canary Islands, Macaronesia, Spain but outside the risk assessment area) the species was first observed in Corralejo in 2003 and expanded its range in the period 2013–2018 to cover the entire island (1.658 km²) (SEO/Birdlife 2017; Nowakowski and Dulisz 2019). There is not a lot of habitat available to the birds on the island, and they are limited to towns and holiday resorts with gardens and parks, but also inhabit agricultural plantations. The first breeding was confirmed in 2018 around Costa Calma. The birds were observed in stands of trees in gardens of a

holiday resort composed of various palm trees, fig trees, oleanders, yuccas, acacias and shrubs, and often visited the dry shrubs, typical for semi-arid vegetation of the open landscape of the island, located outside the resort's gardens (Nowakowski & Dulisz 2019).

A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea

Response:

The red-vented bulbul is currently only present in the Mediterranean biogeographic region (Spain) within the risk assessment area (see Qu. A6b), but has not shown signs of invasiveness (see Qu. A8b).

A12. In which EU Member States has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, the Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden

Response:

The red-vented bulbul has only recently established locally in Spain and has not shown signs of invasiveness (see Qu. A.8b).

A13. Describe any known socio-economic benefits of the organism.

including the following elements:

- Description of known uses for the species, including a list and description of known uses in the Union and third countries, if relevant.
- Description of social and economic benefits deriving from those uses, including a description of

the environmental, social and economic relevance of each of those uses and an indication of associated beneficiaries, quantitatively and/or qualitatively depending on what information is available.

If the information available is not sufficient to provide a description of those benefits for the entire risk assessment area, qualitative data or different case studies from across the Union or third countries shall be used, if available.

Response:

In its native Indian range, the red-vented bulbul has been reported to feed on the cotton bollworm (*Helicoverpa armigera*), a moth that is globally considered as a major pest species because its larvae feed on a wide range of plants, including many important cultivated crops such as cotton (Rana et al., 2014, 2017).

In a cost-benefit analysis done by Daigneault & Brown (2013) on Fiji, 47% of surveyed village focus groups reported benefits of the red-vented bulbul for their community. 18% responded that it is effective at insect control, 12% noted that the red-vented bulbul sends out alarms calls when a mongoose is about to attack chickens, thereby reducing the attacks on chickens and another 12% stated that the bulbul is occasionally eaten by villagers. In addition, in the north-east of India, red-vented bulbul fights were part of a traditional and religious annual celebration, until this practice was banned in January 2016 (Shalet, 2016).

As the species is widely kept as a caged bird within and outside the risk assessment area (see A.2), it represents ornamental, sentimental and aesthetic value as a companion animal. There are no official records on trade volumes, but there are plenty of advertisements for birds online, often sold in pairs. Prices found online vary from € 165 – 250 per bird or € 250 per pair.

SECTION B – Detailed assessment

Important instructions:

- In the case of lack of information the assessors are requested to use a standardized answer: “No information has been found.”
- With regard to the scoring of the likelihood of events or the magnitude of impacts see Annexes I and II.
- With regard to the confidence levels, see Annex III.
- Highlight the selected response score and confidence level in **bold** but keep the other scores in normal text (so that the selected score is evident in the final document).

1 PROBABILITY OF INTRODUCTION

Important instructions:

- **Introduction** is the movement of the species into the risk assessment area (it may be either in captive conditions and/or in the environment, depending on the relevant pathways).
- **Entry** is the release/escape/arrival in the environment, i.e. occurrence in the wild and is treated in the next section (N.B. introduction and entry may coincide for species entering through pathways such as “corridor” or “unaided”).
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used. For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document³ and the provided key to pathways⁴.
- For organisms which are already present in the risk assessment area, only complete this section for current active pathways and, if relevant, potential future pathways.

Qu. 1.1. List relevant pathways through which the organism could be introduced. Where possible give details about the specific origins and end points of pathways as well as a description of any associated commodities.

For each pathway answer questions 1.2 to 1.7 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 1.2a, 1.3a, etc. and then 1.2b, 1.3b etc. for the next pathway.

In this context a pathway is the route or mechanism of introduction of the species.

The description of commodities with which the introduction of the species is generally associated shall include a list and description of commodities with an indication of associated risks (e.g. the volume of trade; the likelihood of a commodity being contaminated or acting as vector).

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions

1.2-1.9

³ <https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf>

⁴ <https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf>

Pathway name:

- a. ESCAPE from confinement: Botanical garden/zoo/aquaria (excluding domestic aquaria)
- b. ESCAPE from confinement: Pet/aquarium/terrarium species (including live food for such species)
- c. TRANSPORT stowaway: Hitchhikers on ship/boat (excluding ballast water and hull fouling)

Deliberate releases of red-vented bulbul for biocontrol of insect pests have also been documented in the Indo-Pacific (Watling, 1978) but these pathways of introduction are rare and considered irrelevant for the risk assessment area. Therefore they are not dealt with here (see also Qu. 2.1).

Qu. 1.2a. ESCAPE from confinement: Botanical garden/zoo/aquaria (excluding domestic aquaria)

Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response:

Intentional because zoological gardens will intentionally buy or acquire one or more red-vented bulbul individuals to put on display.

Qu. 1.3a. How likely is it that large numbers of the organism will be introduced through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in introduction whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Whilst there is no data available on the total captive red-vented bulbul population within all zoological collections within the EU, information was provided by EAZA (European Association of Zoos and Aquaria) on populations kept at approximately 300 of their Member zoos and aquariums in 25 EU Member States (with the exception of Cyprus and Malta), and the United Kingdom. The information provided by EAZA (EAZA datafile 3/10/2019) indicates that the species is kept in low numbers by EAZA Member zoos in Germany, Poland, and the United Kingdom. This data comes from the animal care and management software provided by Species360 Zoological Information Management System (ZIMS) (zims.Species360.org, 2018) whose usage is widespread throughout the EAZA Membership. It must be noted that the actual situation might slightly differ if the species has been recorded under a different/older taxonomic name.

The red-vented bulbul is on display in at least the following EAZA zoos in the risk assessment area: Warsaw Zoological Garden (Poland), Köln Zoologischer Garten (Germany), Plzen Zoo (Czech Republic), Graested Nordsjællands Fuglepark (Denmark), Helsingborg Djurparken / ex. Fågelparken (Sweden), Farnham Birdworld & Underwater World (UK), Thrigby Hall Wildlife Gardens (UK) (www.zootierliste.de). EAZA is the European Association of Zoos and Aquaria and has 300 full members, 21 candidates for membership, 40 corporate members and 38 associate members as of May 2018 (www.eaza.net). This list comprises just a quarter of all zoological gardens and animal parks in Europe (www.zoos.media). Therefore, it is difficult to assess to what extent the species is kept in captivity within the risk assessment area.

Although the import of wild birds into the EU has been illegal since 2005, zoological gardens with a special zoo license can still import them. Indeed, the red-vented bulbul is present in several zoos, but it is only moderately likely that large numbers will be introduced in the zoos within the risk assessment area over the course of one year. However, no information is available as to the total number of zoos that keep red-vented bulbul, the size of the captive population nor how often these are introduced into a new zoo, so the confidence level is low.

Qu. 1.4a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

If a zoo intentionally buys or acquires one or more red-vented bulbul individuals, it is very likely that these animals will survive their transport and storage along this pathway, since they are meant to stay alive. Reproduction however, is very unlikely.

Qu. 1.5a. How likely is the organism to survive existing management practices during transport

and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

When imported legally by a zoological garden with a license, there is no reason why existing management practices would target this species. In the case of illegal import, there are no known existing management practices that target the red-vented bulbul.

Qu. 1.6a. How likely is the organism to be introduced into the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

The receiving zoo will always know that they are introducing the red-vented bulbul. Additionally, when on display, visitors will also detect this bird.

Qu. 1.7a. Estimate the overall likelihood of introduction into the risk assessment area based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

The red-vented bulbul is already introduced in the risk assessment area, as it is present in several zoological gardens. Since zoological gardens can acquire a license to import wild birds and this species is not considered threatened, we can say with high confidence that it is very likely that the red-vented bulbul will be introduced into the risk assessment area through this pathway.

End of pathway assessment, repeat Qu. 1.3 to 1.7 as necessary using separate identifier.

Qu. 1.2b. ESCAPE from confinement: Pet/aquarium/terrarium species (including live food for such species)

Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response:

Intentional because a person will intentionally buy the red-vented bulbul to keep as a pet even if the subsequent escape would be accidental (following IUCN 2017).

Qu. 1.3b. How likely is it that large numbers of the organism will be introduced through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in introduction whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

On several hobbyist websites, it is stated that the red-vented bulbul is among the most easily-kept of the softbills, which is a term applied to non-typical cage birds, such as bulbuls. The species has a confident and inquisitive nature, with a “friendly” personality towards humans, making them popular as pets. As stated in Qu. A13, there are no official records on trade volumes, but there are plenty of advertisements for birds online, often sold in pairs. Prices found online vary from € 165–250 per bird or € 250 per pair. For example, in France, red-vented bulbuls are advertised for sale online at € 250 per pair (www.leboncoin.fr). Captive breeding in outdoor aviaries occurs as French regulations do not require a declaration of detention up to 50 individuals (personal communication J.-F. Maillard, June 2020). Wild caught bird trade (as opposed to captive bred) has been suspended in the EU since 2005, when a temporary ban on wild bird imports was installed to prevent the spread of avian influenza (Reino et al., 2017). The ban was made permanent in 2007 and considers all wild caught bird imports regardless of species’ conservation status. This ban has been effective in reducing propagule pressure (Cardador et al., 2019). We did not find any records of illegal trade into the EU from non-EU countries. At the moment, there are no documented records of illegally imported red-vented bulbuls in the risk assessment area. It is however possible that releases could happen, for instance by activists or pet owners wanting to free their birds, not being able to take care of them, releasing excess birds after overly successful breeding or because of panic releases (cf. Hulme, 2015). However, we know the red-vented bulbul is exchanged/traded between hobbyists within Europe. We did not find information on the number of the red-vented bulbul present in the risk assessment area, which makes it impossible to assess propagule pressure. Neither do we know how many birds are exchanged/traded between hobbyists, so we also have no idea of the market for animals bred in captivity. Our confidence is therefore low.

Qu. 1.4b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

As stated in Qu. 1.3b, we do not know if the red-vented bulbul is imported into the EU illegally, making it very difficult to assess if many birds die during transport. We can assume though that the intention is to bring live animals with the aim to keep them and make them reproduce in captivity. Likewise, in the case of exchange/trade between hobbyists, their intention is to keep the birds alive and well during transport to deliver them so survival is likely. Since we have no official records on this matter, confidence is low.

Qu. 1.5b. How likely is the organism to survive existing management practices during transport

and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

In the case of trade between hobbyists, this is a legal trade pathway with the intention to keep the birds alive and well during transport to deliver them to the buyer. As stated above, we do not know if the red-vented bulbul is imported into the EU illegally. We also do not know of any existing management practices that could possibly kill the red-vented bulbul during transport and storage along this pathway. According to the IUCN, the red-vented bulbul is not subjected to any international management or trade controls (IUCN, 2019). Because of this, we think it is very likely that the red-vented bulbul will survive existing management practices, with medium confidence.

Qu. 1.6b. How likely is the organism to be introduced into the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

The red-vented bulbul has been introduced without being detected at first in several parts of its invasive range in the Pacific and the Middle East (Watling, 1978; Vander Velde, 2002). However, we could not find evidence on any undetected introductions of the red-vented bulbul into the risk assessment area, so confidence is only medium.

Qu. 1.7b. Estimate the overall likelihood of introduction into the risk assessment area based on this pathway?

RESPONSE	very unlikely unlikely moderately likely	CONFIDENCE	low medium high
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	likely very likely		
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Response:

This species is already present as a pet in private collections in several (if not all) countries within the risk assessment area. Several hobbyist websites currently have the species on offer, suggesting it is very likely to be introduced repeatedly in the future within the risk assessment area. However, information on introduction from outside the risk assessment area is lacking, therefore our response is moderately likely with low confidence.

End of pathway assessment, repeat Qu. 1.3 to 1.7 as necessary using separate identifier.

Qu. 1.2c. TRANSPORT stowaway: Hitchhikers on ship/boat (excluding ballast water and hull fouling)

Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response:

If the red-vented bulbul were to hitchhike on cargo ships or fishing boats, then this would be unintentional transport.

Qu. 1.3c. How likely is it that large numbers of the organism will be introduced through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in introduction whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely	CONFIDENCE	low
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	unlikely moderately likely likely very likely		medium high
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Response:

Some of the introductions of red-vented bulbul on the Marshall Islands could possibly be stowaways on container ships or fishing boats (Vander Velde, 2002; Brochier et al., 2010). Locations of first sightings in Majuro were all in close proximity to its major commercial port. Here, containers enter from Hawaii and Asia, where there are resident populations of red-vented bulbul. There are also speculations that introductions in Hawaii and New Zealand (Auckland in the 1950s) could have been assisted by barge or boat (Islam & Williams, 2000; Heather & Robertson, 1996).

Red-vented bulbuls are known to nest in some unusual places, including the motor of a ceiling fan and the end of a curtain rod, both within buildings (Islam & Williams, 2000). Hence, the possibility exists that a few birds stowed away among some heavy equipment or in crevices on board of a ship (Vander Velde, 2002). However, there are no confirmed stowaways so the confidence level of our answer is low.

This type of introductory event is considered to be moderately likely, since it possibly already happened outside of the risk assessment area. However, there are no official records of this, so confidence level is low.

Qu. 1.4c. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

see Qu. 1.3c.

In the past, some birds were stowaways and survived on a ship from Hawaii to Majuro (Marshall Islands), a journey of around 3700 km (Vander Velde et al., 2002). Birds from the population on the Arabian Peninsula could possibly hitchhike to Europe through this pathway (the distance from Dubai to Cyprus is around 6000 km). There is also a population on Fuerteventura, one of the Canary Islands (Spain) (Nowakowski and Dulisz 2019). The journey from Fuerteventura to Cádiz (Spain) is just over 1100 km, which probably is perfectly manageable for the red-vented bulbul.

When it comes to food supply for survival, if a couple of red-vented bulbuls have been able to survive a journey of 3700 km (Vander Velde, 2002), they probably found food or were fed along the way, indicating this could also be possible for journeys of 6000 km or more.

Qu. 1.5c. How likely is the organism to survive existing management practices during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

There are no known management practices for these birds on ships, hence it is likely to survive transport and storage along the pathway.

Qu. 1.6c. How likely is the organism to be introduced into the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

It is unlikely stowaway red-vented bulbul on cargo ships or fishing boats would remain undetected during the journey. These birds are noisy, active and curious (Vander Velde, 2002). Nonetheless, even if the birds would be detected, there is no certainty that this sighting would ever be reported by the sailors. Since there are no official records of this happening, the confidence is low.

Qu. 1.7c. Estimate the overall likelihood of introduction into the risk assessment area based on this pathway?

RESPONSE	very unlikely unlikely	CONFIDENCE	low medium
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	moderately likely likely very likely		high
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Response:

This has possibly happened in the past in the Pacific Ocean, not in the risk assessment area. There are no official records so the confidence is low.

End of pathway assessment, repeat Qu. 1.3 to 1.7 as necessary using separate identifier.

Qu. 1.8. Estimate the overall likelihood of introduction into the risk assessment area based on all pathways and specify if different in relevant biogeographical regions in current conditions.

Provide a thorough assessment of the risk of introduction in relevant biogeographical regions in current conditions: providing insight in to the risk of introduction into the Union.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

This species is already present in zoological gardens and private collections in multiple countries within the risk assessment area. We did not find any quantitative data though. Since there is a ban on the introduction of wild birds into the EU, from which only zoological gardens with a license are exempted, it is most likely that the red-vented bulbul will be introduced through the first pathway. Introductions into private collections are less likely, and introductions as stowaways will be the least likely. There are no indications of differences between biogeographical regions.

Qu. 1.9. Estimate the overall likelihood of introduction into the risk assessment area based on all pathways in foreseeable climate change conditions?

Thorough assessment of the risk of introduction in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)

- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of introduction (e.g. change in trade or user preferences)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely introduction within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

The likelihood of introduction will not change in any climate change scenario since none of the pathways will be affected by any of these scenarios.

2 PROBABILITY OF ENTRY

Important instructions:

- Entry is the release/escape/arrival in the environment, i.e. occurrence in the wild. Entry is not to be confused with spread, the movement of an organism within the risk assessment area.
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used. For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document⁵ and the provided key to pathways⁶.
- For organisms which are already present in the risk assessment area, only complete this section for current active or if relevant potential future pathways. This section need not be completed for organisms which have entered in the past and have no current pathway of entry.

Qu. 2.1. List relevant pathways through which the organism could enter into the environment.

For each pathway answer questions 2.2 to 2.7 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 2.2a, 2.3a, etc. and then 2.2b, 2.3b etc. for the next pathway.

In this context a pathway is the route or mechanism of entry of the species into the environment.

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 2.2-2.8

Pathway name:

- a) ESCAPE from confinement: Botanical garden/zoo/aquaria (excluding domestic aquaria)
- b) ESCAPE from confinement: Pet/aquarium/terrarium species (including live food for such species)
- c) TRANSPORT stowaway: Hitchhikers on ship/boat (excluding ballast water and hull fouling)

Deliberate releases of red-vented bulbul for biocontrol of insect pests have also been documented in the Indo-Pacific (Watling, 1978). As this is not an active pathway in the risk assessment area we do not consider it here. Furthermore, outside the risk assessment area, red-vented bulbul was introduced through other intentional release. For example, the initial introduction in 1903 on the Fiji islands was attributed to Indian immigrants bringing the species for bulbul fighting and releasing them to avoid persecution (Parham, 1955; Watling, 1978; Gill et al., 1995). Since 2016 this practice is however banned (Ratnagar, 2015). Also, in several Asian countries (China, Vietnam, Malaysia, Thailand, Korea, Cambodia, possibly more), people “make merit” by releasing captive animals for religious reasons (McNeely, 2001). Although these prayer releases do occur in Asia (McNeely, 2001; Severinghaus & Chi, 1999), we consider them less relevant for Europe and therefore this pathway is not dealt with here.

⁵ <https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf>

⁶ <https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf>

Qu. 2.2a. ESCAPE from confinement: Botanical garden/zoo/aquaria (excluding domestic aquaria)

Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from confinement)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response:

Escape, so entry into the environment is unintentional with high confidence.

Qu. 2.3a. How likely is it that large numbers of the organism will enter into the environment along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

The red-vented bulbul is present in several EAZA zoological gardens throughout Europe (see Qu. 1.3a). These are all located in Northern or Central Europe, not in Southern Europe, where we expect more suitable habitat and climate for red-vented bulbul. However, as stated above, EAZA only represents a quarter of all European zoological gardens, so there might be some in zoological gardens in Southern Europe.

There is a record of a zoo escape on Fuerteventura (Canary Islands), outside of the risk assessment area. Here, birds escaped from the zoological garden of La Lajita in 2013 (together with common

myna *Acridotheres tristis*), settled and started to breed in the surroundings a few years later (Nowakowski and Dulisz 2019). The myna was eradicated from the surroundings of the zoo, yet the bulbuls were left unattended and spread to the rest of the island (Nowakowski and Dulisz 2019). Considering the potential impact on biodiversity of the Canary Islands ecosystems, including by predation on native and endemic species, there are calls for its eradication (SEO/Birdlife 2017).

Qu. 2.4a. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

It is very unlikely that the red-vented bulbul would be able to escape from a zoological garden without this being noticed. In the event of an escape, the zoo will most likely take measures to recapture the animal. However, a study by Cassey & Hogg (2015) in Australia stated that, compared with mammals and reptiles, bird escapes were significantly less likely to be retrieved, and more likely to remain undetected.

Qu. 2.5a. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Mean annual temperature is the limiting factor for most of the risk assessment area. If the red-vented bulbul would escape in northern Europe, then it would likely enter a habitat which is too cold in autumn, winter and spring. The bird could possibly survive if it would enter into the environment during summer.

For more information on climate suitability see Qu. A7.

Qu. 2.6a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Zoological gardens are often located in urbanized areas, meaning that if the red-vented bulbul would escape, it would easily find an urban garden or park in which to establish. As mentioned in Qu. 2.5a, if the zoological garden is located too northerly in Europe, the climate will be too cold during autumn, winter and spring.

Qu. 2.7a. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

If the red-vented bulbul is present in zoological gardens within its predicted suitable habitat, then it is moderately likely that the species would enter into the environment within the risk assessment area. Given that we do not have information on the presence of the red-vented bulbul in zoological gardens within these suitable areas, the confidence level is low.

End of pathway assessment, repeat Qu. 2.2 to 2.7. as necessary using separate identifier.

Qu. 2.2b. ESCAPE from confinement: Pet/aquarium/terrarium species (including live food for such species)

Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from confinement)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response:

Escape, so entry into the environment is unintentional, with a high confidence level.

Qu. 2.3b. How likely is it that large numbers of the organism will enter into the environment along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

The red-vented bulbul is probably kept as a pet in most (if not all) countries within the risk assessment area (see Qu. 1.7b) and the species can easily be found on display for sale online, often under the name “Kala buulbuul” (e.g. www.vogelspecialclub.nl). For example, in the Netherlands, the species is kept and bred by members of a bird breeders association specialized in insect and fruit-eating species who adopted a code of best practice for keeping of red-vented bulbul (NBvB, 2014). It is known as a hardy species to keep in aviaries, which is easy to breed on a variety of mealworms and insects when they have young. Several hobby-keeper websites indicate that red-vented bulbul wings should not be clipped, since they exercise by flying, not by climbing. Some websites indicate the species should be kept in a large walk-in aviary, preferably outdoors, implying a higher chance of escape compared to birds kept inside in small cages. Additionally, red-vented bulbuls are often sold and kept in pairs, which implies that there is a high chance of breeding when they would escape. Shieh et al. (2006) reported that Pycnonotidae (with Sturnidae, Timaliidae and Cacatuidae) have significantly higher probabilities of escaping from captivity in Asia, in comparison to other birds families.

Considering the locations where birds were observed in the Netherlands and Belgium (see Qu. A6) and the fact that the species is kept in captivity, it can be assumed escapes have happened here in the past.

Qu. 2.4b. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

If the red-vented bulbul would escape, it is likely that the keeper will alarm neighbours and maybe even animal rescue centres nearby.

Qu. 2.5b. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

The red-vented bulbul is a species of equatorial climate, and will most likely only be able to establish in the Mediterranean biogeographical region within the risk assessment area. For more information on climate suitability see Qu. A7 and the SDM.

If this species would escape from its confinement in the south of Europe, then any moment of the year will probably be appropriate for establishment. The species is known to settle in urban areas, where temperatures are higher and food is more readily available, increasing its chances of establishment (Vander Velde, 2002).

In fact, there have been two successful entry events for the red-vented bulbul in the risk assessment area: in Málaga and in Valencia. In Valencia, there has been establishment following the entry of the species. It is possible that these birds escaped, but this has not been recorded.

Confidence level is medium because of the lack of official reports on the matter within the risk assessment area.

Qu. 2.6b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:
 See Qu. 2.6a.

Qu. 2.7b. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

This sort of event has happened (and was recorded) multiple times in the past outside the risk assessment area. Moreover, this has happened inside the risk assessment area too, but records of this are scarce and not official. The confidence level of our response is still high because of the records outside of the risk assessment area.

End of pathway assessment, repeat Qu. 2.2 to 2.7. as necessary using separate identifier.

Qu. 2.2c. TRANSPORT stowaway: Hitchhikers on ship/boat (excluding ballast water and hull fouling)

Is entry into the environment intentional (e.g. the organism is released for a specific purpose) or unintentional (e.g. the organism escapes from confinement)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response:

Birds that hitchhike on a ship/boat would enter unintentionally.

Qu. 2.3c. How likely is it that large numbers of the organism will enter into the environment along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of entry into the environment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in entry whereas for others high propagule pressure (many thousands of individuals) may not).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

This sort of event has allegedly happened before in the Pacific (Vander Velde, 2002). However, the number of birds that would actually be able to enter the environment through this pathway over the course of one year will be very low, therefore we score unlikely. Since we have no official records of this happening, our confidence is low.

For more information, see Qu. 1.3c.

Qu. 2.4c. How likely is the organism to enter into the environment within the risk assessment area undetected?

RESPONSE	very unlikely	CONFIDENCE	low
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	unlikely moderately likely likely very likely		medium high
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Response:

See response Qu. 1.6b.

Qu. 2.5c. How likely is the organism to enter into the environment during the months of the year most appropriate for establishment?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

The red-vented bulbul is a species of equatorial climate, and will most likely only be able to establish in the Mediterranean biogeographical region within the risk assessment area. For more information on climate suitability see Qu. A7 and the SDM (Annex VII).

There are populations of the red-vented bulbul on Fuerteventura (Canary Islands) and on the Arabian Peninsula, which are in fact closest to the areas within the risk assessment area where it is most likely that the red-vented bulbul could establish. For additional information regarding months of the year most appropriate for establishment, see response Qu. 2.5b.

Qu. 2.6c. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host in the environment?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

See response to Qu. 2.6a.

Qu. 2.7c. Estimate the overall likelihood of entry into the environment within the risk assessment area based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

This sort of event has probably happened in the past (not in the RA area), but has not been recorded very well, hence the low confidence.

End of pathway assessment, repeat Qu. 2.2 to 2.7. as necessary using separate identifier.

Qu. 2.8. Estimate the overall likelihood of entry into the environment within the risk assessment area based on all pathways in current conditions and specify if different in relevant biogeographical regions.

Provide a thorough assessment of the risk of entry into the environment in relevant biogeographical regions in current conditions.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Entry has happened in several locations within the risk assessment area (at least in the Netherlands, Belgium, Spain, see Qu. A8). Since the red-vented bulbul is kept as a pet and is on display in zoological gardens, escape and release events are bound to occur in the future. Additionally, it is possible that some individuals or pairs will hitchhike with boats coming from areas with a red-vented bulbul population. Entry could happen in all biogeographical regions within the risk assessment area, but establishment will only be possible in the Mediterranean region. For additional information, see Qu. A7 and the SDM.

Qu. 2.9. Estimate the overall likelihood of entry into the environment within the risk assessment area based on all pathways in foreseeable climate change conditions and specify if different in relevant biogeographical regions.

Thorough assessment of the risk of entry in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if likelihood of entry is likely to increase or decrease for specific pathways.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Climate change will not alter the possibility of entry into the environment, so we score this question the same as we scored the question under current climate. For additional information, see Qu. A7 and SDM (Annex VII).

3 PROBABILITY OF ESTABLISHMENT

Important instructions:

- For organisms which are already established in parts of the risk assessment area, answer the questions with regard to those areas, where the species is not yet established.

Qu. 3.1. How likely is it that the organism will be able to establish in the risk assessment area based on the history of invasion by this organism elsewhere in the world (including similarity between other abiotic conditions within it and the organism’s current distribution)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

The red-vented bulbul is a species of an equatorial climate but is also established in cooler and drier climate regions (including the Mediterranean biogeographical region), both in and outside of the risk assessment area. It has so far primarily established in urban areas, of which there is no shortage within the risk assessment area.

Qu. 3.2. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area?

RESPONSE	very isolated isolated moderately widespread widespread ubiquitous	CONFIDENCE	low medium high
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Response:

The climatic niche of the red-vented bulbul corresponds to an equatorial climate according to the Koppen-Geiger classification (Kottek et al., 2006). In the Mediterranean biogeographical region, suitable habitat for the red-vented bulbul is moderately widespread. Under current climate the area predicted suitable represents only 2% of the entire Mediterranean bioregion but is likely to increase under future climatic conditions (see 7a,b; Annex VII, figure 9). Especially Cyprus, the southern

islands of Greece, Southern Andalucia (Spain) and the area around Gibraltar, and a small part of France are at risk. With a non-native range that is still expanding, there is a possibility that the potential climatic niche hence the predicted potential distribution is underestimated.

The red-vented bulbul feeds on a variety of fruits, berries, flowers, buds, insects and small vertebrate prey (Bhatt & Kumar 2001). This broad diet that includes cultivated plants allows the red-vented bulbul to find food easily. Red-vented bulbul builds its nest in trees and bushes at different heights, either on the forks of trees, in the middle or at the top (Vijayan, 1980; Zia et al., 2014). According to several studies, preferred nest height varies from 1 - 4 metres and preferred trees are thorny and very close to each other (Vijayan, 1980; Watling, 1983; Zia et al., 2014). The study done by Zia et al. (2014) in the native range in India found that the percentage of failed nests was highest for treetop nests, with most nests being destroyed due to heavy wind, rain or predators. Such thorny trees are omnipresent in the Mediterranean, not the least on the Greek islands, therefore red-vented bulbul has plenty of suitable breeding habitat available in the Mediterranean biogeographical region.

It is unlikely that the red-vented bulbul would be able to establish in the northern Atlantic, Boreal, Pannonian, Steppe, Black Sea, Alpine or Continental biogeographical regions, due to the cold winter (Annex VII). This is corroborated by reports from a keeper in The Netherlands who says that several young died when kept outside due to the cold weather during the night (<http://www.buulbuul.nl/Mijn%20Kala%20buulbuul.html>).

Qu. 3.3. If the organism requires another species for critical stages in its life cycle then how likely is the organism to become associated with such species in the risk assessment area?

RESPONSE	N/A very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

The red-vented bulbul does not require another species for any critical stage of its life cycle.

Qu. 3.4. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely	CONFIDENCE	low medium high
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	very likely		
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Response:

Potentially, the red-vented bulbul could compete for food and space (e.g. for nesting) with other bird species. However, as establishment often occurs in (sub)urban areas with gardens, parks and artificial habitat (see A8, A10), usually depleted in local fauna, very low levels of competition with native species can be expected and it is very likely establishment will occur despite this. In the risk assessment area, competition with highly successful native species such as corvids, gulls and starlings could impact establishment in some cases, but there have been no studies that have assessed this so far hence medium confidence.

It should be noted that the red-whiskered bulbul (*P. jocosus*) established around Valencia shortly after the first observation was made in 2003 in the lower Rio Turia basin area (Santos 2015). The red-whiskered bulbul has been reproducing for more than a decade here and was estimated at 100-150 individuals in 2016 (Santos 2015). Detailed impact studies for Spain are equally lacking for this species. However, as the two species of bulbul are now sympatric, there is the possibility of cumulated impact of an entire invasive bird community. Introduced species may act in concert, facilitating one another's invasion, and increasing the likelihood of successful establishment, spread and impact. Such positive interactions among introduced species are relatively common (e.g. between birds/mammals and plants), but few have been studied in detail (Traveset & Richardson 2014). No information on such mechanisms is available for bulbuls.

The red-vented bulbul is known to be aggressive towards other birds in its preferred forage trees, especially during the breeding season (Sherman & Fall, 2010; Blanvillain et al., 2003; Gorman 1972). Competition with other (native) bird species is in fact one of the three serious impact categories associated with red-vented bulbul, so it is unlikely that competition will be limiting for this bird (Thibault et al., 2018d). For example, in Tahiti, red-vented bulbuls compete with the Tahiti monarch (*Pomarea nigra*), an endemic and critically endangered passerine (Blanvillain et al., 2003). Another study, done by Thibault et al. (2018d) in New-Caledonia found that nine out of ten native bird species monitored in man-modified habitats were less abundant when the red-vented bulbul was present. The impact of the red-vented bulbul appears to be restricted to niche contraction of the native species (Thibault et al., 2018d).

A study in New Caledonia (Thibault et al., 2018d) states that there are no indications for interspecific competition with other invasive species present on the island, such as common myna (*Acridotheres tristis*) and red-whiskered bulbul. It is possible that these species show some sort of niche segregation in their invaded range, as shown for red-vented bulbul and common myna in French Polynesia by Bates et al. (2014).

Qu. 3.5. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?

RESPONSE	N/A	CONFIDENCE	low
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	very unlikely unlikely moderately likely likely very likely		medium high
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Response:

Predators

The red-vented bulbul is not particularly vulnerable to predation. In its native range, a study carried out by Zia et al. (2017) found that predation rate on red-vented bulbul was only 6% in eggs and 9% in nestlings. Predators in the study area included bird species such as crested eagle (*Morphnus guianensis*) and barn owl (*Tyto alba*), and rodents such as the black rat (*Rattus rattus*). Breeding success in the study was 82% and 86% for eggs and fledglings, respectively. The red-vented bulbul has specific behavioural adaptations to avoid predatory impact during nesting, notably through broken-wing display, that one or both parents would perform when a predator is seen near the nest (Kumar 2004, 2010).

Parasites

In its native range, the red-vented bulbul is known to host the internal parasite *Isospora* spp. known to cause isosporiasis in passerine birds (Boughton et al., 1938), lice species such as *Menacanthus eurysternus* (Price 1975), *Bruelia guldum*, *Sturnidoecus guldum* (Ansari 1957), the mite *Pteroherpis pycnonoti* (Constantinescu et al., unpublished) as well as disease carrying ticks (Islam & Williams, 2000; Vander Velde & Vander Velde 2013; Thibault et al., 2018c). Vander Velde and Vander Velde (2013) considered the constant influx of red-vented bulbuls on Micronesia a potential risk for the spread of tick-borne diseases.

Pathogens

In 1996, Jarvi et al. (2003) detected no avian malaria (*Plasmodium* spp.) in blood smears, and Atkinson et al. (2006) found no evidence of *Plasmodium*, *Trypanosoma*, *Atoxoplasma* or microfilaria. Red-vented bulbuls in Tahiti, however, have been found to carry the zoonotic disease *Chlamydia* sp. (Blanvillain et al., 2013). Grewal (1964) experimentally infected red-vented bulbuls with *Plasmodium praecox* (= *relictum*), the most widespread malaria parasite of birds. The birds developed typical infections about a week after inoculation and survived without apparent. The species may therefore be a carrier in nature (Grewal 1964).

Qu. 3.6. How likely is the organism to establish despite existing management practices in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely	CONFIDENCE	low medium high
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	very likely		
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Response:

There are no known existing management practices against this species in the risk assessment area.

Qu. 3.7. How likely are existing management practices in the risk assessment area to facilitate establishment?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

There are no known existing management practices against this species in the risk assessment area.

Qu. 3.8. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

As stated before, the red-vented bulbul is a loud species and it is thus unlikely to stay undetected. It is in fact this biological property that will make it more susceptible to eradication campaigns.

The most suitable habitat for the red-vented bulbul are Cyprus and the Greek islands. Invasive alien species control tends to be more achievable on islands than on the continent (Myers et al., 2000; McGeoch et al., 2016).

For more information, see the Annex with control measures.

Qu. 3.9. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?

including the following elements:

- a list and description of the reproduction mechanisms of the species in relation to the environmental conditions in the Union
- an indication of the propagule pressure of the species (e.g. number of gametes, seeds, eggs or propagules, number of reproductive cycles per year) of each of those reproduction mechanisms in relation to the environmental conditions in the Union.

If relevant, comment on the likelihood of establishment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

The red-vented bulbul lives in anthropogenic landscapes in all of its alien range in the Pacific, Houston, Fuerteventura, Málaga and Valencia, so it can be assumed that the highly fragmented and anthropogenic landscape in southern Europe will not hamper its establishment.

If a pair escapes or is released from confinement, a population could establish from just this pair given that genetic diversity is sufficiently high. This is allegedly what happened in the Republic of the Marshall Islands, when a pair may have hitchhiked on a ship and established a population (Vander Velde, 2002).

The red-vented bulbul often has two to three broods per year, that consist of two to five eggs (Long, 1981; Vander Velde, 2002), with an incubation period of about 14 days (Berger, 1972). Consequently, population size is likely to increase fast, leading to establishment.

Qu. 3.10. How likely is the adaptability of the organism to facilitate its establishment?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

The red-vented bulbul is a species that thrives in urban and suburban gardens and parks, indicating that it is an adaptable species (Brooks, 2013; Thibault, 2018a). Thibault (2018a) showed that densities in suburban areas vary along an urbanization gradient, but can go up to 120 individuals/km². In their native range, bulbuls are found from 0 to 2,000m, along forest edges, as well as in gardens and cultivated areas. These habitats have plenty of exotic plant species available to red-vented bulbul, usually not or little consumed by local wildlife, a resource that can easily be exploited by these adaptable birds and offering some advantage over native passerines. Virtually all of the bulbuls in Houston are found in residential gardens at sea level, with the only other cases being fragments of secondary habitat in edge situations.

Qu. 3.11. How likely is it that the organism could establish despite low genetic diversity in the founder population?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

No genetic diversity studies have been done on the red-vented bulbul, but several studies on singular breeding species (i.e., species that breed in pairs on a defended territory) show that these do not avoid random mating (Van Tienderen & van Noordwijk, 1988; Keller & Arcese, 1998; Hansson et al., 2007). However, Kruuk et al. (2002) noticed severe inbreeding depression in collared flycatchers, indicating species-specific differences in inbreeding tolerance.

Even with a small founder population, the red-vented bulbul has established in several parts of the world where it is thriving (also see Qu. A5).

Qu. 3.12. If the organism does not establish, then how likely is it that casual populations will continue to occur?

Consider, for example, a species which cannot reproduce in the risk assessment area, because of unsuitable climatic conditions or host plants, but is present because of recurring introduction, entry and release events. This may also apply for long-living organisms.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Since the red-vented bulbul is a popular pet bird and is present in zoological gardens, there is a continuous risk of release and escape in the future. Actual recurring introduction, entry and release events without establishment will happen in areas with unsuitable climatic conditions, e.g. in many colder parts of Europe, where it is too cold for the red-vented bulbul to reproduce successfully.

Qu. 3.13. Estimate the overall likelihood of establishment in the risk assessment area based on the similarity between climatic conditions within it and the organism’s current distribution under current climatic conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under current climatic conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in current conditions: providing insight in the risk of establishment in (new areas in) the Union.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

As stated in Qu. A7 and the SDM (Annex VII), establishment is most likely in the Mediterranean biogeographical region but only a small proportion of the area is predicted suitable. Indeed, establishment has already happened in Valencia (Spain). The ensemble model (Annex VII) suggested that the suitable distribution area for red-vented bulbul was most strongly determined by Annual mean temperature (Bio1), accounting for 43.5% of variation explained, followed by Mean temperature of the warmest quarter (Bio10) (24.7%). Annual mean temperature (Bio1) was also the most strongly limiting factors for establishment of red-vented bulbul in most of Europe and the Mediterranean region in current climatic conditions. In some Mediterranean areas (southern Iberia, Balearic islands, Sicily and Sardinia, Greece and Aegean islands, Cyprus), Precipitation of the wettest month (Bio13) was the most limiting factor. Other climatic variables such as Precipitation of the wettest month (Bio13) (8.8%), Minimum temperature of the coldest month (Bio6) (8.3%), Annual precipitation (Bio12) (5.9%) and Precipitation of the driest month (Bio14) (2.3%) explained much less of the variation in the species distribution model. The considered Non-climatic factors Human influence index (HII) (5.6%) and Global tree cover (Tree) (0.9%) explained only little of the observed variation. For information about important non-climatic variables, see Qu. 3.14.

Qu. 3.14 Estimate the overall likelihood of establishment in the risk assessment area under foreseeable climate change conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under foreseeable climate change conditions should be

provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of establishment (e.g. increase in average winter temperature, increase in drought periods)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely establishment within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

As stated in Qu. A7 and the SDM (Annex VII), establishment is estimated to be possible in the Mediterranean biogeographical regions under both RCP 2.6 and RCP 4.5. The Mediterranean will remain the most vulnerable region under climate change. The proportion of the area suitable for establishment in the Mediterranean biogeographical region is predicted to double under RCP 2.6 and to triple under RCP 4.5 by 2070. Therefore, we scored very likely.

4 PROBABILITY OF SPREAD

Important instructions:

- Spread is defined as the expansion of the geographical distribution of an alien species within the risk assessment area.
- Repeated releases at separate locations do not represent continuous spread and should be considered in the probability of entry section. In other words, intentional anthropogenic “spread” via release or escape (“jump-dispersal”), should be dealt within the entry section. However, as repeated releases contribute to the spread of the target organism in the risk assessment area, the relevant pathway(s) should be briefly discussed here too, with an explicit reference to the entry section for additional details.

Qu. 4.1. How important is the expected spread of this organism within the risk assessment area by natural means? (List and comment on each of the mechanisms for natural spread.)

including the following elements:

- a list and description of the natural spread mechanisms of the species in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of each of those spread mechanisms in relation to the environmental conditions in the Union.

The description of spread patterns should include elements of the species life history and behavioural traits able to explain its ability to spread, including: reproduction or growth strategy, dispersal capacity, longevity, dietary requirements, environmental and climatic requirements, specialist or generalist characteristics.

RESPONSE		CONFIDENCE	
	minimal		low
	minor		medium
	moderate		high
	major		
	massive		

Response:

For information on spread outside of the risk assessment area, see Qu. A5. For information on spread inside of the risk assessment area, see Qu. A8(b). There is a discrepancy between spread rate inside and outside of the risk assessment area, the rate being higher outside than inside. The rate of spread will be dependent on propagule pressure and the size of the population that has established.

Life history traits important for spread: the breeding season in the red-vented bulbul starts in February and lasts till September (Zia et al., 2014). Nest construction period is only 2 - 5 days, which is notably faster than other Pycnonotids like yellow-throated bulbul and grey-headed bulbul for which nest building takes 3 - 8 days (Balakrishnan, 2010). According to a study performed by Zia et al. (2014), preferred nest-building vegetation of the red-vented bulbul was beri (*Zizyphus nummularia*) (31%) followed by guava (*Psidium guajava*) (22%), sheesham (*Dalbergia sissoo*) (18%), snatha (*Dodonea viscosa*) (16%) and date palm (*Phoenix dactylifera*) (13%). Clutch size in general in Pycnonotids is two and rarely three (Ali & Ripley, 1987). Clutch size of the red-vented bulbul varies from 1 - 4, and

there are indications that this varies between regions, as studies from different regions of the species' range have partially different results (Zia et al., 2017; Prajapati et al., 2011; Rao et al., 2013).

The study done by Zia et al. (2014) also recorded the predators of the red-vented bulbul nests. Mostly, rodents and raptors were responsible for failed nests, e.g. brown rat (*Rattus rattus*), barn owl (*Tyto alba*) and crested eagle (*Morphnus guianensis*). Another interesting found in the study was that nests made in beri plants were more likely to fail which could be connected to their location near residential areas, where human disturbance and urban pollution could have an effect on red-vented bulbul reproductive success.

Qu. 4.2. How important is the expected spread of this organism within the risk assessment area by human assistance? (List and comment on each of the mechanisms for human-assisted spread and provide a description of the associated commodities.)

including the following elements:

- a list and description of the anthropogenic spread mechanisms of the species in relation to the environmental conditions in the Union.
- an indication of the rate of each of those spread mechanisms in relation to the environmental conditions in the Union.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

The red-vented bulbul is known to nest in unusual sites (see Qu. 1.3b), and it could therefore spread by human assistance on cargo ships or fishing boats within the risk assessment area. It appears that this species preferentially spreads through urban corridors, possibly facilitating its spread in most European countries.

Qu. 4.2a. List and describe relevant pathways of spread. Where possible give detail about the specific origins and end points of the pathways. For each pathway answer questions 4.3 to 4.9 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 4.3a, 4.4a, etc. and then 4.3b, 4.4b etc. for the next pathway.

including the following elements:

- a list and description of pathways with an indication of their importance and associated risks (e.g. the likelihood of spread in the Union, based on these pathways; likelihood of survival, or reproduction, or increase during transport and storage; ability and likelihood of transfer from the pathway to a suitable habitat or host). Where possible details about the specific origins and end points of the pathways shall be included.

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication.
- All relevant pathways should be considered. The classification of pathways developed by the Convention of Biological Diversity shall be used.

Pathway name:

TRANSPORT (stowaway) - Hitchhikers on ship/boat (excluding ballast water and hull fouling)

Qu. 4.3a. Is spread along this pathway intentional or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response:

Unintentional.

Qu. 4.4a. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?
including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Introduction, establishment and spread of the red-vented bulbul has allegedly happened through this pathway in the Pacific, suggesting that this could happen in the risk assessment area as well (Vander Velde et al., 2002). The red-vented bulbul was first sighted on the Marshall Islands in 2000 near the major commercial dock of Majuro and in 2002, there were already several breeding populations (Vander Velde, 2002).

The Mediterranean region and the Greek islands in particular are vulnerable given the large amount of islands and boat (commercial and leisure) traffic between them.

Also see response to Qu 1.3c and 1.4c.

Qu. 4.5a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

This sort of event has allegedly already happened in the Pacific (Vander Velde et al., 2002), but evidence is scarce, hence the low level of confidence. See Qu. 1.3c & 1.4c.

Qu. 4.6a. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

There are no known existing management practices on ships that would target the red-vented bulbul.

Qu. 4.7a. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Also see Qu. 2.4a.

Qu. 4.8a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread? (including, where possible, details about the specific origins and end points of the pathway)

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

If the organism would spread through the Mediterranean by boat transport, it would enter new areas through harbours, which are often close to urbanisation. This would enhance its chances of spread, since the species is known to settle in urban areas (Vander Velde, 2002), where temperatures are higher and food is more readily available, increasing its chances of establishment.

Qu. 4.9a. Estimate the overall potential rate of spread within the Union based on this pathway? (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response:

Spread through this pathway has possibly happened in the Pacific, and could happen in the risk assessment area, especially in the Mediterranean region. However, there are no official records of this so confidence is low.

End of pathway assessment, repeat Qu. 4.3 to 4.9. as necessary using separate identifiers.

Qu. 4.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?

RESPONSE	very easy easy with some difficulty difficult very difficult	CONFIDENCE	low medium high
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Response:

The species is easily recognisable and will probably be detected when present on a ship. However, even if conspicuous, the species does have a track-record as a stowaway (Vander Velde et al., 2002). Should the red-vented bulbul get more firmly established in the risk assessment area, it would take considerable effort to implement biosecurity procedures preventing stowaways, for example through controls on shipping routes between Mediterranean islands. We found no information on standard measures that sailors take to prevent the birds from escaping the ship. We did find some recommendations from the Australian government for sailors that encounter a stowaway animal on a ship: closing container or vessel doors, creating barriers; isolating the affected cargo in an area away from other goods; using blankets to restrict animal movement; taking photos of the animal or try to catch it. As these sets of measures are complex, we assume this would be implemented with some difficulty at least.

Qu. 4.11. Estimate the overall potential rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (indicate any key issues and provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in current conditions, providing insight in the risk of spread into (new areas in) the Union.

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response:

Groups and families of the red-vented bulbul can be seen in Valencia, but their range is small (Carlos Gutiérrez-Expósito, personal communication 2018), suggesting that (mainland) spread is slow. For additional information, see Qu. A8. The relatively slow spread could however be context dependent (e.g. environmental conditions, lack of suitable dispersal corridors, food availability) and therefore it is difficult to generalize the Valencia case to the whole risk assessment area. Invasion histories elsewhere in the world illustrate the species is certainly able to spread in insular contexts where highly attractive habitat is linked by boats.

Qu. 4.12. Estimate the overall potential rate of spread in relevant biogeographical regions in foreseeable climate change conditions (provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if rates of spread are likely slowed down or accelerated.

RESPONSE		CONFIDENCE	
	very slowly		low
	slowly		medium
	moderately		high
	rapidly		
	very rapidly		

Response:

The species is classified as a rather sedentary species, showing possible movements depending on environmental conditions (del Hoyo et al., 2005). The invasion on Fuerteventura, where the species invaded the entire island in a few decades and has shown important range expansion in the period 2013–2018 (Nowakowski and Dulisz 2019) indeed shows spread could be very context-dependent and the species is certainly able to spread moderately rapidly or rapidly in insular contexts.

5 MAGNITUDE OF IMPACT

Important instructions:

- Questions 5.1-5.5 relate to biodiversity and ecosystem impacts, 5.6-5.8 to impacts on ecosystem services, 5.9-5.13 to economic impact, 5.14-5.15 to social and human health impact, and 5.16-5.18 to other impacts. These impacts can be interlinked, for example a disease may cause impacts on biodiversity and/or ecosystem functioning that leads to impacts on ecosystem services and finally economic impacts. In such cases the assessor should try to note the different impacts where most appropriate, cross-referencing between questions when needed.
- Each set of questions starts with the impact elsewhere in the world, then considers impacts in the risk assessment area (=EU excluding outermost regions, and the United Kingdom) separating known impacts to date (i.e. past and current impacts) from potential future impacts (including foreseeable climate change).
- Only negative impacts are considered in this section (socio-economic benefits are considered in Qu. A.7)

Biodiversity and ecosystem impacts

Qu. 5.1. How important is the impact of the organism on biodiversity at all levels of organisation caused by the organism in its non-native range excluding the risk assessment area?

including the following elements:

- Biodiversity means the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems
- impacted chemical, physical or structural characteristics and functioning of ecosystems

RESPONSE		CONFIDENCE	
	minimal		low
	minor		medium
	moderate		high
	major		
	massive		

Comment:

Impacts on biodiversity were comprehensively reviewed by Thibault et al. (2018a) and include impact on native fauna through competition and community changes due to dispersal of invasive alien plant seeds. The authors note the lack of quantified impact studies and based on this, challenge the inclusion of red-vented bulbul on the list of the world's worst invasive species (Lowe et al., 2004). Evans et al. (2016), in their EICAT assessment of 415 bird species, scored impact of the red-vented bulbul as Moderate (MO) (i.e. *it causes declines in the population size of native species, but no changes to the structure of communities or to the abiotic or biotic composition of ecosystems*) with high confidence and list competition and interaction with other alien species (spreading the seeds of alien plants and thereby mediating alien plant invasions) as the impact mechanisms causing the most severe impacts. Martin-Albarracin et al. (2015) and Baker et al. (2014), in a global analysis of alien bird impact, note

competition and interactions with other non-native species as impact mechanisms. According to their scoring system used (an adapted scheme based on Kumschick and Nentwig (2010) and Blackburn et al. (2014), where 0 indicates no impact detected and 5 massive impact), the red-vented bulbul scores 2 on competition (i.e. *competition with several native species by exploitation competition, without large impact on affected species or decline of their populations*) and 4 on interactions with other species (i.e. *dispersal of seeds of non-native plants facilitates local or population extinction of at least one native species, and produces changes in community composition that are reversible but would not have occurred in the absence of the species*).

Impact mechanisms:

1: Frugivory (spreading the fruit/seeds of alien plants)

Thibault et al. (2018c) compiled lists of 110 plant species consumed and 33 plant species dispersed by red-vented bulbul. In the literature there are at least 56 mentions of problematic seed dispersal by the species from eight locations (six countries) (Thibault et al., 2018c). Red-vented bulbul is considered a major vector of some notoriously problematic invasive alien plant species on islands, such as the invasive tree miconia (*Miconia calvescens*) and lantana (*Lantana camara*) in French Polynesia (Meyer, 1996; Spotswood et al., 2012; 2013), lantana (*Lantana camara*), prickly solanum (*Solanum torvum*) and cape gooseberry (*Physalis angulata*) on Fiji (Fox, 2011), ivy gourd (*Coccinia grandis*) on Oahu and Brazilian peppertree (*Schinus terebinthifolius*) in New Caledonia (Spotswood et al., 2012; Thouzeau-Fonseca, 2013). Watling (1978) described the early invasion in the Indo-Pacific region and noted that the distribution of the red-vented bulbul on islands could be determined by the presence of three weed species the species used as principal food: spiked pepper (*Piper aduncum*), turkey berry (*Solanum torvum*) and Cape gooseberry (*Physalis peruviana*). All of these introduced, weedy species subsequently spread on islands with bulbuls present (Watling 1978).

2: Competition

Blanvillain et al. (2003) provide evidence of competition with Tahiti monarch (*Pomarea nigra*), a forest bird endemic to Tahiti (French Polynesia). They noted aggressive interactions (e.g. alarm calls and chasing) between flycatchers and red-vented bulbuls during and outside reproductive activity and suggest this interspecific competition for nest sites and territories might be in part responsible for low breeding success of the Tahiti flycatchers. In contrast, interactions with common myna (*Acridotheres tristis*) were more common when the birds had chicks and eggs in the nest and therefore mynas had a more important direct impact as nest predators of the monarchs (Blanvillain et al., 2003). Competition between red-vented bulbul and other remaining native birds in Fiji forests was also suspected based on reported interspecific aggressive interactions towards at least four native species mostly during the bulbul's breeding season. This caused habitat shifts in native birds (Watling 1978, 1983). Thibault et al. (2018d) provide evidence for competition in man-modified habitats in New Caledonia showing a negative relationship between red-vented bulbul and the abundance of nine native (including endemic) species with which its distribution range overlaps, hence red-vented bulbul is believed to drive reassembly of native species toward sub-optimal locations along an urban-rural gradient, by its aggressive behaviour enabling it to out-compete native species and dominate access to food (Thibault et al., 2018d). Interestingly, the abundance of other introduced alien species (*Acridotheres tristis*, *Passer domesticus*, *Spilopelia chinensis*) was not affected.

3: Hybridisation

Also, some studies report the presence of hybrids due to cross-breeding with native related bulbuls, such as white-cheeked bulbul (*Pycnonotus leucogenys*), white-eared bulbul (*P. leucotis*) and yellow-

vented bulbul (*P. xanthopygos*) in the Middle East (Khamis, 2010; Thibault et al., 2018ab). In Bahrain, two local bird ringers (Brendan Kavanagh and Abdulla Al-Khaabi) recorded a cross-breeding incident between the red-vented bulbul and the white-cheeked bulbul (*P. leucogenys*), where they observed hybrid chicks in a nest. A culturally important species, the local population of the white-cheeked bulbul is under the continuous threat of habitat degradation and poaching. Hence, cross-breeding, which leads to sterile offspring, forms an additional threat to this already vulnerable species and should be considered seriously (Khamis, 2010). Hybridisation of red-vented bulbul with common bulbul (*P. barbatus*), which is one of the commonest birds in Africa that only recently started breeding in southern Spain around Tarifa, has not been reported. Common bulbul is however known to hybridize with four other species, from Africa (*P. capensis*, *P. nigricans*, *P. tricolor*) and the Middle East (*P. xanthopygos*) (<https://avibase.bsc-eoc.org/>).

4: Predation

Other impacts include predation on invertebrates such as Hemiptera, Coleoptera, Odonata, Lepidoptera (Fox, 2011), Hymenoptera and Diptera, as well as small reptiles including geckos and skinks (Brooks et al., 2013; Thibault et al., 2019). On Hawaii, predation by red-vented bulbul and red-whiskered bulbul on larvae and adults of the iconic monarch butterfly *Danaus plexippus* (Lepidoptera: Nymphalidae) led to changes in the proportions of colour morphs in the population (Stimson and Kasuya 2000). Clearly, bulbuls are not deterred by the cardiac glycosides in the monarchs' tissues and can exert heavy predation on larvae feeding on their host plant milkweed (Stimson and Berman 1990). However, we could find no evidence of population level impacts on these species.

Based on and in line with these assessments of alien birds and with species-specific studies we consider the current impact of red-vented bulbul outside the risk assessment area as moderate. Although it is noted that its harmful effects on agricultural systems and native fauna could be highly context-dependent (Thibault, 2018a), there is good evidence of the species altering ecosystems through the spread of seeds of other alien and invasive plant species, and for competition with other bird species, but there is no evidence of red-vented bulbul causing extinctions. This is in line with Thibault et al. (2018d) who suggest that red-vented bulbul causes niche contractions rather than mortality in native species, especially in human-modified landscapes where native birds are already under pressure. No documented cases of recovery of native species after *Pycnonotus* eradication was found, but presumably, as native species are rather displaced and pushed out of optimal habitat, this process is also reversible. Hence a moderate impact score but with high confidence as there are a good number of reliable impact studies on red-vented bulbul from several populations in its invasive range.

Qu. 5.2. How important is the current known impact of the organism on biodiversity at all levels of organisation (e.g. decline in native species, changes in native species communities, hybridisation) in the risk assessment area (include any past impact in your response)?

Discuss impacts that are currently occurring or are likely occurring or have occurred in the past in the risk assessment area. Where there is no direct evidence of impact in the risk assessment area (for example no studies have been conducted), evidence from outside of the risk assessment area can be used to infer impacts within the risk assessment area.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Comment:

No direct evidence is available on impact of red-vented bulbul in the risk assessment area. Since the only established population in Spain is presumably still small, impact can be assumed to be minimal but with low confidence due to the lack of impact studies in the RA area.

Qu. 5.3. How important is the potential future impact of the organism on biodiversity at all levels of organisation likely to be in the risk assessment area?

See comment above. The potential future impact shall be assessed only for the risk assessment area.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Comment:

The species could have impacts on native biodiversity at places where it becomes more widely established and numerous, such as in peri-urban habitats, but also more natural vegetation types such as maquis, open dry scrubland, and forest edge habitat. Here, following the same categorisation of impact mechanisms as in the alien range (see Qu. 5.1), we identified a number of potentially sensitive receptors (species, habitats, protected areas) within the risk assessment area which could be impacted upon, considering the areas where the species is already established or areas predicted suitable for establishment (Iberia, Mediterranean, Mediterranean islands).

1: Frugivory (spreading the fruit/seeds of alien plants)

Because of its frugivorous diet, the red-vented bulbul is a possible disperser of invasive alien plant seeds (see Q5.1), and could thus facilitate invasions of invasive plants (Traveset, 2006; Traveset and Richardson 2014; MacFarlane et al., 2016). It is well known that island ecosystems are especially sensitive to the impacts of invasive alien species and because of high levels of endemism (e.g. Tershy et al., 2015; Bellard et al., 2016). The Mediterranean basin is particularly vulnerable to invasive alien plant invasions because its climatic conditions potentially allow the establishment of sub-tropical and tropical species (Lambdon et al., 2008; Brunel et al., 2010; Brundu 2015). Mediterranean islands are especially vulnerable (Lloret et al., 2005; Vila et al., 2006, 2008; Hulme et al., 2008). A number of established and emerging invasive alien plants for Mediterranean countries produce fleshy fruits and could therefore potentially be spread by birds such as the red-vented bulbul in the risk assessment area

(cf. Gosper et al., 2005; Spotswood et al., 2012, 2013). American pokeweed (*Phytolacca americana*) and Indian pokeweed (*P. acinosa*) produce fleshy purple berries that are spread by birds (McDonnell et al., 1984). Other examples of (potentially) invasive alien plants that might be spread by red-vented bulbuls in the risk assessment area include Brazilian peppertree (*Schinus terebinthifolius*), a commonly planted ornamental in the Mediterranean which is reported to be spread by the species (Thibault et al., 2018c), silver-leaved nightshade (*Solanum elaeagnifolium*), a common agricultural weed (Brunel et al., 2010), sticky nightshade (*S. sisymbriifolium*), a weed of pastures and irrigated crops, roseleaf bramble (*Rubus rosifolius*), Chinese privet (*Ligustrum sinense*) and garden privet (*L. ovalifolium*) (Tanner 2017). Some of these species typically occur in human-modified areas such as parks, gardens and ruderal terrains. By comparison, also red-whiskered bulbuls (*P. jocosus*), a species with comparable ecology, are notorious for spreading invasive weeds including *Lantana* spp., *Rubus* spp., *Phytolacca* spp., *Chrysanthemoides* spp. and *Ligustrum* spp. in Mediterranean parts of Australia and this is considered their biggest impact on ecosystems (Mo 2015). In Spain, red-whiskered bulbuls have been reported feeding on seeds and fruits of native as well as non-native plant species e.g. kurrajong (*Brachychiton populneus*), fig (*Ficus carica*), strawberry tree (*Arbutus unedo*), loquat (*Eriobotrya japonica*), privet (*Ligustrum vulgare*), chinaberry tree (*Melia azedarach*), pomegranate (*Punica granatum*), Peruvian pepper (*Schinus molle*), date palm (*Phoenix dactylifera*), oleander (*Nerium oleander*) flowers, feijoa (*Acca sellowiana*) and yucca (*Yucca* sp.). On Mauritius, red-whiskered bulbuls also have similar species in their diets e.g. *Ligustrum robustum*, *Rubus rosifolius*, *Rubus alceifolius* (Linnebjerg et al., 2010). Spread of typical garden ornamentals by red-vented bulbul could be an issue in the Mediterranean considering the habitat preference of the species for gardens and man-made habitat, for example *Trachycarpus fortunei*, *Mahonia aquifolium*, non-native *Ribes* sp., *Parthenocissus* sp., *Cotoneaster* sp., *Rosa* sp., *Elaeagnus* sp., *Ziziphus jujuba*, *Morus* sp., *Pittosporum* sp., *Myoporum* sp., *Mirabilis jalapa*, *Opuntia* sp., *Lycium* sp., *Lonicera* sp., *Aralia* sp. and *Hedera* sp. With regards to IAS of Union concern (Union list as it stands in 2019), *Persicaria perfoliata* is the only species that produces berry-like fruits (personal communication G. Brundu, 23/10/2019).

2: Competition

Impact on native bird species will mostly occur through competition for food or space including harassment of native birds by (groups of) red-vented bulbul, being chased away or on the nest through territorial interactions (see Qu. 5.1). However, as the species mostly occurs in urban, human influenced landscapes, most of the passerine species it would compete with are relatively common (e.g. blackcap *Sylvia atricapilla*, Sardinian warbler *S. melanocephala*, common blackbird *Turdus merula*, house sparrow *Passer domesticus*) and the presence of the bulbul is expected to lead mostly to niche contraction rather than declines or extinctions (cf. the reported impact of the species on Tahiti monarch, Blanvillain et al., 2003) hence a score of moderate. Other potentially impacted species could include the Iberian magpie (*Cyanopica cooki*), Iberian grey shrike (*Lanius meridionalis*) and ortolan bunting (*Emberiza hortulana*). Iberian magpies are a typical element of Iberian avifauna and have been split from their Asian conspecific based on genetic evidence (Kryukov et al., 2004). The species is highly valued by birdwatchers as a flagship species for Mediterranean agrosilvopastoral systems (e.g. dehesas) (see Q 5.7). Iberian magpies roam in groups in open woodland with grassy clearings, including orchards and olive groves. Their diet consists mainly of acorns and pine nuts, supplemented by invertebrates, soft fruits and berries, and also human-provided scraps in parks and towns. They are in the same feeding niche as red-vented bulbul.

3: Hybridisation

Hybridisation with common bulbul (*P. barbatus*) could potentially occur in the risk assessment area. However, hybrids have not yet been reported. Also, common bulbul is one of the commonest birds in Africa but it only recently expanded its range and started breeding in southern Spain around Tarifa so this risk of hybridisation is currently limited.

4: Predation

Red-vented bulbul is known to be a predator of insects and smaller (or juvenile) vertebrate prey like geckos and lizards (Thibault et al., 2018c). Using Speybroeck et al. (2016) and data compiled on native reptiles on Mediterranean islands Ficetola et al. (2014) we compiled a list of lizards and geckos that could potentially be predated upon. A lot of those have restricted, endemic ranges within Mediterranean islands. These include: Greek Algyroides (*Algyroides moreoticus*) (endemic), Dalmatian Algyroides (*A. nigropunctatus*) and Peloponnese slow worm (*Anguis cephallonica*) on Ionian islands; Sicilian wall lizard (*Podarcis waglerianus*) (endemic) on Sicily and the threatened Aeolian wall lizard (*Podarcis raffoneae*) (endemic) on some smaller Aeolian islets; Milos wall lizard (*Podarcis milensis*) (endemic), Balkan green lizard (*Lacerta trilineata hansschweizeri*) (endemic subspecies), Skyros wall lizard (*Podarcis gaigeae*) (endemic) and Erhard's wall lizard (*Podarcis erhardii*) on the Aegean islands; Cretan wall lizard (*Podarcis cretensis*) (endemic), Kotschy's gecko (*Mediodactylus kotschy bartoni*) (endemic subspecies), Balkan green lizard (*L. trilineata polylepidota*) (endemic subspecies) and Pori wall lizard (*Podarcis levendis*) (endemic) on Crete; Pygmy algyroides (*A. fitzingeri*) (endemic), Tyrrhenian wall lizard (*Podarcis tiliguerta*) (endemic) and European leaf-toad gecko (*Euleptes europaea*) (endemic) on the Thyrrenian islands; Ibiza wall lizard (*Podarcis pityusensis*) (endemic) and Lilford's wall lizard (*Podarcis lilfordi*) (endemic) on the Balearic islands; Kotschy's gecko (*Mediodactylus kotschy*) on Cyprus and the eastern Mediterranean; the more widespread Moorish gecko (*Tarentola mauritanica*) and Turkish gecko (*Hemidactylus turcicus*) across the Mediterranean. Likewise, the list of insects (Lepidoptera, Coleoptera, Orthoptera) that could be predated upon by bulbuls is very long. Considering documented predation on monarch butterfly (*Danaus plexippus*) on Hawaii (see Q 5.1), this species could also be impacted in southern Spain where it is established yet rare (Gil 2006; Lafranchis 2004). Also similar species of Nymphalidae (and other families such as Papilionidae) with conspicuous colours and/or conspicuous larvae could be impacted in Spain and the Mediterranean, such as the African queen (*Danaus chrysippus*) but documented information is lacking for the risk assessment area.

However, although in theory population declines of native insects and reptiles are possible, it is unlikely predation on (mostly nocturnal) geckos or lizards would effectively lead to species extinctions and there are no documented examples of extinctions caused by red-vented bulbuls elsewhere in its invasive range. Hence, score is moderate but with low confidence because of lack of specific impact studies relating to the risk assessment area.

Qu. 5.4. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism currently in the risk assessment area?

including the following elements:

- native species impacted, including red list species, endemic species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats

- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Comment:

We found no documented impact information for the population around Valencia, which is breeding in suburban gardens and sympatric with the more widely established red-whiskered bulbul (*P. jocosus*) (personal communication Carlos Gutiérrez-Expósito, 2018), nor the individuals in Torremolinos, Málaga (Spain) or the population on Fuerteventura which established around 2000. Currently, in the risk assessment area, there is no evidence that red-vented bulbul occurs or is spreading in high conservation value habitats. As no studies have been conducted on this subject, the confidence on this response is low, however it is generally difficult to provide proof of impact in early invasion stages.

Qu. 5.5. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism likely to be in the future in the risk assessment area?

including the following elements:

- native species impacted, including red list species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Comment:

Based on what is known of its ecological amplitude and habitat characteristics, the red-vented bulbul could potentially establish and spread in a variety of habitats, mostly (peri)urban habitats as is the case in Torremolinos (Málaga) and in Valencia where the species frequents gardens in family groups. Here,

impact (e.g. through competition) would occur on rather common species (see Q 5.3). However, if the species becomes more widespread, also conservation value habitats could be invaded where the bulbuls could affect species of concern. Habitats protected by the Habitats Directive which could potentially be invaded and impacted upon through seed dispersal of invasive alien plants include sub-Mediterranean and temperate scrub, Mediterranean arborescent matorral (maquis), Mediterranean sclerophyllous forests (e.g. wild olive woodland, cork-oak forests), garrigue (also known as phrygana in the eastern Mediterranean) and maquis shrubland, which is a complex of several possible vegetation types but characterized by densely growing evergreen shrubs. Several of these vegetation types have unique representation as specific habitats of the Habitats Directive on islands in the Mediterranean (e.g. Tyrrhenian islands, Ionian islands, Cyprus, Malta) (based on European Commission 2013). The Mediterranean scrub biome is also home to a number of breeding birds that could be affected by the red-vented bulbul through competition for nesting space and food. These include a range of songbirds (shrikes, warblers, buntings etc.) and include many species protected by the Birds Directive as well as species listed as vulnerable on the IUCN Red List e.g. Iberian grey shrike (*Lanius meridionalis meridionalis*), bunting species such as ortolan bunting (*Emberiza hortulana*) or commoner species of similar habitats like European stonechat (*Saxicola rubicola*). Currently, the red-vented bulbul is present in Valencia (established) and Torremolinos (not established but status remains unclear) (Spain), both areas with typical Mediterranean vegetation that fall within the modelled distribution area for the species. The vulnerable species listed above are also present in that area. Other vertebrate species that could potentially be affected include geckos and lizards, many of which are endemic or have very restricted ranges and are listed on the Annexes II and IV of the EU Habitats Directive.

Ecosystem Services impacts

Qu. 5.6. How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?

- For a list of relevant services use the CICES classification V5.1 provided as an annex.
- Impacts on ecosystem services build on the observed impacts on biodiversity (habitat, species, genetic, functional) but focus exclusively on reflecting these changes in relation to their links with socio-economic well-being.
- Quantitative data should be provided whenever available and references duly reported.
- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Comment:

The red-vented bulbul is a known pest species on horticultural and agricultural produce outside its native range, e.g. in plant nurseries or on fruits and vegetables (see Qu. 5.9). It has become a pest in agriculture and an active disperser of invasive alien plants (Shine et al., 2003). In 1999, Decree N°171CM, prepared by the Délégation à l'Environnement listed red-vented bulbul among three other alien birds as a threat to biodiversity in French Polynesia (Shine et al., 2003).

1 / Provisioning ecosystem services: The species may have an impact on provisioning ecosystem services such as cultivated terrestrial plants grown for nutritional purposes and as ornamentals. The red-vented bulbul can feed on unripe fruits and buds in large flocks (Fox, 2011), and as a consequence, damage to cultivated plants is the most frequently reported impact of the red-vented bulbul in its alien range. However, these studies were conducted in a limited number of places, such as on Hawaii and in New Caledonia (Thibault et al., 2018b). In a global literature review, Thibault et al. (2018c) report damage to at least 52 plant species belonging to 25 families with 67% (35 species) being food plants (fruits and legumes such as papayas, bananas, lychee, mangos, spinach, cucumber, courgette, aubergine, dragon fruit) and 33% (17 species) being ornamental plant species (e.g. orchids, *Hibiscus* spp.). These numbers are underestimations as many reports of consumption by red-vented bulbul do not consider the type of impact (damage to production or seed dispersal). The impact of the red-vented bulbul appears to be particularly serious on Oahu (Hawaii), where the birds are reported consuming several species of fruits, vegetables and flowers, leading to considerable economic losses. In New Caledonia, significant impacts have been recorded for some crops and plant nurseries with up to 35% losses (Caplong and Barjon, 2010). Significant impacts were observed on production fruit trees, with no less than 35% loss in attacked orchards. Damage was recorded on lychee and peach production but also on papaya and other fruits, sometimes up to the total destruction of the orchard (Metzdorf and Brescia, 2008). Moreover, damage to red fruits in general (tomatoes, strawberries) was reported with losses on tomato production of 500 kg per week as is damage to buds and flowers (Metzdorf and Brescia, 2008). Damage to aubergine crops has also been reported, but also other crops such as dragon fruit (*Pitaya sp.*) (Thouzeau-Fonseca, 2013). Conversely, the red-vented bulbul is not considered an agricultural pest in Fiji (Watling, 1979), nor in Houston (Texas, USA) where it was found to consume mainly introduced tropical plant species (Brooks, 2013).

2 / Cultural ecosystem services: Impacts on cultural ecosystem services may include disturbance of the heritage of isolated island ecosystems in case red-vented bulbul establishment and spread comes at the expense of endemic species. This could occur through changes in abundance of native bird species driving a spatial reassembly of the avifauna. Also, this would especially occur when the species would cause changes in vegetation by promoting seed dispersal of unwanted invasive alien plant species, which could alter ecosystem structure and species composition (Watling 1979; Blanvillain, et al., 2003; Thibault et al. 2019) and make landscapes less attractive for recreation and wildlife watching, or impact the qualities of ecosystems with cultural importance. For instance, a study conducted by Spotswood et al. (2013) in French Polynesia showed that the red-vented bulbul prefers the fruit of a highly invasive tree (*Miconia calvescens*) over that of three other species (one alien, two native). Also in New Caledonia, the red-vented bulbul showed preference for non-native species, including the highly invasive *S. terebinthifolius* (Thibault et al., 2018b). In addition, gut transition led to enhanced germination rates and could represent an “invasional meltdown”, a mutualistic relationship between invasive seed dispersers and invasive plant species leading to higher numbers/faster spread rates of both species and possibly major conservation issues, particularly in ecosystems that host a large number of endemic plant species (Thibault et al., 2018b).

These impacts are mostly documented on Pacific islands, not in other areas. In addition, no studies have addressed ecosystem services impact specifically, but there is evidence of economic and ecosystem impact, hence we score confidence as medium.

Qu. 5.7. How important is the impact of the organism on provisioning, regulating, and cultural services currently in the different biogeographic regions or marine sub-regions where the species has established in the risk assessment area (include any past impact in your response)?

- See guidance to Qu. 5.6.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Comment:

No information has been found on ecosystem services impact of the red-vented bulbul where it established in the RA area (Spain). The population in Valencia is breeding in gardens of urbanized areas surrounding the city (personal communication Carlos Gutiérrez-Expósito, 2018).

1 / Provisioning ecosystem services: there may be impacts on provisioning ecosystem services by damage to plants, fruits and legumes grown in gardens or commercial produce (e.g. citrus and tomato around Valencia) but this would probably be very localized (see Q 5.11).

2 / Cultural ecosystem services: If the red-vented bulbul would establish on isolated Mediterranean islands harbouring endemic species, similar effects could occur as documented on Pacific island ecosystems through decreasing ecosystem qualities for observational interactions such as wildlife watching, birding, ecotourism etc. (see Qu. 5.6). As an example, through competition with native bird species a red-vented bulbul invasion could transform avian assemblages in Iberia (see Qu 5.3). Maritime pine (*Pinus pinaster*) forests are a protected European Habitat (Annex I habitat type 2270 - wooded dunes with *P. pinea* and/or *P. pinaster*), and dehesa (Annex I habitat type 6310 - Dehesas with evergreen *Quercus* spp), open parklands of *Quercus ilex rotundifolia* used for cattle grazing, are a well-known, traditional, culturally highly valued landscape in rural Iberia. A large proportion of the surface area (35.3%) of this typically Mediterranean agrosilvopastoral ecosystem is classified by UNESCO as a Biosphere Reserve (Dehesas de Sierra Morena) since 2002 and Sierra Morena is also part of the Natura 2000 network (site code ES0000090) of protected areas since 1997 (Massot 2016). This ecosystem contains a number of typical bird species that are highly valued by birdwatchers (see Qu 5.3). The presence of large populations of bulbuls, which often flock together in noisy family groups, could cause changes in such valued native bird assemblages, including in parks and gardens where people go to appreciate native wildlife.

Qu. 5.8. How important is the impact of the organism on provisioning, regulating, and cultural

services likely to be in the different biogeographic regions or marine sub-regions where the species can establish in the risk assessment area in the future?

- See guidance to Qu. 5.6.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Comment:

Within the Mediterranean biogeographic region, the red-vented bulbul will likely impact on several ecosystem services, including:

- Provisioning - Biomass - Cultivated terrestrial plants - Cultivated terrestrial plants (including fungi, algae) grown for nutritional purposes
- Regulation & Maintenance - Regulation of physical, chemical, biological conditions - Lifecycle maintenance, habitat and gene pool protection - Seed dispersal
- Cultural - Direct, in-situ and outdoor interactions with living systems that depend on presence in the environmental setting - Physical and experiential interactions with the natural environment - Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through passive or observational interactions
- Cultural - Direct, in-situ and outdoor interactions with living systems that depend on presence in the environmental setting - Physical and experiential interactions with the natural environment - Intellectual and representative interactions with natural environment - Characteristics of living systems that enable aesthetic experiences
- Cultural - Indirect, remote, often indoor interactions with living systems that do not require presence in the environmental setting - Other biotic characteristics that have a non-use value - Characteristics or features of living systems that have an option or bequest value

Besides crop or ornamental plant damage in the endangered area in the Mediterranean (including citrus, tomatoes and strawberry relevant to Iberia, see Qu 5.6), the red-vented bulbul also impacts seed dispersal and could act as a vector for seeds of invasive plant species causing changes in ecosystem structure and species composition of vulnerable Mediterranean island ecosystems harbouring endemic species (for examples see Qu 5.3). This could impact on culturally valued qualities of ecosystems (structure, species composition etc.) that make them attractive to ecotourism, recreation, wildlife watching, or that have cultural and intrinsic value, particularly on Mediterranean islands.

Lastly, since the red-vented bulbul could have an impact on the distribution of less territorial native species, it could alter the species composition in the invasive range, impacting on several cultural ecosystem services as listed above (see Qu 5.6, 5.7).

Economic impacts

Qu. 5.9. How great is the overall economic cost caused by the organism within its current area of distribution (excluding the risk assessment area), including both costs of / loss due to damage and the cost of current management.

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the world these should be reported here. The assessment of the potential costs of / loss due to damage shall describe those costs quantitatively and/or qualitatively depending on what information is available. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Comment:

As stated in Qu. 5.8, the red-vented bulbul is considered an agricultural pest species in several parts of its invasive range. The estimated value of the damage to Oahu’s Orchid industry in 1989 was \$300,000 (Fox, 2011) when the red-vented bulbul together with the Japanese white-eye (*Zosterops japonicus*) reportedly destroying up to 75% of Hawaiian *Dendrobium* orchid and *Anthurium* plantations. This also prompted investigations into chemical repellents to keep the birds off orchid plantations (Cummings et al., 1994). Also in New Caledonia, Thibault et al. (2019) report an economic loss of approximately \$18,355 USD for September 2016 alone in tomato plots.

The species is a well-known agricultural pest species in many parts of the world and there are many, although mostly anecdotal, records of economic damages. Many more probably remain unreported, so we assume yearly damages can easily mount up to more than € 1,000,000 and scored major.

Qu. 5.10. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism currently in the risk assessment area (include any past costs in your response)?

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the EU these should be reported here. Assessment of the potential costs of damage on human health, safety, and the economy, including the cost of non-action. A full economic assessment at EU scale might not be possible, but qualitative data or different case studies from across the EU (or third countries if relevant) may provide useful information to inform decision making. In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal minor moderate major	CONFIDENCE	low medium high
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	massive		
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Comments:

No information has been found on the issue.

Qu. 5.11. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 5.10.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Comments:

Although Thibault et al. (2019) report considerable economic damages outside the native range (e.g. on tomato production, *Hibiscus* and orchids) (see Qu 5.8 and 5.9), Brooks (2013) reports no damage of that kind in Houston and states that flock sizes remain small (average 2.3 birds/flock, range 1–22) compared to the native range which also limits the potential for damage. Also, although this would involve extra costs, bulbul damage can be mitigated using nets, repellents or other methods (see Annex IV). Spain is an important producer of fresh fruit and vegetables which are mostly exported to other EU Member States and the United Kingdom (<https://wits.worldbank.org/>), with the area of Valencia where the species is currently established as an important citrus region. Tomatoes are also of great economic importance in Spain, as it is the world’s 8th producer, with a production of 5,163 million kilograms, grown on an area of 60,852 hectares (www.hortoinfo.es). In Torremolinos, Málaga, the main crops of economic importance that could potentially be impacted include almonds, sunflowers and olives (Massot 2016), apart from small agricultural produce of vegetables in gardens. In the case where the red-vented bulbul establishes more widely in the risk assessment area, economic damages could occur. However, they would probably be localized and context dependent. Also, methods are available to mitigate or prevent bird damages to sensitive crops (e.g. Tracey et al., 2007).

Another vulnerable receptor is the orchid industry, but damage to the orchid industry seems unlikely, given that most orchids in the risk assessment area are produced in The Netherlands in greenhouses, which is outside of the predicted distribution area of the red-vented bulbul and greenhouses are not easily accessible to birds.

In line with other examples of damages outside the native range, but considering the importance of vegetable production in the Mediterranean, we scored moderate.

Qu. 5.12. How great are the economic costs / losses associated with managing this organism currently in the risk assessment area (include any past costs in your response)?

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Comments:

The species is currently not under management in the risk assessment area, therefore we can say with confidence the current management cost is minimal.

Qu. 5.13. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 5.12.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Comments:

There are examples of bulbul eradications on islands using a variety of methods (mostly mist netting with or without supplementary shooting, see Annex IV) (Bunbury et al., 2019, Saavedra & Reynolds, 2019, <http://diise.islandconservation.org/>). On Fuerteventura, seven birds were caught in 2010 (Saavedra & Reynolds, 2019). However, the costs of such eradication/control efforts are not documented. As a crude proxy, Holmes et al. (2015) provide costings for island eradications of predators, and show that although the implementation costs per ha can be relatively low, the planning phase, isolation and the presence of human habitation (often the case with red-vented bulbul invasions) can add up to great expense. As a comparison, Parkes et al. (2006) estimated that the costs to achieve eradication of common myna (*A. tristis*) from Mangaia (Cook Islands, 5180 ha) with appropriate levels of monitoring would be about NZ\$100,000 (c. € 55,000), with 80% of that budget needed for preparation and training and surveillance, including detecting surviving birds. However, here, the method proposed was toxic baiting.

As stated in Qu. 5.11, mitigation of bird damage to produce will also involve additional management costs.

Considering the costs described both above and in Holmes et al. (2015) and assuming the red-vented bulbul would establish more widely in the RA area in suburban mainland areas and/or on islands, we scored moderate but since data on costs from the literature are not species specific, confidence is low.

Social and human health impacts

Qu. 5.14. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism for the risk assessment area and for third countries, if relevant (e.g. with similar eco-climatic conditions).

The description of the known impact and the assessment of potential future impact on human health, safety and the economy, shall, if relevant, include information on

- illnesses, allergies or other affections to humans that may derive directly or indirectly from a species;
- damages provoked directly or indirectly by a species with consequences for the safety of people, property or infrastructure;
- direct or indirect disruption of, or other consequences for, an economic or social activity due to the presence of a species.

Social and human health impacts can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Comments:

No social or human health impact has been caused by the red-vented bulbul so far. However, red-vented bulbuls in Tahiti have been found to carry the zoonotic disease *Chlamydia sp.* (Blanvillain et al., 2013).

Qu. 5.15. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism in the future for the risk assessment area.

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	minimal minor	CONFIDENCE	low medium
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	moderate major massive		high
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Comments:

See Qu. 5.14. This is not expected to change in the future.

Other impacts

Qu. 5.16. How important is the impact of the organism as food, a host, a symbiont or a vector for other damaging organisms (e.g. diseases)?

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Comments:

Dispersal of parasites by red-vented bulbul is not well documented in its alien range. In its native range, the red-vented bulbul is known to host *Isoospora* spp. (Boughton et al., 1938), *Menacanthus eurysternus* (Price 1975), *Bruelia guldum* and *Sturnidoecus guldum* (Ansari 1957) and *Pteroherpis pycnonoti* (Constantinescu et al., unpublished). In 1996, Jarvi et al. (2003) detected no avian malaria (*Plasmodium* spp.) in blood smears, and Atkinson et al. (2006) found no evidence of *Plasmodium*, *Trypanosoma*, *Atoxoplasma* or microfilaria. *Plasmodium* is however present in the south Pacific area. Red-vented bulbuls in Tahiti, however, have been found to carry the zoonotic disease *Chlamydia* (Blanvillain et al., 2013).

Qu. 5.17. How important might other impacts not already covered by previous questions be resulting from introduction of the organism?

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Comments:

No other possible impacts were found.

Qu. 5.18. How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area?

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Comments:

The red-vented bulbul is not particularly vulnerable to predation. In its native range, a study carried out by Zia et al. (2017) found that predation rate on the red-vented bulbul was only 6% in eggs and 9% in nestlings. Predators in the study area included bird species like eagles and barn owl (*Tyto alba*), and rodents like the black rat (*Rattus rattus*). These species or similar species are also present in the risk assessment area, possibly impacting the red-vented bulbul. However, if these only have minor effects in the native range, it is unlikely that their impact will be higher in the introduced range, where they are not used to hunt on the red-vented bulbul. Likely predators of the red-vented bulbul in Valencia and Torremolinos, and by extension the risk assessment area, include the booted eagle (*Hieraetus pennatus* Gmelin, 1788), Bonelli's eagle (*Aquila fasciata* Vieillot, 1822), the northern goshawk (*Accipiter gentilis* Linnaeus, 1758) and the sparrowhawk (*Accipiter nisus* Linnaeus, 1758). The latter might become the most important predator of the red-vented bulbul in its invasive range, since it mainly preys upon species similar to the red-vented bulbul regarding size, behaviour and habitat, such as the house sparrow (*Passer domesticus* Linnaeus, 1758), common blackbird (*Turdus merula* Linnaeus, 1758), starlings and pigeons. Other birds of prey may take the eggs of the red-vented bulbul, as will mammalian predators like the stone martin (*Martes foina* Erxleben, 1777) and European pine marten (*Martes martes* Linnaeus, 1758). Since the red-vented bulbul nests in trees, it will not be vulnerable to ground predators such as foxes or stoats.

Although the red-vented bulbul is a known host to several parasites in its native range, including *Isoospora* spp. (Boughton et al., 1938), *Menacanthus eurysternus* (Price 1975), *Bruelia guldum* and *Sturnidoecus guldum* (Ansari 1957) and *Pteroherpis pycnonoti* (Constantinescu et al., unpublished), no impacts of these parasites on the red-vented bulbul were found.

No information was found on impact of pathogens on the red-vented bulbul.

For more information, see Qu. 3.5.

Qu. 5.19. Estimate the overall impact in the risk assessment area under current climate conditions. In addition, details of overall impact in relevant biogeographical regions should be

provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, in current conditions.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

See answers to Qu. 5.3, 5.5, 5.8, 5.11, 5.13, 5.15 and 5.16.

Qu. 5.20. Estimate the overall impact in the risk assessment area in foreseeable climate change conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, under future conditions.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

The proportion of the area suitable for establishment in the Mediterranean biogeographical region is predicted to double under RCP 2.6 and to triple under RCP 4.5 by 2070 (see Qu. 3.14, Annex VII). Therefore, it is moderately likely that the overall impacts will increase too.

RISK SUMMARIES

	RESPONSE	CONFIDENCE	COMMENT
Summarise Introduction*	very unlikely unlikely moderately likely likely very likely	low medium high	The species is already present in zoological gardens and private collections in multiple countries within the risk assessment area. There is also the possibility that the species is introduced for private bird collections although quantitative data are lacking. Introductions as stowaways are less likely.
Summarise Entry*	very unlikely unlikely moderately likely likely very likely	low medium high	The red-vented bulbul is kept as a pet and is on display in zoological garden. Escapes and releases have happened before in several countries in the risk assessment area and entry is likely to occur in the future. Stowaways on ships might enter the risk assessment area as well, as the species is established on the Canary islands.
Summarise Establishment*	very unlikely unlikely moderately likely likely very likely	low medium high	The species already established on at least two locations in the risk assessment area (Spain). The Mediterranean bioregion, including Mediterranean islands, is generally vulnerable to invasion by red-vented bulbul, both under current and future climatic conditions. The red-vented bulbul is most likely to establish in (peri)urban areas with a mediterranean or (semi-)arid climate.
Summarise Spread*	very slowly slowly moderately rapidly very rapidly	low medium high	The species is classified as a rather sedentary species, showing possible movements depending on environmental conditions. Indeed, spread is limited in Spanish mainland populations, although densities are still limited here and the species has to compete with red-whiskered bulbul. However, the invasion on Fuerteventura, where the species invaded the entire island in a few decades and has shown important range expansion in a short period of time, as well as the Pacific

			invasion, show red-vented bulbul is able to spread moderately rapidly or rapidly in insular contexts.
Summarise Impact*	minimal minor moderate major massive	low medium high	The red-vented bulbul can impact on native species and ecosystems through competition, frugivory, by spreading alien plants, hybridising with bulbul species, by predating on (in)vertebrates and by pathogen transmission. It is also an agricultural pest in parts of its alien range. However, although declines and niche contraction in sensitive and protected species are possible, no extinctions caused by red-vented bulbul have been documented so far.
Conclusion of the risk assessment (overall risk)	low moderate high	low medium high	Considering the potential of red-vented bulbul to cause population declines in native species, documented contractions of niches of native species in its alien range but no extinctions, moderate spread capacity, the limited area suitable for establishment in the risk assessment area but presence of sensitive island biota, local and reversible effects on ecosystem services and the potential for moderate economic damages, we scored moderate impact. This score is in line with a recent, global, environmental impact assessment of alien birds using EICAT.

*in current climate conditions and in foreseeable future climate conditions

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Distribution Summary

Please answer as follows:

- Yes if recorded, established or invasive
- if not recorded, established or invasive
- ? Unknown; data deficient

The columns refer to the answers to Questions A5 to A12 under Section A.

For data on marine species at the Member State level, delete Member States that have no marine borders. In all other cases, provide answers for all columns.

Member States and the United Kingdom

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Austria	-	-	-	-	-
Belgium	Yes	-	-	-	-
Bulgaria	-	-	-	-	-
Croatia	-	-	-	-	-
Cyprus	-	-	Yes	Yes	-
Czech Republic	-	-	-	-	-
Denmark	-	-	-	-	-
Estonia	-	-	-	-	-
Finland	-	-	-	-	-
France	-	-	Yes	Yes	-
Germany	-	-	-	-	-
Greece	-	-	Yes	Yes	-
Hungary	-	-	-	-	-
Ireland	-	-	-	-	-
Italy	-	-	?	?	?
Latvia	-	-	-	-	-
Lithuania	-	-	-	-	-
Luxembourg	-	-	-	-	-
Malta	-	-	?	?	?
Netherlands	Yes	-	-	-	-
Poland	-	-	-	-	-
Portugal	-	-	?	?	?
Romania	-	-	-	-	-
Slovakia	-	-	-	-	-
Slovenia	-	-	-	-	-
Spain	Yes	Yes	Yes	Yes	
Sweden	-	-	-	-	-
United Kingdom	-	-	-	-	-

Biogeographical regions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Alpine	-	-	-	-	-
Atlantic	-	-	-	-	-
Black Sea	-	-	-	-	-
Boreal	-	-	-	-	-
Continental	-	-	-	-	-
Mediterranean	-	-	Yes	Yes	-
Pannonian	-	-	-	-	-
Steppic	-	-	-	-	-

ANNEX I Scoring of Likelihoods of Events

(taken from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Description	Frequency
Very unlikely	This sort of event is theoretically possible, but is never known to have occurred and is not expected to occur	1 in 10,000 years
Unlikely	This sort of event has not occurred anywhere in living memory	1 in 1,000 years
Moderately likely	This sort of event has occurred somewhere at least once in recent years, but not locally	1 in 100 years
Likely	This sort of event has happened on several occasions elsewhere, or on at least one occasion locally in recent years	1 in 10 years
Very likely	This sort of event happens continually and would be expected to occur	Once a year

ANNEX II Scoring of Magnitude of Impacts

(modified from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Biodiversity and ecosystem impact	Ecosystem Services impact	Economic impact (Monetary loss and response costs per year)	Social and human health impact, and other impacts
	<i>Question 5.1-5</i>	<i>Question 5.6-8</i>	<i>Question 5.9-13</i>	<i>Question 5.14-18</i>
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected ⁷	Up to 10,000 Euro	No social disruption. Local, mild, short-term reversible effects to individuals.
Minor	Some ecosystem impact, reversible changes, localised	Local and temporary, reversible effects to one or few services	10,000-100,000 Euro	Significant concern expressed at local level. Mild short-term reversible effects to identifiable groups, localised.
Moderate	Measurable long-term damage to populations and ecosystem, but reversible; little spread, no extinction	Measureable, temporary, local and reversible effects on one or several services	100,000-1,000,000 Euro	Temporary changes to normal activities at local level. Minor irreversible effects and/or larger numbers covered by reversible effects, localised.
Major	Long-term irreversible ecosystem change, spreading beyond local area	Local and irreversible or widespread and reversible effects on one / several services	1,000,000-10,000,000 Euro	Some permanent change of activity locally, concern expressed over wider area. Significant irreversible effects locally or reversible effects over large area.
Massive	Widespread, long-term population loss or extinction, affecting several species with serious ecosystem effects	Widespread and irreversible effects on one / several services	Above 10,000,000 Euro	Long-term social change, significant loss of employment, migration from affected area. Widespread, severe, long-term, irreversible health effects.

⁷ Not to be confused with “no impact”.

ANNEX III Scoring of Confidence Levels

(modified from Bacher et al., 2017)

Each answer provided in the risk assessment must include an assessment of the level of confidence attached to that answer, reflecting the possibility that information needed for the answer is not available or is insufficient or available but conflicting.

The responses in the risk assessment should clearly support the choice of the confidence level.

Confidence level	Description
Low	There is no direct observational evidence to support the assessment, e.g. only inferred data have been used as supporting evidence <i>and/or</i> Impacts are recorded at a spatial scale which is unlikely to be relevant to the assessment area <i>and/or</i> Evidence is poor and difficult to interpret, e.g. because it is strongly ambiguous <i>and/or</i> The information sources are considered to be of low quality or contain information that is unreliable.
Medium	There is some direct observational evidence to support the assessment, but some information is inferred <i>and/or</i> Impacts are recorded at a small spatial scale, but rescaling of the data to relevant scales of the assessment area is considered reliable, or to embrace little uncertainty <i>and/or</i> The interpretation of the data is to some extent ambiguous or contradictory.
High	There is direct relevant observational evidence to support the assessment (including causality) <i>and</i> Impacts are recorded at a comparable scale <i>and/or</i> There are reliable/good quality data sources on impacts of the taxa <i>and</i> The interpretation of data/information is straightforward <i>and/or</i> Data/information are not controversial or contradictory.

ANNEX IV Ecosystem services classification (CICES V5.1, simplified) and examples

For the purposes of this risk assessment, please feel free to use what seems as the most appropriate category / level / combination of impact (Section – Division – Group), reflecting information available.

Section	Division	Group	Examples (i.e. relevant CICES “classes”)
Provisioning	Biomass	Cultivated <i>terrestrial</i> plants	<p>Cultivated terrestrial plants (including fungi, algae) grown for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from cultivated plants, fungi, algae and bacteria for direct use or processing (excluding genetic materials); Cultivated plants (including fungi, algae) grown as a <u>source of energy</u></p> <p><i>Example: negative impacts of non-native organisms to crops, orchards, timber etc.</i></p>
		Cultivated <i>aquatic</i> plants	<p>Plants cultivated by in- situ aquaculture grown for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from in-situ aquaculture for direct use or processing (excluding genetic materials); Plants cultivated by in- situ aquaculture grown as an <u>energy source</u>.</p> <p><i>Example: negative impacts of non-native organisms to aquatic plants cultivated for nutrition, gardening etc. purposes.</i></p>
		Reared animals	<p>Animals reared for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from reared animals for direct use or processing (excluding genetic materials); Animals reared to provide <u>energy</u> (including mechanical)</p> <p><i>Example: negative impacts of non-native organisms to livestock</i></p>
		Reared <i>aquatic</i> animals	<p>Animals reared by in-situ aquaculture for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from animals grown by in-situ aquaculture for direct use or processing (excluding genetic materials); Animals reared by in-situ aquaculture as an <u>energy source</u></p> <p><i>Example: negative impacts of non-native organisms to fish farming</i></p>
		Wild plants (terrestrial and aquatic)	<p>Wild plants (terrestrial and aquatic, including fungi, algae) used for <u>nutrition</u>; <u>Fibres and other materials</u> from wild plants for direct use or processing (excluding genetic materials); Wild plants (terrestrial and aquatic, including fungi, algae) used as a <u>source of energy</u></p> <p><i>Example: reduction in the availability of wild plants (e.g. wild berries, ornamentals) due to non-native organisms (competition, spread of disease etc.)</i></p>
		Wild animals (terrestrial and aquatic)	<p>Wild animals (terrestrial and aquatic) used for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from wild animals for direct use or processing (excluding genetic materials); Wild animals (terrestrial and aquatic) used as a <u>source of energy</u></p> <p><i>Example: reduction in the availability of wild animals (e.g. fish stocks, game) due to non-native organisms (competition,</i></p>

			<i>predations, spread of disease etc.)</i>
	Genetic material from all biota	Genetic material from plants, algae or fungi	<p><u>Seeds, spores and other plant materials</u> collected for maintaining or establishing a population; Higher and lower plants (whole organisms) used to <u>breed new strains or varieties</u>; Individual genes extracted from higher and lower plants for the <u>design and construction of new biological entities</u></p> <p><i>Example: negative impacts of non-native organisms due to interbreeding</i></p>
		Genetic material from animals	<p>Animal material collected for the purposes of maintaining or establishing a population; Wild animals (whole organisms) used to breed new strains or varieties; Individual genes extracted from organisms for the design and construction of new biological entities</p> <p><i>Example: negative impacts of non-native organisms due to interbreeding</i></p>
	Water⁸	Surface water used for nutrition, materials or energy	<p>Surface water for <u>drinking</u>; Surface water used as a material (<u>non-drinking purposes</u>); Freshwater surface water, coastal and marine water used as an <u>energy source</u></p> <p><i>Example: loss of access to surface water due to spread of non-native organisms</i></p>
		Ground water for used for nutrition, materials or energy	<p>Ground (and subsurface) water for <u>drinking</u>; Ground water (and subsurface) used as a material (<u>non-drinking purposes</u>); Ground water (and subsurface) used as an <u>energy source</u></p> <p><i>Example: reduced availability of ground water due to spread of non-native organisms and associated increase of ground water consumption by vegetation.</i></p>
Regulation & Maintenance	Transformation of biochemical or physical inputs to ecosystems	Mediation of wastes or toxic substances of anthropogenic origin by living processes	<p>Bio-remediation by microorganisms, algae, plants, and animals; <u>Filtration/sequestration/storage/accumulation</u> by microorganisms, algae, plants, and animals</p> <p><i>Example: changes caused by non-native organisms to ecosystem functioning and ability to filtrate etc. waste or toxics</i></p>
		Mediation of nuisances of anthropogenic origin	<p><u>Smell reduction</u>; noise attenuation; visual screening (e.g. by means of green infrastructure)</p> <p><i>Example: changes caused by non-native organisms to ecosystem structure, leading to reduced ability to mediate nuisances.</i></p>
	Regulation of physical, chemical, biological conditions	Baseline flows and extreme event regulation	<p>Control of <u>erosion</u> rates; Buffering and attenuation of <u>mass movement</u>; <u>Hydrological cycle and water flow regulation</u> (Including flood control, and coastal protection); <u>Wind</u> protection; <u>Fire</u> protection</p> <p><i>Example: changes caused by non-native organisms to ecosystem functioning or structure leading to, for example, destabilisation of soil, increased risk or intensity of wild fires etc.</i></p>

⁸ Note: in the CICES classification provisioning of water is considered as an abiotic service whereas the rest of ecosystem services listed here are considered biotic.

		<p>Lifecycle maintenance, habitat and gene pool protection</p>	<p><u>Pollination</u> (or 'gamete' dispersal in a marine context); <u>Seed dispersal</u>; Maintaining <u>nursery populations and habitats</u> (Including gene pool protection)</p> <p><i>Example: changes caused by non-native organisms to the abundance and/or distribution of wild pollinators; changes to the availability / quality of nursery habitats for fisheries</i></p>
		<p>Pest and disease control</p>	<p>Pest control; Disease control</p> <p><i>Example: changes caused by non-native organisms to the abundance and/or distribution of pests</i></p>
		<p>Soil quality regulation</p>	<p><u>Weathering processes</u> and their effect on soil quality; <u>Decomposition and fixing processes</u> and their effect on soil quality</p> <p><i>Example: changes caused by non-native organisms to vegetation structure and/or soil fauna leading to reduced soil quality</i></p>
		<p>Water conditions</p>	<p>Regulation of the <u>chemical condition</u> of freshwaters by living processes; Regulation of the chemical condition of salt waters by living processes</p> <p><i>Example: changes caused by non-native organisms to buffer strips along water courses that remove nutrients in runoff and/or fish communities that regulate the resilience and resistance of water bodies to eutrophication</i></p>
		<p>Atmospheric composition and conditions</p>	<p>Regulation of <u>chemical composition</u> of atmosphere and oceans; Regulation of <u>temperature and humidity</u>, including ventilation and transpiration</p> <p><i>Example: changes caused by non-native organisms to ecosystems' ability to sequester carbon and/or evaporative cooling (e.g. by urban trees)</i></p>
Cultural	Direct, in-situ and outdoor interactions with living systems that depend on presence in the environmental setting	<p>Physical and experiential interactions with natural environment</p>	<p>Characteristics of living systems that that enable activities promoting health, recuperation or enjoyment through <u>active or immersive interactions</u>;</p> <p>Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through <u>passive or observational interactions</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that make it attractive for recreation, wild life watching etc.</i></p>
		<p>Intellectual and representative interactions with natural environment</p>	<p>Characteristics of living systems that enable <u>scientific investigation</u> or the creation of traditional ecological knowledge;</p> <p>Characteristics of living systems that enable <u>education and training</u>;</p> <p>Characteristics of living systems that are resonant in terms of <u>culture or heritage</u>;</p> <p>Characteristics of living systems that enable <u>aesthetic experiences</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have cultural importance</i></p>

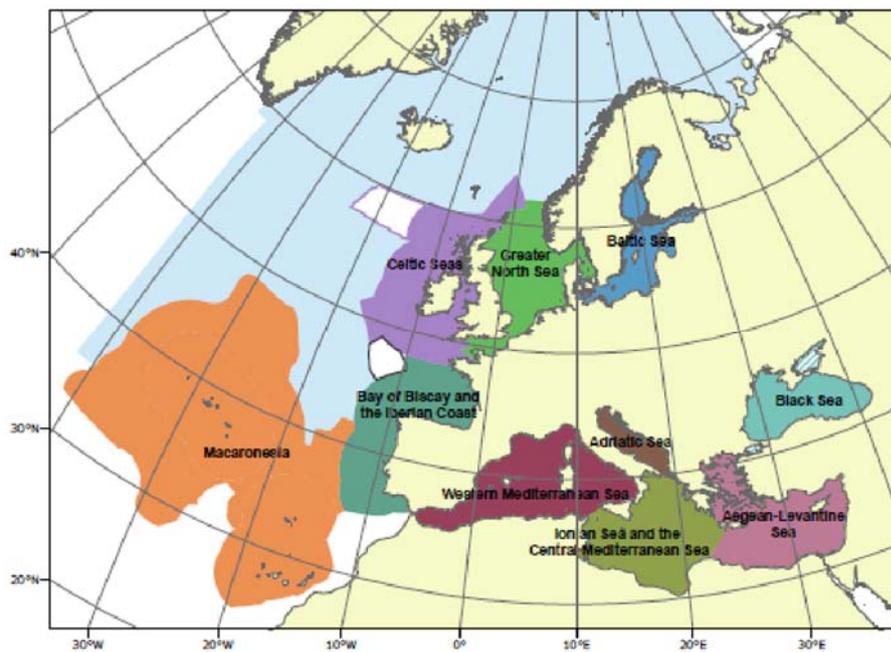
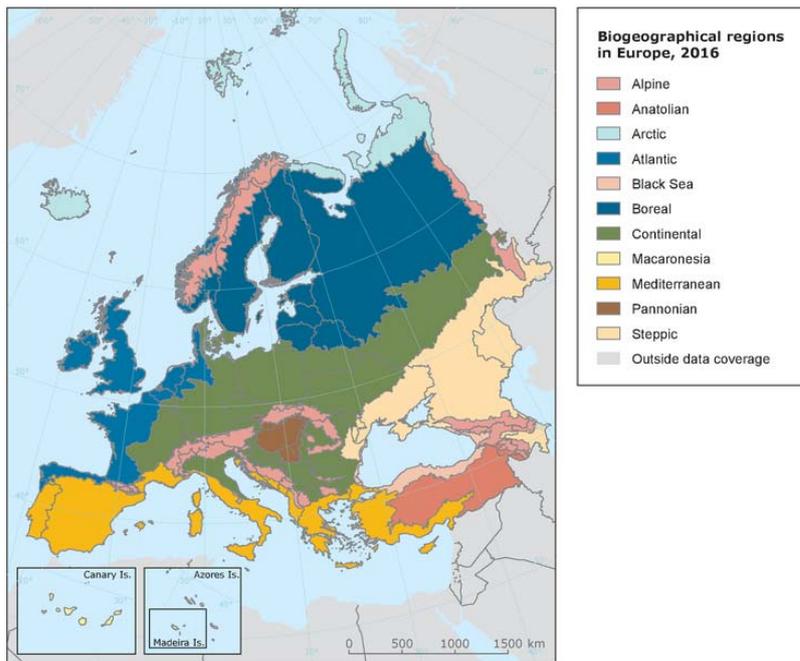
<p>Indirect, remote, often indoor interactions with living systems that do not require presence in the environmental setting</p>	<p>Spiritual, symbolic and other interactions with natural environment</p>	<p>Elements of living systems that have <u>symbolic meaning</u>; Elements of living systems that have <u>sacred or religious meaning</u>; Elements of living systems used for <u>entertainment or representation</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have sacred or religious meaning</i></p>
	<p>Other biotic characteristics that have a non-use value</p>	<p>Characteristics or features of living systems that have an <u>existence value</u>; Characteristics or features of living systems that have an <u>option or bequest value</u></p> <p><i>Example: changes caused by non-native organisms to ecosystems designated as wilderness areas, habitats of endangered species etc.</i></p>

ANNEX V EU Biogeographic Regions and MSFD Subregions

See <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2>,
http://ec.europa.eu/environment/nature/natura2000/biogeog_regions/

and

<https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions-1/technical-document/pdf>



ANNEX VI Delegated Regulation (EU) 2018/968 of 30 April 2018

see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>

ANNEX VII Projection of climatic suitability for *Pycnonotus cafer* establishment

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31 October 2019

Aim

To project the suitability for potential establishment of *Pycnonotus cafer* in Europe, under current and predicted future climatic conditions.

Data for modelling

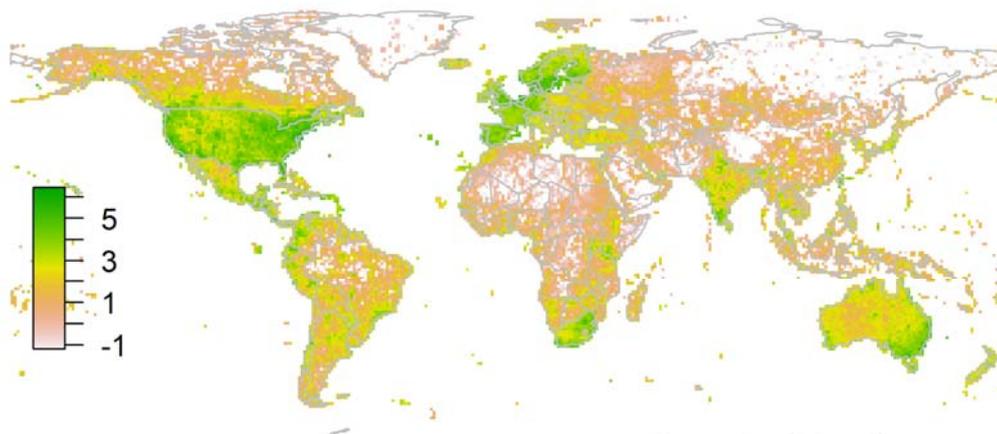
Species occurrence data were obtained from the Global Biodiversity Information Facility (GBIF) (245716 records), the Biodiversity Information Serving Our Nation database (BISON) (8660 records), iNaturalist (358 records), the Integrated Digitized Biocollections (iDigBio) (42 records), and a small number of additional records from the risk assessment team. We scrutinised occurrence records from regions where the species is not known to be established and removed any dubious records (e.g. fossils, captive records) or where the georeferencing was too imprecise (e.g. records referenced to a country or island centroid) or outside of the coverage of the predictor layers (e.g. small island or coastal occurrences). The remaining records were gridded at a 0.25 x 0.25 degree resolution for modelling, yielding 3381 grid cells with occurrences (Figure 1a). As a proxy for recording effort, the density of Aves records held by GBIF was also compiled on the same grid (Figure 1b).

Figure 1. (a) Occurrence records obtained for *Pycnonotus cafer* and used in the modelling, showing native and invaded distributions. (b) The recording density of Aves on GBIF, which was used as a proxy for recording effort.

(a) Species distribution used in modelling



(b) Estimated recording effort (log₁₀-scaled)



Climate data were selected from the 'Bioclim' variables contained within the WorldClim database (Hijmans et al., 2005), originally at 5 arcminute resolution (0.083 x 0.083 degrees of longitude/latitude) and aggregated to a 0.25 x 0.25 degree grid for use in the model.

Based on the biology of *Pycnonotus cafer*, the following climate variables were used in the modelling:

- Annual mean temperature (Bio1)
- Minimum temperature of the coldest month (Bio6)
- Mean temperature of the warmest quarter (Bio10)
- Annual precipitation (Bio12)
- Precipitation of the wettest month (Bio13)
- Precipitation of the driest month (Bio14)

To estimate the effect of climate change on the potential distribution, equivalent modelled future climate conditions for the 2070s under the Representative Concentration Pathways (RCP) 2.6 and 4.5 were also obtained. These represent low and medium emissions scenarios, respectively. The above variables were obtained as averages of outputs of eight Global Climate Models (BCC-CSM1-1, CCSM4, GISS-E2-R, HadGEM2-AO, IPSL-CM5A-LR, MIROC-ESM, MRI-CGCM3, NorESM1-M), downscaled and calibrated against the WorldClim baseline (see http://www.worldclim.org/cmip5_5m).

The following habitat layers were also used:

- Tree cover (Tree): This was estimated from the MODerate-resolution Imaging Spectroradiometer (MODIS) satellite continuous tree cover raster product, produced by the Global Land Cover Facility (<http://glcf.umd.edu/data/vcf/>). The raw product contains the percentage cover by trees in each 0.002083 x 0.002083 degree grid cell. We aggregated this to the mean cover in our 0.25 x 0.25 degree grid cells.
- Human influence index (HII): As many non-native invasive species associate with anthropogenically disturbed habitats. We used the Global Human Influence Index Dataset of the Last of the Wild Project (Wildlife Conservation Society - WCS & Center for International Earth Science Information Network - CIESIN - Columbia University 2005), which is developed from nine global data layers covering human population pressure (population density), human land use and infrastructure (built-up areas, nighttime lights, land use/land cover) and human access (coastlines, roads, railroads, navigable rivers). The index ranges between 0 and 1 and was ln+1 transformed for the modelling to improve normality.

Species distribution model

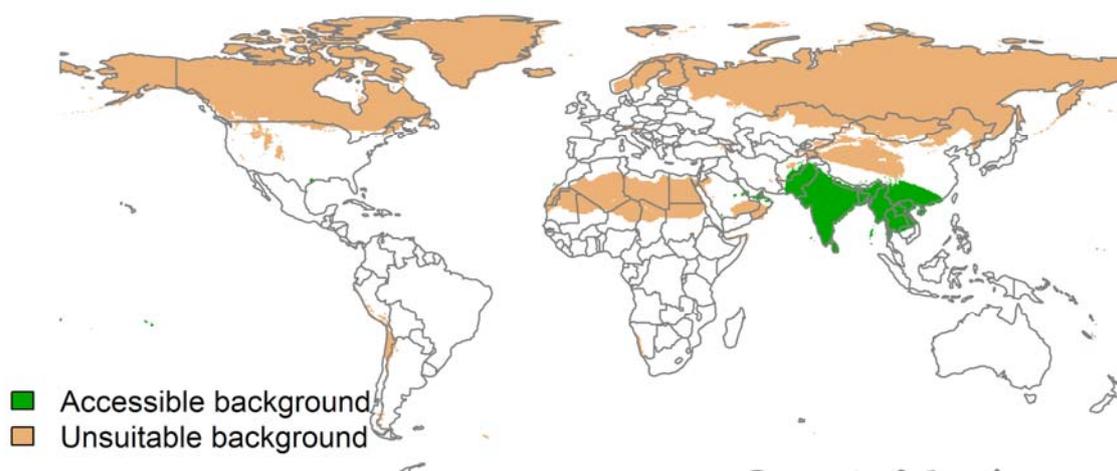
A presence-background (presence-only) ensemble modelling strategy was employed using the BIOMOD2 R package v3.3-7.1 (Thuiller et al., 2019, Thuiller et al., 2009). These models contrast the environment at the species' occurrence locations against a random sample of the global background environmental conditions (often termed 'pseudo-absences') in order to characterise and project suitability for occurrence. This approach has been developed for distributions that are in equilibrium with the environment. Because invasive species' distributions are not at equilibrium and subject to dispersal constraints at a global scale, we took care to minimise the inclusion of locations suitable for the species but where it has not been able to disperse to (Chapman et al., 2019). Therefore the background sampling region included:

- The area accessible by native *Pycnonotus cafer* populations, in which the species is likely to have had sufficient time to disperse to all locations. Based on presumed maximum dispersal distances, the accessible region was defined as a 300km buffer around the native range occurrences; AND
- A 50km buffer around the non-native occurrences, encompassing regions likely to have had high propagule pressure for introduction by humans and/or dispersal of the species; AND
- Regions where we have an *a priori* expectation of high unsuitability for the species so that absence is assumed irrespective of dispersal constraints (see Figure 2). The following rules were applied to define a region expected to be highly unsuitable for *Pycnonotus cafer* at the spatial scale of the model:
 - Annual mean temperature (Bio1) < 4°C
 - Annual precipitation (Bio12) < 4 (i.e. <55mm, as the Bioclim variable is on a natural log scale)

Altogether, only 0.5% of occurrence grid cells were located in the unsuitable background region.

Within the background region, 10 samples of 5000 randomly sampled grid cells were obtained, weighting the sampling by recording effort (Figure 2).

Figure 2. The background from which pseudo-absence samples were taken in the modelling of *Pycnonotus cafer*. Samples were taken from a 300km buffer around the native range and a 50km buffer around non-native occurrences (together forming the accessible background), and from areas expected to be highly unsuitable for the species (the unsuitable background region). Samples were weighted by a proxy for recording effort (Figure 1(b)).



Each dataset (i.e. combination of the presences and the individual background samples) was randomly split into 80% for model training and 20% for model evaluation. With each training dataset, seven statistical algorithms were fitted with the default BIOMOD2 settings and rescaled using logistic regression, except where specified below:

- Generalised linear model (GLM)
- Generalised boosting model (GBM)
- Generalised additive model (GAM) with a maximum of four degrees of freedom per smoothing spline
- Artificial neural network (ANN)
- Multivariate adaptive regression splines (MARS)
- Random forest (RF)
- Maxent

Since the background sample was larger than the number of occurrences, prevalence fitting weights were applied to give equal overall importance to the occurrences and the background. Normalised variable importance was assessed and variable response functions were produced using BIOMOD2's default procedure.

Model predictive performance was assessed by the following three measures:

- AUC, the area under the receiver operating characteristic curve (Fielding & Bell 1997). Predictions of presence-absence models can be compared with a subset of records set aside for model evaluation (here 20%) by constructing a confusion matrix with the number of true positive, false positive, false negative and true negative cases. For models generating non-dichotomous scores (as here) a threshold can be applied to transform the scores into a dichotomous set of presence-absence predictions. Two measures that can be derived from the confusion matrix are sensitivity (the proportion of observed presences that are predicted as such, quantifying omission errors), and specificity (the proportion of observed absences that are predicted as such, quantifying commission errors). A receiver operating characteristic (ROC) curve can be constructed by using all possible thresholds to classify the scores into confusion matrices, obtaining sensitivity and specificity for each matrix, and plotting sensitivity against the corresponding proportion of false positives (equal to $1 - \text{specificity}$). The use of all possible thresholds avoids the need for a selection of a single threshold, which is often arbitrary, and allows appreciation of the trade-off between sensitivity and specificity. The area under the ROC curve (AUC) is often used as a single threshold-independent measure for model performance (Manel, Williams & Ormerod 2001). AUC is the probability that a randomly selected presence has a higher model-predicted suitability than a randomly selected absence (Allouche et al., 2006).
- Cohen's Kappa (Cohen 1960). This measure corrects the overall accuracy of model predictions (ratio of the sum of true presences plus true absences to the total number of records) by the accuracy expected to occur by chance. The kappa statistic ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random. Advantages of kappa are its simplicity, the fact that both commission and omission errors are accounted for in one parameter, and its relative tolerance to zero values in the confusion matrix (Manel, Williams & Ormerod 2001). However, Kappa has been criticised for being sensitive to prevalence (the proportion of sites in which the species was recorded as present) and may therefore be inappropriate for comparisons of model accuracy between species or regions (McPherson, Jetz & Rogers 2004, Allouche et al., 2006).
- TSS, the true skill statistic (Allouche et al., 2006). TSS is defined as sensitivity + specificity - 1, and corrects for Kappa's dependency on prevalence. TSS compares the number of correct forecasts, minus those attributable to random guessing, to that of a hypothetical set of perfect forecasts. Like kappa, TSS takes into account both omission and commission errors, and success as a result of random guessing, and ranges from -1 to +1, where +1 indicates perfect agreement and values of zero or less indicate a performance no better than random (Allouche et al., 2006).

An ensemble model was created by first rejecting poorly performing algorithms with relatively extreme low AUC values and then averaging the predictions of the remaining algorithms, weighted by their AUC. To identify poorly performing algorithms, AUC values were converted into modified z-scores based on their difference to the median and the median absolute deviation across all algorithms (Iglewicz & Hoaglin, 1993). Algorithms with $z < -2$ were rejected. In this way, ensemble projections were made for each dataset and then averaged to give an overall suitability, as well as its standard deviation. The projections were then classified into suitable and unsuitable regions using the 'minROCDist' method, which minimizes the distance between the ROC plot and the upper left corner of the plot (point (0,1)).

We also produced limiting factor maps for Europe following Elith et al. (2010). For this, projections were made separately with each individual variable fixed at a near-optimal value. These were chosen as the median values at the occurrence grid cells. Then, the most strongly limiting factors were identified as the one resulting in the highest increase in suitability in each grid cell.

Results

The ensemble model suggested that suitability for *Pycnonotus cafer* was most strongly determined by Annual mean temperature (Bio1), accounting for 42.9% of variation explained, followed by Mean temperature of the warmest quarter (Bio10) (23.5%), Precipitation of the wettest month (Bio13) (10.2%), Minimum temperature of the coldest month (Bio6) (9.4%), Human influence index (HII) (5.7%), Annual precipitation (Bio12) (5.3%), Precipitation of the driest month (Bio14) (2.2%) and Global tree cover (Tree) (0.8%) (Table 1, Figure 3).

Table 1. Summary of the cross-validation predictive performance (AUC, Kappa, TSS) and variable importance of the fitted model algorithms and the ensemble (AUC-weighted average of the best performing algorithms). Results are the average from models fitted to 10 different background samples of the data.

Algorithm	AUC	Kappa	TSS	Used in the ensemble	variable importance (%)								
					Annual mean temperature (Bio1)	Mean temperature of the warmest quarter (Bio10)	Precipitation of the wettest month (Bio13)	Minimum temperature of the coldest month (Bio6)	Human influence index (HII)	Annual precipitation (Bio12)	Precipitation of the driest month (Bio14)	Global tree cover (Tree)	
GLM	0.840	0.488	0.614	yes	60	7	10	9	12	1	1	0	
GAM	0.841	0.503	0.616	yes	56	16	7	7	8	4	1	1	
ANN	0.847	0.530	0.650	yes	22	31	8	25	5	4	3	2	
GBM	0.839	0.512	0.624	yes	42	29	11	4	2	8	3	1	
MARS	0.837	0.488	0.614	yes	35	34	16	1	1	11	2	0	
RF	0.697	0.478	0.609	no	20	14	13	17	9	10	10	8	
Maxent	0.835	0.500	0.613	no	39	34	7	4	7	6	2	2	
Ensemble	0.846	0.522	0.628		43	24	10	9	6	5	2	1	

Figure 3. Partial response plots from the fitted models. Thin coloured lines show responses from the algorithms in the ensemble, while the thick black line is their ensemble. In each plot, other model variables are held at their median value in the training data. Some of the divergence among algorithms is because of their different treatment of interactions among variables.

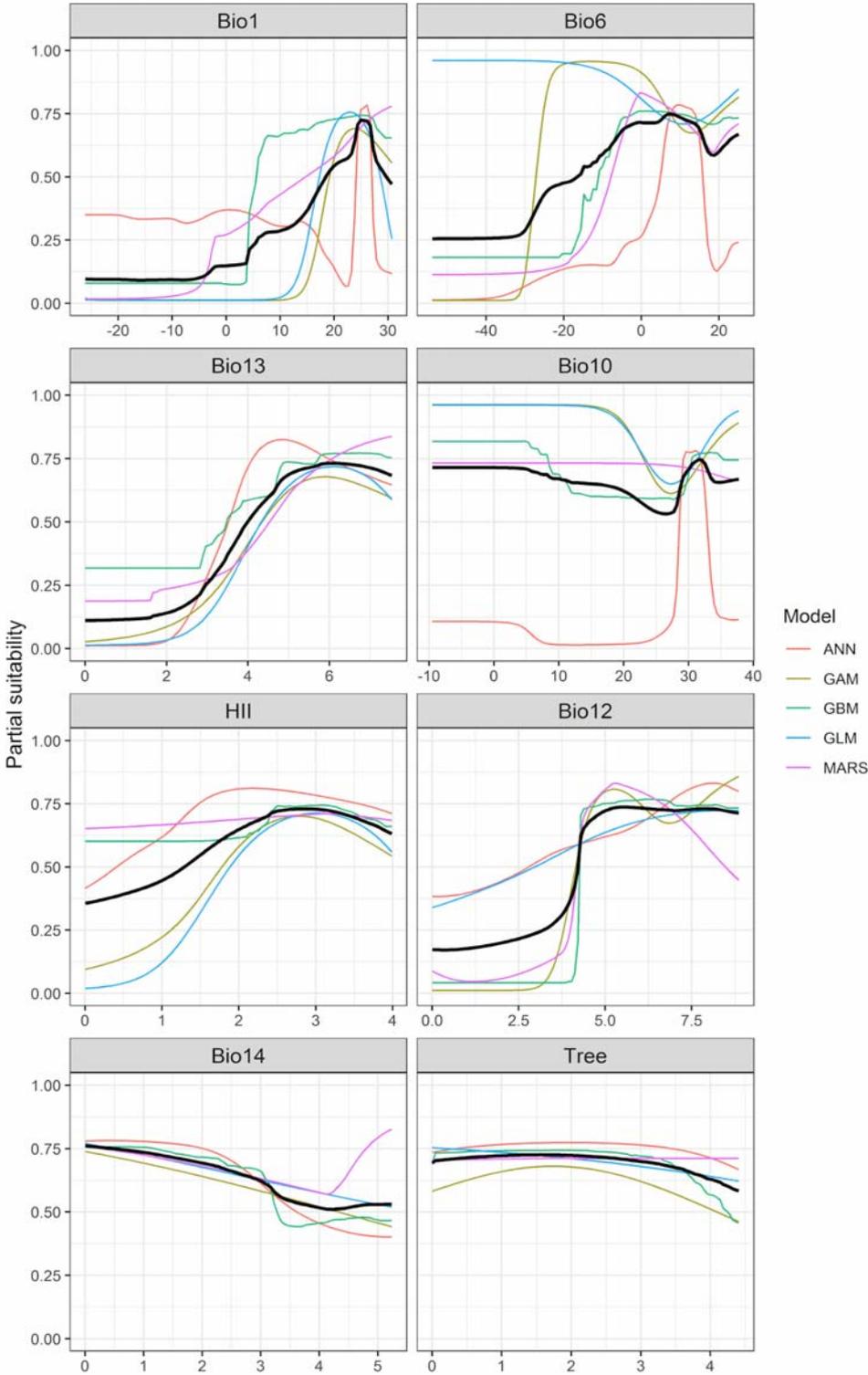
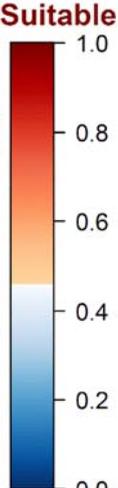
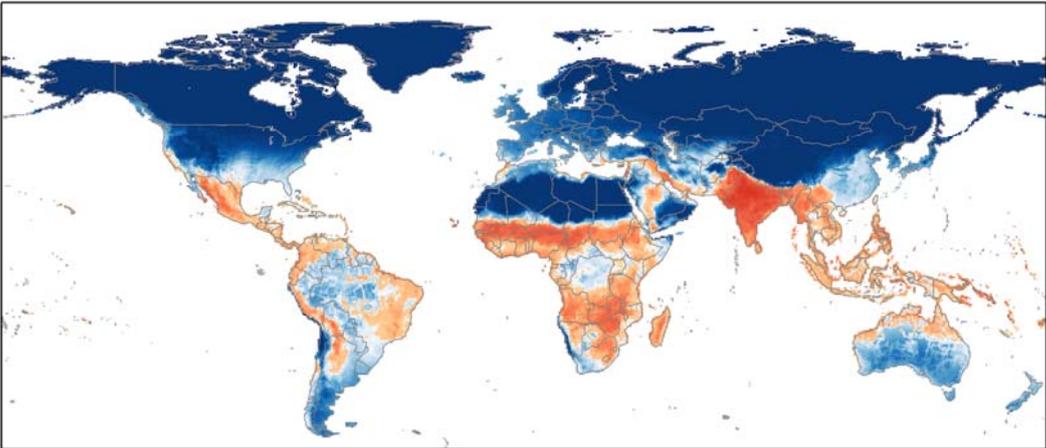


Figure 4. (a) Projected global suitability for *Pycnonotus cafer* establishment in the current climate. For visualisation, the projection has been aggregated to a 0.5 x 0.5 degree resolution, by taking the maximum suitability of constituent higher resolution grid cells. Values > 0.46 may be suitable for the species. Grey areas have missing data in a predictor layer. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

(a) Projected suitability



(b) Standard deviation in projected suitability

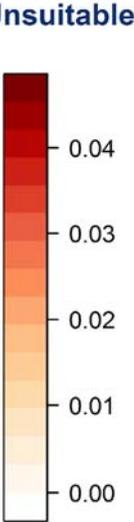
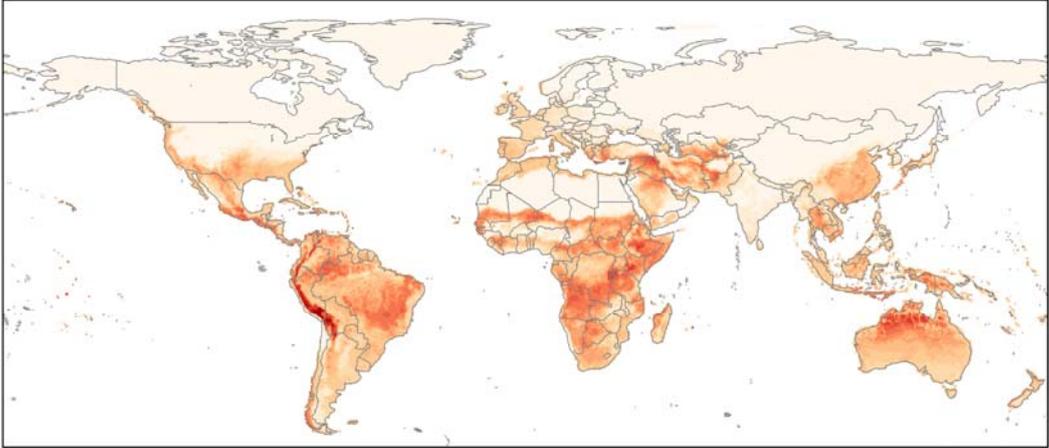


Figure 5. (a) Projected current suitability for *Pycnonotus cafer* establishment in Europe and the Mediterranean region. Grey areas have missing data in a predictor layer. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

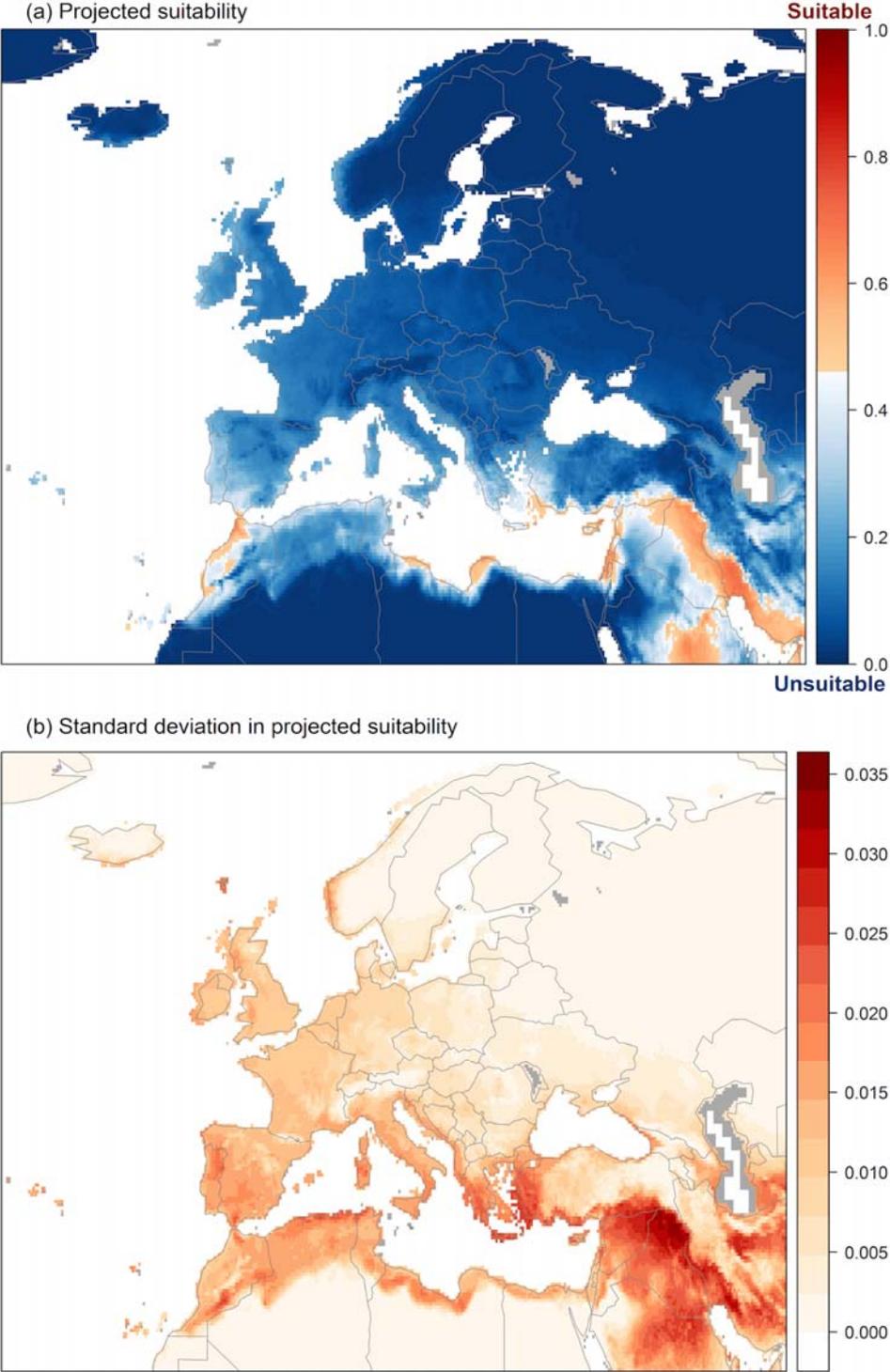


Figure 6. The most strongly limiting factors for *Pycnonotus cafer* establishment estimated by the model in Europe and the Mediterranean region in current climatic conditions.

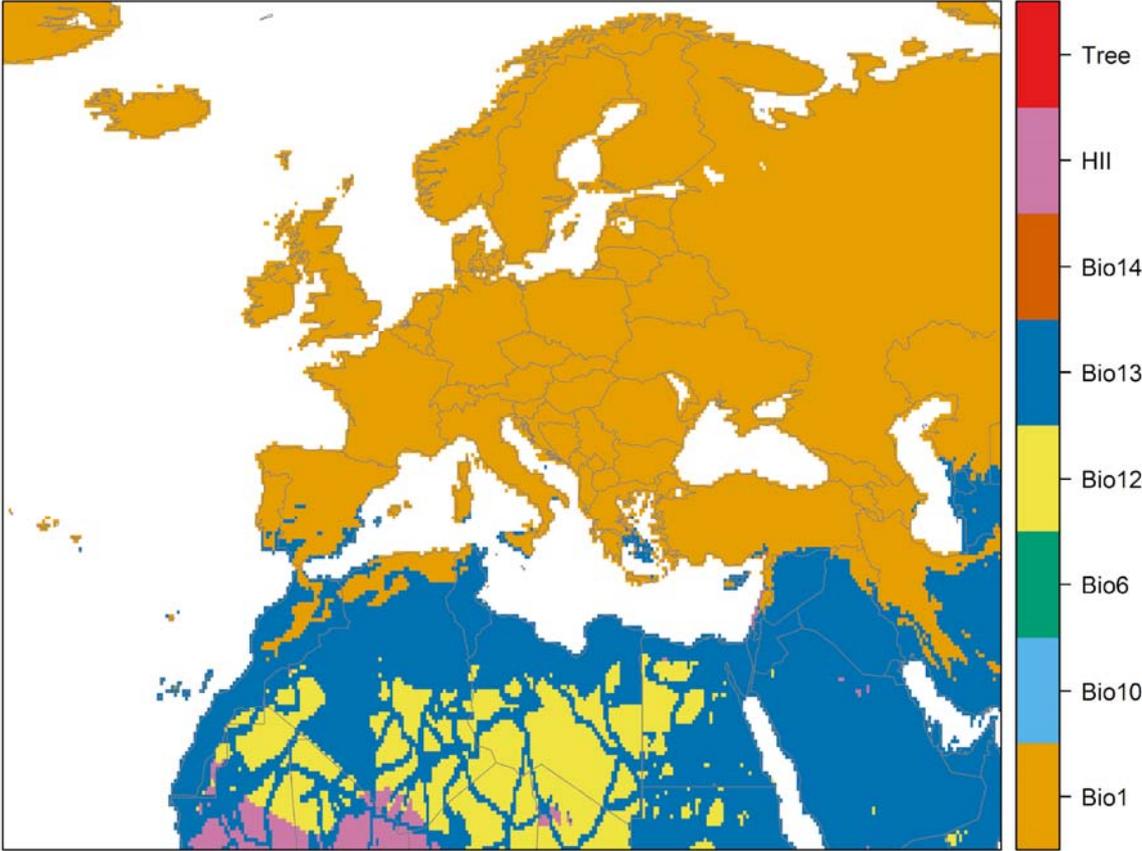


Figure 7. (a) Projected suitability for *Pycnonotus cafer* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP2.6, equivalent to Figure 5. Grey areas have missing data in a predictor layer. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

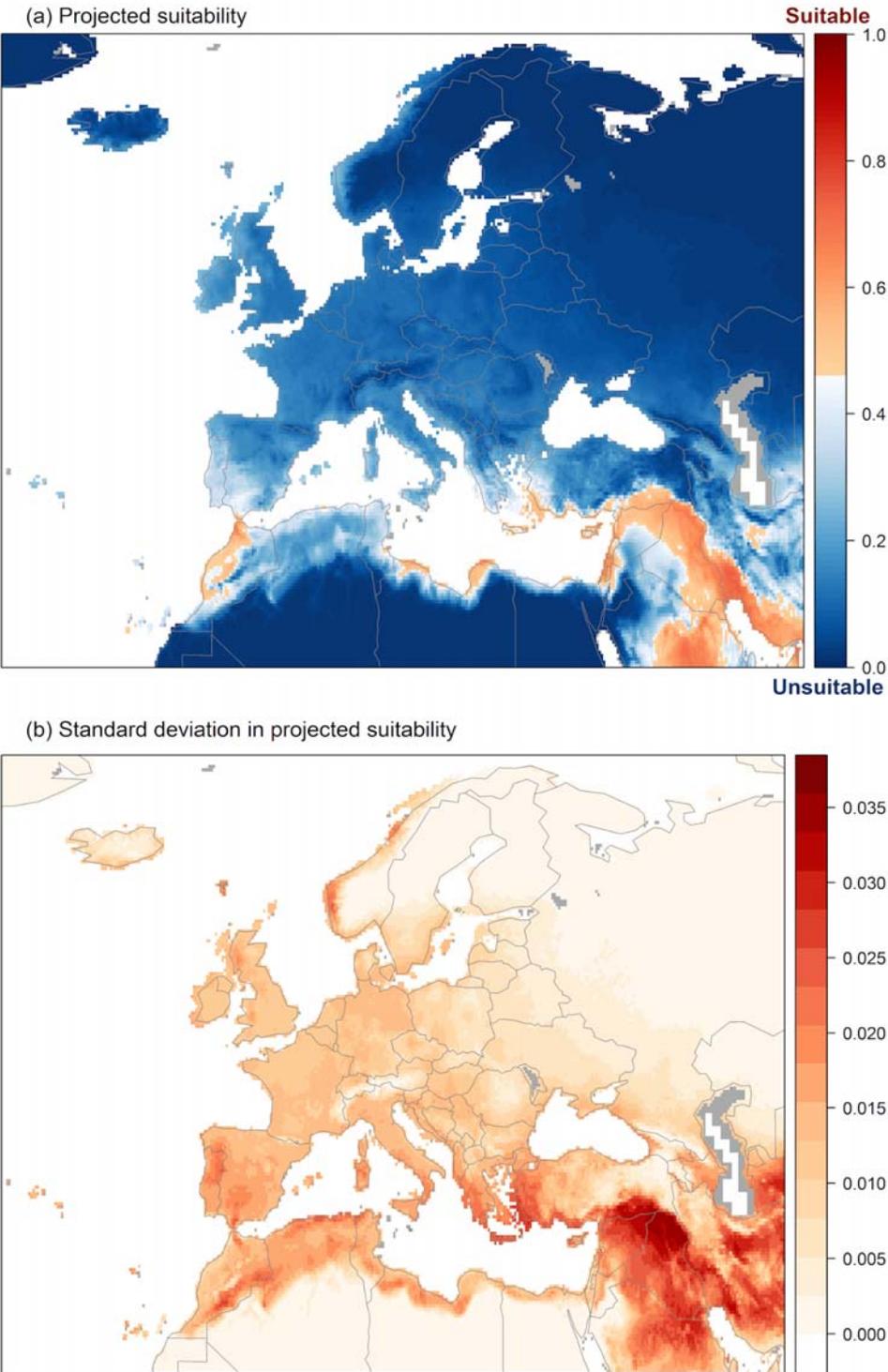


Figure 8. (a) Projected suitability for *Pycnonotus cafer* establishment in Europe and the Mediterranean region in the 2070s under climate change scenario RCP4.5, equivalent to Figure 5. Grey areas have missing data in a predictor layer. (b) Uncertainty in the ensemble projections, expressed as the among-algorithm standard deviation in predicted suitability, averaged across the 10 datasets.

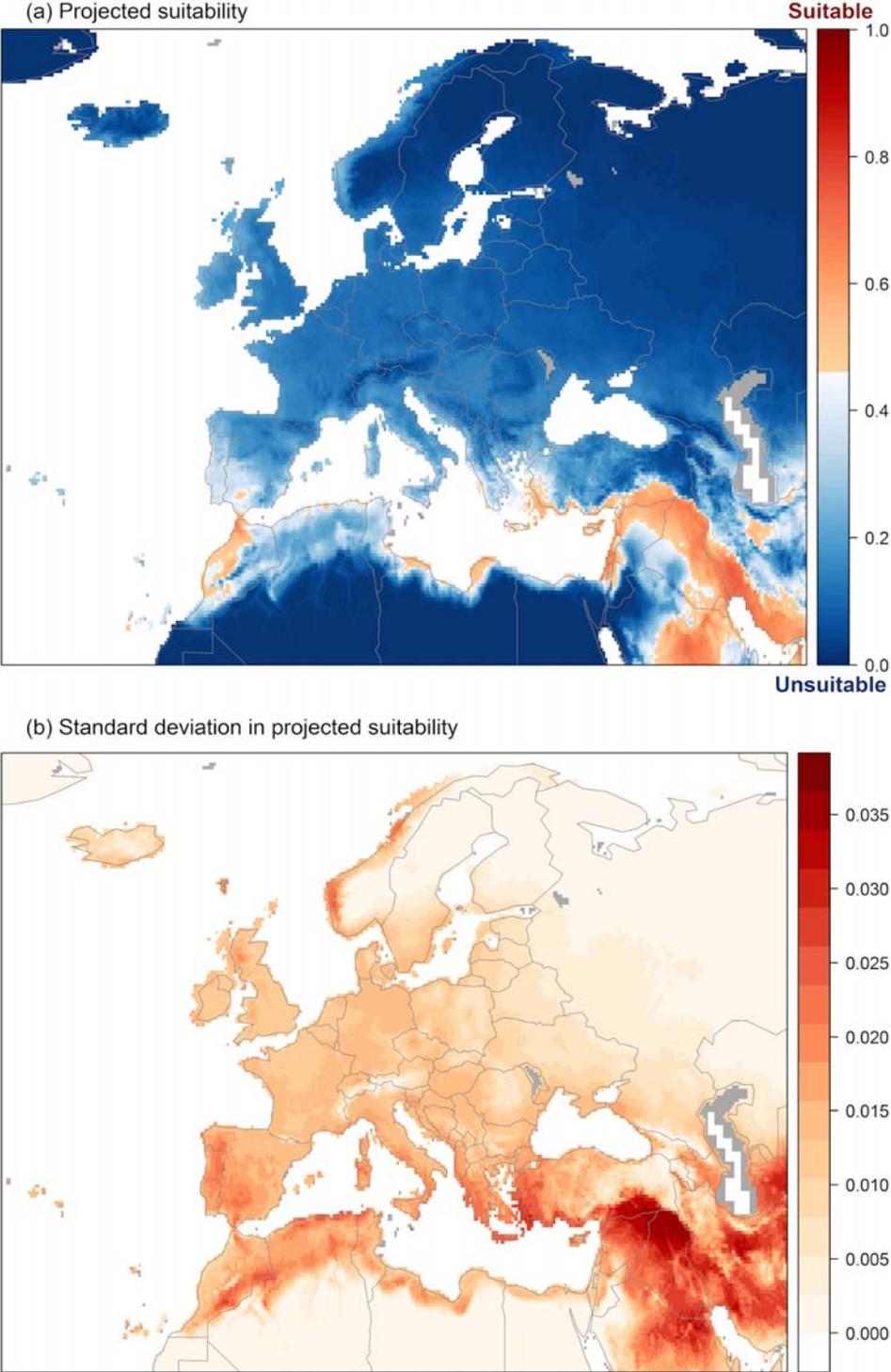


Figure 9. Variation in projected suitability for *Pycnonotus cafer* establishment among Biogeographical regions of Europe (<https://www.eea.europa.eu/data-and-maps/data/biogeographical-regions-europe-3>). The bar plots show the proportion of grid cells in each region classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. The location of each region is also shown. The Arctic and Macaronesian biogeographical regions are not part of the study area, but are included for completeness.

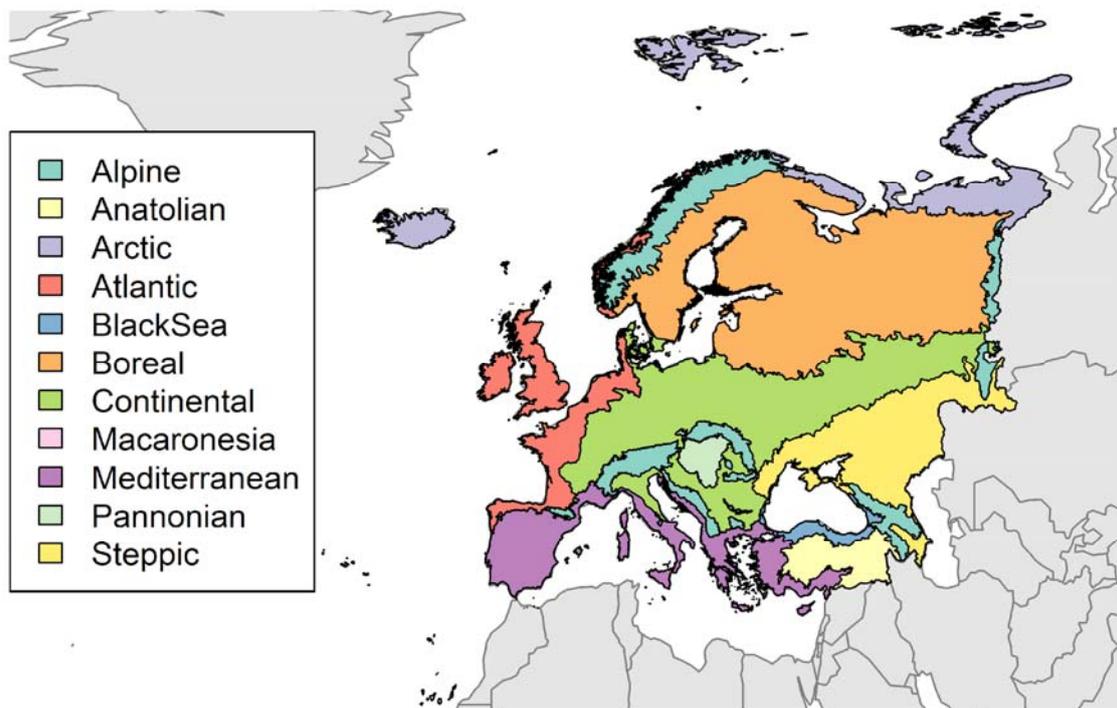
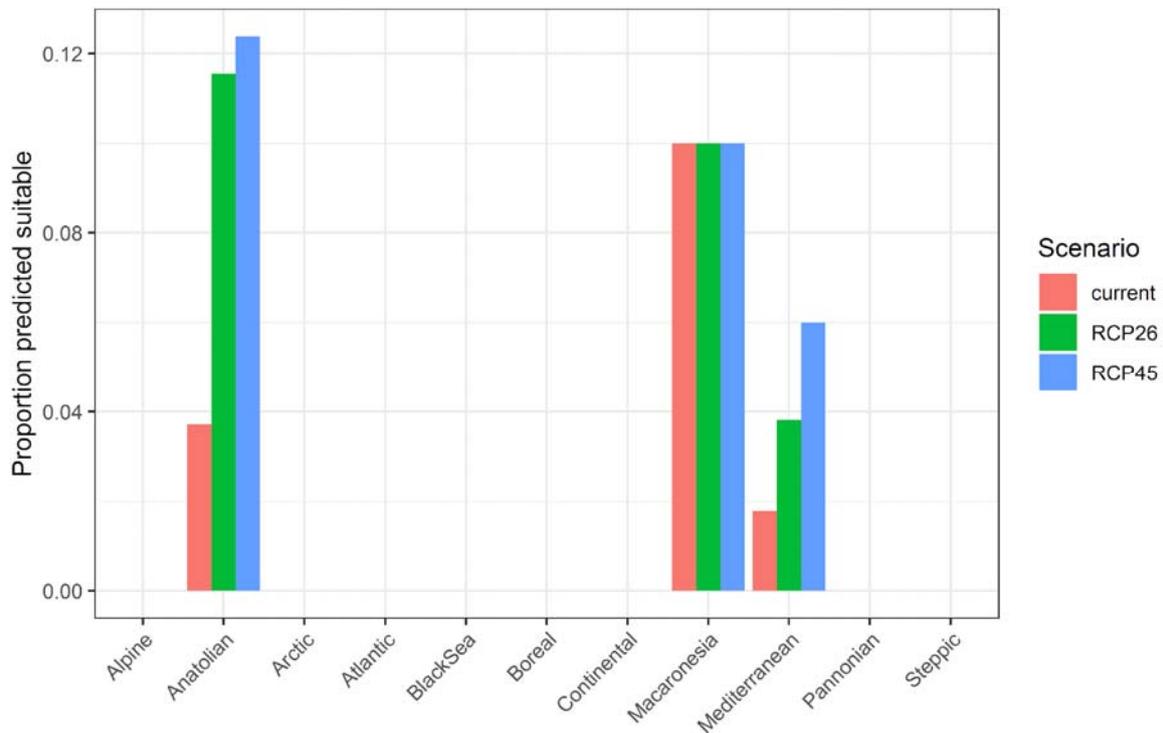
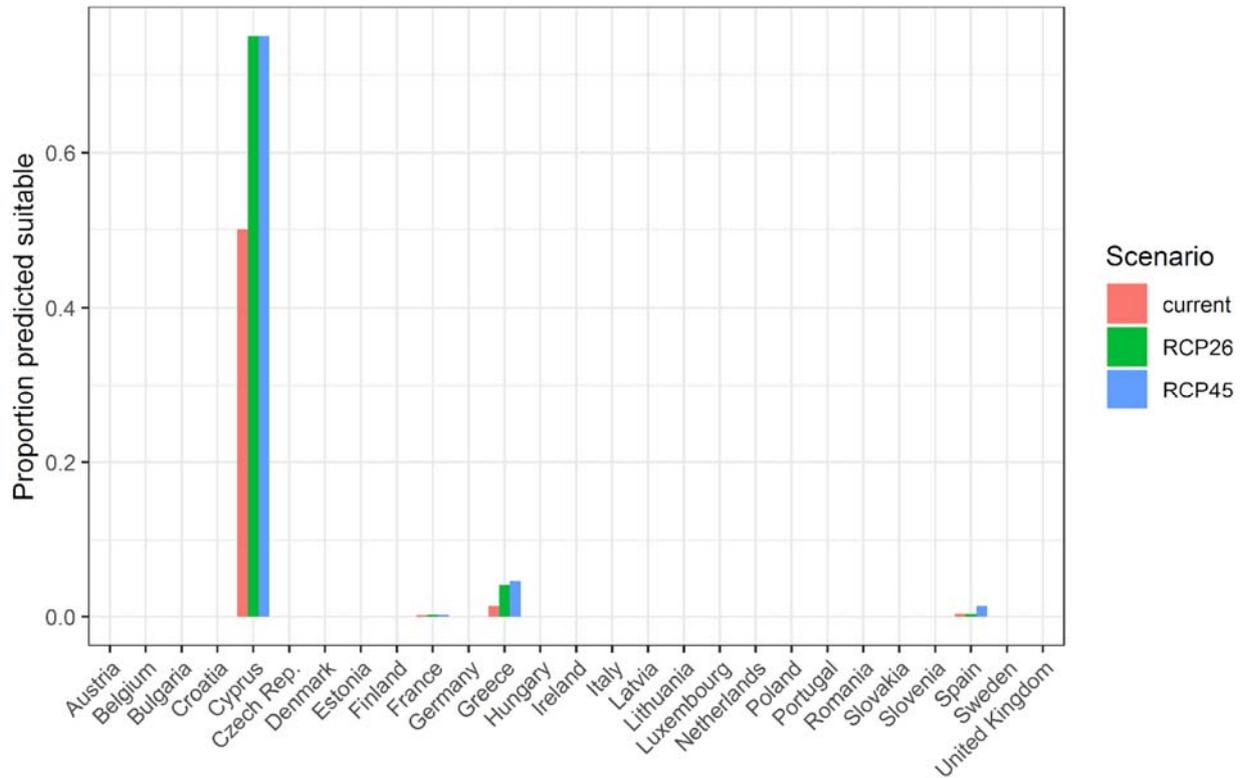


Figure 10. Variation in projected suitability for *Pycnonotus cafer* establishment among European Union countries and the United Kingdom. The bar plots show the proportion of grid cells in each country classified as suitable in the current climate and projected climate for the 2070s under two RCP emissions scenarios. Malta has been excluded because the Human Influence Index dataset lacks coverage for Malta.



Caveats to the modelling

To remove spatial recording biases, the selection of the background sample was weighted by the density of Aves records on the Global Biodiversity Information Facility (GBIF). While this is preferable to not accounting for recording bias at all, it may not provide the perfect measure of recording bias.

There was substantial variation among modelling algorithms in the partial response plots (Figure 3). In part this will reflect their different treatment of interactions among variables. Since partial plots are made with other variables held at their median, there may be values of a particular variable at which this does not provide a realistic combination of variables to predict from.

Other variables potentially affecting the distribution of the species, such as land cover were not included in the model.

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**Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention"
Contract No 07.0202/2019/812602/ETU/ENV.D.2¹**

Name of organism: *Vespa mandarinia* Smith, 1852

Author(s) of the assessment: Marc Kenis and Lukas Seehausen, CABI, Delémont, Switzerland

Risk Assessment Area: The risk assessment area is the territory of the European Union²⁷ and the United Kingdom, excluding the EU-outermost regions.

Peer review 1: Alan Stewart, University of Sussex, Brighton, UK

Peer review 2: Olaf Booy, GB Non-Native Species Secretariat

Date of completion: 15/10/2020

Date of revision: 15/10/2021

¹ This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA). A number of amendments have been introduced to ensure compliance with Regulation (EU) 1143/2014 on IAS and relevant legislation, including the Delegated Regulation (EU) 2018/968 of 30 April 2018, supplementing Regulation (EU) No 1143/2014 of the European Parliament and of the Council with regard to risk assessments in relation to invasive alien species (see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>).

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SECTION A – Organism Information and Screening

A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?

including the following elements:

- the taxonomic family, order and class to which the species belongs;
- the scientific name and author of the species, as well as a list of the most common synonym names;
- names used in commerce (if any)
- a list of the most common subspecies, lower taxa, varieties, breeds or hybrids

As a general rule, one risk assessment should be developed for a single species. However, there may be cases where it may be justified to develop one risk assessment covering more than one species (e.g. species belonging to the same genus with comparable or identical features and impact). It shall be clearly stated if the risk assessment covers more than one species, or if it excludes or only includes certain subspecies, lower taxa, hybrids, varieties or breeds (and if so, which subspecies, lower taxa, hybrids, varieties or breeds). Any such choice must be properly justified.

Response:

Scientific name (Class: Order: Family): *Vespa mandarinia* Smith, 1852 (Insecta: Hymenoptera: Vespidae)

Most common synonyms:

Vespa japonica Rad., 1857

Vespa latilineata Cameron, 1903

Vespa magnifica Smith, 1852

Vespa sonani Matsumura, 1930

Common English name: Asian giant hornet

Other common names: calabrone gigante asiatico (IT), Asiatische Riesenhornisse (GE), frelon géant (FR)

Subspecies: Various sub-species and colour forms with different geographic ranges and varying in colours have been described in the literature. Archer (1995) mentions three sub-species *V. mandarinia mandarinia* (in China, Japan, Russia and Korea); *V. mandarinia magnifica* (Northern India, Nepal, Bhutan, Burma, Laos, Peninsular Malaysia, western China); *V. mandarinia nobilis* (Taiwan). *Vespa mandarinia japonica* is sometimes cited as sub-species but is rather a colour form (Archer 1995). All sub-species will be treated together in this risk assessment. No hybrids with other species are known.

A2. Provide information on the existence of other species that look very similar [that may be detected in the risk assessment area, either in the environment, in confinement or associated with a pathway of introduction]

Include both native and non-native species that could be confused with the species being assessed, including the following elements:

- other alien species with similar invasive characteristics, to be avoided as substitute species (in this case preparing a risk assessment for more than one species together may be considered);
- other alien species without similar invasive characteristics, potential substitute species;
- native species, potential misidentification and mis-targeting

Response:

There are 22 *Vespa* spp. worldwide, all occurring in some parts of Asia (Archer 2008; Smith Pardo et al. 2020). Four of them also occur in Europe: *Vespa crabro* L. and *V. orientalis* L. are native to Europe and *V. velutina* Lepeletier is an invasive alien species from East Asia. The fourth species, *V. bicolor* F., also non-native to Europe, is present in a small area in southern Spain since at least 2013 but does not seem to spread (Castro et al. 2019). All non-native species could potentially be invasive in Europe, but this risk assessment concerns *V. mandarinia*.

Vespa mandarinia can easily be separated from the four species occurring in Europe using morphological characters (Archer 1995, 2012; Smith Pardo et al. 2020). The most obvious character is the size of the wasps, the smallest workers of *V. mandarinia* measuring at least 35 mm (queens can exceed 50 mm) whereas only the largest queens of *V. crabro* reach 35 mm. It is most similar to *Vespa soror* du Buysson (a south-East Asian species) but the two species can be distinguished by the colour of their abdomen. In *V. soror*, the third to the sixth gastral terga in the female and to the seventh gastral terga in the male are black, whereas, in *V. mandarinia*, these terga are at least partly orange (Archer 1995).

A3. Does a relevant earlier risk assessment exist? Give details of any previous risk assessment, including the final scores and its validity in relation to the risk assessment area.

Response:

Two partial risk assessments have been produced for North America. Norderus et al. (2021) conducted a risk assessment of the establishment of *V. mandarinia* specifically on the Pacific Northwest and Zhu et al. (2020) assessed its ecological niche and invasion potential. None of the two, however, provide detailed assessments of the magnitude of impact. USDA (2020) has produced “New Pest Response Guidelines” following the recent observations of *V. mandarinia* in North. In Europe, risk assessments have been produced for *V. velutina* after this species became established (e.g. Marris et al. 2011) and, thus, although helpful, they are not fully relevant for *V. mandarinia* in Europe, where it is not yet present.

A4. Where is the organism native?

including the following elements:

- an indication of the continent or part of a continent, climatic zone and habitat where the species is naturally occurring

- if applicable, indicate whether the species could naturally spread into the risk assessment area

Response:

Vespa mandarinia is native to Asia, occurring in at least 9 Köppen climates from the continental, temperate and tropical groups. It has been recorded in the following countries: China, Japan, Russia, South Korea, India, Buthan, Nepal, Myanmar, Laos, Malaysia, Thailand, Vietnam (Archer 1995; GBIF 2020; Smith Pardo et al. 2020; USDA 2020). Smith Pardo et al. (2020) also includes Sri Lanka but Kumar and Srinivasan (2010) consider this as an error. The distribution records in Asia are indicated in Figure 1.

In Japan, *V. mandarinia* is associated with hilly country (850-1900m), nesting in the ground at a depth of 6-60cm. Nests are started in small mammal burrows and decayed root spaces. These cavities can be enlarged by the workers removing soil particles. A few nests have been found in hollow trees and a mud wall 1-2m above the ground. (Archer 2008). As for all Vespidae, nest building requires a sufficient amount of humidity and water availability (Matsuura and Yamane 1990)

Vespa mandarinia is a good flier but considering the distances involved, it cannot naturally spread on its own from its native area to the risk assessment area.

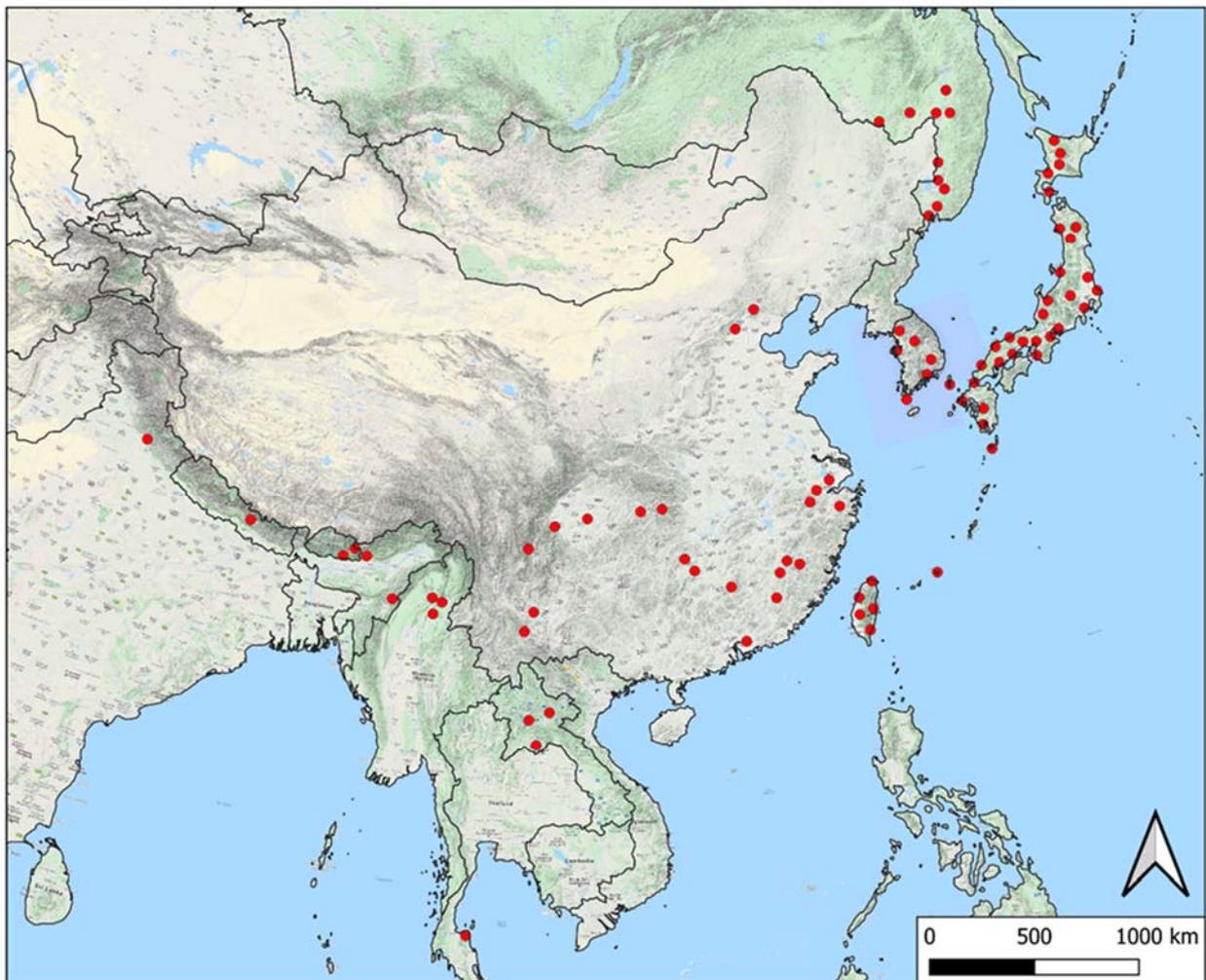


Figure 1. Distribution of *V. mandarinia* in Asia (background map from Google). Only the sites where precise occurrences are known are indicated in the map.

A5. What is the global non-native distribution of the organism outside the risk assessment area?

Response:

The first record outside its native range was in August and September 2019, when workers and a nest were found in Nanaimo, Vancouver Island, British Columbia, Canada. In 2019, workers were also observed on the mainland, in White Rock, south of Vancouver and close-by at two sites (Blaine and Bellingham) in Washington State (USA), suggesting that nests were also present on the mainland (USDA 2020). In May 2020, a live individual queen was captured at Langley, British Columbia and, by September 2020, several queens, workers and a male were also found in Custer, Birch Bay and Bellingham, Washington (Norderud et al. 2021; WSDA 2021). In October 2020, a nest was found and destroyed in Blaine and a new dead male was discovered in June 2021 in near Marysville (all Washington, WSDA 2021). Genetic analyses showed that the nest found on Vancouver Island and the individual found in Washington State belong to different lineages (Wilson et al. 2020).

A6. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species been recorded and where is it established? The information needs to be given separately for recorded (including casual or transient occurrences) and established occurrences. “Established” means the process of an alien species successfully producing viable offspring with the likelihood of continued survival².

A6a. Recorded: List regions

A6b. Established: List regions

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

- Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea.

Comment on the sources of information on which the response is based and discuss any uncertainty in the response.

For delimitation of EU biogeographical regions please refer to <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2> (see also Annex VI).

² Convention on Biological Diversity, Decision VI/23

For delimitation of EU marine regions and subregions consider the Marine Strategy Framework Directive areas; please refer to <https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions/technical-document/pdf> (see also Annex VI).

Response (6a):

No record yet in the risk assessment area. In GBIF (2020), a record from Germany could not be traced to the original source and seems erroneous.

Response (6b):

No establishment yet in the risk assessment area.

A7. In which biogeographic region(s) or marine subregion(s) in the risk assessment area could the species establish in the future under current climate and under foreseeable climate change? The information needs to be given separately for current climate and under foreseeable climate change conditions.

A7a. Current climate: List regions

A7b. Future climate: List regions

With regard to EU biogeographic and marine (sub)regions, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (7a):

Based on present occurrences and the limited bioecological data available about the hornet, a CLIMEX model has been built, which integrates the weekly responses of an organism to climate using a series of annual indices.

The model (Seehansen and Kenis, unpublished) is described in Annex VIII. In CLIMEX, the potential for population growth is modeled combining growth based on temperature and soil moisture during favorable conditions using a Growth Index (GI), and stress indices (cold, wet, hot, dry) to determine the effect of abiotic stress on survival in unfavorable conditions. The growth and stress indices are calculated weekly and then combined into an overall annual index of climatic suitability, the

Ecoclimatic Index (EI). The EI ranges from 0 (no persistence possible) to 100 (optimal conditions), but in temperate climates, the maximum EI value is rather close to 50 and values of >20 are known to be sufficient to support substantial population densities.

The model shows that in its native range, *V. mandarinia* is mainly predicted to be present in humid subtropical and humid oceanic climates (Figure 2). The northern distribution of *V. mandarinia* is mainly limited by cold stress during the harsh winters of the subarctic regions and dry stress in the highlands and Mongolian desert. The southern and western distribution limits are mainly defined by heat stress.

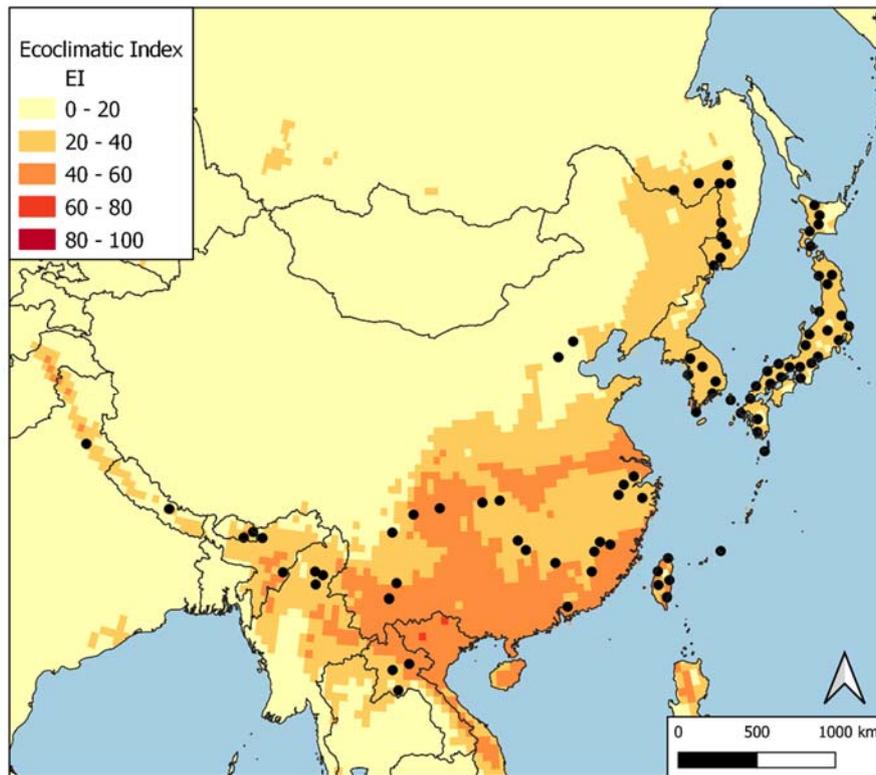


Figure 2: Observed (black points) and potential distribution of *V. mandarinia* in its native Asia as predicted by the Ecoclimatic Index (EI) from the CLIMEX model. Locations with EI>20 can be considered as climatically suitable for establishment and growth of *V. mandarinia*.

Under current climate, the most suitable biogeographic regions of Europe are predicted to be the Continental and Atlantic regions and the most humid parts of the Mediterranean, Black Sea and Pannonian regions (Figure 3). The Arctic, Boreal and Alpine regions will probably be too cold for establishment. The hornet is predicted to experience cold stress in the Alps, and in the subarctic region low mean temperatures, as well as a relatively short and dry growing season restrict population growth. The Steppic regions appear too dry to be suitable for *V. mandarinia*, defining the predicted southern distribution limits through dry stress. However, irrigated areas or humid microclimates may provide favourable areas for the hornet in the south.

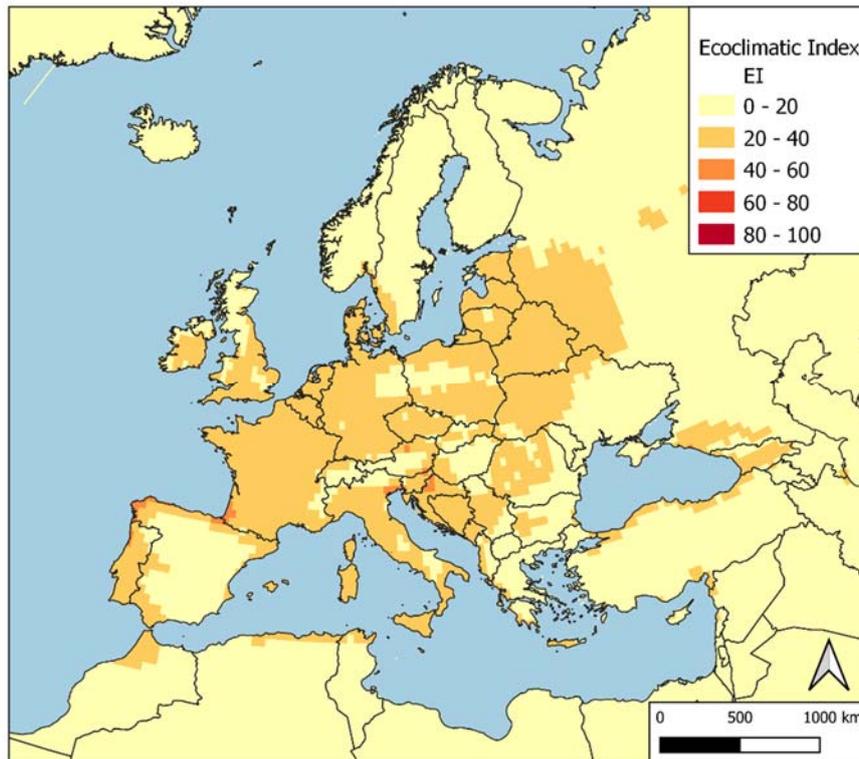


Figure 3: Potential distribution of *V. mandarinia* in Europe and northern Africa as predicted by the Ecoclimatic Index (EI) from the CLIMEX model. Locations with $EI > 20$ can be considered as climatically suitable for establishment and growth of *V. mandarinia*.

Response (7b):

A CLIMEX Ecoclimatic Index map (Figure 4) shows the likely occurrence of *V. mandarinia* in Europe in 2080, following the A1B climate change scenario, which is comparable to the RCP 4.5 scenario (RCP scenarios are not available in CLIMEX). In the future, increases in average temperatures are likely to favour establishment in the warmest areas of the Boreal and Alpine regions. In contrast, increased summer drought in Southern and Eastern Europe will make some parts of the Mediterranean and Continental regions and most of the Pannonian, Black Sea and Steppic regions unsuitable without irrigation. Especially the predicted loss of suitable area in north-eastern Europe are due to dryer summers that may severely restrict population growth of the hornet.

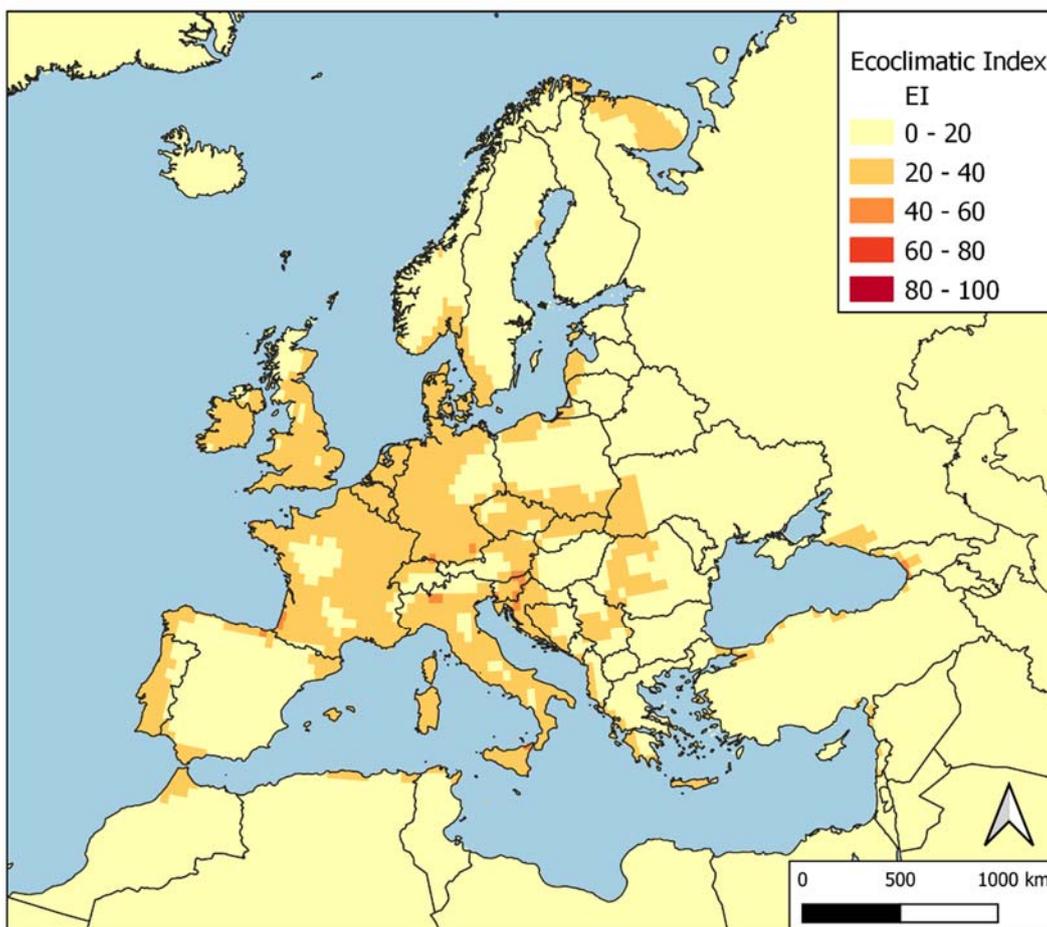


Figure 4: Potential distribution of *V. mandarinia* in Europe and northern Africa in 2080, as predicted by the Ecoclimatic Index (EI) from the CLIMEX model using the A1B scenario. Locations with $EI > 20$ can be considered as climatically suitable for establishment and growth of *V. mandarinia*.

A8. In which EU Member States has the species been recorded and in which EU Member States has it established? List them with an indication of the timeline of observations. The information needs be given separately for recorded and established occurrences.

A8a. Recorded: List Member States

A8b. Established: List Member States

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, United Kingdom

The description of the invasion history of the species shall include information on countries invaded and an indication of the timeline of the first observations, establishment and spread.

Response (8a):

No record yet in the risk assessment area. In GBIF (2020), a record from Germany seems erroneous

Response (8b):

No establishment yet in the risk assessment area

A9. In which EU Member States could the species establish in the future under current climate and under foreseeable climate change? The information needs be given separately for current climate and under foreseeable climate change conditions.

A9a. Current climate: List Member States

A9b. Future climate: List Member States

With regard to EU Member States, see above.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the risk assessment (e.g. increase in average winter temperature, increase in drought periods)

The assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

Response (9a):

Almost all member states are at least partly suitable for establishment. Establishment in Finland and Sweden will probably be limited by cold average temperatures whereas, in Cyprus, drought may hamper establishment (Figure 3).

Response (9b):

In the future, under the A1B scenario (see Q A7b) climate may become suitable at higher altitudes and latitudes, e.g. in Sweden and Finland because of increased average temperatures in particular during brood development, whereas Eastern European and Mediterranean countries may become less suitable because of increased summer droughts (Figure 4).

A10. Is the organism known to be invasive (i.e. to threaten or adversely impact upon biodiversity and related ecosystem services) anywhere outside the risk assessment area?

Response:

No information is available on the species impact in invaded areas.

Since August 2019, a nest and workers have been found at three sites in British Columbia, Canada and a nest and various specimens have been found at several locations in Washington State, USA (USDA 2020; WSDA 2021). However, it is not known whether these populations will become invasive.

Many Vespinae, including *Vespa* spp., are invasive worldwide, threatening biodiversity and various ecosystem services. See Beggs et al. (2011) for a review on the ecological effects of invasive alien Vespidae. The mechanisms of ecological impacts of invasive Vespidae include: direct predation on native species, in particular invertebrates; competition for food (invertebrates, honeydew, sap runs etc.) with other insects but also birds; competition for space, in particular other vespids; predation on honeybees and wild bees affecting pollination services, etc.

A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Freshwater / terrestrial biogeographic regions:

- Alpine, Atlantic, Black Sea, Boreal, Continental, Mediterranean, Pannonian, Steppic

Marine regions:

- Baltic Sea, North-east Atlantic Ocean, Mediterranean Sea, Black Sea

Marine subregions:

Greater North Sea, incl. the Kattegat and the English Channel, Celtic Seas, Bay of Biscay and the Iberian Coast, Western Mediterranean Sea, Adriatic Sea, Ionian Sea, Central Mediterranean Sea, Aegean-Levantine Sea

Response:

The species is not yet present in the risk assessment area.

A12. In which EU Member States has the species shown signs of invasiveness? Indicate the area endangered by the organism as detailed as possible.

Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, United Kingdom

Response:

The species is not yet present in the risk assessment area.

A13. Describe any known socio-economic benefits of the organism.

including the following elements:

- Description of known uses for the species, including a list and description of known uses in the risk assessment area and third countries, if relevant.
- Description of social and economic benefits deriving from those uses, including a description of the environmental, social and economic relevance of each of those uses and an indication of associated beneficiaries, quantitatively and/or qualitatively depending on what information is available.

If the information available is not sufficient to provide a description of those benefits for the entire risk assessment area, qualitative data or different case studies from across the risk assessment area or third countries shall be used, if available.

Response:

In Asia, larvae and pupae of *V. mandarinia* are considered a delicacy. They are traded and consumed in various ways. They are also considered to have medicinal properties (Nonaka 2008; Ying et al. 2008; Ho 2019). To our knowledge, live *V. mandarinia* are not traded in Europe but at least one entire nest containing live larvae and pupae was intercepted in USA (Smith Pardo et al. 2020) suggesting that illegal trade of nests does occur outside Asia.

SECTION B – Detailed assessment

Important instructions:

- In the case of lack of information the assessors are requested to use a standardized answer: “No information has been found.”
- With regard to the scoring of the likelihood of events or the magnitude of impacts see Annexes I and II.
- With regard to the confidence levels, see Annex III.
- Highlight the selected response score and confidence level in **bold** but keep the other scores in normal text (so that the selected score is evident in the final document).

1 PROBABILITY OF INTRODUCTION AND ENTRY

Important instructions:

- **Introduction** is the movement of the species into the risk assessment area (it may be either in captive conditions and/or in the environment, depending on the relevant pathways).
- **Entry** is the release/escape/arrival in the environment, i.e. occurrence in the wild
- Introduction and entry may coincide for species entering through pathways such as “corridor” or “unaided”, but it also may differ. If different, please consider all relevant pathways, both for the introduction into the risk assessment area and the entry in the environment.
- The classification of pathways developed by the Convention of Biological Diversity (CBD) should be used (see Annex IV). For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document³ and the provided key to pathways⁴.
- For organisms which are already present (recorded or established) in the risk assessment area, the likelihood of introduction and entry should be scored as “very likely” by default.
- Repeated (independent) introductions and entries at separate locations in the risk assessment area should be considered here (see Qu. 1.7).

Qu. 1.1. List relevant pathways through which the organism could be introduced into the risk assessment area and/or enter into the environment. Where possible give details about the specific origins and end points of the pathways as well as a description of any associated commodities.

For each pathway answer questions 1.2 to 1.7 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 1.2a, 1.3a, etc. and then 1.2b, 1.3b etc. for the next pathway.

In this context a pathway is the route or mechanism of introduction and/or entry of the species.

³ <https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf>

⁴ <https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf>

The description of commodities with which the introduction of the species is generally associated shall include a list and description of commodities with an indication of associated risks (e.g. the volume of trade; the likelihood of a commodity being contaminated or acting as vector).

If there are no active pathways or potential future pathways this should be stated explicitly here, and there is no need to answer the questions 1.2-1.9.

The pathways considered in this risk assessment are:

- A. Transport - Contaminant (nursery material)
- B. Transport - Contaminant (transportation of habitat material) (other than nursery material)
- C. Transport - Stowaway (container/bulk)
- D. Escaped from confinement (live food and live bait)

Vespa mandarinia presently occurs too far from the risk assessment area to consider that the hornet can enter “unaided”. Thus, all pathways considered here will be human-assisted. There is very limited information on the potential pathways that can be used by *V. mandarinia* to enter new continents. Non-native Vespidae are not listed as quarantine pests in the EU and, therefore, records do not appear in the national and international lists of intercepted pests. Thus, there is hardly any information available from Europe except that the congeneric and invasive alien species *Vespa velutina* probably arrived in Europe in bonsai pottery from Asia (Arca et al. 2015). Smith Pardo et al. (2020) mentioned that, from 2010 to 2018, there have been close to 50 interceptions of *Vespa* spp. and *Vespula* spp. at US ports of entry and that little less than half of those interceptions were hornets, including *V. mandarinia*. They did not mention on which pathways and which commodities these interceptions were made. However, these data, and the recent introductions of *V. mandarinia* in North America (USDA 2020) along with various *Vespa* spp., *Vespula* spp. and *Polistes* spp. worldwide (Beggs et al. 2011) show that vespids, including *V. mandarinia* can be transported between continents. While queens, males and workers can potentially travel, only mated queens will be able to build a new colony. *Vespa mandarinia* queens overwinter singly, usually in cavities excavated in the soil, or occasionally in rotten wood or straw. Thus, the transport of mated queens from autumn to spring (before, during and after overwintering) is the most likely means of long-distance dispersal. Single queens may unintentionally be transported as contaminant of soil (e.g. soil for construction, potted plants) or rotten wood or straw in which they overwinter, or as stowaway in any kind of good in containers. Theoretically, a mated queen could also travel freely, outside containers, in ships or planes but these pathways were considered too unlikely to be mentioned here.

The introduction of a single mated queen is potentially sufficient to establish a colony. The introduction of *V. velutina* in France was due to a single queen that had mated multiple times, or a very small number of overwintering queens that had mated singly (Arca et al. 2015).

One of the interceptions of significance in USA mentioned by Smith Pardo et al. (2020) was an entire nest of *V. mandarinia* containing live brood and pupae that was sent via express courier from Asia, probably for consumption or as traditional medicine (USDA 2020). Thus, intentional importations of nests are considered here as well.

A. Transport - Contaminant (nursery material)

Qu. 1.2a. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
-----------------	-------------------------------------	-------------------	------------------------------

Response:

The introduction and/or entry along this pathway is unintentional. Queens overwinter mainly in a hole that they have excavated in the soil (Matsuura and Sakagami 1973; Archer 1995). Thus, any kind of soil or similar substrate that is transported with nursery material can potentially transport an overwintering queen.

Qu. 1.3a. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
-----------------	--	-------------------	------------------------------

Response:

There is no data on *V. mandarinia* or other *Vespa* spp. travelling in potted plants. The import of soil and related substrates to Europe has been much reduced in recent years. Nevertheless, a large amount of plants still arrive in Europe with soil or in pots (with substrates) from East Asia every year and, although the soil/substrate is supposed to be sterile, it is possible that hornets can enter the soil/substrates just before or during transport.

Qu. 1.4a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
-----------------	--	-------------------	------------------------------

Response:

Queens are very strong insects. They do not feed nor reproduce in their overwintering site (Matsuura and Sakagami 1973; Archer 1995) and can probably survive long ship journeys.

Qu. 1.5a. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

In international horticultural trade, plant material is supposed to be free of pests and soil/substrate should be sterile, but hornets can enter the soil/substrates after treatments, just before or during transport. Only a small portion of the plant material imported to the EU is inspected and the imported pots are quickly dispersed into the EU (Eschen et al. 2015).

Qu. 1.6a. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Although a *V. mandarinia* queen is rather big (4-5 cm), the cavity in which it overwinters is usually plugged (Archer 1995) and it can easily remain undetected during intercontinental transport and dispatching to the final destinations.

Qu. 1.7a. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated	CONFIDENCE	low
	widespread		medium
	ubiquitous		high

Response:

Horticultural products can enter the risk assessment area at various ports of entry and are usually quickly distributed within the EU. Queens are good fliers and can build nests in various environments (Matsuura and Sakagami 1973; Archer 1995). Thus, they would have no difficulty in reaching suitable habitats for nesting.

Qu. 1.8a. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely	CONFIDENCE	low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response:

There is no data on this pathway and it is not clear how frequently queens could enter soil and other substrates accompanying traded potted plants and other plant material. Probably this is a rather infrequent event considering that large amounts of soil or other substrates are transported every year into the EU and no occurrence of *V. mandarinia* has been ever recorded in Europe. However, once a queen has entered the commodity to build its overwintering site, the probability of arriving safely and entering the risk assessment area is rather high, considering that queens are strong and long-lived animals, and that the cavity in which they overwinter is usually plugged.

B. Transport - Contaminant (transportation of habitat material) (other than nursery material)

Qu. 1.2b. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response:

The introduction and/or entry along this pathway is unintentional. Queens overwinter mainly in a hole that they have excavated in the soil, but also occasionally in rotting wood, piles of straw, etc. (Matsuura and Sakagami 1973; Archer 1995). Thus, this pathway covers these overwintering substrates other than soil in potted plants, which is covered in pathway A above.

Qu. 1.3b. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

There is no data on *V. mandarinia* or other *Vespa* spp. travelling in suitable habitats such as straw and rotting wood. While rotting wood is unlikely to be imported intentionally, various amounts of items made of straw arrive in Europe from East Asia every year and it is possible that these can host overwintering hornets. Items made of straw may include handicraft, horticulture and gardening material, packing material, etc.

Qu. 1.4b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Queens are very strong insects. They do not feed nor reproduce in their overwintering site (Matsuura and Sakagami 1973; Archer 1995) and can probably survive long ship journeys.

Qu. 1.5b. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

There is no data on existing management practices on imported straw items. These can be quickly dispatched to the whole risk assessment area.

Qu. 1.6b. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Although a *V. mandarinia* queen is rather big (4-5 cm), the cavity in which it overwinters is usually plugged (Archer 1995) and it can easily remain undetected during transport and dispatching to the final destinations.

Qu. 1.7b. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated	CONFIDENCE	low
	widespread		medium
	ubiquitous		high

Response:

Such commodities can enter the risk assessment area at various ports of entry and can be quickly distributed within the EU. Queens are good fliers and can build nests in various environments (Matsuura and Sakagami 1973; Archer 1995). Thus, they would have no difficulty in reaching suitable habitats for nesting.

Qu. 1.8b. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely	CONFIDENCE	low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response:

There is no data on this pathway and it is not clear how frequently queens could be transported in straw material, rotting wood and similar overwintering habitats. Probably this is an infrequent event. However, once a queen has entered the commodity to build its overwintering site, the probability of arriving safely and entering the risk assessment area is rather high, considering that queens are strong and long-lived animals, and that the cavity in which they overwinter is usually plugged.

C. Transport-Stowaway (container/bulk)

Qu. 1.2c. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response:

The introduction and/or entry along this pathway is unintentional. In their search for overwintering sites, or when emerging from overwintering sites between autumn and spring, queens can easily hide in various type of containers and can be shipped with any kind of good in containers with various transport means (ships and planes).

Qu. 1.3c. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

There is no data to define which goods and containers are the most likely to carry queens. However, the number and variety of commodities and containers that can potentially transport queens is unlimited and, thus, we believe that queens are more likely to be transported as stowaways in containers than as contaminants of soil. At arrival, queens can easily escape and build a nest in the vicinity of the port of entry, or be transported with the container into any region of the risk assessment area.

Qu. 1.4c. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Queens are very strong insects. They do not feed nor reproduce in their overwintering site (Matsuura and Sakagami 1973; Archer 1995). Thus, they can probably survive long journeys, especially if they find an overwintering site in the container. Once the container is open and the commodity has been delivered, the queen can escape and easily find a suitable habitat for nesting since nests are commonly found in various habitats from forests to urban green spaces (Matsuura and Sakagami 1973; USDA 2020).

Qu. 1.5c. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

This will depend on the management practices used in the transport conditions but none will target Vespidae and it is unlikely that any management practice will affect the transported queen along the pathway and within the risk assessment area after transport and dispatching to the final destination.

Qu. 1.6c. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Queens are very big (4-5 cm) and may be quite obvious when they simply hitchhike in a container. However, overwintering queens are usually hidden and are easily missed when transported.

Qu. 1.7c. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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Response:

Containers can enter the risk assessment area at various ports of entry and can also be quickly sent anywhere within the EU. Queens are good fliers and can build nests in various environments, from forests to urban areas (Matsuura and Sakagami 1973; Archer 1995; USDA 2020). Thus, they would have no difficulty in reaching suitable habitats for nesting.

Qu. 1.8c. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

There is no data on this pathway and it is not clear how frequently queens could be transported as stowaway between continents. However, considering that the number and variety of commodities and containers that can potentially transport queens is unlimited, queens are probably more likely to be transported as stowaway than as contaminant of soil or straw.

D. Escaped from confinement (live food and live baits)

Qu. 1.2d. Is introduction and/or entry along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response:

This pathway has been added because among the ca. 50 reported interceptions of Vespidae in ports of entry in USA, one was an entire nest of *V. mandarinia* with live larvae and pupae sent by express courier from Asia (Smith-Pardo et al. 2020). It is not known precisely why this nest was sent to USA, but it is likely that it was for human consumption. In Asia, larvae and pupae of *V. mandarinia* are considered a delicacy, and consumed in various ways. They are also considered to have medicinal properties (Nonaka 2008; Ying et al. 2008; Ho 2019). Larvae and pupae are consumed fresh. People collect nests and leave larvae and pupae alive within the combs of the nests until they are ready to be prepared (Nonaka 2008).

Qu. 1.3d. How likely is it that large numbers of the organism will be introduced and/or enter into the environment through this pathway from the point(s) of origin over the course of one year?

including the following elements:

- discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.
- an indication of the propagule pressure (e.g. estimated volume or number of individuals / propagules, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if relevant, comment on the likelihood of introduction and/or entry based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in subsequent establishment whereas for others high propagule pressure (many thousands of individuals) may not.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Such shipments are probably rare events, but these nests may contain hundreds of larvae and pupae that can become adults and escape, if they are not consumed, representing a potential pathway of introduction (USDA 2020).

Qu. 1.4d. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Depending on the time of year, the larvae and pupae in the nests may be workers, males or reproductive females. However, hornet-hunters usually harvest nests late in the season when workers are rearing sexuals. Thus, it is likely that hornets harvested for consumption would contain high-risk castes, i.e. sexuals (Nonaka 2008; USDA 2020). Larvae are likely to die during transport if they are not yet mature, in the absence of adult hornet caretakers. In contrast, pupae are likely to remain alive and viable for an extended period. These have the potential to mature into adults and escape to establish a population (USDA 2020). The larvae or pupae in nests will not reproduce or increase during transport.

Qu. 1.5d. How likely is the organism to survive existing management practices before and during transport and storage along the pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

There is no particular management in such express courier shipments, which are usually delivered within a few days. However, larvae and pupae are meant to be preserved, cooked and eaten and only if the nests are discarded will the insects escape into the wild. Furthermore, even if they are sexuals, they have to mate to establish in the following year.

Qu. 1.6d. How likely is the organism to be introduced into the risk assessment area or entry into the environment undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Most such express courier shipments are not opened at ports of entry and usually remain undetected until the recipient receives it. However, larvae and pupae are meant to be preserved, cooked and eaten and only if the nests are discarded will the insects escape into the wild. Furthermore, even if they are sexuals, they have to mate to establish in the following year.

Qu. 1.7d. How isolated or widespread are possible points of introduction and/or entry into the environment in the risk assessment area?

RESPONSE	isolated widespread ubiquitous	CONFIDENCE	low medium high
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Response:

The recipient can be anywhere in the risk assessment area.

Qu. 1.8d. Estimate the overall likelihood of introduction into the risk assessment area and/or entry into the environment based on this pathway?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

The likelihood of introduction along this pathway is unlikely because such shipments are probably very rare events, particularly to Europe. Furthermore, if such a nest was delivered to the recipient, it would likely be for human consumption. Finally, if some hornets escaped, they would have to be of both sexes, mate (45% of *V. mandarinia* queens emerging from nests are not fertilized (Archer 2012)), and the queen would need to survive the winter. This, such a pathway of introduction is much less likely than those involving a mated queen arriving as contaminant or as stowaway.

Qu. 1.9. Estimate the overall likelihood of introduction into the risk assessment area or entry into the environment based on all pathways and specify if different in relevant biogeographical regions in current conditions.

Provide a thorough assessment of the risk of introduction in relevant biogeographical regions in current conditions: providing insight in to the risk of introduction into the risk assessment area.

RESPONSE	very unlikely unlikely moderately likely likely	CONFIDENCE	low medium high
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	very likely		
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Response:

Data on pathways of invasion of *Vespa* spp. and Vespidea in general are hardly available. However, considering the high numbers of -detrimental- introductions of invasive Vespidae worldwide in recent years (Beggs et al. 2011; USDA 2020), it is likely that non-intentional transports of wasps and hornets are rather common. The transport of mated *Vespa mandarinia* queens from autumn to spring is the most likely means of long-distance dispersion and only mated queens are able to build a new colony. Single queens may unintentionally be transported as contaminant of soil or rotten wood or straw in which they overwinter, or as stowaway in any kind of good, container or vehicle. Considering that the number and variety of commodities and containers that can potentially transport queens is unlimited, queens are probably more likely to be transported as stowaway.

The likelihood of introduction into the risk assessment area or entry into the environment based on all pathways does not vary among biogeographical regions.

Qu. 1.10. Estimate the overall likelihood of introduction into the risk assessment area or entry into the environment based on all pathways in foreseeable climate change conditions?

Thorough assessment of the risk of introduction in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of introduction (e.g. change in trade or user preferences)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely introduction within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Climate change should not influence negatively or positively the likelihood of introduction into the risk assessment area.

2 PROBABILITY OF ESTABLISHMENT

Important instructions:

- For organisms which are already established in parts of the risk assessment area or have previously been eradicated, the likelihood of establishment should be scored as “very likely” by default.
- Discuss the risk also for those parts of the risk assessment area, where the species is not yet established.

Qu. 2.1. How likely is it that the organism will be able to establish in the risk assessment area based on similarity of climatic and abiotic conditions in its distribution elsewhere in the world?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

The climate model made for this risk assessment (Figures 2-4) suggests that a large part of the risk assessment area is climatically suitable for the establishment of *V. mandarinia*, whose native distribution includes a wide variety of abiotic conditions and climates (present in at least 9 Köppen climates from the continental, temperate and tropical groups) from the Russian Far East in the north to Thailand and Malaysia in the south (Matsuura and Sakagami 1973; Matsuura and Yamane 1990; Archer 1995). The climate model showed that, under current climate, the most suitable biogeographic regions are the Continental and Atlantic regions (Figure 3, see also Annex VIII). Establishment will also be possible in the most humid parts of the Mediterranean, Black Sea and Pannonian regions. The Arctic, Boreal and Alpine regions will probably be too cold for establishment while the Steppic region appear too dry to be suitable for *V. mandarinia*. However, in this last region, irrigated areas or humid microclimates may be favourable for the hornet. The ecological niche model developed by Zhu et al. (2020) mostly for North America also shows a global risk map and the ecological suitability presented for Europe is very similar to the CLIMEX model presented in Figure 3 and Annex VIII, with the exception of Fennoscandia which is presented as largely suitable.

Qu. 2.2. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area? Consider if the organism specifically requires another species to complete its life cycle.

RESPONSE	very isolated isolated moderately widespread widespread ubiquitous	CONFIDENCE	low medium high
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Response:

In its area of origin, *V. mandarinia* is widespread and inhabits a large variety of habitats and is particularly abundant in forests and urban green spaces (USDA 2020). *Vespa mandarinia* does not have particular ecological requirements except that nest building and maintaining require humidity. Nests are built mainly in cavities in the soil, such as rodent or reptile burrows, or in rotten tree root systems. They are polyphagous, feeding on a large variety of prey, mainly insects, as well as sugary food such as tree sap and fruits (Matsuura and Sakagami 1973; Matsuura and Yamane 1990; Archer 1995).

Qu. 2.3. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

In its area of origin, *V. mandarinia* co-occurs with several hornet and other wasps, among which *V. crabro* and *V. velutina*, which are two of the three hornets occurring in Europe. Matsuura and Yamane (1990) provide a detailed review of the competitive interactions among *Vespa* spp. and also with other vespidae genera. In general, they consider that the differences in the life history traits among *Vespa* spp. (e.g. different nesting or foraging behaviour) reduce interspecific competition. However, in most cases, when interspecific competition exists, it is in favour of *V. mandarinia*. For example, in spring, queens eat fermenting sap that seeps from tree wounds, which they fiercely defend against other insects, including other *V. mandarinia* queens (Matsuura and Sakagami, 1973; Matsuura, 1984). Recently, Kwon and Choi (2020) showed that, in Korea, *V. mandarinia* had the highest aggressive score towards other hornet species, including the European hornet *V. crabro*. *Vespa mandarinia* is not only able to attack bee hives but it is also the only hornet that can organize group attacks and complete destructions of nests of other *Vespa* spp. (including *V. crabro*) and *Vespula* spp., as described in Matsuura and Sakagami (1973) and Matsuura and Yamane (1990).

Qu. 2.4. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?

RESPONSE	N/A very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Although *Vespa* spp. have a whole range of natural enemies, most of them are not species-specific (Matsuura and Yamane, 1990). Natural enemies of the European hornet such as parasitic insects and nematodes (Turchi and Derijard 2018) and various birds (Monceau et al. 2014) have not been able to prevent the establishment and spread of the invasive alien *Vespa velutina*. This latter is not controlled by natural enemies in Europe, at least when in the nest. Predation on single queens and workers has been poorly studied.

Qu. 2.5. How likely is the organism to establish despite existing management practices in the risk assessment area? Explain if existing management practices could facilitate establishment.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

In the European countries affected by the invasion of *V. velutina*, management practices in place to control the spread of the invasive alien species (see management annex), including trapping and nest destruction, may facilitate rapid responses against *V. mandarinia* and help prevent its establishment (Turchi and Derijard 2018). However, the two species strongly differ in their nesting behavior, *V. mandarinia*'s nests being underground whereas *V. velutina*, as *V. crabro*, nest are mainly built in trees and buildings (Matsuura and Yamane 2020).

Qu. 2.6. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Eradication campaigns would include (1) the finding and destruction of nests in case individuals are found; (2) trapping campaigns to assess the presence of hornets in the eradication region. Underground nests are not easy to locate but protocols are available (USDA 2020, and see management annex). Depending on the time of the year during which the nests are found, queens may have already left (from October on) and will then be very difficult to localize. One of the main biological properties that makes eradication difficult is the high reproductive and dispersal rate of the organism. It is likely that if early eradication fails, the outbreak would increase in scale to a point that would make eradication very difficult.

Qu. 2.7. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?

including the following elements:

- a list and description of the reproduction mechanisms of the species in relation to the environmental conditions in the risk assessment area
- an indication of the propagule pressure of the species (e.g. number of gametes, seeds, eggs or propagules, number of reproductive cycles per year) of each of those reproduction mechanisms in relation to the environmental conditions in the risk assessment area.
- If relevant, comment on the likelihood of establishment based on propagule pressure (i.e. for some species low propagule pressure (1-2 individuals) could result in establishment whereas for others high propagule pressure (many thousands of individuals) may not.
- If relevant, comment on the adaptability of the organism to facilitate its establishment and if low genetic diversity in the founder population would have an influence on establishment.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

The life cycle of *V. mandarinia* is described in details in Matsuura and Sakagami (1973), Matsuura and Yamane (1990) and Archer (1995) and can be summarized as follows. Mated queens overwinter in moist soil cavities, rotting wood or piles of straw. The entrance of the cavity is plugged and the queen passes the winter hanging from the top of the chamber. In spring, they start feeding to develop their ovaries, and search for a nesting site in soil (e.g. rodent's burrow) or in root systems of rotten trees. Then, queens build their nest, first alone and then with the first workers. In summer, the queens become totally nest-bounded. Mature nests contain an average of 2,712 cells, but this number is highly variable, and cells can be reused two or three times in the season (Matsuura and Koike, 2002). In autumn, the nests start producing sexuals. An average colony will produce about 212 males and 205 females (Archer, 1995). Males emerge before females and wait for females at the nests' entrance to mate. Males die before the winter and mated females overwinter. This biology allows single females to colonize, alone, a new area and produce sufficient amounts of sexuals to ensure their establishment.

It has been shown that the *V. velutina* established in France originated from one or a few queens (Arca et al. 2015), suggesting that a very low propagule pressure is sufficient for the establishment of hornet species.

Qu. 2.8. If the organism does not establish, then how likely is it that casual populations will continue to occur?

Consider, for example, a species which cannot reproduce in the risk assessment area, because of unsuitable climatic conditions or host plants, but is present because of recurring introduction, entry and release events. This may also apply for long-living organisms.

RESPONSE	very unlikely	CONFIDENCE	low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response:

Introductions of hornets are probably rather unlikely events but little information is available on introduction events.

Qu. 2.9. Estimate the overall likelihood of establishment in the risk assessment area under current climatic conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under current climatic conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in current conditions: providing insight in the risk of establishment in (new areas in) the risk assessment area.

RESPONSE	very unlikely	CONFIDENCE	low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response:

If queens are introduced, establishment can be considered very likely in a large part of the risk assessment area, considering the climatic suitability (Figure 3), the availability of suitable habitats, the ability of single mated queens to colonize new areas, the high fecundity and the high competitiveness of the species (Matsuura and Sakagami 1973; Matsuura and Yamane 1990; Archer 1995). Climate models suggest that the establishment would be particularly likely in the Continental and Atlantic regions (Figure 3). Establishment will also be possible in the most humid parts of the Mediterranean, Black Sea and Pannonian regions. The Arctic, boreal and Alpine regions will probably be too cold for

establishment while the Steppic regions appear too dry to be suitable for *V. mandarinia*. However, in this last region, irrigated areas or humid microclimates may be favourable for the hornet.

Qu. 2.10. Estimate the overall likelihood of establishment in the risk assessment area under foreseeable climate change conditions. In addition, details of the likelihood of establishment in relevant biogeographical regions under foreseeable climate change conditions should be provided.

Thorough assessment of the risk of establishment in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk.

With regard to climate change, provide information on

- the applied timeframe (e.g. 2050/2070)
- the applied scenario (e.g. RCP 4.5)
- what aspects of climate change are most likely to affect the likelihood of establishment (e.g. increase in average winter temperature, increase in drought periods)

The thorough assessment does not have to include a full range of simulations on the basis of different climate change scenarios, as long as an assessment of likely establishment within a medium timeframe scenario (e.g. 30-50 years) with a clear explanation of the assumptions is provided. However, if new, original models are executed for this risk assessment, the following RCP pathways shall be applied: RCP 2.6 (likely range of 0.4-1.6°C global warming increase by 2065) and RCP 4.5 (likely range of 0.9-2.0°C global warming increase by 2065). Otherwise, the choice of the assessed scenario has to be explained.

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Considering the wide climatic suitability of *V. mandarinia*, the general likelihood of establishment in Europe will not change under climate change scenarios. However, it is predicted that, with time, northern regions and high elevation areas will become more suitable because increased temperatures will allow the full development of a nest in summer whereas the Mediterranean and Continental Regions will become less suitable because of increased drought whereas humidity is needed for the building and maintenance of vespids nests.

3 PROBABILITY OF SPREAD

Important instructions:

- Spread is defined as the expansion of the geographical distribution of an alien species within the risk assessment area.
- Repeated releases at separate locations do not represent continuous spread and should be considered in the probability of introduction and entry section (Qu. 1.7).

Qu. 3.1. How important is the expected spread of this organism within the risk assessment area by natural means? (List and comment on each of the mechanisms for natural spread.)

including the following elements:

- a list and description of the natural spread mechanisms of the species in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of spread discussed in relation to the species biology and the environmental conditions in the risk assessment area.

The description of spread patterns here refers to the CBD pathway category “Unaided (Natural Spread)”. It should include elements of the species life history and behavioural traits able to explain its ability to spread, including: reproduction or growth strategy, dispersal capacity, longevity, dietary requirements, environmental and climatic requirements, specialist or generalist characteristics.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Vespa mandarinia workers and queens are strong fliers. Workers usually forage in a radius of one to two kilometers around their nest but are capable of foraging up to eight kilometers away from the nest (Matsuura and Sakagami 1973). There is no reliable data on the flying capacities of queens. Abe et al. (1995) reported that *V. mandarinia* can fly at speeds of over 30 kilometers per hour and cover about 100 km in a single day but this information needs verification (USDA 2020). For comparison, queens of the invasive *V. velutina* can fly an average of 25 km/day in flight mill experiments (Sauvard et al. 2018). In nature, queens may not spread that fast, but they may cover very long distances during their extensive flight periods (i.e., before and after hibernation). *Vespa velutina* has spread an average of 78 km/year in the first years of its invasion in France (Robinet et al. 2017), but only 18 km/year in Italy (Bertolino et al. 2016) and 10–20 km/year in South Korea (Choi et al. 2012). Human-mediated dispersal may not be necessarily responsible for the hornet’s rapid range expansion even though the initial jumps to UK, Portugal or the Netherlands are likely due to transport of goods (Robinet et al. 2017, 2019).

Qu. 3.2a. List and describe relevant pathways of spread other than "unaided". For each pathway answer questions 3.3 to 3.9 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 3.3a, 3.4a, etc. and then 3.3b, 3.4b etc. for the next pathway.

including the following elements:

- a list and description of pathways of spread with an indication of their importance and associated risks (e.g. the likelihood of spread in the risk assessment area, based on these pathways; likelihood of survival, or reproduction, or increase during transport and storage; ability and likelihood of transfer from the pathway to a suitable habitat or host) in relation to the environmental conditions in the risk assessment area.
- an indication of the rate of spread for each pathway discussed in relation to the species biology and the environmental conditions in the risk assessment area.
- All relevant pathways of spread (except “Unaided (Natural Spread)”, which is assessed in Qu. 3.1) should be considered. The classification of pathways developed by the Convention of Biological Diversity shall be used (see Annex IV).

Even though workers could be transported by humans, only the transport of mated queens will allow the species to spread. And, because the transport of full, active nests in summer is highly unlikely, only the transport of mated queens from autumn to spring should be considered. Single queens may unintentionally be transported as contaminant of nursery material and transportation of habitat material in which they overwinter or as stowaway in any kind of good, container or vehicle.

The pathways of spread considered in the risk assessment are:

- A. Transport - Contaminant (nursery material)
- B. Transport - Contaminant (other than soil of nursery material)
- C. Transport - stowaway (Container/Bulk)
- D. Transport - stowaway (vehicles)

A. Transport - Contaminant (nursery material)

Qu. 3.3a. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional	CONFIDENCE	low
	unintentional		medium
			high

Response:

Queens overwinter mainly in a hole that they have excavated in the soil. (Matsuura and Sakagami 1973; Archer 1995). Thus, any kind of soil or similar substrate related to the movement of nursery material, in particular soil in potted plants can potentially transport an overwintering queen.

Qu. 3.4a. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Only one mated queen is needed to form a colony in a newly invaded region, as observed in the arrival and spread of *V. velutina* in Europe (Arca et al. 2015; Robinet et al. 2017; 2019), and it is assumed that this could occur also for *V. mandarinia*.

Qu. 3.5a. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Queens are very strong insects. They do not feed nor reproduce in their overwintering site (Matsuura and Sakagami 1973; Archer 1995). Soil of potted plants is likely to remain intact during transport.

Qu. 3.6a. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely	CONFIDENCE	low medium
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	moderately likely likely very likely		high
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Response:

Phytosanitary practices in the horticultural sector may accidentally harm queens hidden in soil of potted plants when plants are treated with insecticides. However only some of the plants traded or moved within EU are treated with insecticides.

Qu. 3.7a. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Although the organism is rather big, the cavity in which a queen overwinters is usually plugged (Archer 1995) and it can easily remain undetected during transport until a viable population is established.

Qu. 3.8a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Queens are good fliers and can build nests in various habitats, from forests to urban green areas (Matsuura and Sakagami 1973; Archer 1995). Thus, they would have no difficulty in reaching suitable habitats for nesting.

Qu. 3.9a. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area. (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response:

The spread of overwintering queens in soil is likely, considering the amount of soil in the horticulture sector transported within the risk assessment area. However, it must be noted that, almost two decades after the invasion of *V. velutina* in Europe, and after having invaded nine countries, little is known about the part of human-mediated dispersal in the fast spread of the hornet and on the underlying mechanisms (Robinet et al. 2017; 2019).

B. Transport - Contaminant (transportation of habitat material) (other than soil of nursery material)

Qu. 3.3b. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response:

Queens overwinter mainly in a hole that they have excavated in the soil, but also occasionally in rotting wood, piles of straw, etc. (Matsuura and Sakagami 1973; Archer 1995). Thus, any kind of soil or similar substrate, except soil of nursery material (e.g. soil for construction), straw, rotting wood, etc. can potentially transport an overwintering queen.

Qu. 3.4b. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication

- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Only one mated *V. mandarinia* queen is needed to form a colony in a newly invaded region, as observed in the arrival and spread of *V. velutina* in Europe (Arca et al. 2015; Robinet et al. 2017; 2019) and it is assumed that this could occur also for *V. mandarinia*.

Qu. 3.5b. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Queens are very strong insects. They do not feed nor reproduce in their overwintering site. Soil is often shaken during transport, which will not favour the survival of the queen. However, other substrates such as straw can be easily transported undisturbed, soil of potted plants is more likely to remain intact.

Qu. 3.6b. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Within the risk assessment area, soil not related to nursery material and straw are usually transported without management practice.

Qu. 3.7b. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Although a *V. mandarinia* queen is very big, the cavity in which a queen overwinters is usually plugged (Archer 1995) and it can easily remain undetected during transport until a viable population is established.

Qu. 3.8b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Queens are good fliers and can build nests in various habitats, from forests to urban green areas (Matsuura and Sakagami 1973; Archer 1995). Thus, they would have no difficulty in reaching suitable habitats for nesting.

Qu. 3.9b. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area. (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately	CONFIDENCE	low medium high
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	rapidly very rapidly		
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Response:

The spread of overwintering queens in soil is likely, considering the amount of soil, straw and other potential overwintering substrates transported within the risk assessment area. However, it must be noted that, almost two decades after the invasion of *V. velutina* in Europe, and after having invaded ten countries, very little is known about the part of human-mediated dispersal in the fast spread of the hornet and on the underlying mechanisms (Robinet et al. 2017; 2019)

C. Transport - stowaway (Container/Bulk)

Qu. 3.3c. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional unintentional	CONFIDENCE	low medium high
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Response:

Mated queens can travel hidden in many sorts of containers containing any kind of goods (e.g. pottery, fruits, meat/fish, furniture, etc.), before or after having built an overwintering site.

Qu. 3.4c. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Only one mated queen is needed to form a colony in a newly invaded region, as observed in the arrival and spread of *V. velutina* in Europe (Arca et al. 2015; Robinet et al. 2017; 2019), and it is assumed that this could occur also for *V. mandarinia*.

Qu. 3.5c. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Queens are very strong insects that can survive harsh conditions. Transports of containers within the risk assessment areas are usually very short. In winter, queens do not feed much and can stay hidden a long time. They do not reproduce during that period (Matsuura and Sakagami 1973).

Qu. 3.6c. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

This will depend on the management practices used in the containers' transport conditions but none will target Vespidae and it is unlikely that management practices will affect the transported queen within the risk assessment area.

Qu. 3.7c. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely	CONFIDENCE	low medium high
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	very likely		
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Response:

Queens are very big (4-5 cm) and are quite obvious when the container is inspected. However, in large containers queens in winter are usually hidden in the transported goods and are easily missed when transported.

Qu. 3.8c. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Queens are good fliers and can build nests in various habitats, from forests to urban green areas (Matsuura and Sakagami 1973; Archer 1995). Thus, from the arrival destination of the container, they would have no difficulty in reaching suitable habitats for nesting or finding overwintering sites, depending in the season.

Qu. 3.9c. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area. (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response:

The spread of queens in winter in any kind of container is likely and may be fast, considering the amount of opportunities to be transported that way. However, it must be noted that, almost two decades after the invasion of *V. velutina* in Europe, and after having invaded ten countries, very little is known about the part of human-mediated dispersal in the fast spread of the hornet and on the underlying mechanisms (Robinet et al. 2017; 2019).

D. Transport - stowaway (vehicles)

Qu. 3.3d. Is spread along this pathway intentional (e.g. the organism is deliberately transported from one place to another) or unintentional (e.g. the organism is a contaminant of translocated goods within the risk assessment area)?

RESPONSE	intentional	CONFIDENCE	low
	unintentional		medium
			high

Response:

Mated queens can travel hidden in many types of vehicles, e.g. trucks, trains, cars, etc., before or after having built an overwintering site.

Qu. 3.4d. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?

including the following elements:

- an indication of the propagule pressure (e.g. estimated volume or number of specimens, or frequency of passage through pathway), including the likelihood of reinvasion after eradication
- if appropriate, indicate the rate of spread along this pathway
- if appropriate, include an explanation of the relevance of the number of individuals for spread with regard to the biology of species (e.g. some species may not necessarily rely on large numbers of individuals).

RESPONSE	very unlikely	CONFIDENCE	low
	unlikely		medium
	moderately likely		high
	likely		
	very likely		

Response:

Only one mated queen is needed to form a colony in a newly invaded region, as observed in the arrival and spread of *V. velutina* in Europe (Arca et al. 2015; Robinet et al. 2017; 2019), and it is assumed that this could occur also for *V. mandarinia*.

Qu. 3.5d. How likely is the organism to survive, reproduce, or increase during transport and storage along the pathway (excluding management practices that would kill the organism)?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Queens are very strong and long-lived insects. Journeys in vehicles within the risk assessment areas can be very short and queens would surely survive such short journeys. Mated females will start building nests only in spring (Matsuura and Sakagami 1973; Archer 1995).

Qu. 3.6d. How likely is the organism to survive existing management practices during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

This will depend on the management practices used in the vehicles (usually very little in the EU) but none will target Vespidae and it is unlikely that management practices will affect the transported queen.

Qu. 3.7d. How likely is the organism to spread in the risk assessment area undetected?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Queens are very big (4-5 cm) and are quite obvious when they simply hitchhike in a vehicle. However, in winter queens are usually not moving as actively as workers and, thus, a hidden queen can be transported undetected in large vehicles (trucks, trains), but less so in cars.

Qu. 3.8d. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host during spread?

RESPONSE	very unlikely unlikely moderately likely likely very likely	CONFIDENCE	low medium high
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Response:

Queens are good fliers and can build nests in various habitats, from forests to urban green areas (Matsuura and Sakagami 1973; Archer 1995). Thus, from the arrival destination of the vehicle (or during transport), they would have no difficulty in reaching suitable habitats for nesting or finding overwintering sites, depending in the season.

Qu. 3.9d. Estimate the overall potential rate of spread based on this pathway in relation to the environmental conditions in the risk assessment area. (please provide quantitative data where possible).

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response:

The spread of *V. mandarinia* queens in winter in any kind of vehicle is possible and may be fast. However, it must be noted that, almost two decades after the invasion of *V. velutina* in Europe, and after having invaded ten countries, very little is known about the part of human-mediated dispersal in the fast spread of the hornet and on the underlying mechanisms (Robinet et al. 2017; 2019).

End of pathway assessment, repeat Qu. 3.3 to 3.9. as necessary using separate identifiers.

Qu. 3.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?

RESPONSE	very easy easy	CONFIDENCE	low medium
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	with some difficulty difficult very difficult		high
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Response:

Despite numerous efforts, all countries have failed to contain the spread of *V. velutina* in Europe, with the potential exception of UK and Mallorca where the species may have been eradicated (Monceau et al. 2014; Robinet et al. 2019; Jones et al. 2020; Leza et al. 2021), so it is doubtful that they would be more successful with *V. mandarinia*. Eradication may be easier than containment. Eradication successes against invasive alien Vespidae have been reported elsewhere in the world, e.g. against *Vespula germanica* in Western Australia (Tennant et al. 2011), suggesting that eradication would be possible if carried out at a very early stage of the invasion. However, once there is an established and spreading population it will be very difficult to contain its spread.

Qu. 3.11. Estimate the overall potential rate of spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (indicate any key issues and provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in current conditions, providing insight in the risk of spread into (new areas in) the risk assessment area.

RESPONSE	very slowly slowly moderately rapidly very rapidly	CONFIDENCE	low medium high
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Response:

Considering the strong flying capacities of the queens, their ability to travel as soil contaminant or as stowaway in various types of containers, and based on the fast spread of *V. velutina* in Europe, which invaded 10 countries in 16 years, it is highly likely that *V. mandarinia* would spread very rapidly in the risk assessment area, in particular in the most favourable biogeographical regions such as continental and atlantic regions.

Qu. 3.12. Estimate the overall potential rate of spread in relevant biogeographical regions in foreseeable climate change conditions (provide quantitative data where possible).

Thorough assessment of the risk of spread in relevant biogeographical regions in foreseeable climate change conditions: explaining how foreseeable climate change conditions will influence this risk, specifically if rates of spread are likely slowed down or accelerated.

RESPONSE	very slowly slowly	CONFIDENCE	low medium
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	moderately rapidly very rapidly		high
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Response:

The general risk of spread in the risk assessment area should not be influenced by changing climatic conditions, but spread may become more or less likely depending on whether regions become more or less suitable for the organism (see section on likelihood of establishment). It may also become more rapid spread better survival, faster growth.

4 MAGNITUDE OF IMPACT

Important instructions:

- Questions 4.1-4.5 relate to biodiversity and ecosystem impacts, 4.6-4.8 to impacts on ecosystem services, 4.9-4.13 to economic impact, 4.14-4.15 to social and human health impact, and 4.16-4.18 to other impacts. These impacts can be interlinked, for example, a disease may cause impacts on biodiversity and/or ecosystem functioning that leads to impacts on ecosystem services and finally economic impacts. In such cases the assessor should try to note the different impacts where most appropriate, cross-referencing between questions when needed.
- Each set of questions starts with the impact elsewhere in the world, then considers impacts in the risk assessment area (=EU excluding outermost regions) separating known impacts to date (i.e. past and current impacts) from potential future impacts (including foreseeable climate change).
- Only negative impacts are considered in this section (socio-economic benefits are considered in Qu. A.7)

Biodiversity and ecosystem impacts

Qu. 4.1. How important is the impact of the organism on biodiversity at all levels of organisation caused by the organism in its non-native range excluding the risk assessment area?

including the following elements:

- Biodiversity means the variability among living organisms from all sources, including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems
- impacted chemical, physical or structural characteristics and functioning of ecosystems

RESPONSE	N/A	CONFIDENCE	low
	minimal		medium
	minor		high
	moderate		
	major		
	massive		

Response:

Vespa mandarinia has never been studied outside its native range. It was only recently observed invading Western North America. Therefore, it is too early to assess impacts in its non-native range.

Qu. 4.2. How important is the current known impact of the organism on biodiversity at all levels of organisation (e.g. decline in native species, changes in native species communities, hybridisation) in the risk assessment area (include any past impact in your response)?

Discuss impacts that are currently occurring or are likely occurring or have occurred in the past in the risk assessment area. Where there is no direct evidence of impact in the risk assessment area (for example no studies have been conducted), evidence from outside of the risk assessment area can be used to infer impacts within the risk assessment area.

RESPONSE	N/A minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Vespa mandarinia is not present in the risk assessment area.

Qu. 4.3. How important is the potential future impact of the organism on biodiversity at all levels of organisation likely to be in the risk assessment area?

See comment above. The potential future impact shall be assessed only for the risk assessment area.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Vespa mandarinia can directly affect biodiversity in two ways: as predator of invertebrates or as competitors of other organisms, in particular Vespidae. Based on our knowledge of the insect in its native range, where it is a voracious predator and strong competitor, the impact on biodiversity may be major or massive. However, since it has not yet been invasive anywhere (the new introduction into North America is too recent to gather any information on impact) an assessment on impact on biodiversity can only be made with very low confidence.

Firstly, *Vespa mandarinia* is a polyphagous predator on a whole spectrum of large invertebrates such as caterpillars, spiders, mantids, beetles, etc. (USDA 2020). However, the most spectacular predatory behaviour of *V. mandarinia* is its mass attacks against nests of other social Hymenoptera, such as bees and wasps of the genera *Vespa*, *Vespula* and *Polistes*, during which they attack, kill and occupy nests, sometimes during several days, to feed their own larvae (Matsuura and Sakagami 1973; Matsuura and Yamane 1990). Such behaviour has also been observed against *V. crabro* in Japan (Matsuura 1984).

Secondly, it competes with other vespids for nesting sites and food. Data from its native range suggests that it can outcompete other vespids (Matsuura and Yamane 1990). In spring, queens eat fermenting sap that seeps from tree wounds, which they fiercely defend against other insects,

(Matsuura and Sakagami 1973; Matsuura 1984). Differences in nesting behaviour reduce interspecific competition among Vespidae. In Europe, *V. mandarinia* would not compete with *V. velutina* that usually nests in tree branches. But *Vespula* spp. and *V. crabro* are more generalist and partly nest in soil cavities or tree holes and could therefore be adversely affected by *V. mandarinia*. Competition with other organisms is also likely. For example, the invasion of the European *Vespula vulgaris* in New Zealand resulted in severe competition for food (sap runs, honeydew etc.) and in the decline of several bird and insect species (Beggs et al. 2011). In Japan, *V. mandarinia* also feeds abundantly on tree sap and is known to occupy the highest position in terms of interspecific relationship and to monopolize tree sap and other food sources by chasing off all visitors, not only other *Vespa* species but any other kind of insect, including beetles, butterflies and moths (Matsuura and Yamane 1990)

The potential impact of its likely predation on honey bees also may have an impact on biodiversity because it may affect pollination services (see Q. 4.8).

Qu. 4.4. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism currently in the risk assessment area?
including the following elements:

- native species impacted, including red list species, endemic species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats
- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	N/A	CONFIDENCE	low
	minimal		medium
	minor		high
	moderate		
	major		
	massive		

Response:

Vespa mandarinia is not present in the risk assessment area.

Qu. 4.5. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism likely to be in the future in the risk assessment area?
including the following elements:

- native species impacted, including red list species and species listed in the Birds and Habitats directives
- protected sites impacted, in particular Natura 2000
- habitats impacted, in particular habitats listed in the Habitats Directive, or red list habitats

- the ecological status of water bodies according to the Water Framework Directive and environmental status of the marine environment according to the Marine Strategy Framework Directive

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

At present, it is very difficult to assess which native species would be most affected by *V. mandarinia* and, thus, whether a decline in conservation value will occur. However, it can be assumed that one of the most likely affected species may be *V. crabro* (see Qu. 4.3), which is a red list species in some countries and regions (e.g. in Germany, and in Austria, States of Steiermark and Oberösterreich). More generally, *V. mandarinia* is known to prey on large insects such as scarab and longhorn beetles (Matsuura 1988), mantids (Matsuura 1984), and large caterpillars (Matsuura and Sakagami 1973) and it is likely that, if introduced into Europe, they will prey on red list and protected insects, including species listed in the Habitats directive.

It is not yet possible to assess whether protected sites and habitats will be particularly invaded by the hornet, but it is highly likely that many of them will be invaded, in particular those in the Atlantic and Continental biogeographic regions containing forest areas.

Ecosystem Services impacts

Qu. 4.6. How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?

- For a list of services use the CICES classification V5.1 provided in Annex V.
- Impacts on ecosystem services build on the observed impacts on biodiversity (habitat, species, genetic, functional) but focus exclusively on reflecting these changes in relation to their links with socio-economic well-being.
- Quantitative data should be provided whenever available and references duly reported.
- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	N/A minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Vespa mandarinia has never been studied outside its native range. It was only recently observed invading Western North America.

Qu. 4.7. How important is the impact of the organism on provisioning, regulating, and cultural services currently in the different biogeographic regions or marine sub-regions where the species has established in the risk assessment area (include any past impact in your response)?

- See guidance to Qu. 4.6.

RESPONSE	N/A minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Vespa mandarinia is not present in the risk assessment area.

Qu. 4.8. How important is the impact of the organism on provisioning, regulating, and cultural services likely to be in the different biogeographic regions or marine sub-regions where the species can establish in the risk assessment area in the future?

- See guidance to Qu. 4.6.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Vespa mandarinia may affect provisioning and regulating ecosystem services mainly through its potential impact on honeybees and wild pollinators, i.e. on pollination services. Impacts on provisioning services include effects on biomass of cultivated and wild plants and reared animals; impacts on regulating services include effects on life cycle maintenance. The effect on honeybees in Europe may be particularly important. In Asia, it is considered as a major predator of honey bees (see Qu. 4.9). Its impact is much higher than that of *V. velutina*, which is threatening apiculture in Western Europe (Monceau et al. 2014). *Vespa mandarinia*, being an additional threat to the European apiculture and pollinators, may have the following consequences. European beekeeping and honey

production are already threatened by various factors, such as parasites, viruses, insecticides, climate change, habitat fragmentation, small hive beetle (*Aethina tumida*) and *Vespa velutina*. The additional threat of *V. mandarinia* may lead to the abandonment of beekeeping activity, which would affect the European honey production. In addition, many crops rely mainly on honey bees and other bees (which may also be affected) for pollination. A further decline of honey bees and wild bees may have serious effects on yields. Fedele et al. (2019) provide a method to assess the impact on apple, pear and peach production through pollination reduction, but their numbers provided are based on a pollination reduction of 65% which is probably exaggerated. In fact, there is presently no reliable quantification on the effect of *V. velutina* on honeybee populations and, as a result, on pollination of cultivated and also of wild plants. In a recent study in Spain, it was observed that *V. velutina* affects pollination of a wild native plant, *Mentha suaveolens*, through changes in abundance and behaviour of floral visitors, including honeybees, bumblebees, small hymenopterans and syrphids (Rojas-Nossa and Calviño-Cancela 2020).

Economic impacts

Qu. 4.9. How great is the overall economic cost caused by the organism within its current area of distribution (excluding the risk assessment area), including both costs of / loss due to damage and the cost of current management.

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the world these should be reported here. The assessment of the potential costs of / loss due to damage shall describe those costs quantitatively and/or qualitatively depending on what information is available. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage. As far as possible, it would be useful to separate costs of / loss due to the organism from costs of current management.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

To our knowledge, there is no quantitative data on economic costs related to *V. mandarinia* in its area or origin. But there are at least two categories of damage that lead to economic costs.

Firstly, according to Matsuura and Yamane (1990), *Vespa mandarinia* is considered as “the most serious enemy of both cultivated and wild honeybees in Japan”. In Asia, it is considered to have a much higher impact on honey bees than *V. velutina*, which is threatening apiculture in Western Europe. As for *V. velutina* and other hornets, workers can attack and kill bees singly at the entrance to hives. However, the most damaging attack strategy, called the “slaughter strategy”, consists in attacking a hive in groups of a few dozen workers. An entire bee hive of 30’000 bees can be destroyed in a few hours. The hornets first kill the guard bees to enter the hive, then kill the other bees to finally retrieve the bee larvae and pupa to bring them to their own larvae. This process can take several days

during which the attacked hive will be occupied by the hornets. This strategy has been described in details by Matsuura and Sakagami (1973). In Japan, many beekeepers transfer hives in summer from mountains to plains to avoid the attacks of *V. mandarinia* (Matsuura and Sakagami, 1973). It must be noted that *V. mandarinia* is particularly damaging for the European honey bee *Apis mellifera* while the Asian species *Apis cerana* has developed defense mechanisms such as passive retreat and rapid mass attacks to heat up and kill the hornets (Matsuura and Sakagami 1973).

Economic costs are also related to *V. mandarinia* being a human nuisance. In particular, its impact on human health through its painful stings may trigger serious costs (see Qu 4.14). Furthermore, the economics of recreational activities and tourism in places where the hornets could be prevalent.

Qu. 4.10. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism currently in the risk assessment area (include any past costs in your response)?

- Where economic costs of / loss due to the organism have been quantified for a species anywhere in the EU these should be reported here. Assessment of the potential costs of damage on human health, safety, and the economy, including the cost of non-action. A full economic assessment at EU scale might not be possible, but qualitative data or different case studies from across the EU (or third countries if relevant) may provide useful information to inform decision making. In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”. Cost of / loss due to damage within different economic sectors can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	N/A	CONFIDENCE	low
	minimal		medium
	minor		high
	moderate		
	major		
	massive		

Response:

Vespa mandarinia is not present in the risk assessment area.

Qu. 4.11. How great is the economic cost of / loss due to damage (excluding costs of management) of the organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.10.

RESPONSE	minimal	CONFIDENCE	low
	minor		medium
	moderate		high
	major		

	massive		
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Response:

Given that *Vespa mandarinia* is a serious pest in its native range through its impact on honey bees and human health, there is no reason to think that impacts would be lower in climatically suitable areas in Europe. *Vespa velutina* became a more serious predator of honey bees in Europe than in East Asia, where *V. mandarinia* is considered as much more problematic for honey bees and human health than *V. velutina*. In addition, *V. mandarinia* is particularly damaging for the European honey bee *Apis mellifera* while the Asian species *Apis cerana* has developed defence mechanisms such as passive retreat and rapid mass attacks to heat up and kill the hornets (Matsuura and Sakagami 1973). European beekeeping and honey production are already threatened by a series of various factors, such as parasites, viruses, insecticides, climate change, habitat fragmentation, small hive beetle and *Vespa velutina*. The additional threat of *V. mandarinia* may lead to the abandonment of bee hives, which would affect the European production of honey. In France alone, the annual revenue from apiculture was €135M in 2015 (Barbet-Massin et al. 2020). Even a small reduction in honey production by *V. mandarinia* may lead to substantial losses for the sector in Europe. In addition, many crops rely mainly on honey bees and other bees (which may also be affected) for pollination. A further decline of honey bees and wild bees may have serious effects on crop yields, as estimated for *V. velutina* (Fedele et al. 2019). It is estimated that the yearly pollination services to agriculture are worth €2 billion in France alone (Gallai et al. 2009), so, even a very small effect of *V. mandarinia* on pollination services would lead to severe losses.

Qu. 4.12. How great are the economic costs / losses associated with managing this organism currently in the risk assessment area (include any past costs in your response)?

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	N/A	CONFIDENCE	low medium high
	minimal minor moderate major massive		

Response:

Vespa mandarinia is not present in the risk assessment area.

Qu. 4.13. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?

- See guidance to Qu. 4.12.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Management costs will depend on the magnitude of impact in the risk assessment area, which cannot be assessed precisely for the moment, since the organism is not yet in the risk assessment area and is just starting to invade another region for the first time. To assess management costs, it is best to use the invasion of *V. velutina* in Europe as comparison. Management costs related to *V. velutina* have never been assessed globally. They mainly consist of (1) surveillance programmes and information campaigns in the recently invaded areas, (2) locating and destroying nests, (3) trapping campaigns to capture queens in spring (these are not always considered effective but widely practiced), (4) various management strategies to protect hives; and (5) funding research programmes. So far, only the cost of nest removal has been precisely quantified. Barbet-Massin et al. (2020) estimated that nest destruction cost €23 million between 2006 and 2015 in France. The yearly cost is increasing as the species keeps spreading and could reach €11.9 million in France, €9.0 million in Italy and €8.6 million in the United Kingdom, if the species fills its current climatically suitable distribution. The authors also stated that, “although more work will be needed to estimate the cost of the Asian yellow-legged hornet on apiculture and pollination services, they likely exceed the current costs of control with nest destruction. It could thus be worth increasing control efforts by aiming at destroying a higher percentage of nests”.

The introduction of *V. mandarinia* in Europe would require similar management strategies, keeping in mind that *V. mandarinia* is considered as being more problematic than *V. velutina* in East Asia. The cost of nest removal (100-400 Eur/nest) for *V. velutina* may be different for *V. mandarinia*, which nests in the soil rather than in trees and buildings.

Social and human health impacts

Qu. 4.14. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism for the risk assessment area and for third countries, if relevant (e.g. with similar eco-climatic conditions).

The description of the known impact and the assessment of potential future impact on human health, safety and the economy, shall, if relevant, include information on

- illnesses, allergies or other affections to humans that may derive directly or indirectly from a species;
- damages provoked directly or indirectly by a species with consequences for the safety of people, property or infrastructure;
- direct or indirect disruption of, or other consequences for, an economic or social activity due to the presence of a species.

Social and human health impacts can be a direct or indirect consequence of the earlier-noted impacts on ecosystem services. In such case, please provide an indication of the interlinkage.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Vespa mandarinia has a powerful sting, which is a hazard to human health. Workers can be aggressive when they feel that their nest is threatened and, since nests are in the ground, they are easily overlooked by walkers. In Japan, fatalities due to *V. mandarinia* stings are estimated to range from 30 to 50 persons each year (Yanagawa et al. 2008). While most victims are allergic and die from anaphylaxis or sudden cardiac arrest, others die from multiple organ failure including rhabdomyolysis, renal failure, liver dysfunction, respiratory failure, and disseminated intravascular coagulopathy. In China, in a three months period in July - October 2013, *V. mandarinia* killed 42 and injured 1,675 people in three cities in the southern part of Shaanxi Province (Liu et al. 2016).

Qu. 4.15. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism in the future for the risk assessment area.

- In absence of specific studies or other direct evidences this should be clearly stated by using the standard answer “No information has been found on the issue”. This is necessary to avoid confusion between “no information found” and “no impact found”.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

In some European countries, the invasion of *V. velutina* in Europe led to a significant increase in casualties due to hornet stings (e.g. in Spain, Feás 2021; Vidal 2021) (): *Vespa mandarinia*'s stings are more painful and dangerous, and workers can be more aggressive because the nests are more accessible and easily overlooked. If, as observed for *V. velutina*, *V. mandarinia* becomes more abundant in the invaded range than in its native range, this hornet could become a serious human health issue.

Other impacts

Qu. 4.16. How important is the organism in facilitating other damaging organisms (e.g. diseases) as food source, a host, a symbiont or a vector etc.?

RESPONSE	N/A minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

No information found.

Qu. 4.17. How important might other impacts not already covered by previous questions be resulting from introduction of the organism?

RESPONSE	N/A minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

No information found.

Qu. 4.18. How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area?

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Although *Vespa* spp. have a whole range of natural enemies, such as parasitic insects and nematodes and birds, most of them are not species-specific (Matsuura and Yamane, 1990). Natural enemies of the European hornet have not been able to prevent the invasion of *Vespa velutina* in Europe (Monceau et al. 2014; Turchi and Derijard 2018). Colonies of the latter do not seem to be controlled by natural enemies in Europe. Predation on single queens and workers has been poorly studied and may be more important. It is not clear how the impact of *V. velutina* would be without natural enemies.

Qu. 4.19. Estimate the overall impact in the risk assessment area under current climate conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, in current conditions.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

If introduced into the risk assessment area, *V. mandarinia* is likely to cause severe impacts on the economy, the environment and human health, mainly through its aggressive predatory behaviour towards honey bees and other insects and its harmful stings to humans. The severity of these impacts remains difficult to assess. However, in Asia, *V. mandarinia* is considered, in all aspects, more harmful than *V. velutina*, which is already regarded a serious invasive alien species. Impacts are likely to be higher in regions that are most favourable for *V. mandarinia*, in particular the Continental and Atlantic regions and part of the Mediterranean region (see section 2 on likelihood of establishment).

Qu. 4.20. Estimate the overall impact in the risk assessment area in foreseeable climate change conditions. In addition, details of overall impact in relevant biogeographical regions should be provided.

Thorough assessment of the overall impact on biodiversity and ecosystem services, with impacts on economy as well as social and human health as aggravating factors, under future conditions.

RESPONSE	minimal minor moderate major massive	CONFIDENCE	low medium high
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Response:

Climate change is likely to modify the geographical distribution of impact magnitude (i.e. increase in the north, decrease in the south and east), but not the average magnitude of impact for the risk assessment area in general.

RISK SUMMARIES			
	RESPONSE	CONFIDENCE	COMMENT
Summarise Introduction and Entry*	very unlikely unlikely moderately likely likely very likely	low medium high	Data on pathways of invasion of <i>Vespa</i> spp. are rare. However, considering the high numbers of introductions of invasive Vespidae worldwide in recent years, it is likely that non-intentional transports of wasps and hornets are rather common. The transport of mated <i>Vespa mandarinia</i> queens from autumn to spring is the most likely mean of long-distance dispersal. Single queens may unintentionally be transported as contaminants of soil or straws in which they overwinter, or as stowaways in any kind of good, container or vehicle. Climate change should not affect the likelihood of introduction.
Summarise Establishment*	very unlikely unlikely moderately likely likely very likely	low medium high	If queens are introduced, establishment can be considered very likely in a large part of the risk assessment area, considering the climatic suitability, the availability of suitable habitats, the ability of single mated queens to colonize new areas, the high fecundity and the high competitiveness of the species. Climate models suggest that the establishment would be particularly likely in the Continental and Atlantic regions, and parts of the Mediterranean region. The general likelihood of establishment in Europe will not change under climate change scenarios, even though some regions may become less and others more favourable.

Summarise Spread*	very slowly slowly moderately rapidly very rapidly	low medium high	Considering the strong flying capacities of the queens, their ability to travel as soil contaminants or as stowaways in various types of containers, and based on the fast spread of <i>V. velutina</i> in Europe, it is highly likely that <i>V. mandarinia</i> would spread rapidly in the risk assessment area. The general risk of spread in the risk assessment area will not be influenced by changing climatic conditions, but spread may become more or less easy depending on whether regions become more or less suitable for the organism.
Summarise Impact*	minimal minor moderate major massive	low medium high	If introduced into the risk assessment area, <i>V. mandarinia</i> is likely to cause severe impacts on the economy, the environment and human health, mainly through its aggressive predatory behaviour towards honey bees and other insects and its harmful stings to humans. The severity of these impacts remains difficult to assess. However, in Asia, <i>V. mandarinia</i> is considered, in all aspects, more harmful than <i>V. velutina</i> , which is already regarded a serious invasive species. Climate change is likely to modify the geographical distribution of impact magnitude but not the average magnitude of impact for the risk assessment area.
Conclusion of the risk assessment (overall risk)	low moderate high	low medium high	The likelihood of introduction of <i>V. mandarinia</i> can only be assessed with low confidence because of a lack of quantitative data on interceptions and pathways of entry. In contrast, establishment is highly likely in a

			large part of the risk assessment area and, if the organism becomes established, there is little doubt that it will spread fast and cause significant environmental, economic and social impacts.
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*in current climate conditions and in foreseeable future climate conditions

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Distribution Summary

Please answer as follows:

- Yes if recorded, established or invasive
- if not recorded, established or invasive
- ? Unknown; data deficient

The columns refer to the answers to Questions A5 to A12 under Section A.

For data on marine species at the Member State level, delete Member States that have no marine borders.

In all other cases, provide answers for all columns.

Member States and the United Kingdom

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Austria	-	-	Yes	Yes	-
Belgium	-	-	Yes	Yes	-
Bulgaria	-	-	Yes	?	-
Croatia	-	-	Yes	Yes	-
Cyprus	-	-	?	?	-
Czech Republic	-	-	Yes	Yes	-
Denmark	-	-	Yes	Yes	-
Estonia	-	-	?	?	-
Finland	-	-	?	?	-
France	-	-	Yes	Yes	-
Germany	-	-	Yes	Yes	-
Greece	-	-	Yes	Yes	-
Hungary	-	-	Yes	?	-
Ireland	-	-	Yes	Yes	-
Italy	-	-	Yes	Yes	-
Latvia	-	-	Yes	Yes	-
Lithuania	-	-	Yes	Yes	-
Luxembourg	-	-	Yes	Yes	-
Malta	-	-	Yes	?	-
Netherlands	-	-	Yes	Yes	-
Poland	-	-	Yes	Yes	-
Portugal	-	-	Yes	Yes	-
Romania	-	-	Yes	Yes	-
Slovakia	-	-	Yes	Yes	-
Slovenia	-	-	Yes	Yes	-
Spain	-	-	Yes	Yes	-
Sweden	-	-	Yes	Yes	-
United Kingdom	-	-	Yes	Yes	-

Biogeographical regions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Alpine	-	-	?	Yes	-
Atlantic	-	-	Yes	Yes	-
Black Sea	-	-	Yes	Yes	-
Boreal	-	-	?	Yes	-
Continental	-	-	Yes	Yes	-
Mediterranean	-	-	Yes	?	-
Pannonian	-	-	Yes	Yes	-
Steppic	-	-	?	?	-

Marine regions and subregions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Baltic Sea					
Black Sea					
North-east Atlantic Ocean					
Bay of Biscay and the Iberian Coast					
Celtic Sea					
Greater North Sea					
Mediterranean Sea					
Adriatic Sea					
Aegean-Levantine Sea					
Ionian Sea and the Central Mediterranean Sea					
Western Mediterranean Sea					

ANNEX I Scoring of Likelihoods of Events

(taken from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Description	Frequency
Very unlikely	This sort of event is theoretically possible, but is never known to have occurred and is not expected to occur	1 in 10,000 years
Unlikely	This sort of event has occurred somewhere at least once in the last millenium	1 in 1,000 years
Moderately likely	This sort of event has occurred somewhere at least once in the last century	1 in 100 years
Likely	This sort of event has happened on several occasions elsewhere, or on at least once in the last decade	1 in 10 years
Very likely	This sort of event happens continually and would be expected to occur	Once a year

ANNEX II Scoring of Magnitude of Impacts

(modified from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Biodiversity and ecosystem impact	Ecosystem Services impact	Economic impact (Monetary loss and response costs per year)	Social and human health impact, and other impacts
	<i>Question 5.1-5</i>	<i>Question 5.6-8</i>	<i>Question 5.9-13</i>	<i>Question 5.14-18</i>
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected ⁵	Up to 10,000 Euro	No social disruption. Local, mild, short-term reversible effects to individuals.
Minor	Some ecosystem impact, reversible changes, localised	Local and temporary, reversible effects to one or few services	10,000-100,000 Euro	Significant concern expressed at local level. Mild short-term reversible effects to identifiable groups, localised.
Moderate	Measureable long-term damage to populations and ecosystem, but reversible; little spread, no extinction	Measureable, temporary, local and reversible effects on one or several services	100,000-1,000,000 Euro	Temporary changes to normal activities at local level. Minor irreversible effects and/or larger numbers covered by reversible effects, localised.
Major	Long-term irreversible ecosystem change, spreading beyond local area	Local and irreversible or widespread and reversible effects on one / several services	1,000,000-10,000,000 Euro	Some permanent change of activity locally, concern expressed over wider area. Significant irreversible effects locally or reversible effects over large area.
Massive	Widespread, long-term population loss or extinction, affecting several species with serious ecosystem effects	Widespread and irreversible effects on one / several services	Above 10,000,000 Euro	Long-term social change, significant loss of employment, migration from affected area. Widespread, severe, long-term, irreversible health effects.

⁵ Not to be confused with “no impact”.

ANNEX III Scoring of Confidence Levels

(modified from Bacher et al. 2017)

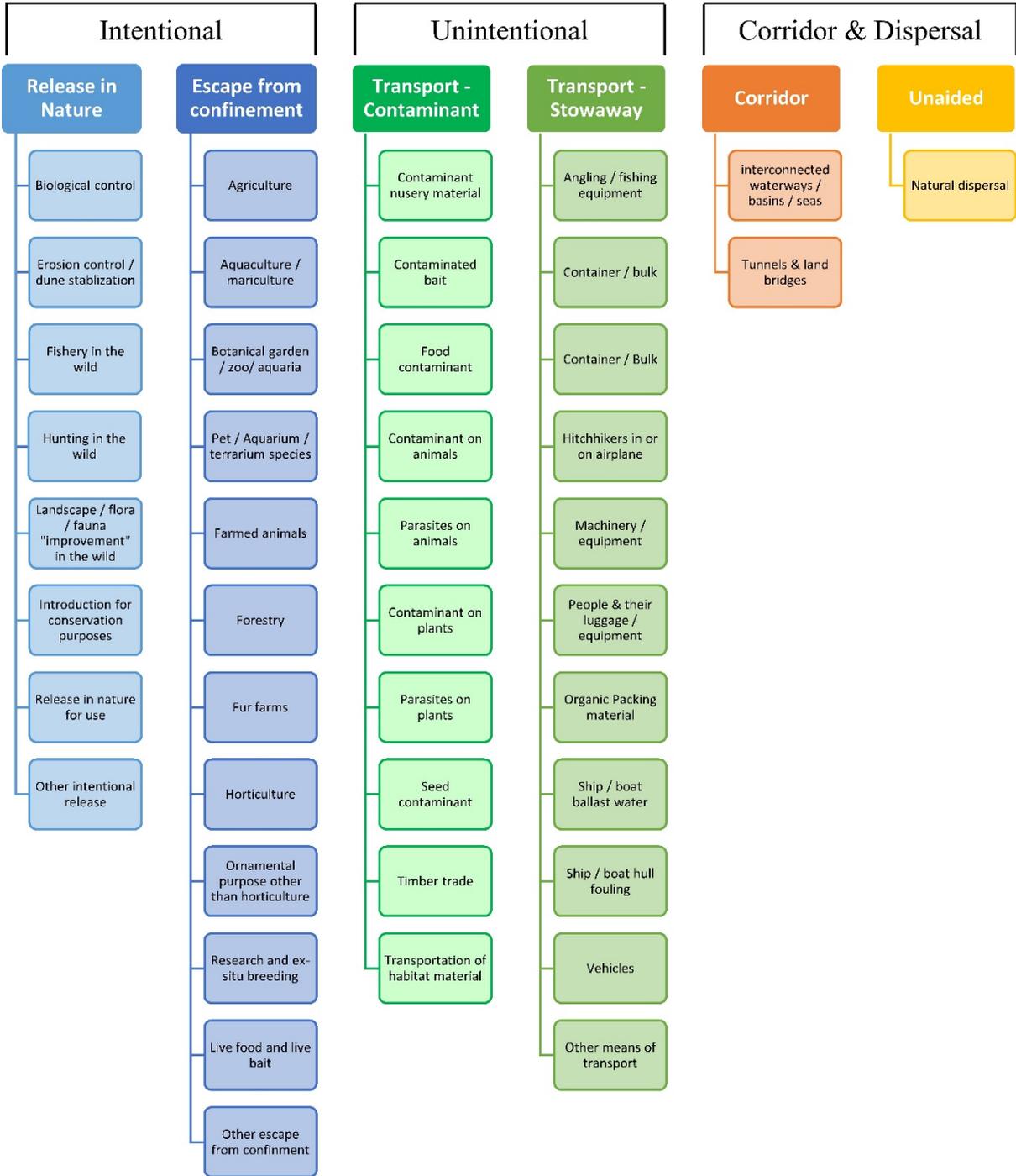
Each answer provided in the risk assessment must include an assessment of the level of confidence attached to that answer, reflecting the possibility that information needed for the answer is not available or is insufficient or available but conflicting.

The responses in the risk assessment should clearly support the choice of the confidence level.

Confidence level	Description
Low	There is no direct observational evidence to support the assessment, e.g. only inferred data have been used as supporting evidence <i>and/or</i> Impacts are recorded at a spatial scale which is unlikely to be relevant to the assessment area <i>and/or</i> Evidence is poor and difficult to interpret, e.g. because it is strongly ambiguous <i>and/or</i> The information sources are considered to be of low quality or contain information that is unreliable.
Medium	There is some direct observational evidence to support the assessment, but some information is inferred <i>and/or</i> Impacts are recorded at a small spatial scale, but rescaling of the data to relevant scales of the assessment area is considered reliable, or to embrace little uncertainty <i>and/or</i> The interpretation of the data is to some extent ambiguous or contradictory.
High	There is direct relevant observational evidence to support the assessment (including causality) <i>and</i> Impacts are recorded at a comparable scale <i>and/or</i> There are reliable/good quality data sources on impacts of the taxa <i>and</i> The interpretation of data/information is straightforward <i>and/or</i> Data/information are not controversial or contradictory.

ANNEX IV CBD pathway categorisation scheme

Overview of CBD pathway categorisation scheme showing how the 44 pathways relate to the six main pathway categories. All of the pathways can be broadly classified into 1) those that involve intentional transport (blue), 2) those in which the taxa are unintentionally transported (green) and 3) those where taxa moved between regions without direct transportation by humans and/or via artificial corridors (orange and yellow). **Note that the pathways in the category “Escape from confinement” can be considered intentional for the introduction into the risk assessment area and unintentional for the entry into the environment.**



ANNEX V Ecosystem services classification (CICES V5.1, simplified) and examples

For the purposes of this risk assessment, please feel free to use what seems as the most appropriate category / level / combination of impact (Section – Division – Group), reflecting information available.

Section	Division	Group	Examples (i.e. relevant CICES “classes”)
Provisioning	Biomass	Cultivated <i>terrestrial</i> plants	<p>Cultivated terrestrial plants (including fungi, algae) grown for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from cultivated plants, fungi, algae and bacteria for direct use or processing (excluding genetic materials); Cultivated plants (including fungi, algae) grown as a <u>source of energy</u></p> <p><i>Example: negative impacts of non-native organisms to crops, orchards, timber etc.</i></p>
		Cultivated <i>aquatic</i> plants	<p>Plants cultivated by in- situ aquaculture grown for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from in-situ aquaculture for direct use or processing (excluding genetic materials); Plants cultivated by in- situ aquaculture grown as an <u>energy source</u>.</p> <p><i>Example: negative impacts of non-native organisms to aquatic plants cultivated for nutrition, gardening etc. purposes.</i></p>
		Reared animals	<p>Animals reared for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from reared animals for direct use or processing (excluding genetic materials); Animals reared to provide <u>energy</u> (including mechanical)</p> <p><i>Example: negative impacts of non-native organisms to livestock</i></p>
		Reared <i>aquatic</i> animals	<p>Animals reared by in-situ aquaculture for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from animals grown by in-situ aquaculture for direct use or processing (excluding genetic materials); Animals reared by in-situ aquaculture as an <u>energy source</u></p> <p><i>Example: negative impacts of non-native organisms to fish farming</i></p>
		Wild plants (terrestrial and aquatic)	<p>Wild plants (terrestrial and aquatic, including fungi, algae) used for <u>nutrition</u>; <u>Fibres and other materials</u> from wild plants for direct use or processing (excluding genetic materials); Wild plants (terrestrial and aquatic, including fungi, algae) used as a <u>source of energy</u></p> <p><i>Example: reduction in the availability of wild plants (e.g. wild berries, ornamentals) due to non-native organisms (competition, spread of disease etc.)</i></p>
		Wild animals (terrestrial and aquatic)	<p>Wild animals (terrestrial and aquatic) used for <u>nutritional purposes</u>; <u>Fibres and other materials</u> from wild animals for direct use or processing (excluding genetic materials); Wild animals (terrestrial and aquatic) used as a <u>source of energy</u></p>

			<i>Example: reduction in the availability of wild animals (e.g. fish stocks, game) due to non-native organisms (competition, predations, spread of disease etc.)</i>
	Genetic material from all biota	Genetic material from plants, algae or fungi	<u>Seeds, spores and other plant materials</u> collected for maintaining or establishing a population; Higher and lower plants (whole organisms) used to <u>breed new strains or varieties</u> ; Individual genes extracted from higher and lower plants for the <u>design and construction of new biological entities</u> <i>Example: negative impacts of non-native organisms due to interbreeding</i>
		Genetic material from animals	Animal material collected for the purposes of maintaining or establishing a population; Wild animals (whole organisms) used to breed new strains or varieties; Individual genes extracted from organisms for the design and construction of new biological entities <i>Example: negative impacts of non-native organisms due to interbreeding</i>
	Water ⁶	Surface water used for nutrition, materials or energy	Surface water for <u>drinking</u> ; Surface water used as a material (<u>non-drinking purposes</u>); Freshwater surface water, coastal and marine water used as an <u>energy source</u> <i>Example: loss of access to surface water due to spread of non-native organisms</i>
		Ground water for used for nutrition, materials or energy	Ground (and subsurface) water for <u>drinking</u> ; Ground water (and subsurface) used as a material (<u>non-drinking purposes</u>); Ground water (and subsurface) used as an <u>energy source</u> <i>Example: reduced availability of ground water due to spread of non-native organisms and associated increase of ground water consumption by vegetation.</i>
Regulation & Maintenance	Transformation of biochemical or physical inputs to ecosystems	Mediation of wastes or toxic substances of anthropogenic origin by living processes	<u>Bio-remediation</u> by micro-organisms, algae, plants, and animals; <u>Filtration/sequestration/storage/accumulation</u> by micro-organisms, algae, plants, and animals <i>Example: changes caused by non-native organisms to ecosystem functioning and ability to filtrate etc. waste or toxics</i>
		Mediation of nuisances of anthropogenic origin	<u>Smell reduction</u> ; <u>noise attenuation</u> ; <u>visual screening</u> (e.g. by means of green infrastructure) <i>Example: changes caused by non-native organisms to ecosystem structure, leading to reduced ability to mediate nuisances.</i>
	Regulation of physical, chemical, biological conditions	Baseline flows and extreme event regulation	Control of <u>erosion</u> rates; Buffering and attenuation of <u>mass movement</u> ; <u>Hydrological cycle and water flow regulation</u> (Including flood control, and coastal protection); <u>Wind</u> protection; <u>Fire</u> protection

⁶ Note: in the CICES classification provisioning of water is considered as an abiotic service whereas the rest of ecosystem services listed here are considered biotic.

			<p><i>Example: changes caused by non-native organisms to ecosystem functioning or structure leading to, for example, destabilisation of soil, increased risk or intensity of wild fires etc.</i></p>
		<p>Lifecycle maintenance, habitat and gene pool protection</p>	<p>Pollination (or 'gamete' dispersal in a marine context); <u>Seed dispersal</u>; Maintaining <u>nursery populations and habitats</u> (Including gene pool protection)</p> <p><i>Example: changes caused by non-native organisms to the abundance and/or distribution of wild pollinators; changes to the availability / quality of nursery habitats for fisheries</i></p>
		<p>Pest and disease control</p>	<p>Pest control; Disease control</p> <p><i>Example: changes caused by non-native organisms to the abundance and/or distribution of pests</i></p>
		<p>Soil quality regulation</p>	<p><u>Weathering processes</u> and their effect on soil quality; <u>Decomposition and fixing processes</u> and their effect on soil quality</p> <p><i>Example: changes caused by non-native organisms to vegetation structure and/or soil fauna leading to reduced soil quality</i></p>
		<p>Water conditions</p>	<p>Regulation of the <u>chemical condition</u> of freshwaters by living processes; Regulation of the chemical condition of salt waters by living processes</p> <p><i>Example: changes caused by non-native organisms to buffer strips along water courses that remove nutrients in runoff and/or fish communities that regulate the resilience and resistance of water bodies to eutrophication</i></p>
		<p>Atmospheric composition and conditions</p>	<p>Regulation of <u>chemical composition</u> of atmosphere and oceans; Regulation of <u>temperature and humidity</u>, including ventilation and transpiration</p> <p><i>Example: changes caused by non-native organisms to ecosystems' ability to sequester carbon and/or evaporative cooling (e.g. by urban trees)</i></p>
Cultural	Direct, in-situ and outdoor interactions with living systems that depend on presence in the environmental setting	<p>Physical and experiential interactions with natural environment</p>	<p>Characteristics of living systems that that enable activities promoting health, recuperation or enjoyment through <u>active or immersive interactions</u>;</p> <p>Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through <u>passive or observational interactions</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that make it attractive for recreation, wild life watching etc.</i></p>
		<p>Intellectual and representative interactions with natural environment</p>	<p>Characteristics of living systems that enable <u>scientific investigation</u> or the creation of traditional ecological knowledge;</p> <p>Characteristics of living systems that enable <u>education and training</u>;</p> <p>Characteristics of living systems that are resonant in terms of <u>culture or heritage</u>;</p> <p>Characteristics of living systems that enable <u>aesthetic experiences</u></p>

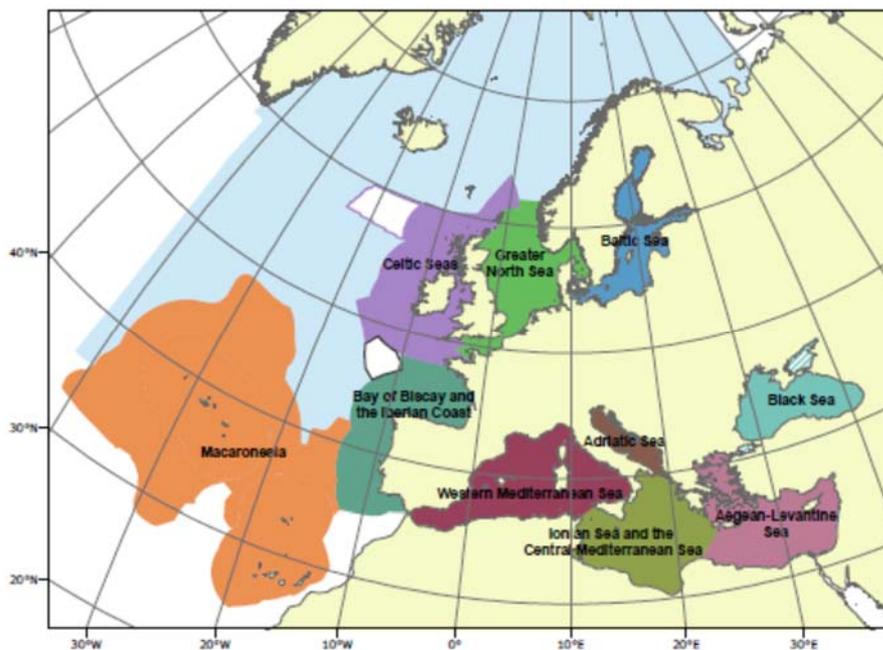
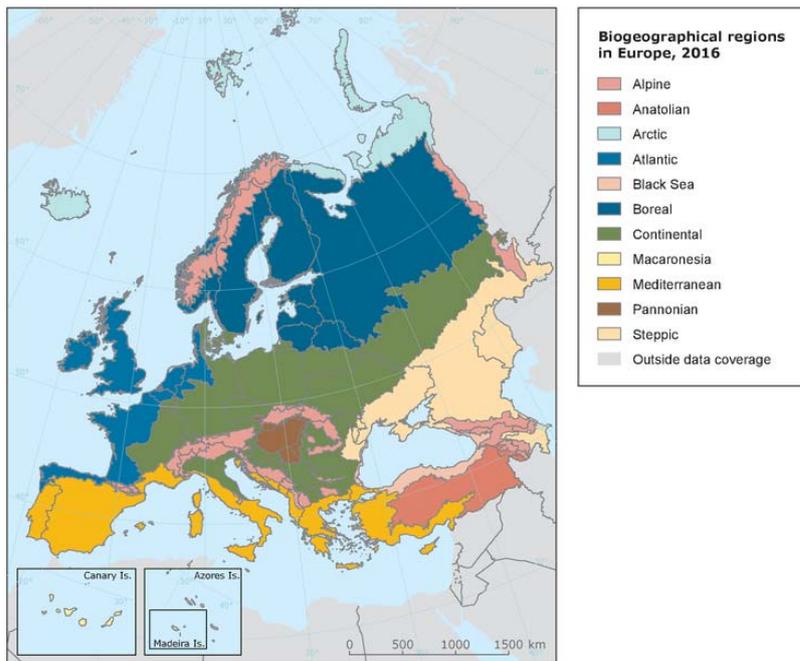
			<i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have cultural importance</i>
	Indirect, remote, often indoor interactions with living systems that do not require presence in the environmental setting	Spiritual, symbolic and other interactions with natural environment	<p>Elements of living systems that have <u>symbolic meaning</u>;</p> <p>Elements of living systems that have <u>sacred or religious meaning</u>;</p> <p>Elements of living systems used for <u>entertainment or representation</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have sacred or religious meaning</i></p>
		Other biotic characteristics that have a non-use value	<p>Characteristics or features of living systems that have an <u>existence value</u>;</p> <p>Characteristics or features of living systems that have an <u>option or bequest value</u></p> <p><i>Example: changes caused by non-native organisms to ecosystems designated as wilderness areas, habitats of endangered species etc.</i></p>

ANNEX VI EU Biogeographic Regions and MSFD Subregions

See <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2> ,
http://ec.europa.eu/environment/nature/natura2000/biogeog_regions/

and

<https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions-1/technical-document/pdf>



ANNEX VII Delegated Regulation (EU) 2018/968 of 30 April 2018

see <https://eur-lex.europa.eu/legal-content/en/TXT/?uri=CELEX%3A32018R0968>

ANNEX VIII Modelling the potential geographic distribution of the Asian giant hornet *Vespa mandarinia* using CLIMEX

M. Lukas Seehausen & Marc Kenis

Methods

About CLIMEX

The bioclimatic models were developed using CLIMEX 4.1 (Kriticos et al. 2016) that integrates the weekly responses of a population to climate using a series of annual indices. The bioclimatic modelling process has been previously described in numerous publications (Vera et al. 2002; Sutherst and Maywald 2005; Poutsma et al. 2008; Olfert et al. 2012), and thus, only a brief description of the CLIMEX program and parameters is provided here. CLIMEX uses an annual Growth Index (GI) to describe the potential for population growth as a function of temperature and soil moisture during favorable conditions, and stress indices (cold, wet, hot, dry) to determine the effect of abiotic stress on survival in unfavorable conditions. The weekly GI is a function of temperature (TI), diapause (DI) and moisture (MI). The growth and stress indices are calculated weekly and then combined into an overall annual index of climatic suitability, the Ecoclimatic Index (EI), which ranges from EI = 0 for locations where the species is not able to persist to EI = 100 for locations that are optimal for the species. However, in temperate climates, the maximum EI value is rather close to 50 and values of >20 are usually considered sufficient to support substantial population densities (Sutherst et al. 2007).

Fitting CLIMEX parameters

Very little is known about the ecoclimatic responses of *V. mandarinia*. Therefore, initial model parameter values were based on the slightly modified values for *V. germanica*, published for the congeneric Asian hornet *Vespa velutina* by Ibáñez-Justicia and Loomans (2011) and Park and Jung (2016). However, because *V. mandarinia* is clearly more cold-tolerant, as especially indicated by its northern distribution in Russia (Siberian Zoological Museum), we further modified the values, especially of the cold stress parameters, until the simulated potential geographical distribution indicated by the Ecoclimatic Index (EI) agreed with the currently known native distribution of *V. mandarinia*.

The CliMond10 climate data set was used as input into the model to predict the native Asian and the potential distribution in Europe and North America (Kriticos et al. 2012). The CliMond data set was developed for species bioclimatic modelling, including both correlative and process-based mechanistic models. The 10' gridded data set includes a hybrid historical data set (based on CRU CL2.0 and WorldClim; centered on 1975). A climate data set with a lower resolution, 30' gridded data, but including more recent data, centered on 1995, was used for modeling the potential distribution and stresses at the global scale. The potential distribution of *V. mandarinia* in 2080 as predicted by the Ecoclimatic Index was modelled using the A1B scenario included in the CLIMEX software.

The CLIMEX parameter values for the present models were calibrated as follows: The limiting low and lower optimal soil moisture were almost the same as for the *V. germanica* models, allowing development above the permanent wilting point. However, upper optimal and limiting high soil moisture were decreased, reflecting the fact that hornet activity is decreased at heavy rainfalls (Rodríguez-Flores et al. 2018). Wet stress starting at 200% (index=2.0) water holding capacity (i.e., significant water run-off) with a relatively steep increase also reflects this fact, while allowing for heavy rain falls that the hornet may experiences in southern Asia. Dry stress parameters were kept the same as specified for the *V. germanica* model.

Observations of *V. velutina*'s colonies activity in France indicated that no activity outside the nest takes place at air temperatures <10°C (Perrard et al. 2009). However, to include *V. mandarinia*'s presence in

south-eastern Russia and northern Japan, the limiting low temperature and also the day-degree threshold temperature for the accumulation of cold stress was decreased to 8°C. The lower optimal temperature was kept as for the *V. germanica* model, however, the upper optimal temperature and the limiting high temperature for development were slightly increased (by 2°C), allowing for the hornet's southern distribution limits and reflecting the fact that this species rather nests in concealed places, such as in the ground at a depth of 6-60cm, e.g. in small mammal burrows or decayed root spaces, or in hollow trees (Matsuura & Sakagami 1973; Archer 1995). The limiting high temperature for development is relatively difficult to measure for hornets, because they are known to actively adjust air temperature in nests through behaviors such as ventilation, regurgitation of water, and wing vibration (Perrard et al. 2009). Therefore, values for limiting high temperatures and heat stress were adjusted in an iterative manner until the native distribution was accurately predicted by the model. However, the resulting values are similar to those previously published for *Vespa velutina* (Ibáñez-Justicia and Loomans 2011; Park and Jung 2016).

The cold stress threshold temperature and its rate of increase were estimated based on the information that mated queens overwinter protected in special cavities excavated into moderately moist soil. The entrance of the cavity is plugged and the queen passes the winter hanging from the top of the chamber (Matsuura and Sakagami 1973; Archer 1995). Thus, the hornet can withstand temperatures well below the freezing point and would only be affected by cold stress if the soil under the snow freezes deep enough. However, once the temperature falls below a critical threshold, queens are expected to freeze to death rather quickly. Like for heat stress, the values for the cold stress threshold and its rate were adjusted in an iterative manner until the native distribution was accurately predicted by the model. The resulting cold stress threshold was -30°C with a rate of -0.0007. It has to be understood that this value is a threshold value for air temperature, and does not reflect soil temperatures under the snow.

Degree-days per generation are not used in this model because like other hornets, foundresses of *V. mandarinia* produce offspring continuously over the season without discrete generations.

Results

The resulting parameters (Table 1) lead to an accurately predicted native distribution (Figure A1). The global distribution of the Ecoclimatic Index indicating the potential distribution of *V. mandarinia*, as well as the heat stress, cold stress, and dry stress that are limiting its potential distribution are shown in Figure A2. Figure A3 represents the potential distribution of *V. mandarinia* in Europe and northern Africa as predicted by the Ecoclimatic Index from the CLIMEX model and Figure A4 shows the potential distribution of *V. mandarinia* in the same area in 2080 using the A1B scenario.

Results are discussed in the risk assessment.

Table 1: CLIMEX model parameters used to predict the distribution of *Vespa mandarinia* in non-native areas around the world.

Group	Parameter	Description	Value
Moisture Index	SMO	Limiting low soil moisture	0.15
	SM1	Lower optimal soil moisture	0.6
	SM2	Upper optimal soil moisture	1
	SM3	Limiting high soil moisture	2
Temperature Index	DV0	Limiting low temperature	8°C
	DV1	Lower optimal temperature	18°C
	DV2	Upper optimal temperature	28°C
	DV3	Limiting high temperature	34°C
Cold Stress	TTCS	Cold stress threshold	-30°C
	THCS	Cold stress temperature rate	-0.0007
	DTCS	Cold stress degree-day threshold	8°C
	DHCS	Cold stress degree-day rate	-0.00001
	TTCSA	Average cold stress degree-day threshold	8
	THCSA		0
Heat Stress	TTHS	Heat stress temperature threshold	34°C
	THHS	Heat stress temperature rate	0.003
	DTHS		0
	DHHS		0
Dry Stress	SMDS	Dry stress threshold	0.15
	HDS	Dry stress rate	-0.008
Wet Stress	SMWS	Wet stress threshold	2.0
	HWS	Wet stress rate	0.002
Day-degree accumulation above DV0	DV0	Limiting low temperature	8°C
	DV3	Limiting high temperature	34°C
	MTS	Model time step	7
Day-degree accumulation above DVCS	DVCS	Cold stress DD threshold temperature	8°C
	DV4	Dummy parameter	100
	MTS	Model time step	7
Day-degree accumulation above DVHS	DVHS	Heat stress DD threshold temperature	34°C
	DV4	Dummy parameter	100
	MTS	Model time step	7
Degree-days per generation	PDD		0

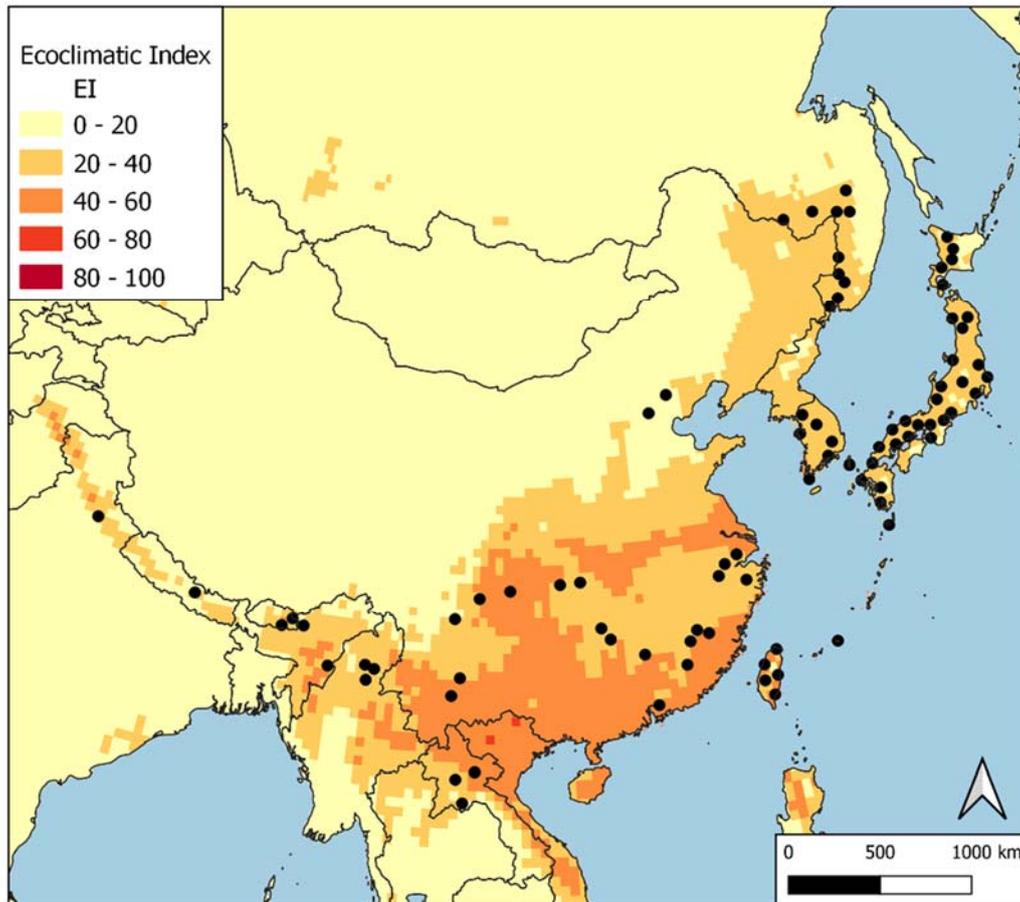


Figure A1: Ecoclimatic Index (EI) from the CLIMEX model for *Vespa mandarinia* in Asia. Black points represent the known native distribution of *V. mandarinia* in Asia from the Global Biodiversity Information Facility (GBIF) and the literature. Locations with $EI > 20$ can be considered as climatically suitable for establishment and growth of *V. mandarinia*

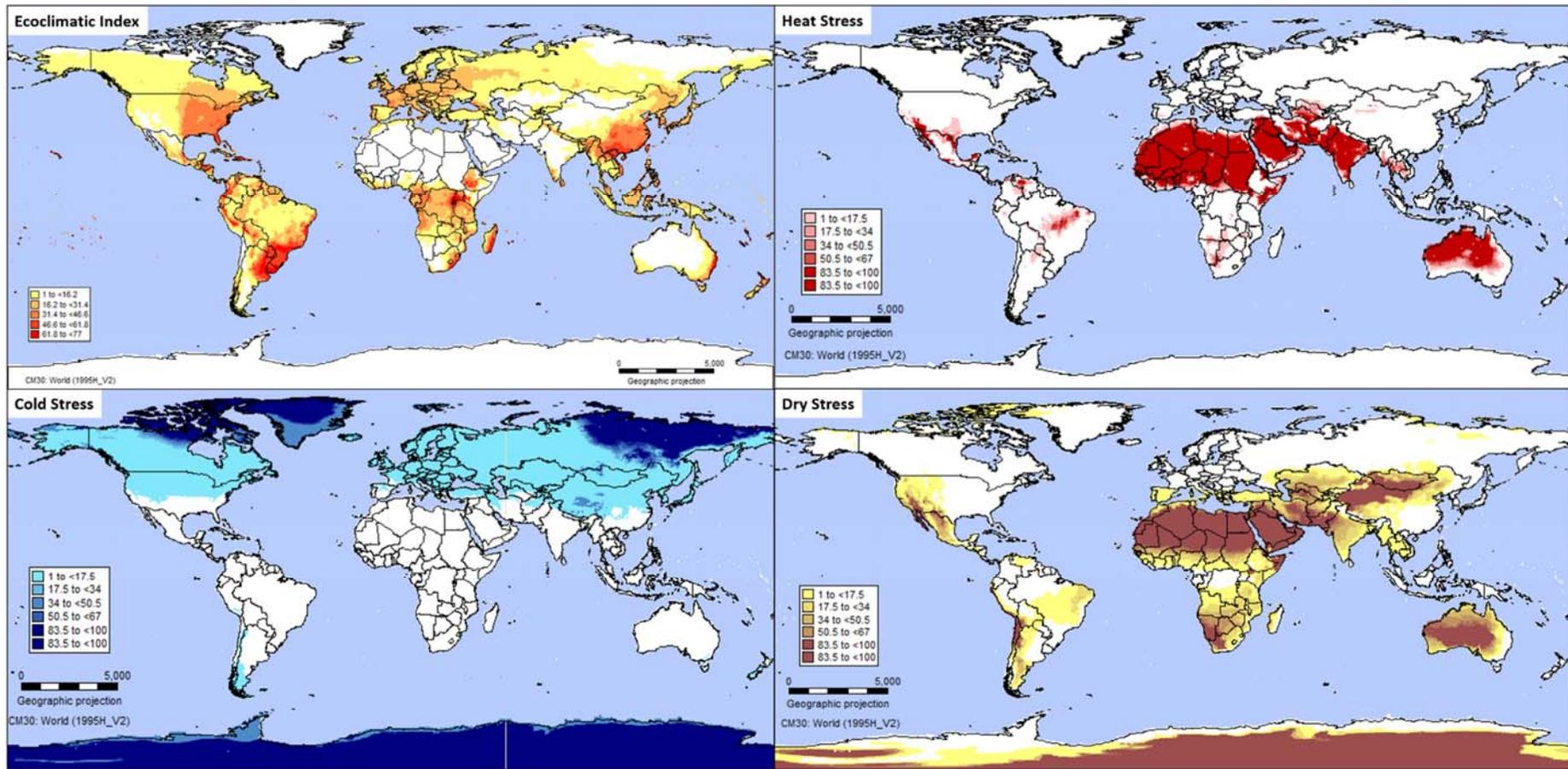


Figure A2: Global distribution of the Ecoclimatic Index (EI), heat stress, cold stress, and dry stress as predicted by the CLIMEX model for *Vespa mandarinia*.

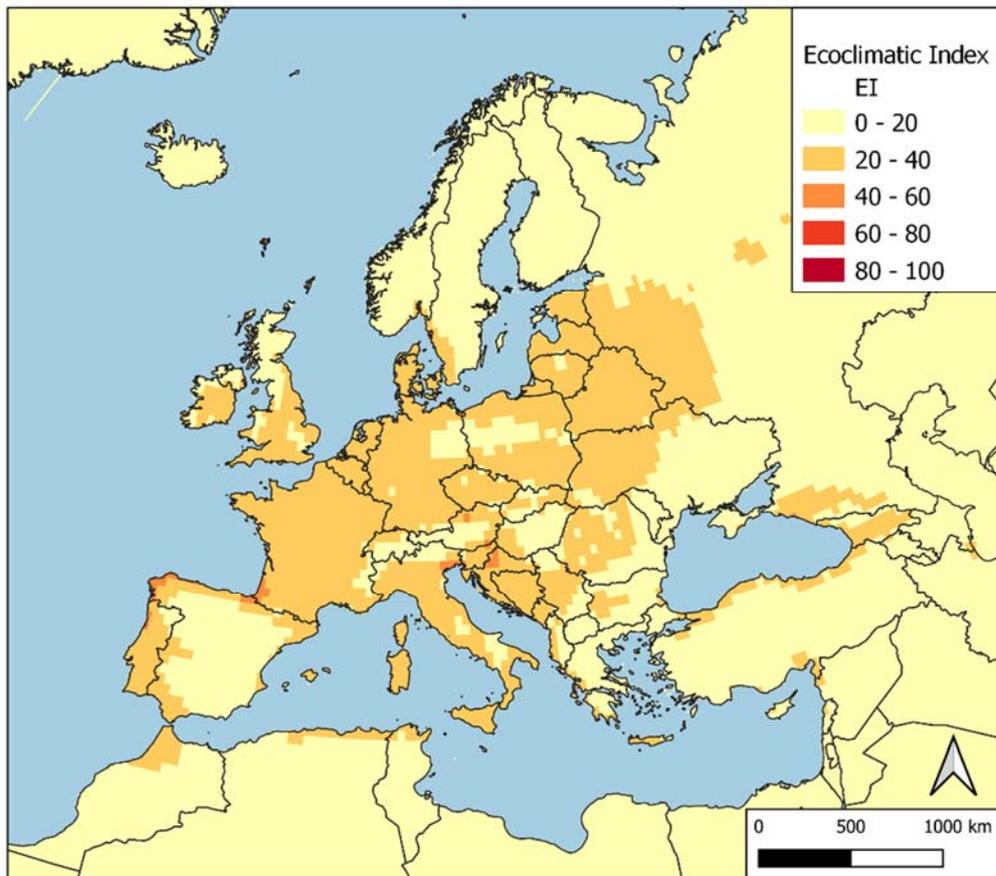


Figure A3: Potential distribution of *V. mandarinia* in Europe and northern Africa as predicted by the Ecoclimatic Index (EI) from the CLIMEX model. Locations with $EI > 20$ can be considered as climatically suitable for establishment and growth of *V. mandarinia*.

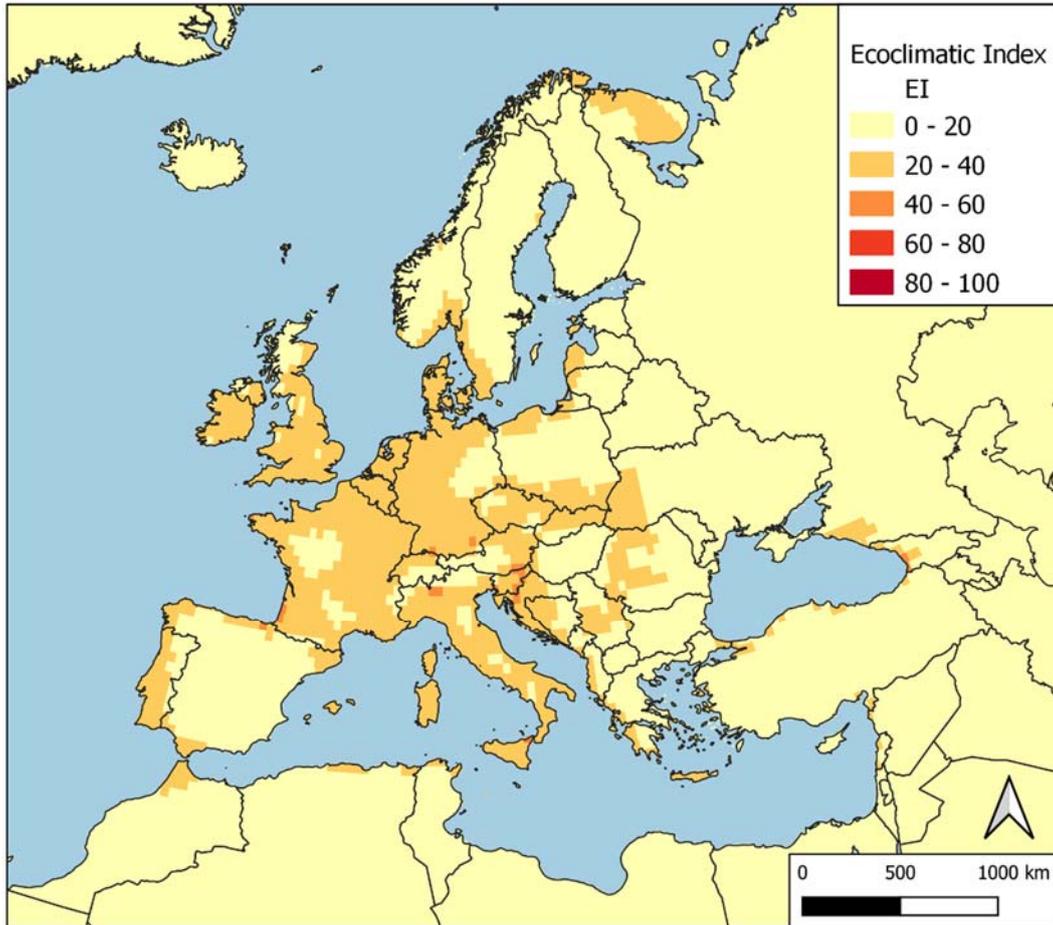


Figure A4: Potential distribution of *V. mandarinia* in Europe and northern Africa in 2080, as predicted by the Ecoclimatic Index (EI) from the CLIMEX model using the A1B scenario. Locations with $EI > 20$ can be considered as climatically suitable for establishment and growth of *V. mandarinia*.

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Risk assessment template developed under the "Study on Invasive Alien Species – Development of risk assessments to tackle priority species and enhance prevention" Contract No 07.0202/2017/763379/ETU/ENV.D.2¹

Name of organism: *Xenopus laevis* (Daudin, 1802)

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Risk Assessment Area: The risk assessment area is the territory of the European Union (excluding the outermost regions) and of the United Kingdom.

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Date of completion of first draft: 25/10/2018

Date of completion of revised version following comments from Scientific Forum and stakeholders: 04/03/2021

¹ This template is based on the Great Britain non-native species risk assessment scheme (GBNNRA).

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RISK SUMMARIES			
	RESPONSE	CONFIDENCE²	COMMENT
Summarise Entry³	very likely	high	The species is already present in the risk assessment area via two main pathways (pet trade and research) which are still active. Facilities hosting captive populations are already present in several countries, hence the risk of entry is very high.
Summarise Establishment⁴	very likely	high	The species is already successfully established in the risk assessment area. There is also evidence that suitable conditions for the species are present in other countries where there are not yet established populations.
Summarise Spread⁵	rapidly	medium	Many studies have shown that the species may rapidly disperse by natural means (i.e. through ecological corridors) in the risk assessment area, facilitated by both the occurring environmental conditions and the species' intrinsic dispersal capacities.
Summarise Impact⁶	moderate	medium	<i>Xenopus laevis</i> can affect native species by competition and predation. In particular there is substantial evidence that it predated on amphibians (either eggs, tadpoles and adults), fish, and several

² In a scale of low / medium / high, see Annex II

³ In a scale of very unlikely / unlikely / moderately likely / likely / very likely, see Annex I

⁴ In a scale of very unlikely / unlikely / moderately likely / likely / very likely, see Annex I

⁵ In a scale of very slowly / slowly / moderately / rapidly / very rapidly

⁶ In a scale of minimal / minor / moderate / major / massive, see Annex II

			groups of invertebrates, (particularly on macro-invertebrate communities), although there is no conclusive evidence of impact on their populations. Furthermore, there is evidence of <i>Xenopus laevis</i> potentially functioning as a reservoir for <i>Bd</i> and other pathogens. However, to date there is no evidence that <i>Xenopus laevis</i> has caused impact on native amphibians through pathogen transmission.
Conclusion of the risk assessment⁷	moderate	medium	The species is known to be invasive in the risk assessment area. Further releases, escapes (or spread) may occur in areas which are not yet colonised, leading to the successful establishment of new populations, hence increasing the overall impact associated with the occurrence of <i>Xenopus laevis</i> in the wild.

⁷ In a scale of low / moderate / high

Distribution Summary:

The columns refer to the answers to Questions A6 to A12 under Section A. See also Annex VI.

The answers in the tables below indicate the following:

- Yes recorded, established or invasive
- not recorded, established or invasive
- ? Unknown; data deficient

Member States and the United Kingdom

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Austria				Yes	
Belgium	Yes		Yes	Yes	
Bulgaria				Yes	
Croatia				Yes	
Cyprus				Yes	
Czech Republic				Yes	
Denmark			Yes	Yes	
Estonia				Yes	
Finland				Yes	
France	Yes	Yes	Yes	Yes	Yes
Germany	Yes		Yes	Yes	
Greece			Yes	Yes	
Hungary				Yes	
Ireland			Yes	Yes	
Italy	Yes	Yes	Yes	Yes	Yes
Latvia				Yes	
Lithuania				Yes	

Luxembourg				Yes	
Malta				Yes	
Netherlands	Yes		Yes	Yes	
Poland				Yes	
Portugal	Yes	Yes	Yes	Yes	Yes
Romania				Yes	
Slovakia				Yes	
Slovenia				Yes	
Spain	Yes		Yes	Yes	
Sweden	Yes		Yes	Yes	
United Kingdom	Yes	?	Yes	Yes	

Biogeographical regions of the risk assessment area

	Recorded	Established (currently)	Possible establishment (under current climate)	Possible establishment (under foreseeable climate)	Invasive (currently)
Alpine			Yes	Yes	
Atlantic	Yes	Yes	Yes	Yes	Yes
Black Sea				Yes	
Boreal				?	
Continental			Yes	Yes	
Mediterranean	Yes	Yes	Yes	Yes	Yes
Pannonian				Yes	
Steppic				Yes	

SECTION A – Organism Information and Screening	
Organism Information	RESPONSE
<p>A1. Identify the organism. Is it clearly a single taxonomic entity and can it be adequately distinguished from other entities of the same rank?</p>	<p>This risk assessment covers only one species, <i>Xenopus laevis</i> (Daudin, 1802), the African Clawed Frog or Common Platanna (Class: Amphibia; Order: Anura; Family: Pipidae; Genus: <i>Xenopus</i>).</p> <p>The African Clawed Frog is also known as Platanna, Common Platanna, Common Clawed Frog, Clawed Toad, Clawed Frog, Upland Clawed Frog, Smooth Clawed Frog, African Clawed Toad, Upland Clawed Frog, Common Clawed Frog, Common Clawed Toad, African Clawed Frog (Frost 2018).</p> <p><i>X. laevis</i> belongs to a genus that comprises at least 29 species, half of which occur in Central Africa (Evans et al. 2015). According to Evans et al. (2015) although <i>Xenopus</i> is easily distinguished from other frog genera, discriminating the relevant species based solely on morphological characters can be difficult.</p> <p>Distinguishing <i>X. laevis</i> from other species of the same genus is usually no problem where they occur, as no other species is as large as <i>X. laevis</i>. Otherwise, some difficulties may be faced in the case of hybrids (John Measey pers. comm. 2018). As summarised by Measey (2016) <i>X. laevis</i> has undergone significant taxonomic revision following a comprehensive molecular study by Furman et al. (2015). The result of this revision is that what was previously known as <i>X. l. laevis</i> is now known as <i>X. laevis</i> with all other subspecies being recognised as full species, and some newly described species included as well (Evans et al., 2015).</p> <p>In fact, as reported by Furman et al. (2015) within <i>X. laevis</i> sensu lato, the analyses show at least four lineages: <i>X. laevis</i> (southern Africa, including Malawi and South Africa), <i>X. poweri</i> (Central Africa, including Nigeria, Cameroon, Zambia, and Botswana), <i>X. petersii</i> (West Central Africa, including the Republic of Congo, western DRC, and Angola) and <i>X. victorianus</i></p>

(East Africa, including Kenya, Uganda, Rwanda, Burundi, eastern DRC, and Tanzania). The data potentially support the transfer of *X. l. sudanensis* to the synonymy of *X. poweri* (instead of *X. laevis*), while *X. l. bunyoniensis* should be tentatively considered a synonym of *X. victorianus*.

Reciprocal crosses between individuals of *X. laevis* sensu lato (that were probably from South Africa), and individuals from Uganda or Botswana, both produced fertile offspring, thus gene flow between these species is possible (Furman et al. 2015). *X. laevis* is also known to hybridise with *Xenopus gilli*. The hybrids of these species pose no intrinsic invasive threat, except for the conservation of the latter species, which is also affected by predation and competition by *X. laevis* (Measey et al. 2017). Hybrids are also known in the wild, in hybrid zones, with *X. poweri* (conjecture) and *X. muelleri* (Fischer et al 2000). However, since we cannot exclude the possibility that hybrids are present in trade and/or in the populations established in the wild, this risk assessment should apply to all *X. laevis* hybrids as well. This is justified by the fact that while some physiological features may be different, the overall impact would be the same. As a remark, this assessment is for *X. laevis*, but unless otherwise stated, all statements apply to any hybrids as well as albino *X. laevis* (albino individuals belong exactly to the same *X. laevis* species).

Here follows a list of the most common synonym names of *X. laevis* according to Frost (2018):

- *Bufo laevis* Daudin, 1802
- *Dactylethera boiei* (Wagler, 1827)
- *Dactylethra bufonia* (Merrem, 1820)
- *Dactylethra capensis* Cuvier, 1830
- *Dactylethra delalandii* Cuvier, 1849
- *Dactylethra laevis* (Daudin, 1802)
- *Engystoma laevis* (Daudin, 1802)
- *Leptopus boiei* (Wagler, 1827)
- *Leptopus oxydactylus* Mayer, 1835
- *Pipa africana* Mayer, 1835
- *Pipa bufonia* Merrem, 1820

	<ul style="list-style-type: none"> • <i>Pipa laevis</i> (Daudin, 1802) • <i>Tremeropugus typicus</i> Smith, 1831 • <i>Xenopus boiei</i> Wagler, 1827 • <i>X. laevis</i> ssp. <i>bunyoniensis</i> Loveridge, 1932 • <i>X. laevis</i> ssp. <i>sudanensis</i> Perret, 1966
<p>A2. Provide information on the existence of other species that look very similar [that may be detected in the risk assessment area, either in the wild, in confinement or associated with a pathway of introduction]</p>	<p><i>X. laevis</i> is not difficult to distinguish from other anurans occurring in Europe (either native or alien ones). In general, the body of <i>X. laevis</i> has a flattened shape. Adult males measure around 90 mm in males, and females 100 mm, although larger individuals are known (John Measey pers. comm. 2018). The skin is smooth and slippery, with peculiar lateral lines along its sides. The eyes are positioned at the top of a small head, which lacks a tongue and eardrums. The hind legs are very developed and webbed (with black claws on the first three toes), while the front limbs are rather small. Colour varies from yellowish to olive grey or dark brown with spots (but albino forms are also common in trade). Tadpoles are easily distinguished from other (native) anurans, particularly because of their distinctive barbells next to the mouth, mid-water suspension feeding and often near transparent, especially when small.</p> <p>Other <i>Xenopus</i> species may be found in the trade. Examples are <i>X. tropicalis</i> and <i>X. epitropicalis</i>, which Tinsley & McCoid (1996) considered regularly imported to Europe with tropical fish from West Africa (although the source seems a bit outdated in this context). However, there is no evidence about the occurrence of such species in the wild in the risk assessment area.</p>
<p>A3. Does a relevant earlier risk assessment exist? (give details of any previous risk assessment and its validity in relation to the risk assessment area)</p>	<p>Some risk assessments exist for the species, i.e. for Great Britain, USA, and Australia.</p> <p>In the risk assessment for Great Britain the risk attributed to the species is low, with a medium level of uncertainty (NNSS 2011). According to this assessment “<i>X. laevis</i> was having very minimal impacts in the UK, given the very few populations occurring in the wild” (which in fact are considered currently extinct, see details below), and the reduced ability to reproduce and further spread in the country (even if climate change would facilitate this). The only concern would be the unknown impact related to the possible spread of diseases (including the chytrid fungus) to native amphibians, and of possible unforeseen scenarios due to future climate</p>

changes. However the conclusions of the GB risk assessment cannot be extended to other EU regions, given the different climate and habitat conditions, which are definitely more suitable for the species, e.g. in the Mediterranean area.

In the USA an Ecological Risk Screening Summary was made by the U.S. Fish and Wildlife Service (Anonymous 2017). The overall risk assessment category attributed to the species is “high”, as the climate matched well in many states, and the certainty of the assessment was deemed medium (given the taxonomic uncertainties noted for the target species). Similarly, in Australia *X. laevis* has been assigned an establishment risk rank of “extreme” (Page et al. 2008). Since these assessments focus on the occurrence of the species in the US and Australia, their validity is limited by the difference in ecological, geographical and climatic conditions compared to the EU situation. Moreover, in the case of the US, the assessment should be revised in the light of the noted taxonomic uncertainties that are now resolved (John Measey pers. comm. 2018).

A number of studies aimed at ranking the impact of amphibians were also carried out at either the global level (e.g. Kumschick et al. 2017a, Kumschick et al. 2017b, Kraus 2015, Measey et al. 2016, Measey et al. 2020) or EU level (e.g. Kopecký et al. 2016). For example, Kumschick et al. (2017b) discussed the application of the Environmental Impact Classification for Alien Taxa (EICAT, see Hawkins et al. 2015 for details on the methodology) on amphibians following two independent assessments made by Kraus (2015) and Kumschick et al. (2017a). The results showed that the impact classification is relatively “high” for *X. laevis*, despite some minor difference between the two assessments: respectively Massive (MV, irreversible community-level changes) and Major (MR, impact on a native community that is reversible). This difference seems justified by the practical interpretation and assignment of disease impacts in the absence of direct evidence of transmission from alien to native species, especially in relation to chytridiomycosis. The study was recently repeated (Measey et al. 2020) and the impact score for *Xenopus laevis* was confirmed as Major, while the confidence score changed from medium to high. It is also worth mentioning that the SEICAT assessment found little documented evidence for socio-economic impacts, except in the species native range where it can be a predator in aquaculture (Bacher et al. 2018).

	<p>Measey et al. (2016) used the generic impact scoring system (GISS) to carry out a global assessment of alien amphibians. In particular <i>X. laevis</i> was the second top scoring amphibian for impact on native ecosystems (considering the sum for environmental and economic scores together) only after the invasive Cane toad <i>Rhinella marina</i>.</p> <p>Finally, it is worth mentioning Kopecký et al. (2016) who applied a risk assessment model (RAM) to ornamental amphibians traded in the EU. <i>X. laevis</i> was used as a reference species (together with <i>L. catesbeianus</i>) and was considered to have a moderate risk (the RAM value is 0.365), with an AmphISK invasion score of 10 (on a scale -10 to 33). This system however does not provide overwhelming evidence of risk, because as pointed out by the authors the RAM establishment value cannot be viewed as a precise estimation of the probability of establishment, but rather provides a relative ranking of ornamental amphibians traded in the EU.</p> <p>As a general remark regarding the scoring of the impact discussed above, it is important to consider that the categories used by this risk assessment (see Annex II) are different to those used by EICAT (according to which a major impact is reversible, contrary to the definition in Annex II which considers a major impact as irreversible). For example, as confirmed by John Measey (pers. comm. 2018) on the data used for this assessment made by Kumschick et al. (2017b), <i>X. laevis</i> was scored Major (within the EICAT scheme) on the basis of two studies (i.e. Lillo et al 2011, Grosselet et al 2005) both regarding predation, which suggest local extinction of native species (both were given medium confidence). However, the same impact under this risk assessment is considered as Moderate according to guidance in Annex II.</p>
<p>A4. Where is the organism native?</p>	<p>The full range of <i>X. laevis</i> covers much of southern Africa: South Africa, Lesotho, Swaziland, Namibia, parts of Botswana, Zimbabwe, parts of Mozambique and extending north into Malawi (Measey 2016, Ihlow et al. 2016).</p> <p>It means that native populations are distributed from winter rainfall regions in the south-western Cape region to summer rainfall regions in the north; and from sea level to 3,000 m in Lesotho (Measey 2004, De Busschere et al. 2016). As summarised by the Global Invasive Species Database (2015) <i>X. laevis</i> is a water-dependent species occurring in a very wide range of</p>

	<p>habitats, including heavily modified anthropogenic habitats. It lives in all sorts of water bodies, including streams, but tends to avoid large rivers, and water bodies with predatory fish. It reaches its highest densities in eutrophic water. It has very high reproductive potential. It is a highly opportunistic species, and colonizes newly created, apparently isolated, water bodies with apparent ease. <i>Xenopus laevis</i> exhibits high tolerance to salty water, pH variation (5-9, but there is evidence of the species breeding below pH 4, according to John Measey pers. comm. 2018), temperature variation (2-35+), and is capable of aestivation during dry periods.</p> <p>The species cannot spread naturally from its native range into the risk assessment area.</p>
<p>A5. What is the global non-native distribution of the organism outside the risk assessment area?</p>	<p>As reviewed by Measey et al. (2012) the global non-native distribution of the species is known to include four continents: North America, South America, Asia and Europe. In particular, established populations are present in different states of the USA, Chile, Japan. The species was recently recorded in China (Wang et al. 2019). There are historic records also for Mexico, Java, Israel, and Ascension Island, but with the notable exception of Mexico (where the species occurrence was recently confirmed, see Peralta-García et al. 2014) the presence of the species in these countries is not confirmed. Regarding Europe, see details under points A7-A9 below.</p>
<p>A6. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species been recorded and where is it established?</p>	<p>The species was recorded and is established in both the Mediterranean and Atlantic biogeographic regions (see details in A8).</p>
<p>A7. In which biogeographic region(s) or marine subregion(s) in the risk assessment area could the species establish in the future under current climate and under foreseeable climate change?</p>	<p>Current climate conditions: Mediterranean, Atlantic, Continental and Alpine biogeographic regions</p> <p>Foreseeable climate change conditions: all biogeographic regions, with the exception of the Boreal and Arctic, may be suitable (at least in part) depending on the different models used (see ANNEX VI). However the confidence level is very low, as we were not able to retrieve precise information on future climate, given the methodological constraints and the lack of accurate information available on the species location and taxonomy. For details on the assumptions</p>

made in relation to climate change see annex VI: projection of climatic suitability.

Recent studies have shown that native phylogeographic lineages have contributed differently to invasive *X. laevis* populations, but most of the introductions have probably been from the Mediterranean climate zone in the southwest of the Western Cape Province, South Africa, where *X. laevis* occurs naturally. For example, according to genetic and historical data the populations established in Europe, and in particular in Italy (Sicily), Portugal and France, seem to involve individuals from the south-western Cape region in South Africa (De Busschere et al. 2016, Lillo et al 2013). In France however another distinct native phylogeographic lineage is involved, i.e. from other regions of South Africa (De Busschere et al. 2016, Rödder et al. 2017). The identification of source populations is particularly relevant for the purpose of this document, because phenotypic as well as genotypic traits of colonizing individuals might influence the invasion process and success, particularly in such cases where there is extensive population differentiation within the native range (De Busschere et al. 2016).

To assess the future distribution under current climate, Measey et al. (2012) used a single lineage of the species from the southwestern Cape of South Africa for their species distribution models (SDMs). As a result, the optimal uninvasion bioclimatic space was identified in isolated parts of France and Portugal only, while a large suitable climatic potential was identified for most of southern Portugal and adjoining Spain, as well as central and southern France, and mainland Italy. Such data are consistent with the finding of Ihlow et al. (2016), who used the entire range as well as invasive populations and who predicted particularly high probabilities in Europe, namely in Portugal, eastern Spain, southern France, and Italy. Furthermore, Ihlow et al. (2016) highlight areas in Spain (including the Balearic Islands), mainland Italy (including Sardinia), and southern France (including Corsica) to be highly vulnerable to potential invasions, as these regions exhibit suitable climatic conditions for *X. laevis* and are adjacent to established invasive populations. According to Measey et al. (2012) a few suitable areas were found also in the United Kingdom outside southern coastal areas, plus Greece, Ireland, Germany, Belgium, Denmark and the Netherlands (for details see maps developed by Measey et al. 2012). On the other hand, Ihlow et al. (2016) predict only moderate probability for Great Britain, where populations from Wales and Lincolnshire have recently been extirpated. Therefore, while the optimal area would fall within the Mediterranean and Atlantic

biogeographic regions only, the maps annexed to the study seem to suggest the presence of suitable areas also within the Continental and Alpine regions.

Under foreseeable climate change, using species distribution models (SDMs), Ihlow et al. (2016) assessed the global invasion potential of this species for 2070 following four IPCC scenarios (i.e. RCP2.6, RCP4.5, RCP6, RCP8.5). In particular, the potential range size was predicted to expand in north-western Europe, especially in France and Great Britain, where new regional conditions may promote new invasions or the spread of established invasive populations. The Mediterranean area was already considered suitable and still is under all climate change scenarios. The maps shown in the paper by Ihlow et al. (2016) do not allow for a precise identification of the biogeographic regions where the species could establish in the future under foreseeable climate change. However, it seems that most biogeographic regions in Europe will become suitable for the species.

Rödger et al. (2017) demonstrated that invasive populations of *X. laevis* are established well beyond the species' multivariate realized niche in southern Africa. Hybridization of different lineages may have enabled a shift in the species' fundamental niche. Given the magnitude of the detected niche shifts, the usefulness of climate matching approaches to assess invasion risk for this species is challenged, as it might frequently underestimate the true potential distribution when a geographic subset of the species' realized distribution is used for model training. It can be expected that the true invasion potential for *X. laevis* is larger than its estimated potential distribution based on its currently realized niche (Rödger et al. 2017).

Similarly, in a recent study, Ginal et al. (2020) stressed that *X. laevis* is a species with a strongly underestimated invasive potential. They point out that the correlative approaches which characterized the previous SDMs (Ihlow et al., 2016; Measey et al., 2012, Rödger et al., 2017) can be vulnerable to extrapolation errors when projecting species' distributions in nonnative ranges. Therefore, to better assess the species' full invasive potential, the authors developed a process-based model based on physiological data like critical thermal limits and temperature-dependent performance. The study suggested a high risk of invasion for most parts of Europe that could be assessed. In particular, according to Ginal et al. (2020), large parts of western and southern Europe as well as many Mediterranean islands are predicted as being climatically

	<p>suitable for <i>X. laevis</i>. This also includes the currently (or formerly) occupied areas of Portugal, France, and Wales. Sicily is predicted as unsuitable, which the authors state is probably due to the low density of waterbodies, preventing assessment by their waterbody availability layer. Extensive “MESS” areas, which highlight possible extrapolation errors, occur in central to eastern Europe as well as in cold mountain ranges such as the Alps and Pyrenees, where predictions should be treated with caution.</p>
<p>A8. In which EU member states has the species been recorded and in which EU member states has it established? List them with an indication of the timeline of observations.</p>	<p>Recorded in the following Member States: Belgium, France, Portugal, Italy, Spain, Sweden, Germany, Netherlands; and in the United Kingdom.</p> <p>Established: France, Portugal, Italy.</p> <p>In general, the situation has been quite dynamic in the last years, mostly due to the fact that introductions are usually followed by a lag of around 15 years between the export of animals and the rise in invasive populations (van Sittert and Measey 2016). In general, it can take between 2 and 25 years or more for first reports of introductions to be released (Measey et al. 2012). As discussed in detail below, the species is currently considered established in France, Portugal and Italy (Sicily). Until recently, the species was considered established also in the UK, where it is currently considered extinct.</p> <p>According to Frazer (1964) the first introduction in the UK occurred in Kent in 1955, but did not succeed. The UK was also home of the first invasive population established in Europe, namely on the Isle of Wight, due to an introduction around 1962 (Tinsley et al. 2015a, Tinsley & McCoid, 1996, van Sittert and Measey 2016). In the UK, in addition to the population on the Isle of Wight, now probably extinct, there have also been two established populations, namely in Glamorgan (Wales), and Lincolnshire (England). They were both the subject of an eradication programme and are considered recently extinct, although the causative factors were possibly the exceptional weather conditions (in conjunction with specific habitat and population characteristics) (Tinsley et al. 2015a). In any case, follow-up monitoring is still required in South Wales (John Measey, pers. comm. 2018). The species was also reported in 1987 and 1990 in two ponds to the southeast of London, although these do not appear to have survived,</p>

and in the southwest of England, but no established populations have been found (see review made by NNSS 2011).

In Portugal the species was first found in 2006 and first reported in 2007, but the first introduction may have occurred in Oeiras in 1979 (Sousa et al. 2018).

In Italy, the only known population of *X. laevis* is on the island of Sicily where the date, site and cause of first release are all unknown (Measey et al. 2012). The first documented occurrence dates back to 1999, while the first report was in 2004. In peninsular Italy, the presence of two adults of *X. laevis* was reported in 2017 for the Lombardia region, in the Groane park (see <http://www.parcogroane.it/wordpress/wp-content/uploads/2017/08/Report-censimento-anfibi-Progetto-LIFE-GESTIRE-2020-1.pdf>).

In France animals were officially first reported in 1998. However, residents of the area suggested that this frog had been present since the early 1980s (Fouquet 2001, Fouquet and Measey 2006, Measey et al. 2012). A new population of *X. laevis* has been discovered in Toulouse in 2018 (see <http://especes-exotiques-envahissantes.fr/decouverte-xenope-lisse-occitanie/>). As reported on the mentioned link, this discovery brings to four the number of known sites of occurrence of *X. laevis* in France. Indeed, in addition to the main nucleus located in between four departments (Deux-Sèvres, Vienne, Maine-et-Loire, Loire-Atlantique), a population was discovered in 2015 in Ambarès-et-Lagrave, near Bordeaux, then another one in La Chapelle-d'Armentières, near Lille, in 2018. The latter population is close to the Belgium border, where an individual was already reported in 2006 (see below).

In Spain, the presence of *X. laevis* was reported in Montjuïc (Barcelona) in 2007, where about 30 larvae were found. A year later, six individuals were found in the Parc del Laberint d'Horta. Both the larvae and the adults found were removed from the environment (EXOCAT database http://exocatdb.creaf.cat/base_dades/#), and the species is now considered eradicated (Pascual et al. 2007).

In Sweden, a single animal exists in the collection of the Gothenberg Natural History Museum collected in 2007 (Measey et al. 2012). However, this record is not reliable as the locality

	<p>reported by Measey et al. (2012) is in fact a name of a person who received a dead frog from another person (Melanie Josefsson in litt. 2018).</p> <p>In Germany, <i>X. laevis</i> specimens have been collected in the Hamburg area, following a release in 1991 by animal rights activists (Tinsley and McCoid, 1996; Rabitsch et al., 2013). Their current status is unknown, but it is very likely that they have disappeared.</p> <p>In the Netherlands there are records of an adult individual caught near Gorichem in 1974 and of tadpoles collected near Utrecht in 1979 (Tinsley and McCoid, 1996). The National Database Flora and Fauna has no recent records for the species in the Netherlands, confirming that the species has not established populations (www.ndff.nl, see also https://www.verspreidingsatlas.nl/soortenlijst/amfibieen).</p> <p>In Belgium, as reported in the Hyla (amphibian and reptile working group of the ngo Natuurpunt) database, 30 <i>X. laevis</i> larvae were found at Antwerp University pond in 2008 (observer Bart Vervust). Their current status is unknown. Two other individuals were found in Ploegsteert (Wallonia) as reported on observations.be, one in 2006 (an albino specimen) and one in 2016. The area is at the border with the French area where in 2018 a new population of the species was reported, as stated above. However, an eDNA study currently in progress has confirmed that no population occurs on the Flemish side of the border (Van Doorn et al. 2021, unpublished report).</p> <p>According to Measey (2017) wild caught <i>X. laevis</i> are also reported to have been imported into the USA from the Czech Republic and Switzerland over the period 1999-2005. Such records are however presumed anomalies and should be treated with suspicion, as no invasive populations are known in these countries. On the other hand, as the source of information are records from USFW for animals imported into the USA, they could be specimens caught in the wild in South Africa and then exported to USA via the Czech Republic or Switzerland (John Measey, pers. comm. 2018).</p>
<p>A9. In which EU member states could the species establish in the future under current</p>	<p>Current climate conditions: France, Portugal, Italy, Spain, Sweden, Germany, Netherlands, Greece, Ireland, Belgium, Denmark; and the United Kingdom</p>

<p>climate and under foreseeable climate change?</p>	<p>Foreseeable climate change conditions: all EU countries may be suitable (at least in part) depending on the different model used (see ANNEX VI). However, the confidence level is very low, as model projections for the current climate vary widely, and projections for future climates are not available for all models. We could not retrieve precise information on future climate, given the methodological constraints and the lack of accurate information available on the species location and taxonomy. Ginal et al. (2020), in their distribution model paper, did not include future climate scenarios. For details see comments on point A7.</p>
<p>A10. Is the organism known to be invasive (i.e. to threaten or adversely impact upon biodiversity and related ecosystem services) anywhere outside the risk assessment area?</p>	<p>There is evidence of invasiveness in the USA, Chile and Japan (see section on impacts below for more details).</p>
<p>A11. In which biogeographic region(s) or marine subregion(s) in the risk assessment area has the species shown signs of invasiveness?</p>	<p>The species has shown signs of invasiveness in both the Mediterranean and Atlantic biogeographic regions, i.e. in all areas where populations are established. For details see section on “magnitude of impact”, points 2.13-2.30 below.</p>
<p>A12. In which EU member states has the species shown signs of invasiveness?</p>	<p>The species has shown signs of invasiveness in all EU Member States where populations are established, i.e. France, Portugal and Italy (Sicily).</p>
<p>A13. Describe any known socio-economic benefits of the organism.</p>	<p><i>Xenopus laevis</i> is a species substantially important in relation to research activities in the risk assessment area and globally (amongst others in oncology, endocrinology, developmental biology and anatomical studies), see e.g. Gurdon and Hopwood, 2000, Hardwick and Philpott, 2015 and Blum and Ott, 2018). The species was used to diagnose pregnancy, but this is apparently no longer a practice in the risk assessment area, and we may exclude its use for this purpose in other parts of the world too. The species is also traded as pet for aquaria and garden ponds.</p> <p><u>Pet trade</u> In relation to the pet sector, sales and associated ancillary product sales of <i>X. laevis</i> are significant, particularly in UK.</p>

According to data provided by the European Pet Organization to the European Commission (EPO 2018), in the UK, the annual revenue for the pet sector from *X. laevis* is in the estimated range of between 168,500 euros to 3 million euros but they consider this is likely to be a conservative estimate. This species is also likely to be economically important to other Member States where trade in this species (as a pet) is permitted, although EPO acknowledges that this may be to a lesser degree when compared to the UK. Furthermore, EPO consider that an albino morph of *Xenopus laevis* is dominant in the pet trade.

More in detail, according to EPO (2018) from the data from those Member States where *X. laevis* is traded by the pet sector, individual animals are sold for values between 1 euro to 11,30 euros depending on the Member State. Therefore, in terms of the total trade based on the information collated by EPO, between the different countries there is a very broad range in the trade values of this species (based on the number of individuals sold), with numbers ranging from a lowest value of 275 euros per annum to as high as 1 million euros per annum. If ancillary products e.g. aquariums, dry goods etc. are factored in, this figure is significantly higher with a conservative estimate of 3 million euros per year across EU and UK. While EPO (2018) does not provide a breakdown per country, they clarify that “*the proportion of sales of animals (and therefore ancillary products) varies significantly between Member States, with the UK representing the highest values whilst those reported for the Netherlands and France being significantly lower*”. Due to the skewed nature of the raw data (which EPO was unable to provide given that it is highly commercially sensitive), EPO was unable to provide median or average estimated trade revenues. However, EPO noted that the figures provided are likely to be a conservative estimate. In the UK, the annual revenue from *X. laevis* for the pet sector could be anywhere between 168,500 euros to 3 million euros. This represents a very broad range, which means that banning this species could have an impact for pet stores related activities in some EU countries.

The species is available for purchase within the risk assessment area also on the internet, e.g. <https://www.sivissen-onlineshop.be/fr/shop/grenouille-apprendre-plus/>
<https://www.oxyfish.fr/especes-variees/33346-xenopus-laevis-albino-1-5-2-cm.html>
<https://www.ebay.co.uk/itm/Albino-African-Clawed-Frog-Xenopus-Laevis-/264926197064>

(accessed on 05/02/2021).

Research activities

In relation to the use of the species in research activities, this frog had a significant role in the history of 20th century science. It became one of four vertebrate species universally recognised as representing a standard biological model (van Sittert and Measey 2016, Gurdon and Hopwood, 2000). The paramount importance of this species in research activities is well illustrated by the concern expressed by EXRC (2019) calling for a “full socio-economic impact assessment of the effect of a ban on *X. laevis*” to be undertaken, and asking that “complete protection for the use of this species as one of the key experimental organisms of biomedical and environmental research is assured by any actions taken as a result of this risk assessment”. In their call, EXRC (2019) indicate that more than 38000 papers are found by a search for “*Xenopus laevis*” in the online archive of biomedical scientific publications Pubmed. Research based on *Xenopus laevis* includes the work by Sir John Gurdon, awarded in 2012 with the Nobel Prize in Physiology or Medicine⁸.

The use of the species in research activities in the EU is regulated by Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes⁹. According to this, specimens of *Xenopus laevis* and *Xenopus tropicalis* may only be used in procedures where those animals have been bred for use in “procedures”. Animals taken from the wild shall not be used in procedures but exemptions may be granted to this rule. “Procedure” means any use, invasive or non-invasive, of an animal for experimental or other scientific purposes, with known or unknown outcome, or educational purposes, which may cause the animal a level of pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle in accordance with good veterinary practice. Furthermore, Member States shall ensure that all breeders, suppliers

⁸ <https://www.nobelprize.org/prizes/medicine/2012/gurdon/facts/>

⁹ <https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:02010L0063-20190626&from=EN>

and users are authorised by, and registered with, the Member State’s competent authority and keep records of number, origin, source etc. of animals.

Statistical data covering the EU 28 Member States (EU28) data over the period 2015-2017 were published in early 2020¹⁰. These statistics cover both *X. laevis* and *X. tropicalis* (data is collected for both species together). According to these, the number of “all uses” for these two species, i.e. the number of times these species were needed for scientific purposes in a given year in EU28 (first use and any subsequent reuse) were:

- in 2015: 18,990 uses of which 42.9% were reuses
- in 2016: 27,904 uses of which 33.70% were reuses
- in 2017: 21,443 uses of which 36.90% were reuses

For the purpose of the above, the “use” of an animal within a project extends from the time the procedure (or first procedure/technique in a series) is applied to it, to the time when the observations, or the collection of data (or other products) for a particular scientific purpose (usually a single experiment or test), are completed. “Reuse” indicates any subsequent use of an animal, which has already completed a procedure (or series of procedures/techniques) for a particular scientific purpose, and for which any animal would suffice. Reuse of *Xenopus* is relatively high. “All uses” (first use and any subsequent reuse) indicate the number of times these species were needed for scientific purposes in a given year in the EU.

EXRC (2019) estimated on basis of a literature review carried out a decade earlier that somewhere between 300 and 400 labs across Europe rely on *X. laevis* for their work. They furthermore estimate that the population of *X. laevis* used for developmental biology alone is over 52,000 animals based on unpublished survey data of 210 laboratories worldwide. Based on contributions from scientists using *Xenopus* in their research, affiliated to 22 Universities and research institutes in Belgium, Denmark, France, Germany, Ireland, Italy and the United Kingdom, EXRC (2019) also indicated that these scientists had in 2019 32,55million EUR in grant income, supporting over 100 early career-scientists. Furthermore, in a given academic

¹⁰ https://ec.europa.eu/environment/chemicals/lab_animals/reports_en.htm

year in these 22 institutions over 600 students would be taught fundamentals of cell and developmental biology using *X. laevis*. EXRC (2019) consider that for most of the experiments that use *X. laevis*, there are no alternatives (e.g. egg extracts, oocyte-based receptor assays, developmental biology using traditional techniques), for genetic approaches to cell and developmental biology *X. tropicalis* can be used but the investment needed for labs to change to this species would be very considerable.

EXRC (2019) also consider that research using *Xenopus* contributes very strongly to the Commission and Society's aim to replace the use of mammals such as mice in biological and biomedical experimentation (the so-called 3Rs of reduction, refinement and replacement). The use of *Xenopus* in place of mice is a refinement of experimental design that supports Directive 2010/63/EU.

Biomedical research has resulted also to commercial use of *Xenopus* embryos and tadpoles for in vivo drug discovery purposes and the technology has been protected by European and US patents (EP2409149, US Patent 9945845). In addition to its widespread role in biomedical research, *X. laevis* is used for testing environmental contamination and the teratogenic potential of new products. These tests are usually carried out commercially and examples include water testing by "Watchfrog" (<https://www.watchfrog.fr>) and the well-established "Frog Embryo Teratogenesis Assay-Xenopus (FETAX)" which is routinely used since early 2000's as a developmental toxicity screening test for pharmaceutical candidate compounds (Leconte and Mouche, 2013).

According to EXRC (2019), most laboratories import animals either from the CRB *Xenopus* in Rennes, NASCO in the US or the EXRC; only a few breed their own.

Indeed, within the risk assessment area, specialised suppliers of *Xenopus laevis* (and other *Xenopus* species) exist in the risk assessment area, e.g. in France and UK. See for example <http://www.xenbase.org/other/obtain.do>, <https://xenopusresource.org/how-to-order> and <https://xenopus.univ-rennes1.fr/tarifs-0> (assessed on 05/02/2021). Data from Measey (2017) show that there are some imports into the US from other countries with known invasive populations (Chile, UK and France), but in small quantities (< 100 animals), and of captive bred

	<p>animals usually for medical or scientific purposes and are thus presumably not from invasive populations. Animals in France were being harvested by Xenopus Express in France, although it is unknown whether or not this practice continues (John Measey, pers. comm. 2018). According to trade data of the U.S Fish and Wildlife Service presented in Measey (2017), live individuals of <i>X. laevis</i> were imported into the USA from EU countries such as Czech Republic, Germany, and Italy (therefore, including countries without known populations established in the wild, like Czech Republic and Germany). This could involve re-exports (see also section A8 on this).</p>
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SECTION B – Detailed assessment

Important instructions:

- In the case of lack of information the assessors are requested to use a standardized answer: “No information has been found.”
- The classification of pathways developed by the Convention of Biological Diversity shall be used For detailed explanations of the CBD pathway classification scheme consult the IUCN/CEH guidance document¹¹ and the provided key to pathways¹².
- With regard to the scoring of the likelihood of events or the magnitude of impacts see Annexes I and II.
- With regard to the confidence levels, see Annex III.

PROBABILITY OF INTRODUCTION and ENTRY

Important instructions:

- Introduction is the movement of the species into the risk assessment area.
- Entry is the release/escape/arrival in the environment, i.e. occurrence in the wild. Not to be confused with spread, the movement of an organism within the risk assessment area.
- For organisms which are already present in the risk assessment area, only complete this section for current active or if relevant potential future pathways. This section need not be completed for organisms which have entered in the past and have no current pathway of introduction and entry.

QUESTION	RESPONSE [chose one entry, delete all others]	CONFIDENCE [chose one entry, delete all others]	COMMENT
1.1. How many active pathways are relevant to the potential introduction of this organism? (If there are no active pathways or potential future	few	high	In Europe, the occurrence of <i>X. laevis</i> in the wild is thought to be a consequence of its use as a research model in laboratories and as a pet (Measey et al. 2012, Tinsley et al. 2015a, Rebelo

¹¹ <https://circabc.europa.eu/sd/a/738e82a8-f0a6-47c6-8f3b-aeddb535b83b/TSSR-2016-010%20CBD%20categories%20on%20pathways%20Final.pdf>

¹² <https://circabc.europa.eu/sd/a/0aeba7f1-c8c2-45a1-9ba3-bcb91a9f039d/TSSR-2016-010%20CBD%20pathways%20key%20full%20only.pdf>

<p>pathways respond N/A and move to the Establishment section)</p>			<p>et al. 2010). The main active pathways are therefore the following: 1) Pet/aquarium/terrarium species (escape from confinement); 2) Research and ex-situ breeding (in facilities) (escape from confinement).</p> <p>As pointed out by Tinsley & McCoid (1996), it may be due to many factors, such as loss of interest, end of an experiment, misguided ethics or curiosity, which occasionally results in the release of captives. Deliberate release are also reported, along with escapes (Measey et al. 2012). The actual scale of releases and escapes is unknown. In fact, in most cases the exact cause is only inferred retrospectively, as the species is often detected only many years after its deliberate or accidental introduction. For example, in Portugal the species lived undetected for more than 20 years (Sousa et al. 2018). In such cases, it is clear that it is not possible to establish the intentionality of the introduction without the relevant events being appropriately documented.</p>
<p>1.2. List relevant pathways through which the organism could be introduced. Where possible give detail about the specific origins and end points of the pathways as well as a description of any associated commodities.</p> <p>For each pathway answer questions 1.3 to 1.10 (copy</p>	<p>1) Pet/aquarium/terrarium species (escape from confinement) 2) Research and ex-situ breeding</p>		<p>In addition to the use of the species as a research model in laboratories and as a pet (which leads to the main two active pathways already identified), other uses are known.</p> <p>For example, the species has been used in schools for training in labs (e.g. dissections etc.),</p>

<p>and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 1.3a, 1.4a, etc. and then 1.3b, 1.4b etc. for the next pathway.</p>	<p>(in facilities) (escape from confinement)</p>		<p>which can be a source of animals released in the wild. While there is no documented evidence of such releases in Europe, in the US, schools are known to ditch their stock when legislation changed making keeping invasive species illegal. The law changed without making any provision for people already keeping them (John Measey, pers. comm. 2018). In any case, the use of animals in schools is treated here under the pathway “Research and ex-situ breeding (in facilities)”, see point 1.4b below.</p> <p>As reported by Weldon et al. (2007) in South Africa <i>X. laevis</i> is appreciated as live bait for freshwater angling (despite this practice being illegal). As a consequence, fishermen are known to seed dams with <i>X. laevis</i> in order to produce a local supply of live bait (Measey et al. 2017). However, this is not considered an active pathway in Europe.</p> <p>Zoo exhibit of this species is also mentioned as a former pathway in the late 1900s (Vredenburg et al. 2013), and although the species is still present in public zoos/aquaria, zoos (escape from confinement) are not considered as an important pathway of introduction of the species in Europe. Whilst there is no data available on the total population within all zoological collections in the EU, information was provided by EAZA (European Association of Zoos and Aquaria) on populations kept at approximately 300 of their</p>
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			Member zoos and aquariums in 26 EU Member States (with the exceptions of Cyprus and Malta). The information provided by EAZA (EAZA, pers. comm. 2018) indicates that 84 specimens in total are kept by 15 zoo/aquarium EAZA Members in 10 Member States (BE, HU, DK, NL, IE, DE, PT, PL, EE, FR) and the United Kingdom. On top of this, in total 5 specimens of the subspecies <i>Xenopus laevis</i> are kept by 1 zoos/aquariums EAZA Members in one Member State (CZ). This data comes from the animal care and management software provided by Species360 Zoological Information Management System (ZIMS) (zims.Species360.org, 2018) whose usage is widespread throughout the EAZA Membership. It must be noted that the actual situation might slightly differ if the species has been recorded under a different/older taxonomic name (as in the case of <i>X. laevis</i>).
Pathway name:	1) Pet/aquarium/terrarium species (escape from confinement)		
1.3a. Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?	intentional	high	<i>X. laevis</i> is traded by the pet sector in several Member States of the EU as well as in UK, with the albino morph being predominant in this trade. The species is also traded through e-commerce

<p>(if intentional, only answer questions 1.4, 1.9, 1.10, 1.11 – delete other rows¹³)</p>			<p>and other internet platforms (although numbers are unknown). The introduction in the risk assessment area through this pathway is intentional. However, the entry into the environment is either intentional or unintentional, depending on whether it is the result of deliberate releases or accidental escapes.</p> <p>Despite the general lack of documented evidence regarding the significance of this pathway for the entry of the species into the environment, there are indications that this pathway is active in Europe, as well as in other parts of the world. For example, according to Measey et al. (2012) the source of a population once occurring near Scunthorpe, Humberside, in the north-east of England, is thought to be due to the closure of a pet shop and the deliberate release of adults in the mid-1990s. EPO-OFI (2019) on the other hand consider that there seems to be no substantive evidence that the closure of the pet shop led to a deliberate release or that the speculated pet shop ever kept <i>Xenopus</i>. They consider it possible that the population in question existed prior to the closure of this shop.</p> <p>The trade and possession of the species is not regulated in most Member States and EPO</p>
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¹³ While the pathways of introduction are considered intentional, it was considered necessary to answer all questions since the subsequent entry into environment could be either intentional or unintentional.

		<p>(2018) reported that trade is ongoing between different countries in the risk assessment area (the Netherlands, Czech Republic) and the UK. On the other hand, the species is regulated in other Member States, subjecting its trade and/or possession to diverse national measures, e.g.</p> <ul style="list-style-type: none"> • Possession and trading of <i>X. laevis</i> is prohibited in Spain (Real Decreto 630/2013, de 2 de Agosto¹⁴). • In Portugal, the species is included in the national list of invasive alien species under the Decree-law of 1999 and subsequently that of 2019¹⁵. • In France, the release of the species into the environment is forbidden¹⁶. Keeping the species requires an authorisation and a certificate of competence (though this is not limited to professionals) and it is prohibited to exhibit the species for sale¹⁷. <p>In Chile there is evidence of continuous releases (meaning that animals were moved from within the invasion area to a new area) which helped the species spread, and which are presumed to be connected with the use of animals in the pet trade, as private owners were suspected of dumping them (Lobos & Jaksic 2005, Measey et</p>
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¹⁴ <https://www.boe.es/buscar/act.php?id=BOE-A-2013-8565>

¹⁵ Decreto-Lei n.o 565/99 de 21 de Dezembro and Decreto-Lei n.º 92/2019 de 10 de julho

¹⁶ Arrêté du 14 février 2018 propagation des espèces animales exotiques envahissantes sur le territoire métropolitain – Annexe I

¹⁷ Arrêté du 8 octobre 2018 fixant les règles générales de détention d'animaux d'espèces non domestiques – Article 14

			<p>al. 2012). Also in the USA, there is evidence of animal importer dumping unwanted stock, e.g. in Florida (King and Krakauer 1966), or intentionally released by a single person, in Arizona (Somma 2018, Tinsley and McCoid, 1996).</p>
<p>1.4a. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year?</p> <p>Subnote: In your comment discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.</p>	<p>very likely</p>	<p>medium</p>	<p>In general, information on the origin of animals, exact number of individuals in trade and those in captivity, is not available. Also, no information could be retrieved on number of introduction events and number of individuals (either adults or larvae) escaped and/or released in the environment, hence it is not possible to assess the propagule pressure.</p> <p>However, according to the European Pet Organization (EPO 2018) in relation to animal traded in the UK, <i>X. laevis</i> is captive bred and none are wild caught. The extent of breeding in other EU Member States is unknown. In terms of numbers traded the data provided (EPO 2018) suggest a widespread range between the different countries with the lowest value being 100 animals sold per annum to the highest value of tens of thousands of animals being sold per annum. Therefore, due to this wide range of values, EPO was unable to provide median or average numbers/volumes due to the skewed nature of the raw data. EPO did not provide the raw data given that this information is commercially sensitive. It is however evident that</p>

		<p>the highest numbers concern the trade within UK.</p> <p>Information from other regions may help to figure out the dimension of the problem although the pet trade markets in the USA and Europe may not be directly comparable. As regards the USA, it is worth mentioning that across the last decades <i>X. laevis</i> trade has changed dramatically in terms of primary purpose, frogs' origin and numbers of animals traded. The species was originally distributed for pregnancy testing and laboratory use, but in the last 15 years, the size of the trade for medicine and science dropped to only 0.1% of imports, with the pet trade commanding 99.6% (Measey 2017). Trade figures reported by Measey (2017) for the USA are impressive (see also Herrel and van der Meijden, 2014). Whilst trade for medical and scientific purposes is now minimal (a few hundred animals per year), the pet trade imported 1.83 million live animals over the last 15 years (a total of 1,856 shipments which ranged from single animals to 11.5 thousand individuals). Just to give an idea of the global trade network supporting such trade, 75% of these animals are imported from Hong Kong (although it is possible that many animals originate from mainland China or elsewhere). It is also worth noting that all of these animals from China appear to be albinos, and there are currently no published reports of invasive albino populations, despite a single exception recently found in China (John Measey, pers. comm. 2018,</p>
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		<p>Wang et al. 2019). Only 5,600 animals were imported from the native area in South Africa, and this trade ceased in 2003. Nearly 200,000 individuals were imported from Chile and the majority of these were reported as being wild caught, suggesting that the invasive population there is being exported for the US pet trade (Measey 2017).</p> <p>Given the supposed widespread presence of this species in the pet trade in several countries, the risk of reinvasion after eradication is to be considered as likely as a first introduction. There are no specific studies providing an indication of the propagule pressure, but single gravid females can indicatively contain from 1,000 to 27,000 eggs per clutch (noting though that the number of 27,000 is not the norm, numbers are usually much less). Furthermore, females will produce multiple clutches in a season under favourable conditions (Global Invasive Species Database 2015), therefore even a handful of individuals may be sufficient to start a new population.</p> <p>In fact, as shown by Lobos et al. (2014), the invasion of <i>X. laevis</i> in Chile has been successful for at least 30 years, in spite of low genetic variability, few events of introduction, low propagule pressure, and bottlenecks in the founding population. Also according to Measey et al. (2012) propagule pressure plays a pivotal role in the establishment of <i>X. laevis</i>, as some</p>
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			populations became established after the release of large numbers of animals from breeding facilities (laboratory and pet supplies). Other evidence of populations that have established from very few individuals is not available (John Measey, pers. comm. 2018).
1.5a. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)? Subnote: In your comment consider whether the organism could multiply along the pathway.	very likely	medium	The species is able to survive during passage along the pathway, as demonstrated by the fact that it has been frequently traded and that the origin of some populations successfully established after animals being released in (or escaped into) the wild is attributed to this pathway.
1.6a. How likely is the organism to survive existing management practices during passage along the pathway?	very likely	low	No information has been found. Trade is intentional, and as such there is no management practice in place to prevent the species entering the risk assessment area. Also, there are no known specific practices for preventing this species from escaping or being released in the wild. Existing biosecurity guidance concentrates in the prevention of disease transmission when species are transferred (OATA 2012).
1.7a. How likely is the organism to enter the risk assessment area undetected?	very likely	high	While animals intentionally introduced in the risk assessment area for the pet trade are not undetected, those being introduced in the wild as a consequence of accidental escapes or intentional releases can be undetected for many years (see point 2.7a below)

<p>1.8a. How likely is the organism to arrive during the months of the year most appropriate for establishment?</p>	<p>moderately likely</p>	<p>medium</p>	<p>We are not certain whether any particular time of the year is more appropriate for establishment. It is likely that <i>X. laevis</i> could establish during any month of the year. In any case, traded animals may arrive and be released or escape at any time during the year in Europe, but data about frequency and months of the year are unknown.</p>
<p>1.9a. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?</p>	<p>moderately likely</p>	<p>medium</p>	<p>It is likely that people who deliberately release <i>X. laevis</i> into the wild will do it in what they consider the most suitable habitat. As a remark, <i>X. laevis</i> is a vigorously adaptable species which may virtually inhabit any type of water bodies, including lakes and rivers, as well as permanent and temporary ponds, over a wide range of altitudes and temperatures (Measey 1998). Besides <i>X. laevis</i> thrives in disturbed landscapes and artificial habitats, like ponds, wells, dams, irrigation canals and other domestic and agricultural water sources (Tinsley et al. 2015a, Lobos & Jaksic 2005). This clearly increases the likelihood of the species being introduced, either intentionally or accidentally, into suitable habitats. There is also a high likelihood that additional translocations by humans within the risk assessment area may occur (see also Qu. 2.1 and 2.2), hence increasing the opportunities for the species introduction within the risk assessment area.</p> <p>In addition, as pointed out by Measey et al. (2012) biosecurity at breeding facilities is clearly</p>

			<p>of paramount importance.</p> <p>It is reasonable to assume that maintenance staff in pet wholesaler/retailer premises may have received in-house training, as also suggested by EPO 2019. Furthermore, codes of conduct (e.g. Council of Europe, 2011) call for promoting awareness of invasiveness and of the need not to release pets in the environment in general. However, care sheets addressed to keepers of <i>X. laevis</i> as pets available in the public domain concentrate on animal welfare considerations with very little or no information regarding the prevention of escapes and in any case with no advice against releases into environment and the potential environmental impacts of such releases (e.g. Reed 2009, amphibian.co.uk).</p>
1.10a. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?	very likely	high	<p>In current conditions, it is very likely that the species will enter the environment within the risk assessment area through this pathway. The species is known to be present in the pet trade in Europe, and has already been recorded in the wild in the region, possibly also as a consequence of this pathway.</p>

Pathway name:	2) Research and ex-situ breeding (in facilities) (escape from confinement)		
1.3b. Is introduction along this pathway intentional (e.g. the organism is imported for trade) or unintentional (e.g. the organism is a contaminant of imported goods)?	intentional	high	<p>The species is traded as model amphibian in scientific research, and as such the introduction in the risk assessment area through this pathway is intentional. However, entry into the environment is</p>

<p>(if intentional, only answer questions 1.4, 1.9, 1.10, 1.11 – delete other rows)</p>			<p>either intentional or unintentional, depending on whether it is the result of deliberate releases or accidental escapes. Despite the general lack of documented evidence regarding the exact pathway of introduction for this species, there are clues of this pathway being active in Europe, as well as in other parts of the world. For example, in France the suspected origin of the species was a breeding facility of the CNRS in Fronteau, Bouillé St Paul (Fouquet 2001), a laboratory supplier for French research institutions (Measey et al. 2012). In Portugal, the species was likely introduced following a flood of the 1979/1980 winter in the laboratories, where the species was used, although this is unconfirmed (Rebelo 2010, Sousa et al. 2018).</p>
<p>1.4b. How likely is it that large numbers of the organism will travel along this pathway from the point(s) of origin over the course of one year?</p> <p>Subnote: In your comment discuss how likely the organism is to get onto the pathway in the first place. Also comment on the volume of movement along this pathway.</p>	<p>very likely</p>	<p>high</p>	<p>There is evidence linking the occurrence of invasive alien populations with the trade and use of this species for biomedical research, although other secondary pathways seem to be involved as well (van Sittert & Measey 2016).</p> <p>The history of the use and trade of the species started in the 1930s with the use in pregnancy testing until the 1960s, and later for laboratory use as model organism (Gurdon and Hopwood 2000, Measey et al. 2012, Tinsley et al. 2015a, van Sittert & Measey 2016). This led to exports of thousands of live animals from its native South African Cape region to laboratories, first to the United Kingdom and eventually all over the world. By 1970, as</p>

		<p>demonstrated by van Sittert & Measey (2016), <i>X. laevis</i> was the world’s most widely distributed amphibian: institutions in 48 countries were supplied with live animals on all continents except Antarctica. In fact, as summarized by Weldon et al. (2007), the use of this species as a model amphibian in scientific research (i.e. genetics, molecular biology, embryology, biochemistry and ecotoxicology) was increasingly popular in the 1970s, and <i>X. laevis</i> became the most widely used amphibian in research in the 1990s. In terms of numbers, over 10,000 animals were exported annually from South Africa between 1998 and 2004 to 132 facilities situated in 30 countries (Weldon et al. 2007). However, there is still no known information on how many animals were shipped privately and where they were shipped to, during this period. Additionally, the secondary movement between places that were supplied also appears to be important (John Measey, pers. comm. 2018).</p> <p><i>X. laevis</i> was also used for educational and training purposes in schools and universities (e.g. dissection classes). However, this use seems declining markedly due to ethical concerns and financial constraints (Reed 2005).</p> <p>Of note here is the link between tadpoles and home teaching: in many cases, the tadpoles are reared in large numbers and then many are euthanized. Some individuals will give tadpoles over to parents to</p>
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			<p>raise them at home, or liberate tadpoles. This is less likely in the pet trade, but may happen in tertiary education institutes where <i>X. laevis</i> is a teaching model (John Measey, pers. comm. 2018).</p> <p>Given the widespread presence of this species in research facilities in several countries (see section A13), the risk of reinvasion after eradication is to be considered as likely as a first introduction.</p>
<p>1.5b. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)?</p> <p>Subnote: In your comment consider whether the organism could multiply along the pathway.</p>	very likely	medium	<p>The species is able to survive during passage along the pathway. Actually, specimens are intentionally transported for use in laboratories. According to EXRC (2019), most laboratories import animals either from the CRB Xenopus in Rennes, NASCO in the US or the EXRC; only a few breed their own. I.e. every effort is made that specimens survive the transport in the best condition possible.</p>
<p>1.6b. How likely is the organism to survive existing management practices during passage along the pathway?</p>	likely	low	<p>No exhaustive information has been found. Movements of animals for their use in research activities is intentional, and as such there is no management practice in place to prevent the species being introduced into the risk assessment area. Specimens shipped to laboratories may be done mostly by specialised services, but the main reason seems to be the reduction of stress to which the animals will be subject to.</p>
<p>1.7b. How likely is the organism to enter the risk assessment area undetected?</p>	very likely	high	<p>The intentional introduction of animals in the risk assessment area for use in research activities cannot go undetected, but those animals being</p>

			introduced in the wild as a consequence of accidental escapes or intentional releases can be undetected for many years (see point 2.7a below).
1.8b. How likely is the organism to arrive during the months of the year most appropriate for establishment?	moderately likely	medium	We are not certain whether any particular time of the year is more appropriate for establishment. It is likely that <i>X. laevis</i> could establish during any month of the year. In any case, traded animals may arrive and be released or escape at any time during the year in Europe, but data about frequency and months of the year are unknown.
1.9b. How likely is the organism to be able to transfer from the pathway to a suitable habitat or host?	moderately likely	medium	As pointed out in the GB risk assessment for the species (NNS 2011), most of the African clawed frogs that are present in captivity in the UK are owned by commercial laboratories, which will be careful to prevent escapes. These laboratories are generally run by competent people who have an interest in amphibians and who realise the negative consequences of releasing these animals into the wild. Measey et al. (2012) seem less optimistic, as they recognise that biosecurity at breeding facilities is clearly of paramount importance, but the maintenance staff in laboratories may not have appropriate information or relevant training. In fact according to Measey et al. (2012) in at least one case tadpoles of <i>X. laevis</i> were released routinely for many years into a pond of university property, despite the fact that the person releasing these tadpoles was instructed to euthanise them. In another case, tadpoles had been given to local schools and friends for learning purposes (Measey

		<p>et al. 2012). Releases or escapes and successful establishment of populations in the wild have been attributed in the past to this pathway. Laboratory security has been increasingly improving over the years so escapes are now considered much less likely. However, people working with alien species (even when in special facilities) should be made aware of the issues concerning release (John Measey, pers. comm. 2018).</p> <p>In this regard, it should be noted that guidance available in the public domain usually refers to animal welfare considerations with little emphasis to biosecurity rules. Reed (2005) for example states: <i>“Containers must prevent escape and should allow space for sufficient volume and depth of water and for enrichment such as refuges to be added. A tank size of 65 x 45 x 45cms has been recommended ... for holding four to five frogs in 5 to 10 litres of water. A tank of this depth should (depending upon the water depth) prevent Xenopus from jumping out. An overhanging lip will further act to prevent escapes”</i>. Similarly, the UK Code of Practice for the Housing and Care of animals bred for scientific purposes (UK Home Office, 2014) is mostly about animal welfare and not about prevention of environmental impacts due to escapes or releases of animals in the environment.</p> <p>In conclusion, the likelihood of the species being intentionally released in the wild, in a suitable habitat, cannot be excluded. Furthermore, there is</p>
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			<p>always the risk of unexpected events which may cause the escape of the animals, as was the case in Portugal where a laboratory experienced flooding, although this event was not confirmed (Measey et al. 2012, Sousa et al. 2018).</p> <p>As an additional remark, Measey et al. (2012) expressed concern about the future risk of laboratory populations of <i>X. laevis</i> which - due to the possible replacement of this species with <i>X. tropicalis</i> as a research model organism - may be dismissed and dumped in the environment. In fact, although there are no documented instances with respect to academic replacement of model organisms, there are examples of this in the US where school pets became illegal to keep and animals were dumped (John Measey, pers. comm. 2018). Given the instruction to euthanize a large number of animals, many people will still choose to dump living animals into the natural environment, if they do not have specific knowledge of the potential consequences of such release (John Measey, pers. comm. 2018).</p>
<p>1.10b. Estimate the overall likelihood of entry into the risk assessment area based on this pathway?</p>	<p>likely</p>	<p>high</p>	<p>In current conditions, it is likely that the species can still enter into the wild in the risk assessment area based on this pathway. The species is already present in research facilities in Europe, and has already been recorded in the wild in the risk assessment region, possibly as a consequence of this pathway.</p>

<i>End of pathway assessment, repeat as necessary.</i>			
1.11. Estimate the overall likelihood of entry into the risk assessment area based on all pathways and specify if different in relevant biogeographical regions in current conditions (comment on the key issues that lead to this conclusion).	very likely	high	The overall likelihood of entry into the risk assessment area based on all pathways is very high in current conditions, particularly given the fact that the species is present in trade and in breeding facilities in many countries, possibly in all biogeographical regions.
1.12. Estimate the overall likelihood of entry into the risk assessment area based on all pathways in foreseeable climate change conditions?	very likely	medium	<p>As reported by Tinsley et al. (2015a) the species originates from Western Cape, South Africa, and has been introduced on four continents, mostly in areas with a similar Mediterranean climate, but also in cooler environments (where persistence for many decades suggests a capacity for long-term adaptation). This suggests that recent climate warming might enhance invasion ability, favouring range expansion, population growth and negative effects on native faunas (Tinsley et al. 2015a). The introductions occurring well out of the Mediterranean climate zone, show the risk that an increasing number of invasions may occur, and that these are not reported in the literature very quickly (John Measey, pers. comm. 2018).</p> <p>In fact, under foreseeable climate change, the global invasion potential of this species for 2070 assessed by Ihlow et al. (2016) following four IPCC scenarios (i.e. RCP2.6, RCP4.5, RCP6, RCP8.5) may expand in north-western Europe and the Mediterranean area. In fact recent studies show</p>

			<p>that invasive populations of <i>X. laevis</i> are established well beyond the species' multivariate realized niche in southern Africa (Rödder et al. 2017). The maps shown in the paper by Ihlow et al. (2016) and Rödder et al. (2017) do not allow a precise identification of the biogeographic regions where the species could establish in the future under foreseeable climate change.</p> <p>A recent study by Ginal et al. (2020) revealed a much higher risk of invasion, especially for most parts of Europe. However, it does not provide detailed maps for countries and biogeographic regions showing the precise potential invasive range of the species in Europe.</p> <p>In conclusion, the likelihood of entry into the risk assessment area based on all pathways in foreseeable climate change condition is likely to be the same as in current conditions (see above). However, no documented evidence exists to support this statement.</p>
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PROBABILITY OF ESTABLISHMENT			
<p>Important instructions:</p> <ul style="list-style-type: none"> For organisms which are already established in parts of the risk assessment area, answer the questions with regard to those areas, where the species is not yet established. If the species is established in all Member States, continue with Question 1.16. 			
QUESTION	RESPONSE	CONFIDENCE	COMMENT
1.13. How likely is it that the organism will be able to establish in the risk assessment area based on the similarity between climatic conditions within it and the organism's current distribution?	very likely	high	According to Measey et al. (2012) a large suitable climatic potential was identified for most of southern Portugal and adjoining Spain, as well as central and southern France, and mainland Italy. Such data are consistent with the findings of Ihlow et al. (2016), who highlighted areas in the main Mediterranean islands (namely the Balearic Islands, Sardinia, and Corsica) to be highly vulnerable to potential invasions. According to Measey et al. (2012), a few suitable areas were found in the United Kingdom outside southern coastal areas, plus Greece, Ireland, Germany, Belgium, Denmark and the Netherlands (for details see maps developed by Measey et al. 2012). Ihlow et al. (2016) predict only moderate probability for Great Britain. Despite this, the species has had persistent populations in the UK that are now extinct.
1.14. How likely is it that the organism will be able to establish in the risk assessment area based on the similarity between other abiotic conditions within it	very likely	high	<i>X. laevis</i> is a vigorously adaptable species which may virtually inhabit any type of water bodies, including lakes and rivers, as well as

<p>and the organism's current distribution?</p>			<p>permanent and temporary ponds, over a wide range of altitudes and temperatures (Measey 1998). Besides that, <i>X. laevis</i> thrives in disturbed landscapes and artificial habitats, like ponds, wells, dams, irrigation canals and other domestic and agricultural water sources (Tinsley et al. 2015a, Lobos & Jaksic 2005). These habitats are widespread all over the EU, including countries where the species is not yet established. Additionally, Moreira et al (2017) have recently documented that this species can breed in both lotic and lentic environments. The use of lotic habitats may open up even more habitats for breeding, and may contribute to the maintenance of the invasive population even in the absence of lentic sites.</p>
<p>1.15. How widespread are habitats or species necessary for the survival, development and multiplication of the organism in the risk assessment area?</p>	<p>widespread</p>	<p>high</p>	<p><i>Xenopus</i> species in sub-Saharan Africa inhabit virtually all water bodies, including large rivers and lakes, as well as permanent and temporary ponds over a wide range of altitudes and temperatures (Measey 1998). According to John Measey (pers. comm. 2018), animals are often found in very low abundance (and occasionally very high) in natural systems, but numbers can become overwhelming in modified habitats. The latter are normally enriched and eutrophic, which probably helps build up their numbers. Measey et al. (2012) pointed out that comparatively few reports exist of <i>X. laevis</i> in</p>

		<p>its natural habitat, hence the lack of knowledge about the native ecology and natural dispersal of this globally invasive species. However, there are a few studies on the invasive range which provide useful information on this regard. For example, according to a study on habitat suitability carried out in Chile, Lobos et al. (2013) confirm that lentic aquatic environments, with slow drainage and murky waters, highly connected, human-disturbed, and part of an irrigation system of small streams and canals, account for the highest probabilities of successful establishment of <i>X. laevis</i> within the area of invasion. As reported by Lobos & Jaksic (2005) <i>X. laevis</i> in Chile lives from almost sea level up to 620m, and inhabit quite a diverse array of habitats with regard to water temperature, dissolved oxygen, pH, and electric conductivity, indicating a high degree of adaptability and colonization potential.</p> <p>In France, the species is found in a wide range of aquatic habitats and seems to have progressed very quickly along river valleys. Large rivers do not present barriers to the expansion of the species (and may even support further spread). It is likely that it used for its spread the watercourse or the wetlands associated to the river Loire (LIFE CROAA, pers. comm. 2019). Also a recent study by Ginal et al. (2020) has pointed out the</p>
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		<p>importance of river networks in assisting the expansion even in areas with moderately suitable conditions. In the case of the French population, the same authors expressed concern that an ongoing shift in the fundamental niche of the French population combined with scenarios of climate change could lead to further expansion into new ranges, which are currently predicted to be unsuitable for this species. In particular, the French invasive population seems to make use of hydrographic networks and has now reached the Loire River catchment which covers about 20% of the French national territory (Ginal et al. 2020).</p> <p>It is also worth noting that animals are found in isolated ponds that are unconnected, like in France and Sicily, which means that <i>X. laevis</i> is able to move overland (John Measey pers. comm. 2018). Overland movements amongst various types of lentic and lotic water bodies seem to be frequent in this species (Ginal et al. 2021) although the possible contribution of man-mediated releases should be also taken into account in these cases.</p> <p>A study by Moreira et al (2017) in Portugal documented that <i>X. laevis</i> breeds in small streams and ponds, suggesting that while lentic sites are most likely responsible for population booms, the potential reproduction</p>
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			<p>in lotic sites may contribute to the maintenance of the invasive population even in the absence of lentic sites.</p> <p>There are numerous ponds, lakes and other water bodies that are potentially suitable for the survival, development and multiplication of this species in the risk assessment area.</p>
1.16. If the organism requires another species for critical stages in its life cycle then how likely is the organism to become associated with such species in the risk assessment area ?	NA		There is no information suggesting that <i>X. laevis</i> requires another species for critical stages in its life cycle.
1.17. How likely is it that establishment will occur despite competition from existing species in the risk assessment area?	very likely	high	<p><i>X. laevis</i> has only a few competitors that may prevent its establishment in the EU, the most effective being fish (e.g. eels), but the species may find suitable habitats where such competitors are absent (see Tinsley et al. 2015a). According to John Measey (pers. comm. 2018) fish appear to influence where animals will colonise, and it is possible that this could be used to prevent further invasions (e.g. a ring of ponds with introduced fish). Other non-native competitors are mentioned by Prinsloo et al. (1981), i.e. the Chinese silver carp, <i>Hypophthalmichthys molitrix</i>, as both the tadpoles and the silver carp compete for phytoplankton as food, and the Chinese bighead carp <i>Aristichthys nobilis</i> (zooplankton feeder). <i>Xenopus laevis</i> may live in the same ponds as crabs and terrapins, and undergo</p>

			significant predation and mutilation from these groups, but without moving (John Measey, pers. comm. 2018).
1.18. How likely is it that establishment will occur despite predators, parasites or pathogens already present in the risk assessment area?	very likely	high	<p>In Africa, <i>X. laevis</i> have evolved morphological, behavioural, and biochemical predator avoidance strategies, and in extralimital situations, for example in California, it is likely that predatory pressure is considerably reduced (for example by occupying sites lacking predatory fish), thus contributing to the success and spread of <i>X. laevis</i> (McCoid and Fritts, 1980).</p> <p>Despite the lack of dedicated studies on the issue, the situation in the EU may be similar (as demonstrated also by the successful spread of the species in some countries). However, a recent study by Kruger et al. (2019) shows anti-predator response of <i>X. laevis</i> to a Dytiscid beetle (<i>Dytiscus dimidiatus</i>), probably a generic response, and even to the invasive crayfish <i>Procambarus clarkii</i>. These responses were observed in naïve tadpoles suggesting that anti-predator behaviour are evolving in the invasive range, in France at least.</p> <p>In principle, <i>X. laevis</i> can be a prey for several species, including fish, snakes, birds and mammals. For example, in the UK, <i>X. laevis</i> may be eaten by fish and amphibian predators</p>

		<p>including herons, American mink (<i>Neovison vison</i>) and, possibly, grass snakes (<i>Natrix helvetica</i>). Eels are potential predators too (Tinsley et al. 2015a). During a study in western France, Eggert and Fouquet (2006) showed that predation by the polecat (<i>Mustela putorius</i>) was deemed the major adult mortality factor, together with (assumed) freezing. Predation by gulls has been observed on a site with high density in a wastewater treatment unit (LIFE CROAA, pers. comm. 2019).</p> <p>In its native range in South Africa, <i>X. laevis</i> is eaten by large fish, turtles, frogs, snakes, aquatic insects, and birds (Lafferty & Page, 1997). This list is actually far longer. Almost every predator eats the adults, crabs eat the eggs and larvae, and odonates and fish eat the tadpoles (John Measey, pers. comm. 2018). Similarly, in its introduced range outside the EU, i.e. in Chile, three bird species were observed to prey on <i>X. laevis</i>: Night heron (<i>Nycticorax nycticorax</i>), Kelp gull (<i>Larus dominicanus</i>) and Burrowing owl (<i>Speotyto cunicularia</i>) (Lobos & Jaksic (2005). In the USA <i>X. laevis</i> is preyed upon by Two-striped Garter Snakes (<i>Thamnophis hammondi</i>). Large fish, and the American Bullfrog, (<i>Lithobates catesbeianus</i>) are considered to be potential predators as well (Lafferty & Page, 1997). Additionally, according to Prinsloo et</p>
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			<p>al. (1981), the largemouth bass (<i>Micropterus salmoides</i>) is a known “biological control” against <i>X. laevis</i>. A recent study by Lobos (2020) carried out in Chile through laboratory assays provided evidence of the vulnerability of <i>X. laevis</i> to the eastern mosquitofish (<i>Gambusia holbrooki</i>). The results seemed confirmed by the recent establishment of <i>G. holbrooki</i> in a site which resulted in a collapse in the reproduction and adult populations of <i>X. laevis</i>. <i>Gambusia holbrooki</i> is an invasive alien species in Europe, but its impact on <i>X. laevis</i> was not yet studied in this region.</p> <p>Regarding parasites and pathogens, <i>X. laevis</i> carries a rather diverse parasite load, and does not seem to be particularly affected by any of them. In Portugal for example, this species was found to be infected by autochthonous parasites, probably as spillover from <i>Pelophylax perezi</i> (Rodrigues 2014). However, the parasite burden was not as high as in the species they co-exist with, or as high as in the habitats where it is native, which in fact could enable this species to dominate the streams where it was recently introduced.</p>
<p>1.19. How likely is the organism to establish despite existing management practices in the risk assessment area?</p>	<p>very likely</p>	<p>medium</p>	<p>There are no specific management practices in place in the EU which may prevent the organism from establishing wild populations (as demonstrated by the successful establishment of the species in some</p>

			<p>countries). Fish species released (e.g. for sport and angling) may predate on <i>X. laevis</i>, like the non-native largemouth bass (<i>Micropterus salmoides</i>) but this is not considered as a practice targeted to the management of <i>X. laevis</i> in the risk assessment area. Release of <i>Micropterus salmoides</i> as biological control agent of <i>Xenopus laevis</i> was tried in South Africa (Prinsloo et al. 1981). The species may anyway occupy sites lacking predatory fish.</p>
<p>1.20. How likely are existing management practices in the risk assessment area to facilitate establishment?</p>	<p>moderately likely</p>	<p>low</p>	<p>Overall, the species is known to thrive in highly disturbed habitats. For example, in southern California this is considered a common factor in all established populations (McCoid and Fritts, 1980). Therefore, it is likely that management of water bodies facilitates the establishment of the species by contributing to the creation of suitable habitats.</p> <p>However, there are opposite views. For example, management of UK water bodies and connecting habitats tends to be more intense than in many parts of the natural range of <i>X. laevis</i> which would in fact be more likely to hinder, rather than aid, establishment (NNSS 2011).</p> <p>Certain methods of wastewater treatment seems to facilitate the establishment of the species. In France and South Africa, the</p>

			<p>species thrives in units for the treatment of domestic wastewater. These are a string of connected ponds with no specific treatment but with eutrophic waters and higher temperature than other water bodies. In the context of the ongoing LIFE project CROAA (2016-2022), such ponds were fenced and a single unit yielded several thousand captured individuals in less than a year (LIFE CROAA, pers. comm. 2019).</p>
<p>1.21. How likely is it that biological properties of the organism would allow it to survive eradication campaigns in the risk assessment area?</p>	<p>likely</p>	<p>medium</p>	<p>The species went likely extinct by natural means in the UK. However, eradication at a few sites (Measey et al. 2012) using a deliberate, targeted eradication campaign to eliminate the species fairly rapidly was considered a possibility, although follow-up surveys and control measures would be necessary (NNSS 2011).</p> <p>Small eradication campaigns were carried out successfully in the UK, Spain, and the USA (Measey et al. 2012) but in general this was only possible in small areas and at an early stage of invasion. The chances of success seem related more to the specificities of the water bodies affected (e.g. type, size, and overall network) rather than to the biological properties of the species. For example, care must be taken about when this is done as individuals are capable of surviving in the ground for many months (John Measey, pers.</p>

			<p>comm. 2018). Otherwise it is clear that the appropriate methodologies need to be identified carefully in relation to the species biological properties. For example, high concentrations of Rotenone failed to eradicate clawed frogs in Los Angeles County (St. Amant, 1975), because clawed frogs are air breathers (McCoid and Fritts, 1980). In France, a complete eradication of the species is considered impossible in the valley of the Loire due to the large size of the population and the high landscape connectivity of potential habitats (LIFE CROAA, pers. comm. 2019). Furthermore, survival of a handful of individuals could be enough to prevent complete eradication (a single gravid female can contain from 1,000 to 27,000 eggs per clutch, and will produce multiple clutches in a season under favourable conditions).</p>
<p>1.22. How likely are the biological characteristics of the organism to facilitate its establishment in the risk assessment area?</p>	<p>very likely</p>	<p>high</p>	<p>There are no specific studies providing an indication of the propagule pressure, but a single gravid female can contain from 1,000 to 27,000 eggs per clutch, and will produce multiple clutches in a season under favourable conditions (Global Invasive Species Database 2015), therefore in principle only a handful of individuals may be sufficient to start a new population.</p> <p>As shown by Lobos et al. (2014), the invasion</p>

		<p>of <i>X. laevis</i> in Chile has been successful for at least 30 years, in spite of low genetic variability, few events of introduction, low propagule pressure, and bottlenecks in the founding population (although the number of released frogs is unknown, see Lobos and Jaksic 2005). According to Measey et al. (2012) propagule pressure plays a pivotal role in the establishment of <i>X. laevis</i>, as some populations became established after the release of large numbers of animals from breeding facilities (laboratory and pet supplies). Also De Villiers et al (2016) found that small numbers of <i>X. laevis</i> can produce hundreds of adults within relatively short periods (e.g. 18 months).</p> <p><i>X. laevis</i> is principally aquatic throughout its life. In general, tadpoles take 3 months to metamorphosis, and sexual maturity is achieved within one year (Global Invasive Species Database 2015) although this may happen only in certain circumstances (i.e. this was in California and may even be greater than in its native range in South Africa according to John Measey, pers. comm. 2018). Field studies by Tinsley et al. (2015a) showed that in favourable conditions there may be good recruitment, fast individual growth rates and large body size; maximum longevity exceeds 23 years. After all, the reproductive biology of the species seems very flexible. For</p>
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			<p>example in its alien range in the US <i>X. laevis</i> reproduction reportedly occurred in most months of the year, in contrast to the shorter breeding season in South Africa (McCoid and Fritts, 1980). Also in its native range in South Africa, where the breeding season is poorly reported, seems to cover two distinct areas where the breeding times are opposite (John Measey, pers. comm. 2018).</p> <p>In the UK, <i>X. laevis</i> appeared not to breed prolifically under current climatic conditions, but a large recruitment event was considered possible should suitable weather conditions occur for even one season within the period covered by the occurrence of this species in the wild (NNSS 2011).</p> <p>In France, adults move between neighbouring ponds (about 15% of marked individuals). This means that they can evade local unfavourable condition in a site to move to another. Their good dispersal capacity, physiological resistance, and high productivity seems to make populations rather resilient (LIFE CROAA, pers. comm. 2019).</p>
<p>1.23. How likely is the adaptability of the organism to facilitate its establishment?</p>	<p>very likely</p>	<p>high</p>	<p>As summarized by Measey et al. (2012) <i>X. laevis</i> is characterized by a suite of physiological and behavioural traits which makes this anuran very robust and versatile, enabling it to cope with dehydration, high levels of salinity, starvation and anoxic</p>

		<p>conditions. Both adults and larvae perform well over a wide range of temperatures, and larvae can metamorphose in a wide range of temperatures. Behavioural traits include their capability to migrate overland, to survive drought by burrowing into drying mud and to starve for up to 12 months (see Measey et al. 2012, Tinsley et al. 2015a). For example, during drought in the UK, <i>X. laevis</i> could survive in isolated pools in the river course, in subterranean water bodies and by burying themselves in mud (Tinsley et al. 2015a). Additionally, <i>X. laevis</i> shows specific adaptations to aquatic life, including retention of the lateral line system in adults, aquatic chemoreceptors and a body structure particularly adapted for swimming (Elepfandt 1996).</p> <p><i>X. laevis</i> is a very adaptable species, which may virtually inhabit any type of waterbody, including lakes and rivers, as well as permanent and temporary ponds, over a wide range of altitudes and temperatures (Measey 1998). Besides <i>X. laevis</i> thrives in disturbed landscapes and artificial habitats, like ponds, wells, dams, irrigation canals and other domestic and agricultural water sources (Tinsley et al. 2015a, Lobos & Jaksic 2005).</p> <p>After all, as pointed out by Tinsley & McCoid (1996), the hardiness which has made <i>X. laevis</i></p>
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		<p>ideal for laboratory maintenance, has proved to be a considerable advantage for adaptation to new environments. Recent studies show that invasive populations of <i>X. laevis</i> are established well beyond the species' multivariate realized niche in southern Africa (Rödger et al. 2017). As pointed out by John Measey (pers. comm. 2018) it is worth considering that the native range of <i>X. laevis</i> is tropical to Mediterranean, hence from arid desert areas to high rainfall zones, and from sea level to 3000 m asl: this encompasses a massive climatic envelope but does not include their fundamental niche which is likely to have been much larger at the LGM.</p> <p>In addition, the broad global trophic niche of <i>X. laevis</i> and its ability to adapt its diet according to local conditions further contribute to the strong invasive potential of this species (Courant et al. 2017a). The results of the study by Measey et al. (2016) indicate that no prey categories are strongly selected for, suggesting that <i>X. laevis</i> does not usually specialize its diet and hence does not develop a population specific dietary niche. This characteristic may enhance its capacity to establish and spread in novel environments. Furthermore, field data confirm that adults may rely on their own offspring as a food source, enabling older individuals to survive periods of food shortage by exploiting the</p>
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			<p>algal populations eaten by their tadpoles (Tinsley and McCoid 1996), although tadpoles are limited to a certain niche by being obligate suspension feeders (John Measey, pers. comm. 2018).</p> <p>A recent study by Kruger et al. (2019) shows anti-predator response of <i>X. laevis</i> to a Dytiscidae beetle (<i>Dysticus dimidiatus</i>), probably a generic response, and even to the invasive crayfish <i>Procambarus clarkii</i>. These responses were observed in naïve tadpoles suggesting that anti-predator behaviour are evolving in the invasive range, in France at least.</p>
<p>1.24. How likely is it that the organism could establish despite low genetic diversity in the founder population?</p>	<p>likely</p>	<p>high</p>	<p>As shown by a study by Lobos et al. (2014), the invasion of <i>X. laevis</i> in Chile has been successful for at least 30 years, in spite of low genetic variability, few events of introduction, low propagule pressure, and bottlenecks in the founding population (although such low diversity may be not as meaningful as claimed as the study focused on mtDNA, according to John Measey, pers. comm. 2018). Therefore, low genetic diversity is not expected to be a problem for the species invasion process. It is also worth mentioning that these are tetraploid animals, and that this may mitigate against potential bottlenecks (John Measey, pers. comm. 2018).</p>

<p>1.25. Based on the history of invasion by this organism elsewhere in the world, how likely is it to establish in the risk assessment area? (If possible, specify the instances in the comments box.)</p>	<p>very likely</p>	<p>high</p>	<p>The species has already shown to be able to successfully establish viable populations in the risk assessment area, e.g. in Portugal, France and Italy.</p>
<p>1.26. If the organism does not establish, then how likely is it that casual populations will continue to occur?</p> <p>Subnote: Red-eared Terrapin, a species which cannot re-produce in GB but is present because of continual release, is an example of a transient species.</p>	<p>likely</p>	<p>medium</p>	<p>It is likely that high number of individuals are still kept and bred in captivity in the risk assessment area, which leads to a certain risk of some being intentionally or accidentally released in the wild, building up casual occurrences (like happened in the past and led to the occurrence of the populations recorded in the risk assessment area and beyond).</p>
<p>1.27. Estimate the overall likelihood of establishment in relevant biogeographical regions in current conditions (mention any key issues in the comment box).</p>	<p>very likely</p>	<p>high</p>	<p>According to the studies carried out by Measey et al. (2012) and Ihlow et al. (2016) suitable areas (plus some limited optimal areas) fall within the Mediterranean and Atlantic biogeographic regions, as well as the Continental and Alpine regions. Established populations are already present in the former, but not in the latter. A recent study by Ginal et al. (2020) revealed a much higher risk of invasion, especially for most parts of Europe. However, it does not provide detailed maps for countries and biogeographic regions showing the precise potential invasive range of the species in Europe.</p>
<p>1.28. Estimate the overall likelihood of establishment in relevant biogeographical regions in foreseeable climate change conditions</p>	<p>very likely</p>	<p>high</p>	<p>Under foreseeable climate change, the global invasion potential of this species in 2070 assessed by Ihlow et al. (2016) following four</p>

			<p>IPCC scenarios (i.e. RCP2.6, RCP4.5, RCP6, RCP8.5) may expand into northwestern Europe and the Mediterranean area. The maps shown in the paper by Ihlow et al. (2016) do not allow a precise identification of the biogeographic regions where the species could establish in the future under foreseeable climate change. However, it seems that most biogeographic regions may become suitable for the species.</p> <p>A recent study by Ginal et al. (2020) revealed a much higher risk of invasion, especially for most parts of Europe. However, it does not provide detailed maps for countries and biogeographic regions showing the precise potential invasive range of the species in Europe.</p>
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PROBABILITY OF SPREAD			
<p>Important notes:</p> <ul style="list-style-type: none"> • Spread is defined as the expansion of the geographical distribution of an alien species within the risk assessment area. • Repeated releases at separate locations do not represent spread and should be considered in the probability of introduction and entry section. In other words, intentional anthropogenic “spread” via release or escape should be dealt within the introduction and entry section. 			
QUESTION	RESPONSE	CONFIDENCE	COMMENT
<p>2.1. How important is the expected spread of this organism within the risk assessment area by natural means? (Please list and comment on each of the mechanisms for natural spread.)</p>	<p>moderate</p>	<p>high</p>	<p>As summarized by Ihlow et al. (2016) once introduced, the species may rapidly disperse by natural means using irrigation canals, ponds, and rivers as migration corridors, but also performs terrestrial migrations (even without rainfall). It should be noted, however, that movement through streams and irrigation channels appears to be much faster than overland movement (Fouquet and Measey 2006). In a study that compared <i>X. laevis</i> invading an urban area with 2 other species, Vimercati et al (2017) suggested that they may be slower, but build up densities much higher and are arduous to detect.</p> <p>Indicative figures of estimated rate of dispersal are available from a few studies in both the species native and alien range. For example, estimated annual spread of feral populations varied between 1 and over 2 km in France (Fouquet and Measey 2006, LIFE CROAA, pers. comm. 2019) and 5.4 km in Chile (Lobos & Jaksic 2005).</p>

		<p>In particular, a study by Fouquet and Measey (2006) in France showed that, while lotic corridors are used by this principally aquatic species, most ponds are colonised through overland migration. According to Fouquet and Measey (2006) <i>X. laevis</i> is able to detect the presence of non-colonised ponds at a distance, and orient towards them. According to Fouquet and Measey (2006) the terrestrial spread can be estimated at approximately 1 km per year. In Italy, although the rate of spread is not sufficiently assessed, observations were made of newly colonized ponds at a distance of between 400 and 700 m from the nearest pond occupied by <i>X. laevis</i>, where it is likely that most individuals disperse overland (irrigation ditches are not present in the area and the ponds are not connected with each other) or are facilitated by a few temporary streams (Measey et al. 2012). In France, the network of road ditches is dense and these can be filled with water during some periods of the year, which may contribute to enhance survival during overland migration (LIFE CROAA, pers. comm. 2019).</p> <p>Natural spread in the UK appears to have been very slow or non-existent (NNSS 2011) but animals occurred in a very particular system, and spread out of this area would have been very difficult (John Measey, pers. comm. 2018). Additionally, Measey and Tinsley (1998)</p>
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		<p>reported a female travelling 0.2 km in 48 hours. Also in Portugal, according to the result of a recent study by Ginal et al. (2021) the spread of the <i>X. laevis</i> population was comparatively slow compared to the populations in Sicily and France which seem to be spreading fast. This was likely due to the complex topology affecting the habitat connectivity, along with a generalised poor habitat quality of the streams and/or presence of predatory fish, as well as some inherent features of the Mediterranean climate.</p> <p>Overland dispersal rates appear to be slower, compared to situations with ponds close to downstream dispersal corridors (Measey et al. 2012), but as reported by both Faraone et al. (2008) and Fouquet and Measey (2006), population dispersal by terrestrial movement seems prevalent in Italy and France. In particular, in France overland movements of 0.5 km per year are reported (Grosselet et al. 2006), and an adult female followed by radio telemetry moved overland 80 m from a pond through a pasture, crossing a wooded hedge and reaching a puddle 20 centimetres deep (Eggert and Fouquet 2006). According to Measey (2016) distances moved overland were from 40 m to 2 km (although the 2 km distance could have included use of a river), which is comparable to distances travelled by other terrestrial amphibians. There is no apparent difference between native and invasive ranges, besides, walls and thick vegetation are regularly</p>
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		<p>traversed). In fact, in native populations in South Africa a female moved over 2.3 km in less than 6 weeks (De Villiers and Measey 2017).</p> <p>Loupe et al. (2017) found differences in mobility at the range edge of an expanding invasive population of <i>X. laevis</i> in the west of France; in particular individuals from the range edge had a greater stamina and had longer legs compared to individuals at the centre of the range, suggesting fast evolutionary optimization of dispersal abilities. This of course may have implications for conservation because spatial sorting on the range edge resulting in the evolution of locomotory capacity may lead to an accelerated increase in the spread of this invasive species in France. Also, Courant et al. (2017b) found that the level of resources allocated to reproduction was lower at the periphery of the colonized range compared to the centre and may be the result of changes in trade-offs between life-history traits. Such a pattern could be explained by interspecific competition or enhanced investment in dispersal capacity.</p> <p>There is evidence that additional translocations by humans within the risk assessment area may occur, as has been the case in Chile (Measey, pers. comm), however intentional anthropogenic “spread” via release or escape was dealt with in the introduction and entry section (see guidance in the heading above), to avoid duplication of</p>
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			information regarding the relevant pathways.
<p>2.2. How important is the expected spread of this organism within the risk assessment area by human assistance? (Please list and comment on each of the mechanisms for human-assisted spread) and provide a description of the associated commodities.</p>	<p>Minimal</p>	<p>low</p>	<p>There is no evidence of spread by human assistance in the risk assessment area, with the exception of intentional releases or escapes from captive bred populations, which however pertains to the mechanism of entry (hence this is discussed in the relative section). For example, a new Sicilian population of this species was recently described by Faraone et al. (2017) according to whom the hypothesis of natural expansion along the river basin is doubtful, while the occurrence of a man-mediated introduction event is plausible (although it is not clear whether it could originate from individuals caught in the wild or from labs). Therefore this is to be considered in the context of new entries.</p> <p>On this regard, Lobos & Jaksic (2005) pointed out that all calculations of spread rate should be taken with caution given the possibility that there have been additional translocations by humans.</p> <p>No detailed information was found on the potential transport of <i>X. laevis</i> adults and tadpoles with fish lots, as documented for other amphibian species (e.g. the American bullfrog <i>Lithobates catesbeianus</i>), but there is at least one case in France of suspected translocation of individuals with fish restocking (LIFE CROAA, pers. comm. 2019).</p>

			<p>Sediment transport from waste water units also includes potential possibilities to transport frogs over larger distances (LIFE CROAA, pers. comm. 2019).</p>
<p>2.2a. List and describe relevant pathways of spread. Where possible give detail about the specific origins and end points of the pathways.</p> <p>For each pathway answer questions 2.3 to 2.9 (copy and paste additional rows at the end of this section as necessary). Please attribute unique identifiers to each question if you consider more than one pathway, e.g. 2.3a, 2.4a, etc. and then 2.3b, 2.4b etc. for the next pathway.</p>	<ol style="list-style-type: none"> 1) Corridors (Interconnected waterways / basins / seas). 2) “Unaided (Natural dispersal across borders of invasive alien species that have been introduced)”. 		<p>The following pathway is involved in the spread of the species:</p> <ol style="list-style-type: none"> 1. Corridors (Interconnected waterways / basins / seas). <p>This pathway fully overlaps with “Unaided (Natural dispersal across borders of invasive alien species that have been introduced)”.</p> <p>The main difference between the two pathways is that in the first one the species will move through the man-made infrastructures occurring in the area (i.e. interconnected waterway corridors such as channels, ditches, etc.) serving as Corridors with its own capabilities. Otherwise, in the Unaided category, the species is expected to move without any support from either humans or infrastructures. For example, the species is able to spread through overland movements (see details on point 2.1 above) which by the way are intrinsically part of the movements through the waterway corridors. For this reason both pathways have been covered in the risk assessment under one single heading (Corridors (Interconnected waterways / basins / seas)).</p> <p>The likelihood of spread in the Union based on these pathways is very high, since the likelihood</p>

			<p>of survival and reaching a suitable habitat is also very high, as documented above.</p> <p>There is evidence of mass overland movements of animals, estimated to number several thousand (e.g. when water bodies dry-out), and that may be driven, at least in part, by the existence of populations with high densities (Measey 2016).</p>
<i>Pathway name:</i>	[Corridors (Interconnected waterways / basins / seas)] including [Unaided (Natural dispersal acrossborders of invasive alien species that have been introduced)].		
2.3a. Is spread along this pathway intentional (e.g. the organism i released at distant localities) or unintentional (the organism i a contaminant of imported goods)?	Unintentional	high	
2.4a. How likely is it that a number of individuals sufficient to originate a viable population will spread along this pathway from the point(s) of origin over the course of one year?	Likely	high	<p>As summarized by Ihlow et al. (2016) once introduced, the species may rapidly disperse by natural means using irrigation canals, ponds, and rivers as migration corridors.</p> <p>There are no specific studies providing an indication of the propagule pressure, but the species is known to have used this pathway successfully in the risk assessment area.</p> <p>However, the likelihood of this pathway to contribute effectively to the species spread is also related to the overall suitability of the area colonised. In the UK for example, small-scale migration was recorded but overall <i>X. laevis</i> did not show any evidence of dispersal into apparently favourable ponds connected by</p>

			<p>drainage channels in adjacent farmland. Furthermore, limited migration ability under typical environmental conditions was recorded within the potential overland migration range in Africa and California (Tinsley et al. 2015a). De Villiers and Measey (2017) tested also the idea of migratory movements but found no evidence. According to Tinsley et al. (2015a) the low temperature regime may have some effects on dispersal behaviour, but recent studies show that <i>X. laevis</i> is able to move even in quite cold weather conditions, hence this clearly does not prevent the species invasion (Eggert & Fouquet 2006).</p> <p>There is no evidence of reinvasion after eradication, but of course this cannot be excluded given the species' ability to spread undetected.</p> <p>For details see comments in point 2.1. above.</p>
<p>2.5a. How likely is the organism to survive during passage along the pathway (excluding management practices that would kill the organism)?</p> <p>Subnote: In your comment consider whether the organism could multiply along the pathway.</p>	<p>Likely</p>	<p>high</p>	<p>The species is known for having used this pathway successfully in the risk assessment area, hence the likelihood of survival is probably high if the habitat is suitable.</p> <p>If the interconnected waterways (such as irrigation canals, ponds, and rivers) used as migration corridors coincide with suitable habitats (e.g. lack of predatory fish, etc.) it is possible that the species may reproduce successfully along the pathway.</p>

2.6a. How likely is the organism to survive existing management practices during spread?	Likely	medium	No relevant management practices exist which may prevent the natural spread of the species in Europe. On the contrary, there may be practices that may favour the spread of the species. For example in Chile, the common practice of emptying dams once a year (to extract silt) or the extraction and transport of sediments from wastewater units in France may aggravate the situation by forcing the animals to migrate off periodically (Lobos & Jaksic 2005; LIFE CROAA, pers. comm. 2019).
2.7a. How likely is the organism to spread in the risk assessment area undetected?	Very likely	high	The detection of single individuals or even new populations can be difficult, given the aquatic and elusive nature of the species. Several introduced populations of <i>X. laevis</i> have gone undetected for long time periods, 2–25 years in some cases (Measey et al. 2012). Van Sittert and Measey (2016) estimated that invasion debt rates — lag between the export of African clawed frogs and a rise in invasive populations — were around 15 years.
2.8a. How likely is the organism to be able to transfer to a suitable habitat or host during spread?	Very likely	high	According to Measey et al. (2012) irrigation channels and streams or rivers appear to be the major routes for dispersal for many invasions. When these run close to artificial dams or ponds, large populations quickly become established.
2.9a. Estimate the overall potential for spread within the Union based on this pathway?	Likely	high	As summarized by Ihlow et al. (2016) once introduced, the species may rapidly disperse by natural means using irrigation canals, ponds, and

		<p>rivers as migration corridors.</p> <p>Spread may depend on the presence of canals. For example in Chile, a rapidly expanding viticulture industry has been assumed to have the potential to aid the spread of this invader, through extensive irrigation corridors, into new and previously uncolonized areas (Lobos et al. 2013).</p> <p>However, the likelihood of this pathway to contribute to the species spread is also related to the overall suitability of the area colonised. In the UK for example, <i>X. laevis</i> has been unable to spread far by natural means, despite being established at a small number of sites in the UK for several decades. However, it must be considered that animals occurred in a very particular system, and spread out of this area would have been very difficult (John Measey, pers. comm. 2018). Habitat connectivity is poor in the UK and, in any event, it is rarely simultaneously warm and rainy enough to encourage long distance overland movements by this species (NNSS 2011).</p> <p>In France, to the contrary, the high density of water bodies produces a highly connected landscape for the species. Connectivity, however, also depends on land use and according to experiments, the ability to move varies on different surfaces: individuals have more problems progressing across grass cover than</p>
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			<p>across an area with bare soil or forest litter (Vimercati et al. Manuscript in prep.).</p> <p>Sousa et al. (2018) did speculate that artificial lakes of a golf course built between two sites of occurrence of the species in Portugal may have facilitated the dispersal of the species, although this is not confirmed by any definitive evidence.</p>
<i>End of pathway assessment, repeat as necessary.</i>			
2.10. Within the risk assessment area, how difficult would it be to contain the organism in relation to these pathways of spread?	Very difficult	low	Effective containment measures to prevent the spread of <i>X. laevis</i> through the pathway above are the same as those to control/eradicate the species, hence their applicability is clearly context dependent, and depends on the size of the population and the invasion stage.
2.11. Estimate the overall potential for spread in relevant biogeographical regions under current conditions for this organism in the risk assessment area (using the comment box to indicate any key issues).	Rapidly	medium	According to the studies carried out by Measey et al. (2012) and Ihlow et al. (2016) suitable areas (plus some limited optimal areas) fall within the Mediterranean and Atlantic biogeographic regions as well as the Continental and Alpine regions. Established populations are already present in the former, but not in the latter.
2.12. Estimate the overall potential for spread in relevant biogeographical regions in foreseeable climate change conditions	rapidly	medium	Further warming of the climate due to climate change may benefit the species in colonising new areas through natural dispersal. For example, by the 2070s, climate change is predicted to increase suitability in the risk assessment area, although the maps shown in the paper by Ihlow et al.

			(2016) do not allow for a precise identification of the biogeographic regions where the species could establish in the future under foreseeable climate change. However, it seems that most biogeographic regions may become suitable for the species.
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MAGNITUDE OF IMPACT			
<p>Important instructions:</p> <ul style="list-style-type: none"> • Questions 2.13-2.17 relate to biodiversity and ecosystem impacts, 2.18-2.20 to impacts on ecosystem services, 2.21-2.25 to economic impact, 2.26-2.27 to social and human health impact, and 2.28-2.30 to other impacts. These impacts can be interlinked, for example a disease may cause impacts on biodiversity and/or ecosystem functioning that leads to impacts on ecosystem services and finally economic impacts. In such cases the assessor should try to note the different impacts where most appropriate, cross-referencing between questions when needed. • Each set of questions starts with the impact elsewhere in the world, then considers impacts in the risk assessment area (=EU excluding outermost regions) separating known impacts to date (i.e. past and current impacts) from potential future impacts (including foreseeable climate change). • Only negative impacts are considered in this section (socio-economic benefits are considered in Qu. A.7) 			
QUESTION	RESPONSE	CONFIDENCE	COMMENTS
Biodiversity and ecosystem impacts			
2.13. How important is impact of the organism on biodiversity at all levels of organisation caused by the organism in its non-native range excluding the risk assessment area?	Moderate	medium	<p><i>X. laevis</i> is as a generalist predator able to modify its diet according to available resources (Courant et al. 2017a). Evidence exists of the negative impact on local populations of amphibians, fish, and invertebrates (Measey et al. 2012). In fact <i>X. laevis</i> is known to predate on and compete with native amphibians, including eggs and larvae (Measey et al 2015), and is thought to be a cause of trophic cascades by the consumption of benthic macroinvertebrates (Measey, 1998a; Lobos & Measey, 2002). In contrast, <i>Xenopus</i> tadpoles are primarily phytoplankton feeders (Schramm 1986).</p> <p>In particular, in its native range, competition and</p>

			<p>predation toward other pipid frogs (not present in the EU) was reported — i.e. on the IUCN Endangered Cape platanna (<i>X. gilli</i>) — along with predation on other anurans — i.e. the common Cape River Frog (<i>Amietia fuscigula</i>), the clicking stream frog (<i>Strongylopus grayii</i>) and the Southern Dainty Frog (<i>Cacosternum australis</i>) suggesting a high proportion of anurophagy, of either eggs, tadpole or adults (Vogt et al. 2017).</p> <p>In central Chile, <i>X. laevis</i> preys on essentially three major food types: insects, molluscs and crustaceans, while the only vertebrates found in local diets are <i>Xenopus</i> larvae (Lobos & Jaksic 2005). Indeed, predation on amphibians (including on <i>X. laevis</i> itself) represented the most frequent vertebrate taxon in several studies on the species diet (Measey et al., 2016). Another study by Vogt et al. (2017) found <i>X. laevis</i> to consume large quantities of anuran eggs and larvae.</p> <p>Lastly, fish, like the endangered tidewater goby (<i>Eucyclogobius newberryi</i>), western mosquitofish (<i>Gambusia affinis</i>), and Arroyo Chubs (<i>Gila orcuttii</i>), were found in the gut contents of <i>X. laevis</i> inhabiting the estuary of the Santa Clara River, in California (Lafferty & Page, 1997).</p> <p>Given the lack of evidence of long-term irreversible ecosystem change, the impact was considered moderate (see Annex II and remark in point A3 above).</p>
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<p>2.14. How important is the current known impact of the organism on biodiversity at all levels of organisation (e.g. decline in native species, changes in native species communities, hybridisation) in the risk assessment area (include any past impact in your response)?</p>	<p>Moderate</p>	<p>medium</p>	<p>Overall, the species impact on the risk assessment area is similar to the impact described in regions beyond the EU.</p> <p>For example, according to Measey (1998) <i>X. laevis</i> in South Wales ate a wide variety and size range of prey. Zoobenthos and zooplankton made the largest contribution to diets, followed by terrestrial invertebrates. Vertebrate preys (other than eggs and larvae of the same species) were also present, i.e. a bank vole (<i>Clethrionomys glareolus</i>) eaten alive or recently dead, and a chick (unidentified) probably eaten as a carrion. In fact, it is important to consider that <i>X. laevis</i> is also able to detect and feed on carrion (Measey 1998).</p> <p>Amaral & Rebelo (2012) confirmed the predation by <i>X. laevis</i> on eggs and adults of native amphibians, as well as on native fish in Portugal. The diet included benthic preys, with water snails (Physidae) being the most important, followed by the invasive American crayfish (<i>Procambarus clarkii</i>), but also native amphibians (including <i>Rana perezi</i> skeletons and egg masses) and fish (among which <i>Cobitis paludica</i>, a vulnerable Iberian endemic). In Portugal, during an eradication program in Oeiras carried out within the LIFE project LIFE17/GIE/ES/000515 Invasaqua, a captured juvenile of <i>X. laevis</i> was reported to prey on <i>Iberochondrostoma lusitanicum</i> (https://www.wilder.pt/naturalistas/ra-</p>
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			<p>de-unhas-africana-esta-invasora-e-um-caso-de-sucesso-em-portugal/), a critically endangered species according to IUCN realist (Crivelli 2006).</p> <p>In France, Grosselet et al. (2006) speculated that <i>X. laevis</i> may predate on eggs of large newts (i.e. <i>Triturus cristatus</i> and <i>Triturus marmoratus</i>). Also Courant et al. (2018a) showed that species richness of native amphibians was negatively related to the abundance of <i>X. laevis</i>, despite some methodological bias discussed by the authors themselves. In particular, in France a significant decrease in the proportion of nektonic macroinvertebrates was reported in ponds occupied by <i>X. laevis</i> (Courant et al. 2018b).</p> <p>A study by Faraone et al. (2008) in Sicily shows that the most important prey categories are nektonic and planktonic organisms, and confirmed the presence of <i>X. laevis</i> eggs and larvae as well as terrestrial invertebrates (odonates and mayflies) in the diet. Additionally, Lillo et al. (2010) showed that presence of <i>X. laevis</i> in Sicily is associated with a decline in the reproduction of native amphibians (namely <i>Discoglossus pictus</i>, <i>Hyla intermedia</i> and <i>Pelophylax synklepton esculentus</i>). However, no one native amphibian was present in the diet of the species. Only conspecific tadpoles were found, confirming the significant cannibalistic behaviour of this species. The study by Lillo et al. (2010) also shows that the almost total absence of overlap of the trophic niche</p>
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			suggests the lack of competition for trophic resources between the alien species and the native ones.
2.15. How important is the potential future impact of the organism on biodiversity at all levels of organisation likely to be in the risk assessment area?	moderate	low	In case of a future expansion of the species range, other native species may be affected. While there is no documented evidence of the species being able to cause the extinction of any native one, it is likely that the level of risk will at least be confirmed as “Moderate” also in the future.
2.16. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism currently in the risk assessment area?	moderate	low	In the light of the suggested impact on the amphibian species occurring in Italy and France and protected by the Habitats directive (<i>Triturus cristatus</i> is listed in Annex II and IV, while <i>Triturus marmoratus</i> and <i>Discoglossus pictus</i> are listed in Annex IV), the decline in conservation value caused by <i>X. laevis</i> is considered as “Moderate”.
2.17. How important is decline in conservation value with regard to European and national nature conservation legislation caused by the organism likely to be in the future in the risk assessment area?	moderate	low	In case of a future expansion of the species range, other native species may be affected. While there is no documented evidence of the species causing the extinction of any native species, it is likely that the level of risk will at least be “Moderate” in the future.
Ecosystem Services impacts			
2.18 How important is the impact of the organism on provisioning, regulating, and cultural services in its non-native range excluding the risk assessment area?	moderate	high	The impact of <i>X. laevis</i> on ecosystem services is caused by predation with possible accumulative effects in the ecosystem, including increased competition with other species for food (see point

			<p>2.13 and 2.14) and its functioning as a pathogen vector (see point 2.28).</p> <p><i>X. laevis</i> might also have indirect impacts on aquatic systems such as increasing water turbidity and nutrient release caused by <i>X. laevis</i> disturbing the sediment (Lobos and Measey 2002).</p> <p>Consequently, <i>X. laevis</i> has been reported to negatively affect the invaded ecosystems, and as a consequence has been ranked as having the second greatest impact on native ecosystems by any amphibian (Measey et al., 2016). See also the assessments by Kumschick et al. (2017a), Kumschick et al. (2017b) and Kraus (2015) already discussed in point A3 of this document.</p>
<p>2.19. How important is the impact of the organism on provisioning, regulating, and cultural services currently in the different biogeographic regions or marine sub-regions where the species has established in the risk assessment area (include any past impact in your response)?</p>	moderate	high	<p>Overall, the species impact in the risk assessment area is likely to be similar to the impact in regions beyond the EU, as described above, namely on:</p> <ol style="list-style-type: none"> 1) Provisioning (Biomass: Reared aquatic animals); 2) Regulation & Maintenance (Regulation of physical, chemical, biological conditions: Lifecycle maintenance, habitat and gene pool protection, Pest and disease control, Water conditions).
<p>2.20. How important is the impact of the organism on provisioning, regulating, and cultural services likely to be in the different biogeographic regions or marine sub-regions where the species can establish in</p>	moderate	low	<p>There is no documented evidence of the species being able to cause other types of impact (compared to those reported in current conditions), hence the level of risk can be expected to be</p>

the risk assessment area in the future?			“moderate” in the future.
Economic impacts			
2.21. How great is the overall economic cost caused by the organism within its current area of distribution (excluding the risk assessment area), including both costs of / loss due to damage and the cost of current management	minor	low	<p>Due to increased predation and/or competition for food, <i>X. laevis</i> is known to interfere with aquaculture, leading to possible economic costs.</p> <p>While no quantitative estimates about the economic impacts are available, in South Africa, for example, <i>X. laevis</i> is considered a threat to fresh-water aquaculture of common carp (<i>Cyprinus carpio</i>) and Chinese silver carp (<i>Hypophthalmichthys molitrix</i>) mostly because of competition for food (Schramm 1987). Additionally, it is considered a constraint on the production of the giant freshwater prawn (<i>Macrobrachium rosenbergii</i>), mainly due to predation (Taylor et al., 1992). Outside its native range, in Japan, the African clawed frog was found to have an impact on aquaculture by preying on juvenile carp (Kokuryo, 2009). In particular, a study by Schramm (1987) in South African aquaculture ponds, revealed that farmed fish larvae constituted a large proportion of <i>X. laevis</i> stomach contents (up to 25%), and that small fish <1 g are particularly vulnerable (although it does not necessarily represent the typical diet of native populations, see Courant et al. 2017a). Furthermore, in a study by Schramm (1987), it seemed likely that competition with <i>X. laevis</i> tadpoles was at least partly responsible for the slower growth of <i>H. molitrix</i>.</p>

		<p>In addition to the above, a reported problem in South Africa concerns the mass migrations leading to large numbers of clawed frogs invading houses and clogging up irrigation pipes (Somma 2018, Tinsley et al., 1996), but also in this case no figure is available.</p> <p>Following the SEICAT scheme developed by Bacher et al. (2018), the impact category for this species should therefore fall in between Minimal concern and Minor.</p> <p>No information/data is available on the costs for management, despite the many management activities carried out on the species. The only exception is an estimation of the man days reported for the control of the species in a pond in South Africa (De Villiers et al 2016). According to the authors, 27 person days for 338 <i>X. laevis</i> from one impoundment were needed, while regular seining could require as little as eight person days per year. As a side note, according to John Measey (pers. comm. 2018) the impoundment size was 603 m² (see also Vogt et al 2017).</p> <p>Lastly, a LIFE project aimed at the control of <i>Xenopus laevis</i> - together with the American bullfrog (<i>Lithobates catesbeianus</i>) - is currently in progress (2016-2022) in France: LIFE15 NAT/FR/000864 LIFE CROAA - Control strategies Of Alien invasive Amphibians in France (for details, see https://www.life-croaa.eu). The</p>
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			project, co-funded by the EU through the LIFE program, has a total budget of 3,430,179.00 € (see http://ec.europa.eu/environment/life/project/Projects/index.cfm?fuseaction=search.dspPage&n_proj_id=5842). However, as this also targets species other than <i>X. laevis</i> are targeted, and since the project is still in progress, it is not possible to have clear figures on removal costs for <i>X. laevis</i> in particular.
2.22. How great is the economic cost of / loss due to damage* of the organism currently in the risk assessment area (include any past costs in your response)? *i.e. excluding costs of management	minimal	low	No information/data is available on the economic costs caused by <i>X. laevis</i> . In the UK the economic losses caused by this species, if any, were considered likely to be minimal given the limited distribution and very small numbers of <i>X. laevis</i> that were present (NNSS 2011).
2.23. How great is the economic cost of / loss due to damage* of the organism likely to be in the future in the risk assessment area? *i.e. excluding costs of management	minimal	low	In case of a future expansion of the species range, some economic impact and associated costs may be evidenced, e.g. on aquaculture activities or other sectors. While there is no documented evidence of the species causing this type of impact in the risk assessment area, it is not possible to exclude that this could happen in the future. However, for the time being it should be considered minimal.
2.24. How great are the economic costs / losses associated with managing this organism currently in the risk assessment area (include any past costs in your response)?	moderate	-medium	The only figures that are available in the risk assessment area concern the activities carried out in France through the project LIFE CROAA, Control strategies Of Alien invasive Amphibians in France (LIFE15 NAT/FR/000864). This project

			aims to limit the expansion of <i>X. laevis</i> along dispersal corridors (together with the eradication/control of <i>Lithobates catesbeianus</i> in several sites). The total budget of this project is of 3.43 million euro and it will be carried out in the period 2016-2022 by the Société Herpétologique de France and other partners. In particular, the costs sustained locally to fight against <i>X. laevis</i> in 2017 and 2018 amounted to 81.000 €. The activities were carried out on a small part of the main introduction site, with the aim of preserving a few sites considered to be at stake due to the presence of native species or located near dispersal areas (LIFE CROAA, pers. comm. 2019).
2.25. How great are the economic costs / losses associated with managing this organism likely to be in the future in the risk assessment area?	major	low	In case of a future expansion of the species range in the risk assessment area, the economic costs / losses associated with managing <i>X. laevis</i> may rise accordingly.
Social and human health impacts			
2.26. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism for the risk assessment area and for third countries, if relevant (e.g. with similar eco-climatic conditions).	minimal	low	No information has been found on the issue.
2.27. How important is social, human health or other impact (not directly included in any earlier categories) caused by the organism in the future for the risk assessment area.	minimal	low	No information has been found on the issue.
Other impacts			

<p>2.28. How important is the impact of the organism as food, a host, a symbiont or a vector for other damaging organisms (e.g. diseases)?</p>	<p>minor</p>	<p>low</p>	<p>The most serious impact usually attributed to <i>X. laevis</i> is related to its potential role in the introduction and spread of the chytrid fungus, <i>Batrachochytrium dendrobatidis</i> (<i>Bd</i>), the cause of amphibian deaths and population declines in several parts of the world (Weldon et al. 2004). <i>Bd</i> disease has been implicated in mass mortalities and widespread declines in European amphibian species, like common midwife toad (<i>Alytes obstetricans</i>) (Bosch et al., 2001) and fire salamander (<i>Salamandra salamandra</i>) (Bosch & MartínezSolano, 2006) in Spain. However, to date there is no evidence that <i>X. laevis</i> has functioned in this role of <i>Bd</i> vector or has caused impact on native amphibians through this mechanism. For this reason, the impact of the species was considered “minor” by Kumschick et al. (2017b, see in particular the supporting information annexed to the relevant paper). As a remark, the same authors discussed a previous assessment by Kraus (2015) based on the assumption that <i>X. laevis</i> contributed to the spread of <i>Bd</i> which then caused declines in native species, but which is not demonstrated (De Busschere et al. 2016, John Measey, pers. comm. 2018). Hence a higher score would not be justified.</p> <p><i>Xenopus laevis</i> was also identified as a potential vector of ranavirus (Robert et al., 2007).</p> <p>Although a causal link between <i>X. laevis</i> and the dispersal of these pathogens is not demonstrated</p>
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		<p>(Measey et al. 2012), this frog could play a role in the spread of disease, by acting as an asymptomatic reservoir/vector for both diseases, without being susceptible or just suffering sublethal effects. This seems to be confirmed at least for the chytrid fungus by studies on either wild or captive animals in the UK, Chile, and USA (Tinsley et al. 2015b, Solís et al. 2010, Soto-Azat et al. 2016, Vredenburg et al. 2013), but not in France (Ouellet et al. 2012).</p> <p>Additionally, <i>X. laevis</i> may carry several other parasites and pathogens, like <i>Chlamydia</i> (Howerth et al. 1984, Reed et al. 2000) and many others, in both its native range and the alien range (Kuperman et al. 2004, Tinsley, 1996). For example, according to Lafferty & Page (1997), three internal parasites were observed in or on the gut (although a complete parasitological assessment was not undertaken). The African tapeworm <i>Cephalochlamys namaquensis</i> was found in intensities from 6-25 individuals (including several mature adults) in the anterior duodenum. It was not previously reported outside of Africa, hence it may have entered other areas with this species. Ciliates of the genus <i>Nyctotherus</i> (0.25 mm trophs) were present in abundance posterior to the section of the gut where tapeworms occurred. Larval nematodes were encysted on the outside of the stomach wall (might be transferred to the birds that eat them, potentially leading to some pathology).</p>
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			<p>A recent study (Schoeman et al. 2020) reports seven previously unrecorded nematode species (none of which could be identified to species level) parasitising <i>X. laevis</i> across South Africa. These are adult <i>Capillaria</i> sp. and <i>Falcaustra</i> sp. from the intestine, third stage larvae of <i>Contracaecum</i> sp. encysted in the body cavity, third stage larvae of <i>Paraquimperia</i> sp. and <i>Tanqua</i> sp. from the intestine and two different species of second stage nematode larvae from the lungs and kidneys, respectively. According to Schoeman et al. (2020) this result illustrates that <i>X. laevis</i> is an important parasite reservoir in its native range, with implications for its role in the invasive range.</p> <p>In France, only parasites originating from the frog’s native range have been detected so far. This has been found in other areas suggesting that <i>Xenopus</i> also introduces its own parasites in the assessment area (Schoeman et al. 2019). Parasites of <i>X. laevis</i> on its native range have been detected at a high prevalence in western France (<i>Protopolystoma xenopodis</i>: prevalence 19 %, <i>Cephalochlamys namaquensis</i>: prevalence 63%). The same parasites have been found in distinct invasive <i>Xenopus</i> populations. There has been no attempt to determine whether these alien parasites colonized new amphibian hosts in Europe.</p>
2.29. How important might other impacts not already covered by previous questions be resulting from introduction of the organism? (specify in the	NA		No information has been found on the issue.

comment box)			
2.30. How important are the expected impacts of the organism despite any natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area?	major	low	The natural control by other organisms, such as predators, parasites or pathogens that may already be present in the risk assessment area, is not expected to mitigate the impact of <i>X. laevis</i> in relation to its role as a vector of dangerous parasites and pathogens to the native fauna.

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ANNEX I Scoring of Likelihoods of Events

(taken from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Description	Frequency
Very unlikely	This sort of event is theoretically possible, but is never known to have occurred and is not expected to occur	1 in 10,000 years
Unlikely	This sort of event has not occurred anywhere in living memory	1 in 1,000 years
Moderately likely	This sort of event has occurred somewhere at least once in recent years, but not locally	1 in 100 years
Likely	This sort of event has happened on several occasions elsewhere, or on at least one occasion locally in recent years	1 in 10 years
Very likely	This sort of event happens continually and would be expected to occur	Once a year

ANNEX II Scoring of Magnitude of Impacts

(modified from UK Non-native Organism Risk Assessment Scheme User Manual, Version 3.3, 28.02.2005)

Score	Biodiversity and ecosystem impact	Ecosystem Services impact	Economic impact (Monetary loss and response costs per year)	Social and human health impact
	<i>Question 2.18-22</i>	<i>Question 2.23-25</i>	<i>Question 2.26-30</i>	<i>Question 2.31-32</i>
Minimal	Local, short-term population loss, no significant ecosystem effect	No services affected ¹⁸	Up to 10,000 Euro	No social disruption. Local, mild, short-term reversible effects to individuals.
Minor	Some ecosystem impact, reversible changes, localised	Local and temporary, reversible effects to one or few services	10,000-100,000 Euro	Significant concern expressed at local level. Mild short-term reversible effects to identifiable groups, localised.
Moderate	Measureable long-term damage to populations and ecosystem, but little spread, no extinction	Measureable, temporary, local and reversible effects on one or several services	100,000-1,000,000 Euro	Temporary changes to normal activities at local level. Minor irreversible effects and/or larger numbers covered by reversible effects, localised.
Major	Long-term irreversible ecosystem change, spreading beyond local area	Local and irreversible or widespread and reversible effects on one / several services	1,000,000-10,000,000 Euro	Some permanent change of activity locally, concern expressed over wider area. Significant irreversible effects locally or reversible effects over large area.
Massive	Widespread, long-term population loss	Widespread and irreversible effects on one /	Above 10,000,000 Euro	Long-term social change, significant loss of employment,

¹⁸ Not to be confused with „no impact“.

	or extinction, affecting several species with serious ecosystem effects	several services		migration from affected area. Widespread, severe, long-term, irreversible health effects.
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ANNEX III Scoring of Confidence Levels

(modified from Bacher *et al.* 2017)

Confidence level	Description
Low	There is no direct observational evidence to support the assessment, e.g. only inferred data have been used as supporting evidence <i>and/or</i> Impacts are recorded at a spatial scale which is unlikely to be relevant to the assessment area <i>and/or</i> Evidence is poor and difficult to interpret, e.g. because it is strongly ambiguous <i>and/or</i> The information sources are considered to be of low quality or contain information that is unreliable.
Medium	There is some direct observational evidence to support the assessment, but some information is inferred <i>and/or</i> Impacts are recorded at a small spatial scale, but rescaling of the data to relevant scales of the assessment area is considered reliable, or to embrace little uncertainty <i>and/or</i> The interpretation of the data is to some extent ambiguous or contradictory.
High	There is direct relevant observational evidence to support the assessment (including causality) <i>and</i> Impacts are recorded at a comparable scale <i>and/or</i> There are reliable/good quality data sources on impacts of the taxa <i>and</i> The interpretation of data/information is straightforward <i>and/or</i> Data/information are not controversial or contradictory.

ANNEX IV Ecosystem services classification (CICES V5.1, simplified) and examples

For the purposes of this risk assessment, please feel free to use what seems as the most appropriate category / level / combination of impact (Section – Division – Group), reflecting information available.

Section	Division	Group	Examples (i.e. relevant CICES “classes”)
Provisioning	Biomass	Cultivated <i>terrestrial</i> plants	Cultivated terrestrial plants (including fungi, algae) grown for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from cultivated plants, fungi, algae and bacteria for direct use or processing (excluding genetic materials); Cultivated plants (including fungi, algae) grown as a <u>source of energy</u> <i>Example: negative impacts of non-native organisms to crops, orchards, timber etc.</i>
		Cultivated <i>aquatic</i> plants	Plants cultivated by in- situ aquaculture grown for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from in-situ aquaculture for direct use or processing (excluding genetic materials); Plants cultivated by in- situ aquaculture grown as an <u>energy source</u> . <i>Example: negative impacts of non-native organisms to aquatic plants cultivated for nutrition, gardening etc. purposes.</i>
		Reared animals	Animals reared for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from reared animals for direct use or processing (excluding genetic materials); Animals reared to provide <u>energy</u> (including mechanical) <i>Example: negative impacts of non-native organisms to livestock</i>
		Reared <i>aquatic</i> animals	Animals reared by in-situ aquaculture for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from animals grown by in-situ aquaculture for direct use or processing (excluding genetic materials); Animals reared by in-situ aquaculture as an <u>energy source</u> <i>Example: negative impacts of non-native organisms to fish farming</i>
		Wild plants (terrestrial and aquatic)	Wild plants (terrestrial and aquatic, including fungi, algae) used for <u>nutrition</u> ; <u>Fibres and other materials</u> from wild plants for direct use or processing (excluding genetic materials); Wild plants (terrestrial and aquatic, including fungi, algae) used as a <u>source of energy</u> <i>Example: reduction in the availability of wild plants (e.g. wild berries, ornamentals) due to non-native organisms (competition, spread of disease etc.)</i>
		Wild animals (terrestrial and aquatic)	Wild animals (terrestrial and aquatic) used for <u>nutritional purposes</u> ; <u>Fibres and other materials</u> from wild animals for direct use or processing (excluding genetic materials); Wild animals (terrestrial and aquatic) used as a <u>source of energy</u> <i>Example: reduction in the availability of wild animals (e.g. fish stocks, game) due to non-native</i>

			organisms (competition, predations, spread of disease etc.)
	Genetic material from all biota	Genetic material from plants, algae or fungi	<u>Seeds, spores and other plant materials</u> collected for maintaining or establishing a population; Higher and lower plants (whole organisms) used to <u>breed new strains or varieties</u> ; Individual genes extracted from higher and lower plants for the <u>design and construction of new biological entities</u> <i>Example: negative impacts of non-native organisms due to interbreeding</i>
		Genetic material from animals	Animal material collected for the purposes of maintaining or establishing a population; Wild animals (whole organisms) used to breed new strains or varieties; Individual genes extracted from organisms for the design and construction of new biological entities <i>Example: negative impacts of non-native organisms due to interbreeding</i>
	Water ¹⁹	Surface water used for nutrition, materials or energy	Surface water for <u>drinking</u> ; Surface water used as a material (<u>non-drinking purposes</u>); Freshwater surface water, coastal and marine water used as an <u>energy source</u> <i>Example: loss of access to surface water due to spread of non-native organisms</i>
		Ground water for used for nutrition, materials or energy	Ground (and subsurface) water for <u>drinking</u> ; Ground water (and subsurface) used as a material (<u>non-drinking purposes</u>); Ground water (and subsurface) used as an <u>energy source</u> <i>Example: reduced availability of ground water due to spread of non-native organisms and associated increase of ground water consumption by vegetation.</i>
Regulation & Maintenance	Transformation of biochemical or physical inputs to ecosystems	Mediation of wastes or toxic substances of anthropogenic origin by living processes	<u>Bio-remediation</u> by micro-organisms, algae, plants, and animals; <u>Filtration/sequestration/storage/accumulation</u> by micro-organisms, algae, plants, and animals <i>Example: changes caused by non-native organisms to ecosystem functioning and ability to filtrate etc. waste or toxics</i>
		Mediation of nuisances of anthropogenic origin	<u>Smell reduction; noise attenuation; visual screening</u> (e.g. by means of green infrastructure) <i>Example: changes caused by non-native organisms to ecosystem structure, leading to reduced ability to mediate nuisances.</i>
	Regulation of physical, chemical, biological conditions	Baseline flows and extreme event regulation	Control of <u>erosion</u> rates; Buffering and attenuation of <u>mass movement</u> ; <u>Hydrological cycle and water flow regulation</u> (Including flood control, and coastal protection); <u>Wind</u> protection; <u>Fire</u> protection

¹⁹ Note: in the CICES classification provisioning of water is considered as an abiotic service whereas the rest of ecosystem services listed here are considered biotic.

			<p><i>Example: changes caused by non-native organisms to ecosystem functioning or structure leading to, for example, destabilisation of soil, increased risk or intensity of wild fires etc.</i></p>
		Lifecycle maintenance , habitat and gene pool protection	<p><u>Pollination</u> (or 'gamete' dispersal in a marine context); <u>Seed dispersal</u>; Maintaining <u>nursery populations and habitats</u> (Including gene pool protection)</p> <p><i>Example: changes caused by non-native organisms to the abundance and/or distribution of wild pollinators; changes to the availability / quality of nursery habitats for fisheries</i></p>
		Pest and disease control	<p>Pest control; Disease control</p> <p><i>Example: changes caused by non-native organisms to the abundance and/or distribution of pests</i></p>
		Soil quality regulation	<p><u>Weathering processes</u> and their effect on soil quality; <u>Decomposition and fixing processes</u> and their effect on soil quality</p> <p><i>Example: changes caused by non-native organisms to vegetation structure and/or soil fauna leading to reduced soil quality</i></p>
		Water conditions	<p>Regulation of the <u>chemical condition</u> of freshwaters by living processes; Regulation of the chemical condition of salt waters by living processes</p> <p><i>Example: changes caused by non-native organisms to buffer strips along water courses that remove nutrients in runoff and/or fish communities that regulate the resilience and resistance of water bodies to eutrophication</i></p>
		Atmospheric composition and conditions	<p>Regulation of <u>chemical composition</u> of atmosphere and oceans; Regulation of <u>temperature and humidity</u>, including ventilation and transpiration</p> <p><i>Example: changes caused by non-native organisms to ecosystems' ability to sequester carbon and/or evaporative cooling (e.g. by urban trees)</i></p>
Cultural	Direct, in-situ and outdoor interactions with living systems that depend on presence in the environmental setting	Physical and experiential interactions with natural environment	<p>Characteristics of living systems that that enable activities promoting health, recuperation or enjoyment through <u>active or immersive interactions</u>; Characteristics of living systems that enable activities promoting health, recuperation or enjoyment through <u>passive or observational interactions</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that make it attractive for recreation, wild life watching etc.</i></p>

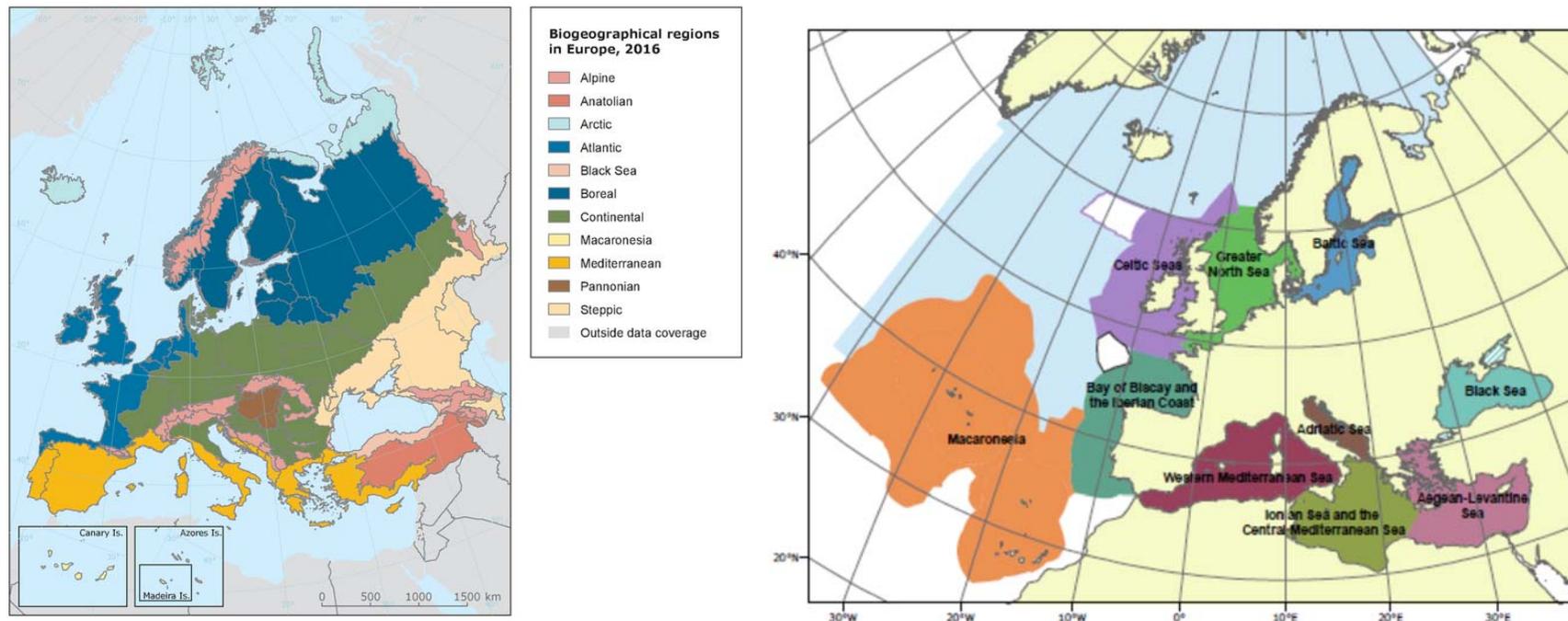
		Intellectual and representative interactions with natural environment	<p>Characteristics of living systems that enable <u>scientific investigation</u> or the creation of traditional ecological knowledge;</p> <p>Characteristics of living systems that enable <u>education and training</u>;</p> <p>Characteristics of living systems that are resonant in terms of <u>culture or heritage</u>;</p> <p>Characteristics of living systems that enable <u>aesthetic experiences</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have cultural importance</i></p>
	Indirect, remote, often indoor interactions with living systems that do not require presence in the environmental setting	Spiritual, symbolic and other interactions with natural environment	<p>Elements of living systems that have <u>symbolic meaning</u>;</p> <p>Elements of living systems that have <u>sacred or religious meaning</u>;</p> <p>Elements of living systems used for <u>entertainment or representation</u></p> <p><i>Example: changes caused by non-native organisms to the qualities of ecosystems (structure, species composition etc.) that have sacred or religious meaning</i></p>
		Other biotic characteristics that have a non-use value	<p>Characteristics or features of living systems that have an <u>existence value</u>;</p> <p>Characteristics or features of living systems that have an <u>option or bequest value</u></p> <p><i>Example: changes caused by non-native organisms to ecosystems designated as wilderness areas, habitats of endangered species etc.</i></p>

ANNEX V EU Biogeographic Regions and MSFD Subregions

See <https://www.eea.europa.eu/data-and-maps/figures/biogeographical-regions-in-europe-2> ,
http://ec.europa.eu/environment/nature/natura2000/biogeog_regions/

and

<https://www.eea.europa.eu/data-and-maps/data/msfd-regions-and-subregions-1/technical-document/pdf>



ANNEX VI Species distribution model

Projected distributions were obtained from the authors of two existing studies reporting three global-scale species distribution models for *Xenopus laevis* (Measey et al 2012; Ihlow et al 2016). Both studies used a similar distribution database, with more record cleaning in the later paper, and a similar set of input variables and modelling methods (Table 1). However, the three models varied considerably in which climate variables were used to predict suitability (Table 1), suggesting high uncertainty in using the outputs of the models for this assessment.

The authors of both papers supplied shapefiles with the projected suitable regions, revealing marked differences in the European regions predicted to be suitable in the current climate (Figure 1). All models predict substantial suitable regions in Portugal, Spain, France and Italy. The models of Ihlow et al (2016) also predict large suitable regions in eastern Europe, based on a minimum training presence threshold. This threshold probably overestimates the suitable region in Europe as the species has been introduced and recorded in marginal conditions in northern Europe and suitability gradient maps shown in the paper suggest moderate to high suitability only in warm western Mediterranean regions. The predictions from the Measey et al (2012) model should be treated with caution as the Maxent model is less reliable than the ensemble model (John Measey pers. comm. 2018). Also, this study did not consider suitability under different emission scenarios (Table 1). Climate change projections supplied for the 2070s from Ihlow et al (2016) differed markedly between emissions scenarios and the Maxent and Ensemble models. However, these projections do not appear consistent across scenarios (e.g. RCP4.5 should be intermediate between RCP2.6 and RCP6.0, but it is not in all cases) and were probably influenced by an overly liberal minimum training threshold choice.

Overall, the information supplied was considered too uncertain to usefully identify suitable regions beyond the currently invaded regions of Europe.

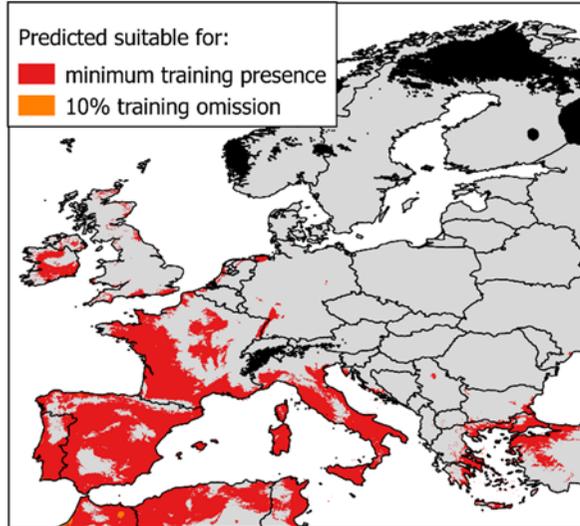
Table 1. Comparison of published species distribution models for *Xenopus laevis*.

	Measey et al (2012)	Ihlow et al 2016 (Maxent)	Ihlow et al 2016 (Ensemble)
Number of native range records	1075	826	826
Number of non-native range records	124	99	99
Spatial resolution	2.5 arcminutes	2.5 arcminutes	2.5 arcminutes
Predictor variables from Worldclim	Isothermality (bio3) Minimum temperature of the coldest month (bio6) Temperature annual range (bio7) Mean temperature of the wettest quarter (bio8) Mean temperature of the driest quarter (bio9) Mean temperature of the warmest quarter (bio10) Precipitation seasonality (bio15) Precipitation of wettest quarter (bio16) Precipitation of driest quarter (bio17) Precipitation of coldest quarter (bio19)	Temperature annual range (bio7) Mean temperature of the wettest quarter (bio8) Mean temperature of the driest quarter (bio9) Mean temperature of the warmest quarter (bio10) Mean temperature of the coldest quarter (bio11) Precipitation of wettest quarter (bio16) Precipitation of driest quarter (bio17) Precipitation of the warmest quarter (bio18) Precipitation of coldest quarter (bio19)	Temperature annual range (bio7) Mean temperature of the wettest quarter (bio8) Mean temperature of the driest quarter (bio9) Mean temperature of the warmest quarter (bio10) Mean temperature of the coldest quarter (bio11) Precipitation of wettest quarter (bio16) Precipitation of driest quarter (bio17) Precipitation of the warmest quarter (bio18) Precipitation of coldest quarter (bio19)
Modelling software	Maxent	Maxent	Biomod
Background definition	Radius of 250 km around the records	Radius of 250 km around the records	Radius of 250 km around the records
Reported predictor importance	Isothermality (27.4%) Minimum temperature of coldest month (19.8%) Precipitation of coldest quarter (11.7%) Mean temperature of warmest quarter (10.4%) Mean temperature of wettest quarter (8.8%) Temperature annual range (6.7%) Precipitation of wettest quarter (6.6%)	Precipitation of driest quarter (27.7%) Mean temperature of wettest quarter (16.8%) Mean temperature of coldest quarter (14.5%) Precipitation of warmest quarter (11.4%) Precipitation of coldest quarter (8.3%) Temperature annual range (7.0%) Mean temperature of driest quarter (6.2%) Precipitation of wettest quarter (6.2%) Mean temperature of warmest quarter (1.9%)	Mean temperature of coldest quarter (19.1%) Precipitation of warmest quarter (16.6%) Mean temperature of warmest quarter (13.9%) Precipitation of driest quarter (12.6%) Precipitation of coldest quarter (8.3%) Mean temperature of driest quarter (8.3%) Precipitation of wettest quarter (8.0%) Mean temperature of wettest quarter (7.6%) Temperature annual range (5.0%)

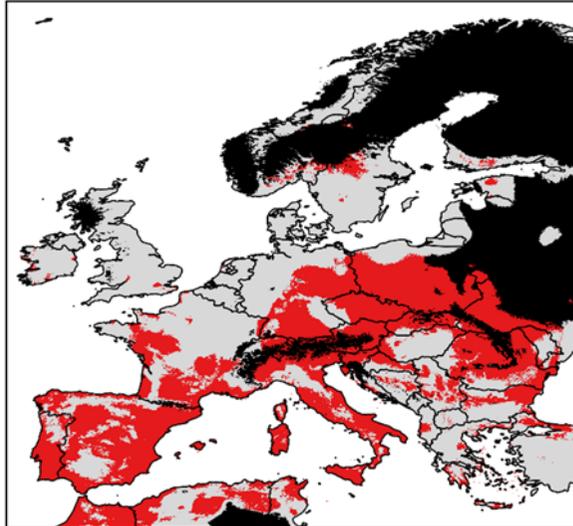
	Measey et al (2012)	Ihlow et al 2016 (Maxent)	Ihlow et al 2016 (Ensemble)
Threshold(s) to project suitable region	Minimum training presence and 10% training omission	Minimum training presence	Minimum training presence
Masking to prevent extrapolation	Multivariate Environmental Similarity Surface (MESS)	Multivariate Environmental Similarity Surface (MESS)	Variable clamping
Climate change scenarios	None	2070s under RCP2.6, RCP4.5, RCP6.0 and RCP8.5	2070s under RCP2.6, RCP4.5, RCP6.0 and RCP8.5

Figure 1. Projected European regions suitable for establishment by *Xenopus laevis* from three modelling approaches. In (a) the suitable region is defined using two thresholds, with almost no parts of Europe projected suitable under the stricter 10% omission threshold. The threshold used in (b) and (c) is the minimum training presence, and suitable areas are shaded red. In all plots, regions where extrapolation prevented prediction are shown in black.

(a) Measey et al (2012) Maxent model



(b) Ihlow et al (2016) Maxent model



(c) Ihlow et al (2016) Ensemble model

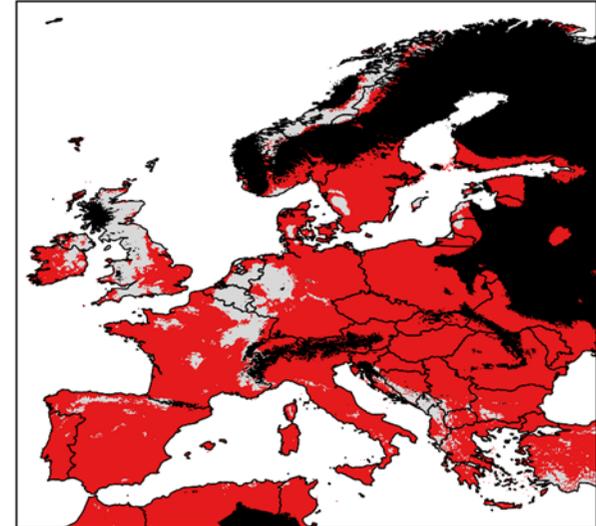


Figure 2. Variation in projected suitability among Biogeographical regions of Europe (Bundesamt für Naturschutz (BfN), 2003) from the different model outputs supplied. The regions are shown in the right hand map. Measey.1 and Measey.2 differ based on thresholding by the minimum training presence or a stricter 10% omission rate, respectively.

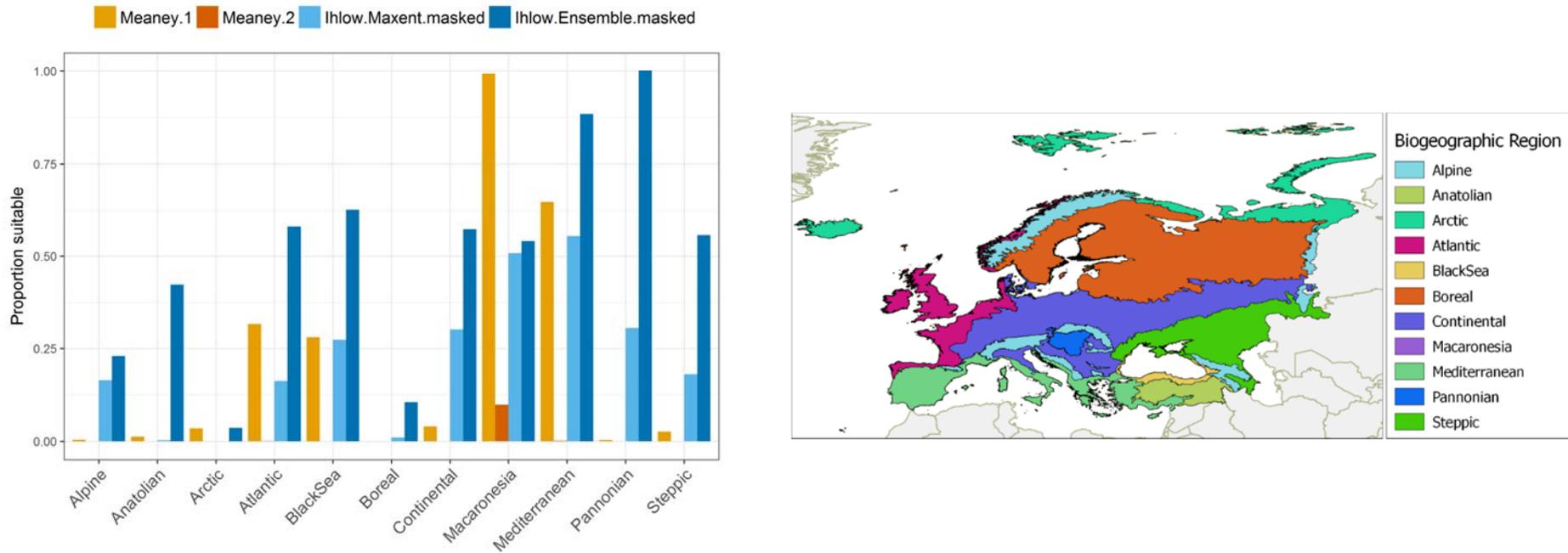
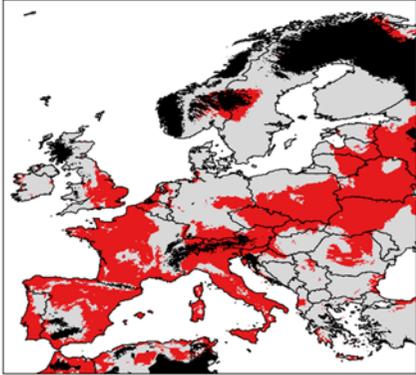
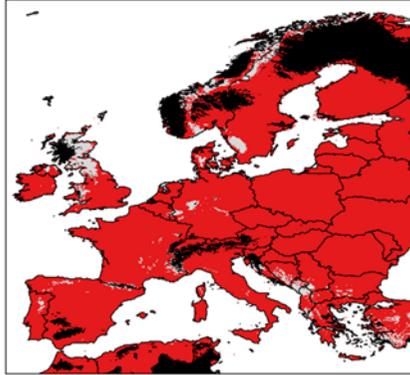


Figure 3. Projected European regions suitable for establishment by *Xenopus laevis* in 2070 under four emissions scenarios. Suitable areas above the suitability of the minimum training presence are shaded red. Regions where extrapolation prevented prediction are shown in black.

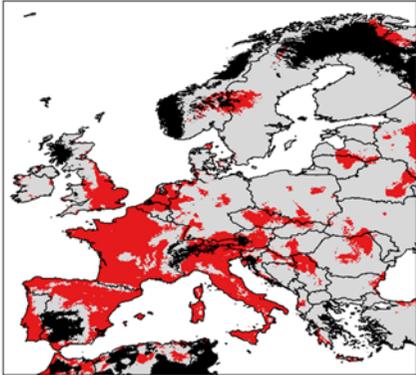
(a) Ihlow et al (2016) Maxent – RCP2.6



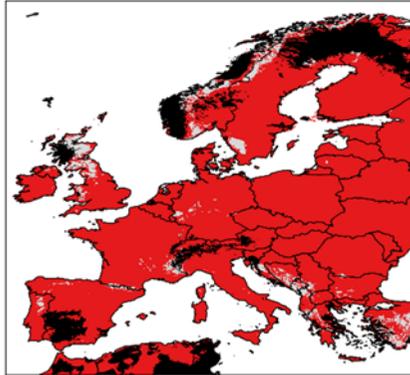
(b) Ihlow et al (2016) Ensemble – RCP2.6



(c) Ihlow et al (2016) Maxent – RCP4.5



(d) Ihlow et al (2016) Ensemble – RCP4.5

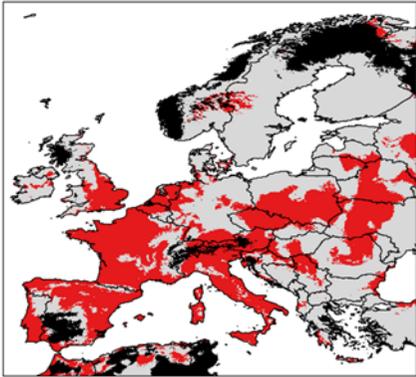


(e) Ihlow et al (2016) Maxent – RCP6.0

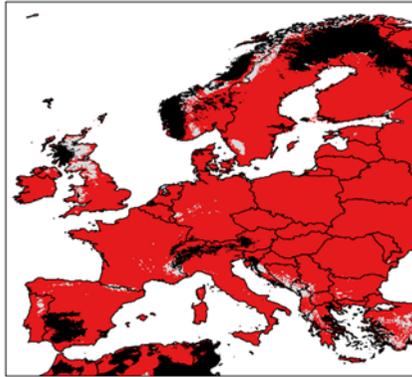


(f) Ihlow et al (2016) Ensemble – RCP6.0

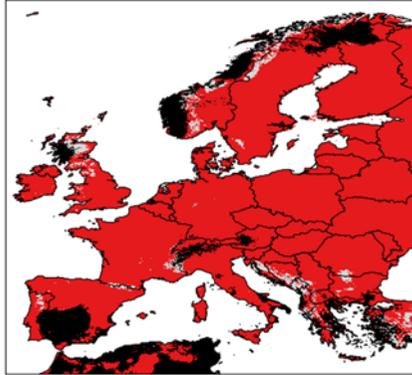
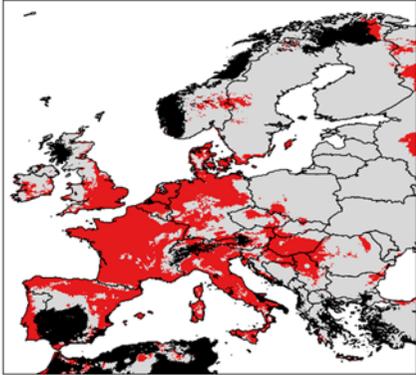




(g) Ihlow et al (2016) Maxent – RCP8.0



(h) Ihlow et al (2016) Ensemble – RCP8.0



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