



HAL
open science

Spill-back events followed by self-sustainment explain the fast colonization of a newly built marina by a notorious invasive seaweed

Mathilde Salamon, Laurent Levêque, Marion Ballenghien, Frédérique Viard

► **To cite this version:**

Mathilde Salamon, Laurent Levêque, Marion Ballenghien, Frédérique Viard. Spill-back events followed by self-sustainment explain the fast colonization of a newly built marina by a notorious invasive seaweed. *Biological Invasions*, 2020, 22 (4), pp.1411-1429. 10.1007/s10530-019-02193-5 . hal-02860993

HAL Id: hal-02860993

<https://hal.science/hal-02860993>

Submitted on 9 Jun 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Spill-back events followed by self-sustainment explain the fast colonization of a newly built**
2 **marina by a notorious invasive seaweed**

3
4 **Authors (names, affiliation, e-mail addresses)**

5 Mathilde Salamon ^(1,2)

6 Laurent Lévêque ⁽³⁾

7 Marion Ballenghien ⁽¹⁾

8 Frédérique Viard ⁽¹⁾

9
10 (1) Sorbonne Université, CNRS, Lab. Adaptation and Diversity in Marine Environments (UMR 7144
11 CNRS SU), Station Biologique de Roscoff, Place G.Teissier, 29680 Roscoff

12 (2) present address : Département des sciences biologiques, Université du Québec à Montréal (UQAM),
13 P.O. Box 8888, Succursale Centre–Ville, Montréal, Québec, Canada, H3C 3P8

14 (3) Sorbonne Université, CNRS, Fédération de Recherche (FR 2424 CNRS SU), Station Biologique de
15 Roscoff, Place G.Teissier, 29680 Roscoff

16 **Author for correspondence:** Frédérique Viard, ORCID no. 0000-0001-5603-9527, [viard@sb-](mailto:viard@sb-roscoff.fr)
17 roscoff.fr

18
19 **Running head**

20 Demo-genetic surveys of the colonization dynamics of *Undaria pinnatifida*

21 **Acknowledgments**

22 The authors gratefully acknowledge the divers the Marine Operations Department (Service Mer &
23 Plongée) of the Roscoff Marine Biological Station (Mathieu Camusat, Yann Fontana, Wilfried Thomas),
24 Caroline Broudin, Ronan Garnier, Sarah Bouchemousse for their help during field surveys and sampling.
25 The authors are also grateful to the marina Director (Frédéric Boccou) and to Patrick Podeur from the
26 Biocéan company who allowed us to conduct these surveys and sampling in the marina and the farm,
27 respectively. We are most grateful to the Biogenouest Genomer core facility for its technical support.
28 This work benefited from the support of the French National Research Agency (ANR) with regards to
29 the IDEALG project (ANR-10-BTBR-04).

31 **Abstract**

32 Biological invasions are occurring at increasing rates since the onset of the 20th century. While ports
33 and marinas have been identified as a major point-of-entry for the spread of marine non-indigenous
34 species (NIS), their relationships with wild habitats however needs further scrutiny. We had the rare
35 opportunity to monitor the real-time colonization dynamics of a newly-built marina by the notorious
36 invasive kelp *Undaria pinnatifida* in the Bay-of-Morlaix, Brittany (France). Field surveys (>20 000
37 individuals geo-localized) were combined with genetic analyses (10 microsatellite loci, N=890
38 individuals) over three years (i.e., 6 generations in the study area). Regarding the colonization dynamics,
39 a dramatic snow-ball effect was documented over time, with local density reaching up to 50 individuals
40 per m after two years. Assignment tests showed that the primary colonizers came from neighboring
41 populations established in natural rocky reefs. A shift towards a self-sustaining population was however
42 observed the following year, with 44% of self-assignment. These processes are best explained by i) life
43 history traits, notably rapid growth and selfing, and ii) natural dispersal within the marina combined
44 with human-mediated dispersal – through leisure boating- over longer distances. Spill-over effects have
45 been previously documented, and here also reported, to explain the expansion of *U. pinnatifida* from
46 marinas to the wild. We showed that the on-going ocean sprawl also offers a perfect arena for spill-back
47 events (i.e., spread from natural habitats to artificial structures), highlighting the need for careful
48 surveillance of newly built infrastructures.

49

50 **Keywords:** *Undaria pinnatifida*, artificial habitats, rocky reefs, dispersal, population genetics, spatial
51 analyses

52

53 **Introduction**

54 The ocean sprawl (i.e., the increase in artificial structures along the coasts; Duarte et al. (2013)) is a major
55 process affecting marine coastal environments, notably by favoring the introduction and establishment of
56 non-indigenous species (NIS) (Firth et al. 2016). In marinas, NIS represent a major component of the flora
57 and fauna (Airoldi et al. 2015; Bishop et al. 2015; Glasby et al. 2007). These marine urban habitats are
58 considered as invasion hubs promoting the spread of NIS towards natural habitats (i.e., spill-over effects,
59 Airoldi et al. 2015; Bulleri, Chapman 2010). Recreational vessels, neglected for a long time, are indeed
60 increasingly recognized as vectors transporting introduced species over a large range of distances (Clarke
61 Murray et al. 2011, Mineur et al. 2008, Ulman et al. 2019). Once marine NIS are established, eradicating
62 them proves to be difficult and cost-inefficient in most cases (Ojaveer et al. 2015; Simberloff et al. 2013).
63 Management of invasive species has been found to be more likely to succeed and less economically
64 damaging when efforts are focused on curtailing the vectors and pathways (Lodge et al. 2016; Pyšek,
65 Richardson 2010). Understanding the interactions between wild and marina populations is critical, notably
66 to define management strategies and prioritize targets in surveillance programs.

67 Field surveys, combined to DNA-based studies, can help uncover the connectivity patterns between
68 populations occupying artificial and natural habitats (Rius et al. 2015). We carried out this approach, using
69 the seaweed *Undaria pinnatifida* Harvey (Suringar, 1873) (Laminariales, Alariaceae) as a case study. This
70 brown alga is an iconic invader: it became established worldwide in less than 30 years, through global
71 shipping and aquaculture (Epstein, Smale 2017; South, Thomsen 2016). In Europe, it was first introduced
72 accidentally in the Mediterranean Sea, presumably by aquaculture imports of the Pacific oyster from Asia,
73 then deliberately transported on the NW coasts of France (Brittany) to be cultivated. In Brittany,
74 colonization of surrounding natural and artificial habitats was rapidly observed nearby these farmed
75 populations (Floc'h et al. 1996), indicating that the cultures were the primary source of new populations in
76 the wild in Brittany. This hypothesis was later confirmed with genetic studies (Voisin et al. 2005). However,
77 in its non-native range, *U. pinnatifida* shows preference for artificial structures (Epstein, Smale 2017;
78 Epstein, Smale 2018a; Epstein, Smale 2018b; Guzinski et al. 2018; South, Thomsen 2016). A recent
79 population genomics study, carried out in Brittany, showed that populations in natural rocky reefs are
80 genetically similar to marinas rather than farms, suggesting on-going spill-over processes from marinas to
81 the wild, rather than from farms to the wild (Guzinski et al. 2018). These DNA-based results are congruent
82 with results obtained from field surveys, documenting spill-over events in areas where no farms exist so far
83 (Epstein, Smale 2018b).

84 *U. pinnatifida* is a short-lived, selfing species (e.g., 2 generations per year in the English Channel),
85 with an annual heteromorphic life cycle of alternating macroscopic diploid sporophytes and microscopic

86 haploid gametophytes. Its opportunistic nature may provide advantages to colonize artificial structures such
87 as marinas, seawalls, buoys etc. (Sliwa et al. 2006). Dispersal of *Undaria* by leisure boating or larger vessels
88 has been documented (South et al. 2017). In addition, spores can survive in bilge water, and gametophytes
89 germinating from spores are able to grow on hulls, anchoring systems, and ropes (Bollen et al. 2017).
90 However, the relative contribution of natural and human-based dispersal needs further scrutiny. Natural
91 dispersal by spores and gametes is indeed expected to occur at very short distances (<10-100m, Forrest et
92 al. 2000), even though spores can survive 1-2 days and be washed away by the tidal currents (Saito 1975b).
93 Drifting mature sporophytes might also be responsible of dispersal over larger distances (1-10km, Sliwa et
94 al. 2006). As pointed out by Guzinski et al. (2018), these natural means of dispersal are nevertheless unlikely
95 to explain the chaotic genetic structure observed at a regional scale, suggesting that dispersal by recreational
96 vessels play a predominant role in connecting distant (> 10 km) sites. Yet, in a context of increasing ocean
97 sprawl, it is unclear to which extent and at which rates novel substrates can be colonized, and through which
98 dispersal means (South et al. 2017).

99 Previous studies (e.g., Epstein, Smale 2018b; Grulois et al. 2011; Guzinski et al. 2018) focused
100 mostly on spatial patterns. Information on the temporal colonization dynamics is, however, important to
101 understand the capacity of *U. pinnatifida* to establish new populations. This study aimed at characterizing
102 the establishment dynamics of *U. pinnatifida* within a newly available artificial site, here a marina, and
103 examining the connectivity between three different population categories (cultivated, established in natural
104 rocky reefs and in marinas), within a bay. Considering the life cycle characteristics and life-history traits of
105 *U. pinnatifida* (Nyberg, Wallentinius 2005), as well as predictions from a model of the population growth
106 dynamics (Murphy et al. 2016a; Murphy et al. 2017), we hypothesized that the colonization dynamics of
107 the marina should be rapid (i.e., within 1-2 years). Given the limited natural dispersal abilities of *U.*
108 *pinnatifida* and the role of leisure boating as a dispersal vector at a regional scale (Guzinski et al. 2018), we
109 also hypothesized that the colonization would rely on diverse sources from nearby populations, inside the
110 study bay, as well as from outside the bay.

111 We chose to test these hypotheses in the bay of Morlaix (Brittany, France), which provides an
112 excellent case study: a farm has been cultivating *U. pinnatifida* since the end of the 1990's, and natural sites
113 of the bay that were considered as suitable habitats for the establishment of *U. pinnatifida* were surveyed
114 starting the early 2000s to detect potential spillovers from the seaweed farm. The first population recorded
115 in natural rocky reefs appeared in 2006 (ca. 10 years after the beginning of the farming activities), at a site
116 located nearby the farm (50m). Until 2013, only three other stable populations were reported in rocky reefs.
117 The building of a marina in 2011-2012 in this bay, close to the farm and known rocky reefs populations,
118 gave us the rare opportunity to conduct a real-time monitoring of the colonization in a relatively well-known

119 historical context of the establishment of this introduced seaweed. We combined field-surveys and a genetic
120 dataset obtained jointly over three years, which correspond to six generations of *U. pinnatifida* in the study
121 area (i.e, two generations per year). Spatio-temporal census data (> 20 000 individuals geo-localized) as
122 well as spatialized genetic data (10 microsatellites loci; N=398 individuals) in the marina allowed us to
123 characterize the colonization patterns (origin of the colonizers, dispersal modes etc.). Temporal genetic data
124 from individuals sampled in the marina and the bay (N = 816) were used to compare the three population
125 categories and their changes over time.

126 **Methods**

127 *Study sites, monitoring and sampling*

128 The study sites are located in the Bay-of-Morlaix (48°41'46"N, 3°54'18"W) in Brittany, France (Fig. 1). The
129 building of the marina started in 2011. No *Undaria pinnatifida* individuals had ever been observed on pilings
130 or breakwater jetties prior to the work, despite intensive survey to record NIS in the context of other research
131 projects (Bouchemousse 2015; FV & LL, unpublished data). The marina structures include 9 floating
132 pontoons (60-205m long) linked by a gateway (25 sections of 12m), and a floating breakwater of 225 m (9
133 sections of 25m) protecting the marina at the south (Fig. 1A). Presence of *U. pinnatifida* on the floating
134 pontoons was checked after their set-up starting in 2012 and detailed monitoring was conducted from 2014
135 onward following the initial observations. Biannual field surveys (December and March, with an additional
136 survey in February 2014) were used to target separate generations ("Winter" and "Spring") as documented
137 in the study region (Grulois 2010; Murphy et al. 2017; Murphy et al. 2016b; Voisin 2007). The number of
138 individuals per 3m long stretches on every artificial structure was recorded, combining diving (for the
139 floating breakwater) and observation from the surface (for the pontoons). Sampling for genetic analyses was
140 carried out during spring surveys: a small piece of tissue (2x2 cm approximatively) was detached from the
141 thalli and stored in silica gel. Until February 2014, all the individuals found in the marina were sampled.
142 After February 2014, because too many individuals were observed, a sub-sampling was done (representative
143 of the spatial distribution and density of the individuals). Each sampled individual was geo-localized, and
144 data integrated in a Geographic Information System (ArcGis). In addition, five nearby natural rocky reefs
145 were surveyed and sampled annually between 2013 and 2015, as well as the farm. The rocky reef
146 populations (Men Guen: MG; Guerh on: GU; Rannic: RA; Roc'h Velen: RV and Enez Touell: ET; Fig. 1)
147 were sampled (23-36 individuals per site) at low tide or by diving. *U. pinnatifida* was not found in RA and
148 GU in 2014, and populations at two localities were newly recorded during the survey (RV in 2014 and ET
149 in 2015). It is important to note that these observations could be due to survey biases (e.g., timing of the
150 survey of this short lived species, unnoticed individuals at the start of the colonization) rather than true
151 absence or colonization of new sites. Cultivated individuals were sampled on the ropes (N=32-36) by diving.

152 ***Spatial analyses***

153 The spatial distribution of individuals in the marina and correlation between abundances of two successive
154 generations were characterized by recording the number of individuals per 3m-long section (defined by one
155 set of coordinates), then analyzed with bivariate mark correlation functions (second-order statistics, Illian
156 et al. (2008)), using the software Programita (Wiegand, Moloney 2014). To look if pairs of abundances
157 between two successive generations and separated by a distance r were smaller or larger than pairs selected
158 at random, the data were first analyzed with the normalized mark correlation function $kmimj(r)$:

159
$$kmimj(r) = \frac{mi * mj}{\mu^2}$$

160 which is the mean of the products of abundance values (counts) m_i and m_j separated by a distance r divided
161 by the normalization constant μ^2 , μ being the average value over all pairs, i and j being indexes for sets of
162 coordinates belonging to distinct successive generations. The correlation between abundances of successive
163 generations was also investigated using the Schlater's correlation function $Imimj(r)$, which is a Moran's I
164 like summary statistic and a spatial analogous of the Pearson correlation coefficient (Schlather et al. 2004):

165
$$Imimj(r) = \frac{[mi - \mu_i(r)] * [mj - \mu_j(r)]}{\sigma_i \sigma_j}$$

166 with $\mu_i(r)$ and $\mu_j(r)$ the average of the counts separated by distance r in each successive generation separately
167 and σ_i, σ_j . the normalization constant with σ_i^2 and σ_j^2 the variances of the counts in each generation. The
168 observed correlation functions were then compared to global envelopes obtained from 199 Monte-Carlo
169 simulations of the null-model (5th lowest and 5th highest values of the estimator, significance level $\alpha = 0.05$),
170 which consists in randomly shuffling the counts of the second (new) generation but preserving the counts
171 of the first generation as well as the x and y coordinates in each generation. To account for the spatial
172 heterogeneity of the distribution of abundances (e.g., east-west gradient), the counts were shuffled locally
173 within a maximal distance r , which preserved the overall heterogeneity ($r = 25$ m from March 2014 to March
174 2015, and $r = 50$ m for December 2015 and March 2016). A bandwidth of 6 m was used when estimating
175 the correlation functions.

176 ***DNA extraction and microsatellites genotyping***

177 DNA was extracted with the Nucleospin® 96 plant kit (Macherey-Nagel, Düren, Germany), from 3-38 mg
178 of dried tissue, following manufacturer's instructions, but with a modified lysis step at room temperature
179 for one hour instead of 65°C for 20 min. Extracted DNA was diluted to 1:100. We chose to use microsatellite
180 markers as a cost-effective technique to examine a large number of individuals. These markers have been
181 shown to provide similar results to ddRad-Sequencing for diversity and connectivity analyses of *U*.

182 *pinnatifida* in the study regions (Guzinski et al. 2018). As compared to ddRad-Sequencing, the use of
183 microsatellites makes also easier the comparison with our previous dataset (Guzinski et al. 2018, Grulois et
184 al. 2011). A total of 816 individuals were genotyped with 10 microsatellite loci, Up-AC-1B2, Up-AC-1B5,
185 Up-AC-1C1, Up-AC-1G2, Up-AC-1H5, Up-AC-2C1, Up-AC-2E8, Up-AC-4G2, Up-AC-4C12, Up-AC-
186 4E9 (Daguin et al. 2005) using multiplex PCRs, as described in Grulois et al. (2011). PCR products were
187 run with a capillary sequencer AB3130XL (Applied Biosystem). Genotypes were scored with GeneMapper
188 4.0 (Applied Biosystem). Missing data represented 2.8% of all data and were distributed across loci and
189 samples.

190 ***Genetic data analyses***

191 Population genetic diversity

192 Genetic diversity indices were estimated for each natural sites and farm samples (detailed in Table S1). For
193 the marina, in 2013 and 2014, all the individuals (N=11 and 91, respectively) were used as a single sampling
194 unit. For the year 2015, indices were calculated for the individuals sampled over the whole marina (N =
195 296), as well as over a subsample of 140 individuals, with 30-40 individuals selected from sections S1 and
196 S8 of the breakwater and on pontoons B and I, which were the locations where the highest densities of *U.*
197 *pinnatifida* were observed in 2014 and thus considered as founding populations. For each sampling unit, we
198 estimated the mean number of alleles per locus (N_a), the allelic richness (A_r), the observed (H_o) and expected
199 heterozygosity under Hardy-Weinberg equilibrium (H_e) as well as the F_{IS} fixation index, using Genepop v.
200 4.3 (Rousset 2008) and Fstat v 2.9.3 (for A_r ; Goudet 1995). The null hypothesis of HW equilibrium was
201 tested for each population unit with an exact G-test using Genepop (100 batches of 5000 iterations).

202 Selfing rates and relatedness analyses

203 *Undaria pinnatifida* being a selfing species (Grulois et al. 2011; Guzinski et al. 2018), selfing rates, s , were
204 estimated from the coefficient g_2 , computed with the software RMES, and using the equation 9 from (David
205 et al. 2007). The null hypothesis « $g_2=s=0$ » was tested with 1000 random resampling of single-locus
206 heterozygosity between individuals. Because aggregation patterns were observed in the marina (see
207 Results), we hypothesized that related individuals might be found close to each other. A parentage analysis
208 was thus conducted with COLONY 2.0.6.2 (Jones, Wang 2009) on the individuals sampled in 2015. We
209 used the full-likelihood method shown to be the most accurate method (Wang 2012). Other parameters were:
210 presence of inbreeding, no parent-candidates, polygamous mating, medium length run and precision. Each
211 analysis was averaged over 5 runs and conducted twice to evaluate the convergence of the results. Sibship
212 category and selfing were considered as true when the probability was > 0.9 over the two independent

213 analyses. We used a one-way ANOVA to test the effect of the Euclidean spatial distances between pairs of
214 individuals on sibship category (full-sib, half-sib and non-related), and differences between categories were
215 assessed with a Tukey HSD test. We finally used the slope b of the regression of the pairwise genetic
216 distance \hat{a}_i and the geographical distance to estimate the axial mean square parent-offspring distance (σ^2)
217 (Rousset 2000). The density value used to estimate σ^2 was 0.06 ind.m^{-2} (4607 individuals counted in Spring
218 2015 over an area of ca. $7.5 \times 10^4 \text{ m}^2$). The confidence interval at 0.95 was obtained for the slope of the
219 regression with the ABC bootstrap method implemented in Genepop.

220 Assignment analyses at the bay scale

221 In order to assess if the sampling sites are connected by recent migration events and determine the most
222 likely origin of the individuals recorded in the marina, we used an assignment procedure, based on the
223 Bayesian method of Rannala and Mountain (1997) implemented in GeneClass v. 2.0 (Piry et al. 2004).
224 Putative sources considered for origin of individuals sampled in 2014 were the populations sampled in 2013.
225 For those sampled in 2015, to account for multi-generational effects, sources were populations sampled in
226 2013 and 2014 (i.e., merged genotypic dataset over the two years). The probability of an individual
227 originating from a given putative source was calculated with the method of Paetkau (2004), from 10000
228 Monte-Carlo simulations of individual genotypes and a type I error rate of 0.01. This probability was used
229 as a threshold to reject ($p < 0.01$) a putative source and thus take into account non-sampled sources in the
230 analysis (i.e., exclusion of all the studied putative sources).

231 Genetic structure at the bay and marina scale

232 Genetic differences were examined by computing the estimator θ of F_{ST} (Weir, Cockerham 1984). The null
233 hypothesis that alleles are drawn from the same distribution in all populations was tested with a G-test in
234 Genepop. We used the Bayesian clustering method implemented in InStruct v. 1.0 to account for selfing
235 (Gao et al. 2007). In this approach, the most likely number K of clusters is assessed with a model that does
236 not assume Hardy-Weinberg equilibrium within cluster: the cluster of origin of individuals (with potential
237 admixture) and the individual's inbreeding coefficients are jointly estimated. The number of MCMC
238 iterations was set at 250000, with a burn-in of 50000 and a thinning of 10 (i.e., 2000 saved iterations). We
239 ran the program for 10 independent chains with values of K between 1 and 20. The Deviance Information
240 Criterion (DIC) was plotted against K to select the best K_{max} (Gao et al. 2011).

241 To characterize the spatial distribution of discrete genetic groups in the population established over the
242 marina in 2015, we combined the Bayesian clustering methods implemented in Instruct and in the R library
243 Geneland v.4.0.5 (Guillot et al. 2005a; Guillot et al. 2005b). We first determined the best number of clusters

244 explaining the overall genetic distribution using Instruct v. 1. 0, with values of K between 1 and 16. We
245 then ran Geneland, with the best K estimated from Instruct, to assess the spatial distribution of the K genetic
246 clusters, using a model with correlated allele frequencies, an uncertainty of the coordinate of 0.5 (accounting
247 for the presence of individuals with identical coordinates) and potential null alleles. The number of MCMC
248 iterations was set at 250000, with a thinning of 100. For the post-processing of the output, the spatial domain
249 was defined with X = 100 et Y = 150 and the burn-in was 500. The results of 5 independent runs were
250 compared to assess the correspondence of the spatial clusters.

251 **Results**

252 **Colonization dynamics of the marina**

253 The survey started in 2012 but *Undaria pinnatifida* did not appear in the marina until February 2013, when
254 a first group of individuals (N = 11, all mature) were found on the hull of a leisure boat located at the east
255 end of the pontoon I (Fig. 1, Fig. 2). The colonization started the year after mainly on the floating breakwater
256 (Fig. 2, February 2014), though three individuals were also observed on pontoons F and I. Over time, empty
257 spots on the breakwater were gradually colonized (Fig. 2, March 2014-March 2016). For pontoons, the
258 colonization really started with the next winter generation (December 2014; Fig. 2), moving from the eastern
259 side (extremity) towards the western side (inside) of each pontoon. In 2016, the marina was fully colonized
260 (Fig. 2, March 2016).

261 Concomitantly to the spatial expansion, the densities increased over successive generations between
262 March 2014 and March 2016 (Fig. 2, Supplementary Material Fig. S1). In March 2016, densities reached 1
263 ind.m⁻¹ at the whole marina scale (8117 m of available substrate, with > 9200 individuals counted overall).
264 Locally, the density reached much higher values: 12, 18 and 50 ind.m⁻¹ found on the gateway, floating
265 pontoons and breakwater respectively (Fig. 2). Population growth followed different trends according to the
266 location within the marina (Fig. S1): on the gateway and along the pontoons the best fit was with an
267 exponential growth curve (Fig. S1C-D) whereas it follows a logarithmic growth curve on the breakwater
268 (Fig. S1B).

269 The mark-correlation functions analyses revealed a clustering pattern of the number of individuals
270 between two consecutive surveys, with the size of the clusters increasing with time. Abundances of
271 individuals observed nearby groups of the previous generation were significantly larger than for pairs of
272 groups selected at random (Fig. 3 B-D-F-H). The spatial extent of this relationship increased over time (0-
273 2 m for March-December 2014 to 0-22m for December 2015-March 2016). Moderate to high positive
274 correlations were found for pairs of groups from two consecutive generations located below 24 m (Fig. 3A-

275 C-E-G). On the contrary, at greater distances, pairs of abundances from groups of two successive
276 generations tend to be smaller or equal to pairs chosen randomly, and negatively or not correlated.

277 **Genetic diversity in the study samples**

278 Regardless of the year and the population category (i.e., marina, farm, rocky reefs), all the study populations
279 were found to be polymorphic, including the set of 11 individuals collected on the ship hull in 2013, which,
280 despite its small census size, show the highest allelic richness (2.7; Table 1). The lowest allelic richness
281 estimates were observed in the cultivated population (1.5 in 2014 and 2015) and in one rocky reef (MG; 1.3
282 to 1.7 across years; Supplementary Material Table S1). Similar results were found for the expected genetic
283 diversity (H_e ; Table 1, Table S1). Altogether, the marina population showed equal or higher genetic diversity
284 than populations sampled in natural rocky reefs, the latter being (with the exception of MG) more genetically
285 diverse than the farm. Ten out of the 19 study populations display significant deviations from expectations
286 under the Hardy-Weinberg equilibrium, all from the marina or wild sites (Table 1, Table S1). These
287 heterozygote deficiencies are likely due to selfing, which is clearly evidenced in 7 out of these 10
288 populations based on the g_2 statistics. Selfing rates varied over time in the marina, null in the colonizing
289 population (2014) but reaching 36% in 2015.

290 **Genetic structure at the bay scale and origin of the colonizers in the marina**

291 High and significant genetic structure was observed at the bay scale, with F_{ST} values of 0.33 ($p < 10^{-5}$), 0.30
292 ($p < 10^{-5}$) and 0.16 ($p < 10^{-5}$) in 2013, 2014 and 2015, respectively. Bayesian clustering analyses (InStruct)
293 showed that the overall genetic variation found in the bay (19 sampling units sampled between 2013 and
294 2015, $N=658$ individuals) was best explained by four genetic clusters ($K=4$; Fig. 4). Individuals collected
295 on pontoons and breakwater showed higher membership to two clusters, pictured in yellow and orange in
296 Fig. 4. Many individuals of pontoon B and breakwater section S1 showed membership to the yellow cluster
297 also characteristic of the individuals from the natural sites RV, ET and RA. Conversely, pontoon I and
298 breakwater section S8, showed high membership to the orange cluster mostly found in rocky reef-GU in
299 2013 (but not in 2015). One rocky reef, MG, displayed a very distinctive genetic background pictured by
300 the purple color in Fig. 4. The farm was also clearly differentiated in 2014 and 2015 (blue color, Fig. 4) but
301 shared membership with the marina and rocky reef-GU in 2013 (orange cluster). The two well established
302 rocky reef populations MG and RA showed a stable genetic background throughout the sampling period.
303 Inspecting results for lower and higher K -values ($K=3$, $K=5$) confirmed the main patterns, with MG and the
304 farm (in 2014 and 2015) well differentiated from all the other samples (Supplementary Material, Fig. S2).

305 InStruct results were confirmed with the assignment analyses carried at the bay scale (Fig. 5). In
306 the marina, the first wave of settlers (February 2014, N = 91) were assigned to different sources, with diverse
307 contributions (Fig. 5A). Almost half of the individuals (48%) were assigned to the rocky reef GU, the
308 geographically closest natural population, whereas 12% seem to originate from the reproduction of the
309 mature sporophytes observed in 2013 on the hull of a boat. A small proportion (9%) could not be assigned
310 to any of the sampled populations, pointing to unknown sources. In 2015, an important change was
311 observed, as most of the individuals (44%) were assigned to the marina population samples of 2013-2014
312 (Fig. 5B). For the rocky reef MG, almost all the individuals were assigned to the same locality sampled in
313 the previous years (97% for 2014 and 100% for 2015; Fig. 5A-B). For the two sites where putative
314 extinction-recolonization occurred (rocky reefs RA and GU), different patterns were observed (Fig. 5B):
315 diverse sources contributed to GU in 2015, whereas RA individuals were mostly assigned to the population
316 found at the same site in 2013. For GU, most of the individuals (68%) were assigned to the nearby located
317 farm. For the putatively new rocky reef populations, RV and ET, the first individuals were assigned to
318 diverse sources. In RV, the largest proportion (32%) of the individuals sampled in 2014 could not be
319 assigned to any of the putative sources sampled in 2013, but the year after, most individuals of RV were
320 assigned to the same site (75% of R15-RV individuals assigned to R14-RV). For ET, newly reported in
321 2015, 26% of the individuals were assigned to the marina.

322 **Spatial and kin aggregation in the marina**

323 Five genetic clusters best explained the genetic variation of the 296 individuals sampled in the marina in
324 2015. Each of these five clusters display a clear spatial aggregation pattern (Fig. 6): individuals belonging
325 to the same genetic group are clumped into relatively high density “nuclei” spread over a small area (mean
326 distance between individuals forming a nucleus: 17.5 ± 14.9 m). The most extreme situation was found with
327 the fifth genetic cluster (Fig. 6F), composed of only 7 individuals, all grouped very closely at the eastern
328 extremity of pontoons F and H. Six of them are full-sibs (see below) and were among the individuals
329 excluded from all the putative sampled sources. For the four other clusters, 8% of the individuals were
330 located farther away from their nuclei in the marina suggesting longer-distance dispersal events (~80-320m).

331 Analyses with COLONY revealed 13 lineages of full-sibs, representing 12% of the 296 sampled
332 individuals. Five lineages were pairs of individuals produced by outcrossing and the other eight (5 pairs and
333 3 groups of 4-6 individuals) were produced by independent selfing events. In addition, a complex half-sib
334 structure was found, with 26 individuals related to one or more individuals. This kinship structure was
335 aggregated in space (Supplementary Material, Fig. S3). Interestingly, the mean Euclidian distances between
336 pairs of individuals decreased inversely to the level of kinship, from unrelated (131.6 ± 77.9 m) to half-sibs

337 (60.1 ± 63.7 m) down to full-sibs (16.6 ± 34.4 m) (Fig. S4). This is congruent with the axial mean parent-
338 offspring distance (σ^2) estimated to 6.8 m (with a population density D of 0.06 ind.m⁻²).

339 **Discussion**

340 Temporal studies of marine invasive species have long been recognized of paramount importance to
341 understand invasion processes (Strayer et al. 2006), as exemplified in the few available temporal genetic
342 studies of marine invaders (e.g., Goldstien et al. (2013) and references therein). Temporal demo-genetic
343 surveys can reveal new introductions, clarify the processes by which introduced species establish
344 sustainably (i.e., immigration vs. local reproduction) and identify sources for the colonization of new
345 habitats. This study illustrates such outcomes in the invasive seaweed *Undaria pinnatifida*. We showed that
346 1) the colonization of the newly-built marina was fast, 2) the initial sources were multiple, including leisure
347 boats, rocky reefs, and cultivated populations; 3) the origin of the successive generations shifted over time
348 in the marina, with populations becoming self-sustaining; a process also observed in putatively newly
349 colonized natural rocky reefs.

350 *A snow-ball colonization dynamics*

351 The first report of the presence of *U. pinnatifida* in the newly built marina were mature sporophytes on the
352 hull of a boat in February 2013, but no individuals were recorded on the floating pontoons and breakwater
353 before February 2014. There was thus a time lag of at least one year and a half between the setting-up of
354 the floating pontoons (June 2012) and the presence of the first sporophytes on these artificial structures,
355 even though farmed populations and rocky reefs populations (RA, GU and MG) were present in the vicinity
356 in 2011 and 2012. This lag could be explained by the requirement of a minimal density of spore settlement
357 and gametophytes, which is needed for the successful fertilization and production of sporophytes (Reed
358 1990). Increased propagule pressure and/or germination of dormant gametophytes (Hoffmann, Santelices
359 1991; Saito 1975a) could explain the sudden establishment observed in February 2014. *U. pinnatifida* then
360 quickly colonized the available structures resulting in very high abundances (> 9000 individuals) after only
361 two years (5 generations). The growth curves (Fig. S1) illustrate this snowball effect. The densities observed
362 in the marina of Bloscon at the end of this study are similar to what have been observed in other marinas in
363 the English Channel (Epstein, Smale 2018a; Epstein, Smale 2018b; Murphy et al. 2016b).

364 A series of life-history traits, including a short life cycle, high fecundity, ability to self, are likely
365 major factors enabling *U. pinnatifida* to quickly colonize available space (Nyberg, Wallentinius 2005). In
366 addition, this kelp also probably benefits from reduced competitive abilities and niche dissimilarities in
367 marinas, notably with native kelps (Bulleri, Chapman 2010; De Leij et al. 2017; Epstein et al. 2019; Farrell
368 et al. 2006). The gametophytes of *U. pinnatifida* have the ability to stay dormant during several months

369 (Hoffmann, Santelices 1991; Saito 1975a) and to emerge as soon as the environmental conditions are
370 favorable. This “seed bank”, combined with the rapid growth of *U. pinnatifida*, could result in a priority
371 effect compared to species which emerge later in the season or have a slower growth (Collinge, Ray 2009;
372 Dickson et al. 2012). The rapid increase of abundance and quick spread over the marina within a two-year
373 period confirm the status of *U. pinnatifida* as a pioneer species (Nyberg, Wallentinus 2005), particularly in
374 anthropogenic habitats.

375
376 ***Local spread results from a combination of gradual and jump dispersal likely modulated by***
377 ***environmental filters***

378 The colonization of the marina presumably results from a mixed-mode of spread, in which primary
379 long-distance saltatory events are followed by gradual dispersal over short distances, producing clumps of
380 individuals. A few long-distance dispersal events (> 100m; i.e., jump dispersal) were evidenced by both the
381 landscape genetic (Geneland, Fig. 6) and the mark-correlation analyses (Fig. 3). Such saltatory dispersal
382 could be due to drifting thalli detached from their substrate, for example during the cleaning of the marina
383 infrastructures (Guzinski et al. 2018; Sliwa et al. 2006; South et al. 2017). In contrast, the gradual dispersal
384 at short distance is most likely explained by spore and gamete dispersal. The field surveys (Fig. 2) and the
385 mark-correlation analysis (Fig.3) indicate an increase in density where individuals of the previous
386 generation were observed. Interestingly, the population established in 2015 displayed substantial selfing
387 rates as compared to the previous year. Together with the clustering of genetically similar individuals over
388 short distance (typically below 25m), this suggests that selfing sustains the gradual colonization and
389 establishment in this habitat.

390 The mixed-mode of dispersal likely explains the erratic spatial pattern of colonization observed over
391 time at the marina scale. However, locally (along pontoons or breakwater), directional spread was observed,
392 and might be due to other factors, notably environmental factors. The first individuals found in March 2014
393 appeared on the breakwater, with 90 % of them located on the southern side of the breakwater (i.e., external
394 side of the marina). The southern side is more exposed to currents and wave motion, but also to a stronger
395 propagule pressure (released from other sites within the bay) which increases the chance of spore settlement.
396 In addition, the southern site is more exposed to incident light, a major environmental factor controlling
397 population growth of *U. pinnatifida* (see Murphy et al. (2017) and references therein). Similarly, inside the
398 marina, a density gradient differentiates the eastern from the western sides of the pontoons. A potential
399 explanatory factor is water motion known to benefit to this seaweed (Peteiro et al. 2016). Current velocities
400 have not been measured, but are known to be higher in the eastern (“outer”) than in the western (“inner”)
401 part of the marina due to its configuration, widely opened to tidal currents (Fig. 1). Reduced mixing of the

402 water occurring in the western side of the marina could also result in lower abundance of nutrients, which
403 can affect negatively the growth of *U. pinnatifida* (Morelissen et al. 2013). Environmental factors
404 determining the heterogeneous pattern of colonization in the marina of Bloscon will require further
405 dedicated experimental studies. However, it is noteworthy that the population dynamics observed in Bloscon
406 was overall qualitatively similar to the outcomes of an agent-based modelling study of the population growth
407 of *U. pinnatifida*, which highlighted the importance of abiotic factors, notably light availability (Murphy et
408 al. 2016a; Murphy et al. 2017).

409 ***Spill-back effects are followed by a shift towards a self-sustaining population***

410 Genetic assignment showed that the first colonizers of the breakwater and floating pontoons in 2014
411 originated from multiple sources, and mainly from rocky reefs populations located at least 1 km away. The
412 role of the rocky reef populations as a primary source of colonizers in a new anthropogenic habitat was
413 somewhat unexpected. Marinas and farms have been targeted as sources of new populations in the wild (i.e.,
414 spillover events, (Epstein, Smale 2018b; Guzinski et al. 2018; James, Shears 2016). The present study shows
415 that, reciprocally, natural populations can act as sources toward newly built artificial sites (i.e., spillback
416 event).

417 Immigration, from farms or rocky reefs, could result from the transport of spores by water currents,
418 from drifting thalli detached from their substrate by storms or human activities (Sliwa et al. 2006), or by
419 recreational vessels (South et al. 2017, Ulman et al. 2019). Although spores only remain viable for a short
420 period of time (1-2 days, Saito (1975a)), the high speed tidal currents in the bay of Morlaix, particularly
421 during spring tides (up to 0.75 m/s in the vicinity of the marina), could carry spores and drifting thalli from
422 nearby rocky reefs to the marina. The role of recreational boating should however not be neglected: it has
423 been proposed to explain the colonization of marinas in England by *U. pinnatifida* (Fletcher, Farrell 1999),
424 and regional chaotic genetic structure reported in the study region Brittany (Guzinski et al. 2018). Mature
425 sporophytes and most likely gametophytes can indeed attach to the hull of leisure boats, ropes and buoys
426 (Bollen et al. 2017; South et al. 2017). In addition, mature sporophytes were observed on the hull of several
427 leisure boats in the marina during the survey (pers. observation). In this context, it is noteworthy that 12%
428 of the colonizers of the marina in 2014 were assigned to the mature sporophytes collected on the hull of a
429 boat the previous year. We can hypothesize that the individuals excluded from all sampled populations in
430 assignment tests might originate from other bays, after their transport by leisure boats infested by the
431 seaweed and moored (but not sampled) in the study marina. This pathway could notably explain the
432 assignment of the group of 6 individuals from 2015 to unknown sources and isolated in a distinct genetic
433 cluster (Fig. 6) and identified as full-sibs (Fig. S3).

434 An important outcome of our temporal genetic survey is to document a major change of the parental
435 (source) population over time. Indeed, conversely to the previous year, in 2015, almost half the individuals
436 were assigned to the population sampled in the marina in 2013-2014, showing that the population shifted
437 toward self-sustaining (Fig. 5B). This could be explained by a priority effect (Fraser et al. 2015) at the intra-
438 specific level, whereby the early growth of locally produced sporophytes at high density could prevent the
439 recruitment of new *Undaria* immigrants arriving later in the season. In addition, selfing, which is reaching
440 36% in this established population (Table 1) can accentuate this effect through rapid space monopolization,
441 as shown by the short dispersal distance between full-sibs (<20m). This local recruitment could have
442 occurred from the presence of dormant gametophytes and/or reproduction of overwintering mature
443 sporophytes (Epstein, Smale 2018a). Dormancy in gametophytes of kelps usually do not exceed a few
444 months (Hoffmann, Santelices 1991), but data on the maximum extent of dormancy in *Undaria*
445 gametophytes is lacking.

446 ***From spill-back to spill-over events at the bay scale***

447 Two populations were recorded for the first time at new rocky reefs sites during this study (RV and
448 ET). It cannot be ascertained with confidence if they were truly absent in the previous years or were simply
449 unnoticed because of the complexity of the rocky shore landscape, especially if the founding population
450 was small. However, the genetic data support the first scenario (i.e., new colonization), in comparison to
451 what have been observed in the marina. The rocky reef populations RV and ET indeed displayed assignment
452 to multiple sources the first year of report. In addition, the source of new recruits quickly shifted to within-
453 site reproduction in RV over the two years of survey (Fig. 5A-B), as also observed in the marina. In contrast,
454 well-established rocky reef populations, such as MG or RA, reported for a long time, showed a high stability
455 in the genetic time series, with self-recruitment being the dominant source of individuals at these sites. Note
456 that this pattern actually makes the presumed absence of RA in 2014 highly doubtful, rather suggesting that
457 this population was present the year before, although not observed (and thus not sampled). The temporal
458 stability of the genetic background documented here is congruent with results from other studies (e.g., over
459 ten years in St-Malo Bay, Guzinski et al. (2018)). It suggests that priority effects are present both in artificial
460 and natural habitats. To which extent self-sustaining populations may evolve local adaptation is unknown.
461 However, for the rocky reef population MG, a genome scan analysis found loci putatively under selection
462 or linked with markers under selection in the population sampled in 2015 (Guzinski et al. 2018).

463 It is noteworthy that the marina contributed substantially to the colonization of both rocky reef
464 populations RV and ET, showing spill-over events from marinas to the wild, as hypothesized in previous
465 studies (Epstein, Smale 2018b; Guzinski et al. 2018). More generally, except MG, all the rocky reef
466 populations studied in 2015 show some assignment to the marina. The capacity of *U. pinnatifida* to build

467 up very high densities in artificial habitats makes the marina a potential important source of propagules to
468 natural rocky reefs of the bay, after only two years since the first colonization. This observation is
469 particularly interesting considering that the colonization of wild habitats by *U. pinnatifida* has been slow in
470 the bay of Morlaix, with the first wild population recorded in GU in 2006 (ca. 10 years after the beginning
471 of the culture of this kelp) even though GU and the farm are separated by only 50m. In comparison, the Bay
472 of St. Malo in Brittany was already heavily colonized 10 years after the set-up of a farm (Castric-Fey et al.
473 1993; Girard-Descatoire et al. 1997). Besides competition with native seaweeds, particularly perennial
474 kelps, which may decrease the establishment success of *U. pinnatifida* in the study region (Epstein et al.
475 2019; Epstein, Smale 2018b), the lag time observed in the Bay of Morlaix could be explained by an initial
476 low propagule pressure, in the absence of a marina where particularly high density of *U. pinnatifida* are
477 reported (this study; Epstein, Smale 2018a).

478 With the presence of *U. pinnatifida* at high density in the newly built marina, one could hypothesize
479 a rapid spread at the bay level, which was actually evidenced by a survey carried out in April 2019 by two
480 of us (FV, LL). As compared to the situation examined in this study, novel populations were reported in the
481 wild, together with an expansion in the port area (i.e., beyond the marina, including concrete substrates such
482 as the ferry jetty). An almost continuous presence of *U. pinnatifida* on all favorable rocky substrates, located
483 in the south of the marina, is now observed. This Pacific kelp is now present (and expands) in the south, the
484 north and the west of the study bay (Supplementary material, Fig. S5). Abundances of *U. pinnatifida* in
485 these natural sites are usually ca. two orders of magnitude lower than in the marina. However, in some sites,
486 the densities are comparable to those of native kelp species, notably in the putatively recently established
487 populations RV and ET, allowing for commercial harvesting of the thalli of this edible alga by local people
488 (as suggested by dozens of specimens cut above the sporophylles observed during the 2019 survey). The
489 combination of the increased propagule pressure and spill-over from the marina with the ability to *U.*
490 *pinnatifida* populations to be rapidly self-sustaining are responsible for the (likely) sustainable establishment
491 of the species at the bay scale.

492 This study is a new evidence supporting the need for careful management of marinas to prevent
493 spread of non-native species into the wild. The evaluation of these risks should be better integrated into
494 Environmental Impacts Assessments (EIA) prior to building new infrastructures. In particular, the NIS
495 already reported in nearby natural rocky habitats should be listed and particularly scrutinized, as they are at
496 risk of colonizing the newly built infrastructure, as exemplified here with *U. pinnatifida*. Areas at high risk
497 of introduction of the kelp from recreational vessels also need to be identified using risk assessment methods
498 (e.g., according to shipping intensity; Shucksmith and Shelmerdine 2015). As advocated in other studies
499 (e.g., Ulman et al. 2019; Epstein, Smale 2017; Peters et al. 2019), our results and surveys call for

500 standardized monitoring and surveillance protocol of NIS in marinas, including the hull of recreational
501 vessels, neglected for a long time. Importantly, for those species, such as *U. pinnatifida*, characterized by
502 rapid population growth, strong monitoring efforts and early response are certainly critical. We indeed
503 showed a snow-ball colonization dynamics and high abundance after only 2 years, that may complicate or
504 even prevent its control and eradication. For instance, Forrest & Hopkins (2013) showed that sustained
505 control (i.e., monthly hand removal on any visible substrates and hulls) is needed to substantially decreased
506 the number of boats infested by *U. pinnatifida* while partial control (i.e., removal targeted over a short time
507 period) resulted in a number of infested boats similar to non-managed marinas. However, even if the species
508 has colonized the marina, one should not neglect management options aiming at decreasing the propagule
509 pressure, by reducing the density and abundance on both the marina infrastructures and leisure boats:
510 reducing the propagule pressure may help in limiting the rapid colonization in nearby sites, particularly in
511 rocky reefs where native species can slow-down the establishment of the NIS through competitive
512 interactions (Epstein and Smale 2018b). Another facet of the present work was to show that many *Undaria*
513 populations are inconspicuous. Considerable monitoring efforts were needed to localize the populations
514 established in rocky reefs because of the complexity of the landscape and location of suitable habitats, which
515 are not easily accessible at low tide and require diving. Nonetheless, a reliable and accurate spatio-temporal
516 mapping is needed to determine high-risks areas. Recently, Jeunen et al. (2019) showed that *U. pinnatifida*
517 presence could be recovered by COI metabarcoding. Such an approach based on environmental DNA is
518 promising to detect this Pacific kelp as well as other hidden NIS.

519

520 **References**

- 521 Airoidi L, Turon X, Perkol-Finkel S, et al. (2015) Corridors for aliens but not for natives: effects of
522 marine urban sprawl at a regional scale. *Divers Distrib* 21:755-768
- 523 Bishop J, Wood CA, Lévêque L, et al. (2015) Repeated rapid assessment surveys reveal contrasting trends
524 in occupancy of marinas by non-indigenous species on opposite sides of the western English
525 Channel. *Mar Pollut Bull* 95:699-706
- 526 Bollen M, Battershill CN, Pilditch CA, et al. (2017) Desiccation tolerance of different life stages of the
527 invasive marine kelp *Undaria pinnatifida*: Potential for overland transport as invasion vector. *J Exp*
528 *Mar Biol Ecol.* 496:1-8
- 529 Bouchemousse S (2015) Dynamique éco-évolutive de deux ascidies congénériques et interfétils, l'une
530 indigène et l'autre introduite, dans leur zone de sympatrie. Doctoral thesis. Université Pierre &
531 Marie Curie (Paris 6). <https://tel.archives-ouvertes.fr/tel-01337034>

532 Bulleri F, Chapman MG (2010) The introduction of coastal infrastructure as a driver of change in marine
533 environments. *J Appl Ecol* 47:26-35

534 Castric-Fey A, Girard A, L'Hardy-Halos MT (1993) The distribution of *Undaria pinnatifida*
535 (Phaeophyceae, Laminariales) on the coast of St Malo (Brittany, France). *Bot Mar* 36:351-358

536 Clarke Murray C, Pakhomov EA, Therriault TW (2011) Recreational boating: a large unregulated vector
537 transporting marine invasive species. *Divers Distrib* 17:1161-1172

538 Collinge SK, Ray CJE (2009) Transient patterns in the assembly of vernal pool plant communities.
539 *Ecology* 90:3313-3323

540 Daguin C, Voisin M, Engel C, et al. (2005) Microsatellites isolation and polymorphism in introduced
541 populations of the cultivated seaweed *Undaria pinnatifida* (Phaeophyceae, Laminariales). *Conserv*
542 *Genet* 6:647-650

543 David P, Pujol B, Viard F, et al. (2007) Reliable selfing rate estimates from imperfect population genetic
544 data. *Mol Ecol* 16:2474-2487

545 De Leij R, Epstein G, Brown MP, et al. (2017) The influence of native macroalgal canopies on the
546 distribution and abundance of the non-native kelp *Undaria pinnatifida* in natural reef habitats. *Mar*
547 *Biol* 164:156

548 Dickson TL, Hopwood JL, Wilsey BJJBI (2012) Do priority effects benefit invasive plants more than
549 native plants? An experiment with six grassland species. *Biol Invasions* 14:2617-2624

550 Duarte CM, Pitt KA, Lucas CH, et al. (2013) Is global ocean sprawl a cause of jellyfish blooms? *Front*
551 *Ecol Environ* 11:91-97

552 Epstein G, Hawkins SJ, Smale DA (2019) Identifying niche and fitness dissimilarities in invaded marine
553 macroalgal canopies within the context of contemporary coexistence theory. *Sci Rep-UK* 9:8816

554 Epstein G, Smale DA (2017) *Undaria pinnatifida*: A case study to highlight challenges in marine invasion
555 ecology and management. *Ecol Evol* 7:8624-8642

556 Epstein G, Smale DA (2018a) Between-habitat variability in the population dynamics of a global marine
557 invader may drive management uncertainty. *Mar Pollut Bull* 137:488-500

558 Epstein G, Smale DA (2018b) Environmental and ecological factors influencing the spillover of the non-
559 native kelp, *Undaria pinnatifida*, from marinas into natural rocky reef communities. *Biol Invasions*
560 20:1049-1072

561 Farrell P, Fletcher RJ (2006) An investigation of dispersal of the introduced brown alga *Undaria*
562 *pinnatifida* (Harvey) Suringar and its competition with some species on the man-made structures of
563 Torquay Marina (Devon, UK). *J Exp Mar Biol Ecol.* 334:236-243

564 Firth LB, Knights AM, Bridger D et al. (2016). Ocean sprawl: challenges and opportunities for
565 biodiversity management in a changing world. *Oceanogr Mar Biol* 54: 193-269.

566 Fletcher RL, Farrell P (1999) Introduced brown algae in the North East Atlantic, with particular respect to
567 *Undaria pinnatifida* (Harvey) Suringar. Helgolander Meeresun 52:259-275

568 Floc'h J-Y, Pajot R, Mouret V (1996) *Undaria pinnatifida* (Laminariales, phaeophyta) 12 years after its
569 introduction into the Atlantic ocean. Hydrobiologia 326/327:217-222

570 Forrest BM, Brown SN, Taylor MD, et al. (2000) The role natural dispersal mechanisms in the spread of
571 *Undaria pinnatifida* (Laminariales, Phaeophyceae). Phycologia 39:547-553

572 Forrest B, Hopkins G (2013). Population control to mitigate the spread of marine pests: insights from
573 management of the Asian kelp *Undaria pinnatifida* and colonial ascidian *Didemnum vexillum*.
574 Manag Biol Invasions 4: 317-326.

575 Fraser CI, Banks SC, Waters JM (2015) Priority effects can lead to underestimation of dispersal and
576 invasion potential. Biol Invasions 17:1-8

577 Gao H, Bryc K, Bustamante CD (2011) On identifying the optimal number of population clusters via the
578 deviance information criterion. PLOS ONE 6:e21014

579 Gao H, Williamson S, Bustamante CD (2007) A Marko Chain Monte Carlo approach for joint inference of
580 population structure and inbreeding rates from multi-locus genotype data. Genetics 76:1635-1651

581 Girard-Descatoire A, Castric-Fey A, L'Hardy-Halos M (1997) Inventaire de la faune et de la flore sur les
582 fonds rocheux de Saint-Malo et de Dinard. Report for Direction Régionale de l'Environnement
583 Bretagne, Conseil Régional de Bretagne. Rennes

584 Glasby T, Connell S, Holloway M, et al. (2007) Nonindigenous biota on artificial structures: could habitat
585 creation facilitate biological invasions? Mar Biol 151:887-895

586 Goldstien SJ, Inglis GJ, Schiel DR, et al. (2013) Using temporal sampling to improve attribution of source
587 populations for invasive species. PLOS ONE 8

588 Goudet J (1995) Fstat (version 1.2): a computer program to calculate F-statistics. J Hered 86:485-486

589 Grulois D (2010) Etude de la dispersion et du recrutement à différentes échelles spatiales chez *Undaria*
590 *pinnatifida*, une macro-algue brune introduite le long des côtes bretonnes. Doctoral Thesis.
591 Université Pierre et Marie Curie (Paris 6). <https://hal.sorbonne-universite.fr/tel-01111061>.

592 Grulois D, Leveque L, Viard F (2011) Mosaic genetic structure and sustainable establishment of the
593 invasive kelp *Undaria pinnatifida* within a bay (Bay of St-Malo, Brittany). Cah Biol Mar 52:485-
594 498

595 Guillot G, Estoup A, Mortier F, et al. (2005a) A spatial statistical model for landscape genetics. Genetics
596 170:1261-1280

597 Guillot G, Mortier G, Estoup A (2005b) Geneland: A computer package for landscape genetics. Mol Ecol
598 Notes 5:708-711

599 Guzinski J, Ballenghien M, Daguin-Thiébaud C, et al. (2018) Population genomics of the introduced and
600 cultivated Pacific kelp *Undaria pinnatifida*: Marinas—not farms—drive regional connectivity and
601 establishment in natural rocky reefs. *Evol Appl* 11:1582-1597

602 Hoffmann AJ, Santelices BJ, Meps O (1991) Banks of algal microscopic forms: hypotheses on their
603 functioning and comparisons with seed banks. *Mar Ecol-Prog Ser* 79:185-194

604 Illian J, Penttinen A, Stoyan H, et al. (2008) Statistical analysis and modelling of spatial point patterns.
605 John Wiley & Sons

606 James K, Shears NT (2016) Proliferation of the invasive kelp *Undaria pinnatifida* at aquaculture sites
607 promotes spread to coastal reefs. *Mar Biol* 163:1-12

608 Jeunen G-J, Knapp M, Spencer HG, et al. (2019) Environmental DNA (eDNA) metabarcoding reveals
609 strong discrimination among diverse marine habitats connected by water movement. *Mol Ecol*
610 *Resour* 19:426-438

611 Jones O, Wang J (2009) COLONY: a program for parentage and sibship inference from multilocus
612 genotype data. *Mol Ecol Resour* 10:551–555

613 Lodge DM, Simonin PW, Burgiel SW, et al. (2016) Risk analysis and bioeconomics of invasive species to
614 inform policy and management. *Annual Review of Environment and Resources* 41:453-488

615 Mineur F, Johnson M, Maggs C (2008). Macroalgal introductions by hull fouling on recreational vessels:
616 seaweeds and sailors. *Environ Manage* 42: 667-676.

617 Morelissen B, Dudley BD, Geange SW, et al. (2013) Gametophyte reproduction and development of
618 *Undaria pinnatifida* under varied nutrient and irradiance conditions. *J Exp Mar Biol Ecol* 448:197-
619 206

620 Murphy JT, Johnson MP, Viard F (2016a) A modelling approach to explore the critical environmental
621 parameters influencing the growth and establishment of the invasive seaweed *Undaria pinnatifida*
622 in Europe. *J Theor Biol* 396:105-115

623 Murphy JT, Johnson MP, Viard F (2017) A theoretical examination of environmental effects on the life
624 cycle schedule and range limits of the invasive seaweed *Undaria pinnatifida*. *Biol Invasions*
625 19:691-702

626 Murphy JT, Voisin M, Johnson M, et al. (2016b) Abundance and recruitment data for *Undaria pinnatifida*
627 in Brest harbour, France: Model versus field results. *Data in Brief* 7:540-5

628 Nyberg CD, Wallentinus I (2005) Can species traits be used to predict marine macroalgal introductions?
629 *Biol Invasions* 7:265-279

630 Ojaveer H, Galil BS, Campbell ML, et al. (2015) Classification of non-indigenous species based on their
631 impacts: Considerations for application in marine management. *PloS Biol* 13:e1002130

632 Paetkau D, Slade R, Burden M, et al. (2004) Genetic assignment methods for the direct, real-time
633 estimation of migration rate: a simulation-based exploration of accuracy and power. *Mol Ecol*
634 13:55-65

635 Peteiro C, Sánchez N, Martínez B (2016) Mariculture of the Asian kelp *Undaria pinnatifida* and the native
636 kelp *Saccharina latissima* along the Atlantic coast of Southern Europe: An overview. *Algal*
637 *Research* 15:9-23

638 Peters K, Sink KJ, Robinson TB (2019). Sampling methods and approaches to inform standardized
639 detection of marine alien fouling species on recreational vessels. *J Environ Manage* 230: 159-167.

640 Piry S, Alapetite A, Cornuet J-M, et al. (2004) GeneClass2: a software for genetic assignment and first
641 generation migrants detection. *J Hered* 95:536-539

642 Pyšek P, Richardson DM (2010) Invasive species, environmental change and management, and health.
643 *Annual Review of Environment and Resources* 35:25-55

644 Rannala B, Mountain JL (1997) Detecting immigration by using multilocus genotypes. *Proc Natl Acad Sci*
645 *USA* 94:9197-9201

646 Reed DC (1990) The effects of variable settlement and early competition on patterns of kelp recruitment.
647 *Ecology* 71:776-787

648 Rius M, Turon X, Bernard G, et al. (2015) Marine invasion genetics: from spatial and temporal patterns to
649 evolutionary outcomes. *Biol Invasions* 17:869-885

650 Rousset F (2000) Genetic differentiation between individuals. *J Evol Biol* 13

651 Rousset F (2008) Genepop'007: a complete re-implementation of the genepop software for Windows and
652 Linux. *Mol Ecol Resour* 8:103-106

653 Saito Y (1975a) Practical significance of algae in Japan: *Undaria*. *Advance of Phycology in Japan*:304-
654 320

655 Saito Y (1975b) *Undaria*. In: Tokida J and Hirose H (eds) *Advance in phycology in Japan*. Junk
656 publishers., The Hague, pp. 304-320

657 Schlather M, Ribeiro Jr PJ, Diggle PJ (2004) Detecting dependence between marks and locations of
658 marked point processes. *J R Stat Soc B* 66:79-93

659 Shucksmith RJ, Shelmerdine RL (2015). A risk based approach to non-native species management and
660 biosecurity planning. *Mar Policy* 59: 32-43.

661 Simberloff D, Martin J-L, Genovesi P, et al. (2013) Impacts of biological invasions: what's what and the
662 way forward. *Trends Ecol Evol* 28:58-66

663 Sliwa C, Johnson CR, Hewitt CL (2006) Mesoscale dispersal of the introduced kelp *Undaria pinnatifida*
664 attached to unstable substrata. *Bot Mar* 49:396-405

665 South PM, Floerl O, Forrest BM, et al. (2017) A review of three decades of research on the invasive kelp
666 *Undaria pinnatifida* in Australasia: An assessment of its success, impacts and status as one of the
667 world's worst invaders. *Mar Environ Res* 131:243-257

668 South PM, Thomsen MS (2016) The ecological role of invading *Undaria pinnatifida*: an experimental test
669 of the driver–passenger models. *Mar Biol* 163

670 Strayer DL, Eviner VT, Jeschke JM, et al. (2006) Understanding the long-term effects of species
671 invasions. *Trends Ecol Evol* 21:646-651

672 Ulman, A, Ferrario, J, Forcada, A, et al. (2019) Alien species spreading via biofouling on recreational
673 vessels in the Mediterranean Sea. *J Appl Ecol*. 00: 1– 10. (Early View)

674 Voisin M (2007) Les processus d’invasions biologiques en milieu côtier marin :le cas de l’algue brune
675 *Undaria pinnatifida*, cultivée et introduite à l’échelle mondiale. Doctoral Thesis. Université Pierre
676 & Marie Curie (Paris 6), Paris, pp. 231. <https://hal.sorbonne-universite.fr/tel-01118270>

677 Voisin M, Engel C, Viard F (2005) Differential shuffling of native genetic diversity across introduced
678 region in a brown alga: aquaculture vs. maritime traffic effects. *Proc Natl Acad Sci USA* 102:5432-
679 5437

680 Wang J (2012) Computationally efficient sibship and parentage assignment from multilocus marker data.
681 *Genetics* 191:183-194

682 Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution*
683 38:1358-1370

684 Wiegand T, Moloney KA (2014) Handbook of spatial point-pattern analysis in ecology. CRC Press /
685 Taylor & Francis, Boca Raton, FL

686

687 **Figure Legends**

688 **Fig. 1. Sampling sites in A) Bloscon Marina and B) and Bay of Morlaix (Brittany, France)**

689 A satellite view (Google Earth) is provided for the marina with names of the pontoons (A to I) and sea-wall
690 sections (0 to 8) surveyed. The marina (BL: 48°42'53.932"N, 3°57'52.258"W), farm (FB: 48°42'41.148"N,
691 3°57'7.919"W) and natural habitats (GU: 48°42'43.704"N, 3°57'8.748"W; RV: 48°42'5.364"N,
692 3°57'43.542"W; ET: 48°42'0.468"N, 3°57'0.936"W; MG: 48°43'46.344"N, 3°58'3.342"W, RA:
693 48°43'40.908"N, 3°58'19.883"W; full name and abbreviations in Suppl. Material Table S1) studied are
694 pictured with triangle, star, and squares, respectively. Other sites were surveyed at the bay scale between
695 2002 and 2016: Black and white circles indicate presence (at least observed once during the survey period)
696 and absence of *U. pinnatifida* in these sites, respectively.

697 **Fig. 2. Colonization dynamics of the marina by *U. pinnatifida***

698 Location and number of individuals recorded on the gateway, pontoons and breakwaters over time (F14:
699 February 2014. M14: March 2014. D14: December 2014. M15: March 2015. D15: December 2015. M16:
700 March 2016). The grey dot in the map F14 indicates the location of 11 individuals observed on the hull of
701 a boat in 2013.

702 **Fig. 3. Mark-correlation analysis of the densities recorded between two consecutive surveys (i.e.,**
703 **distinct generations) in the marina.**

704 A-C-E-G: Bivariate correlation function km_1m_2 (product of the densities), B-D-F-H: Bivariate Moran's *I*
705 like correlation function (correlation coefficient). From top to bottom: (A-B) March 2014-December 2014,
706 (C-D) December 2014-March 2015, (E-F) March 2015-December 2015, (G-H) December 2015-March
707 2016. Black dots: observed values. Red line: mean value expected under the local random marking null
708 model ($r_{max} = 25m$ for A-D and $r_{max} = 50 m$ for E-H). Grey area: global simulation envelope (199 simulations
709 of the null model, 5th lowest and 5th highest values).

710 **Fig. 4. Bayesian clustering analysis (InStruct software) carried out at the bay scale.**

711 Individuals are ordered and grouped according to their habitat, sampling year and locality. Sample codes
712 start with a label for the habitat (F, M and R stands for farm, marina and rocky reef, respectively) and the
713 year of sampling. Each individual is represented by a vertical line divided into coloured segments, the
714 length of which indicates the individual's membership fraction to each of *K* clusters. Results are shown
715 for *K* = 4 (best likelihood). Note that for the marina in 2014, most of the individuals (>60) were found on
716 the breakwater on section S1 and S8 (B1 and B8), and thus included in the analysis, while less than 20

717 were found on other sections and less than 10 individuals on pontoons. For the year 2015, the same
718 sections and two pontoons where most of the individuals were found have been included.

719 **Fig. 5. Assignment analysis (GeneClass software) carried out at the bay scale.**

720 Percentage of individuals collected in 2014 (A) and 2015 (B) assigned to sampled putative sources, in the
721 marina (black, BL), the farm (grey, FB) and natural rocky reefs (white, GU, MG, RV, RA) sampled the
722 previous years (2013 and 2014). Red bubbles (Excl.) are individuals that were excluded from all sampled
723 sources (rejected at the 1% level).

724 **Fig. 6. Spatial clustering analysis of the population established in 2015 in the marina (Geneland
725 analysis).**

726 A) Location of all the 291 individuals sampled in 2015. B) to F) distribution of the isoclines of the
727 membership probabilities of individuals to belong to one of the five genetic clusters identified by
728 INSTRUCT (see text). Maximum probability is in white ($p > 0.9$).

729 **Table 1: Genetic diversity per population categories, for each sampling year (distinct generations).**

730 N: number of individuals; Nall: mean number of alleles per loci; Ar : Allelic richness; Hobs: Observed
 731 heterozygosity; He: Expected heterozygosity under Hardy-Weinberg equilibrium; F_{IS} : fixation index
 732 (inbreeding coefficient); P_{HW}: probability of the exact test for Hardy-Weinberg equilibrium; s_{g2} :
 733 population selfing rate; P_s: P-value of the test 's_{g2} = 0'. For natural rocky reefs, mean values over sites are
 734 indicated, with details per localities provided in Table S1.

Site	Year	N	Nall	Ar	Hobs	He	F _{IS}	P _{HW}	s _{g2}	P _s
Marina	2013	11	2.9	2.7	0.35	0.33	-0.07	0.86	0.00	0.67
	2014	91	2.9	2.4	0.29	0.40	0.27	< 10 ⁻⁴	0.00	0.52
	2015	296	3.2	2.5	0.32	0.45	0.29	< 10 ⁻⁴	0.36	<10 ⁻³
Farm	2013	36	2.5	2.3	0.32	0.36	0.09	0.44	0.18	0.11
	2014	32	1.5	1.5	0.26	0.24	-0.07	0.30	0.14	0.19
	2015	32	1.5	1.5	0.26	0.25	-0.06	0.11	0.06	0.32
Rocky reefs (average)	2013	108	2.1	1.8	0.15	0.24	0.28	n.a.	0.39	n.a.
	2014	63	2.2	2.0	0.23	0.26	0.10	n.a.	0.56	n.a.
	2015	145	2.2	2.0	0.24	0.30	0.20	n.a.	0.20	n.a.

735

736

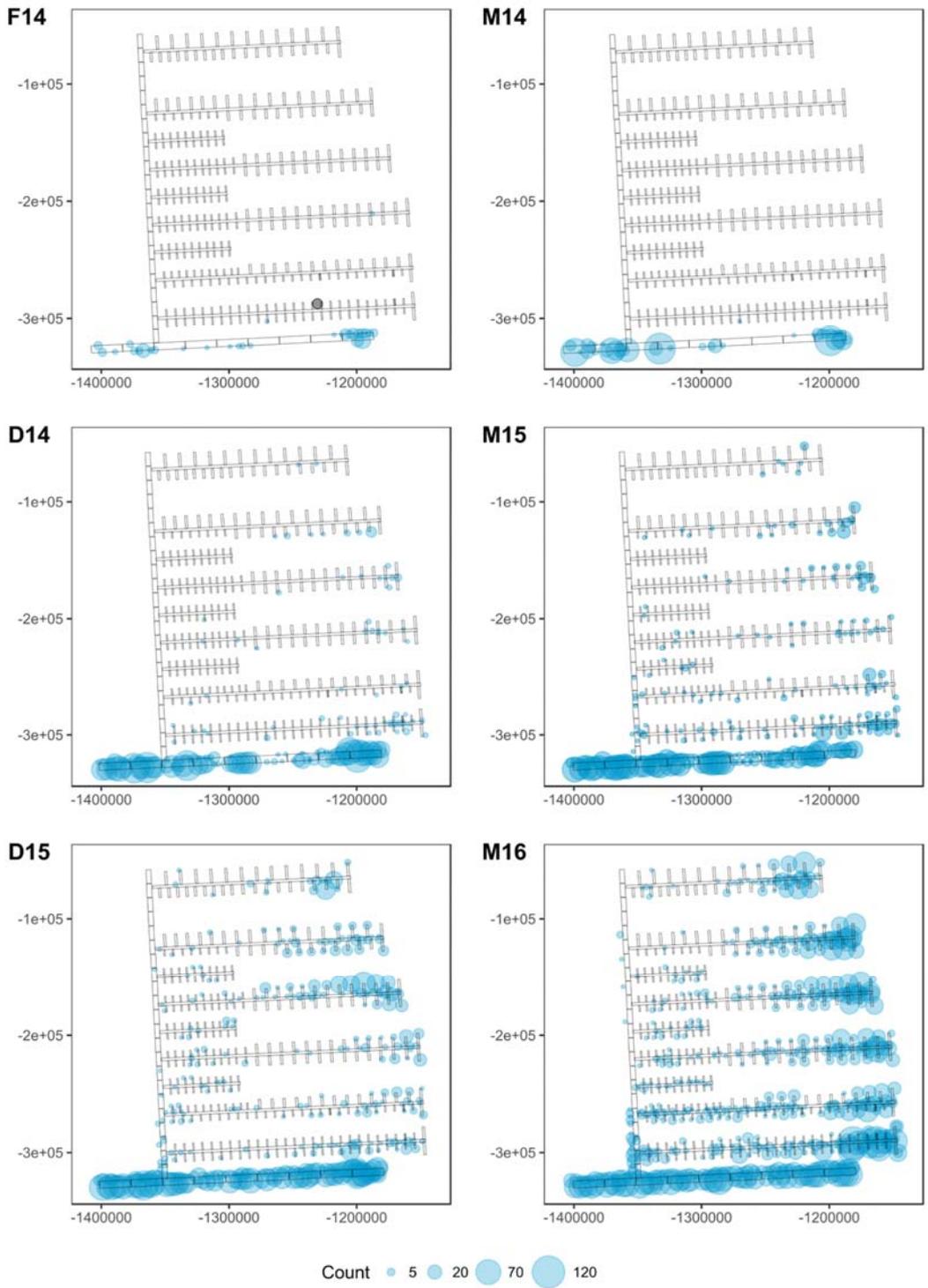
737 **Figure 1.**



738

739

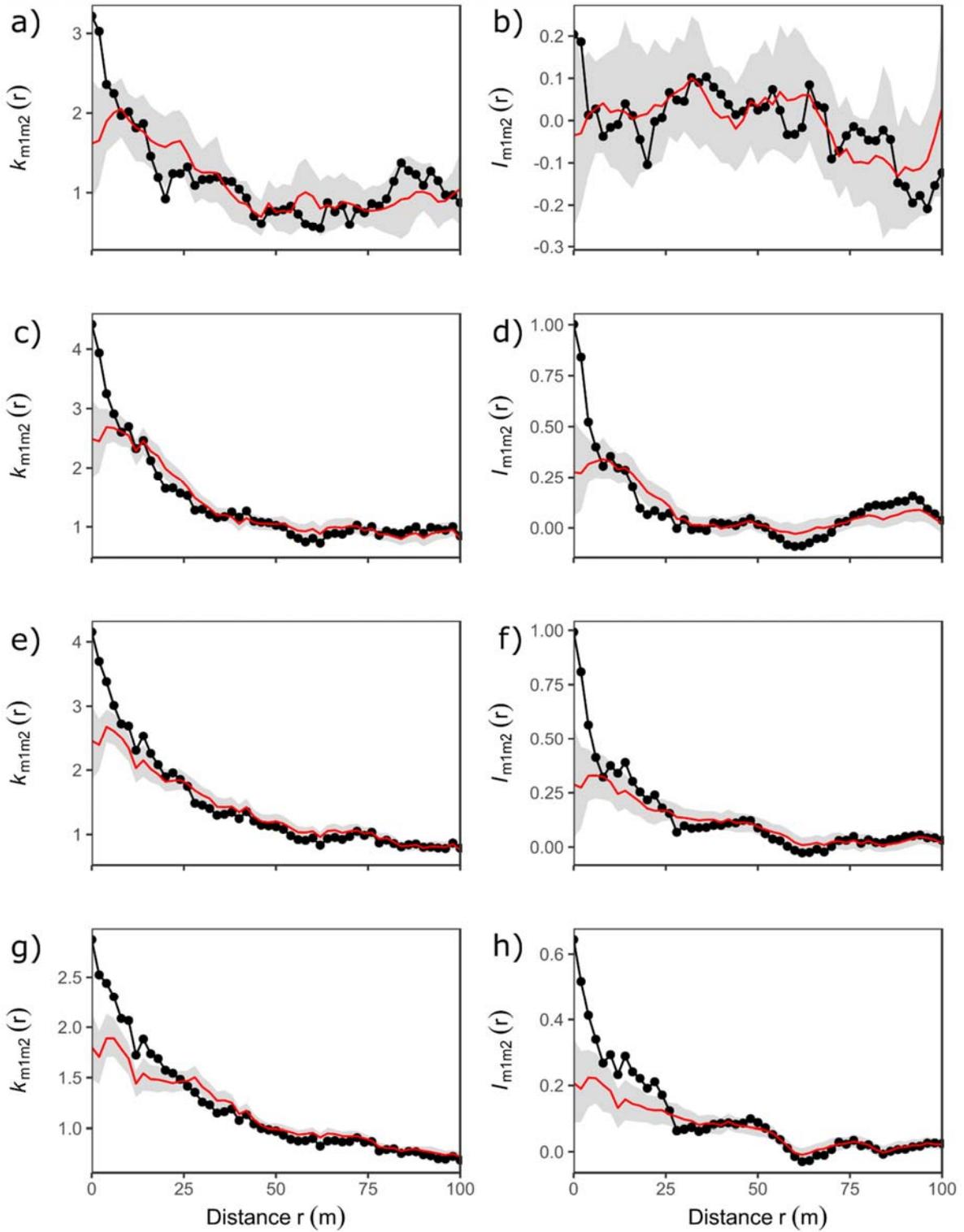
740 **Figure 2**



741

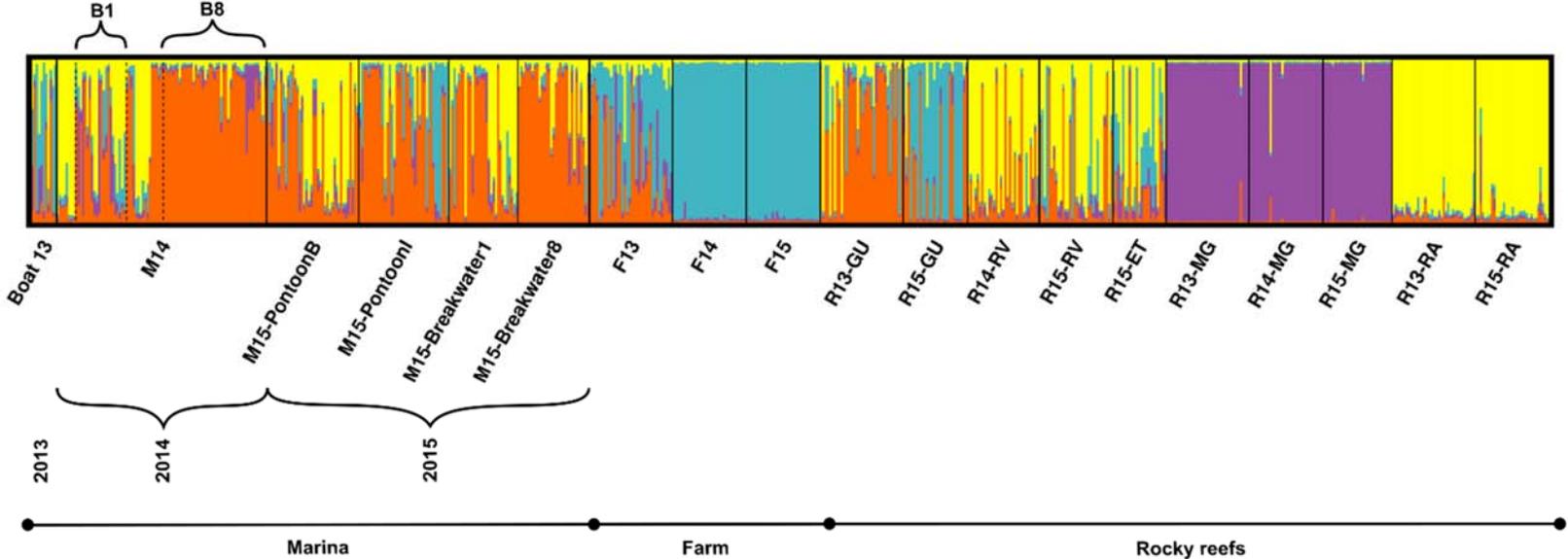
742

743



746 **Figure 4**

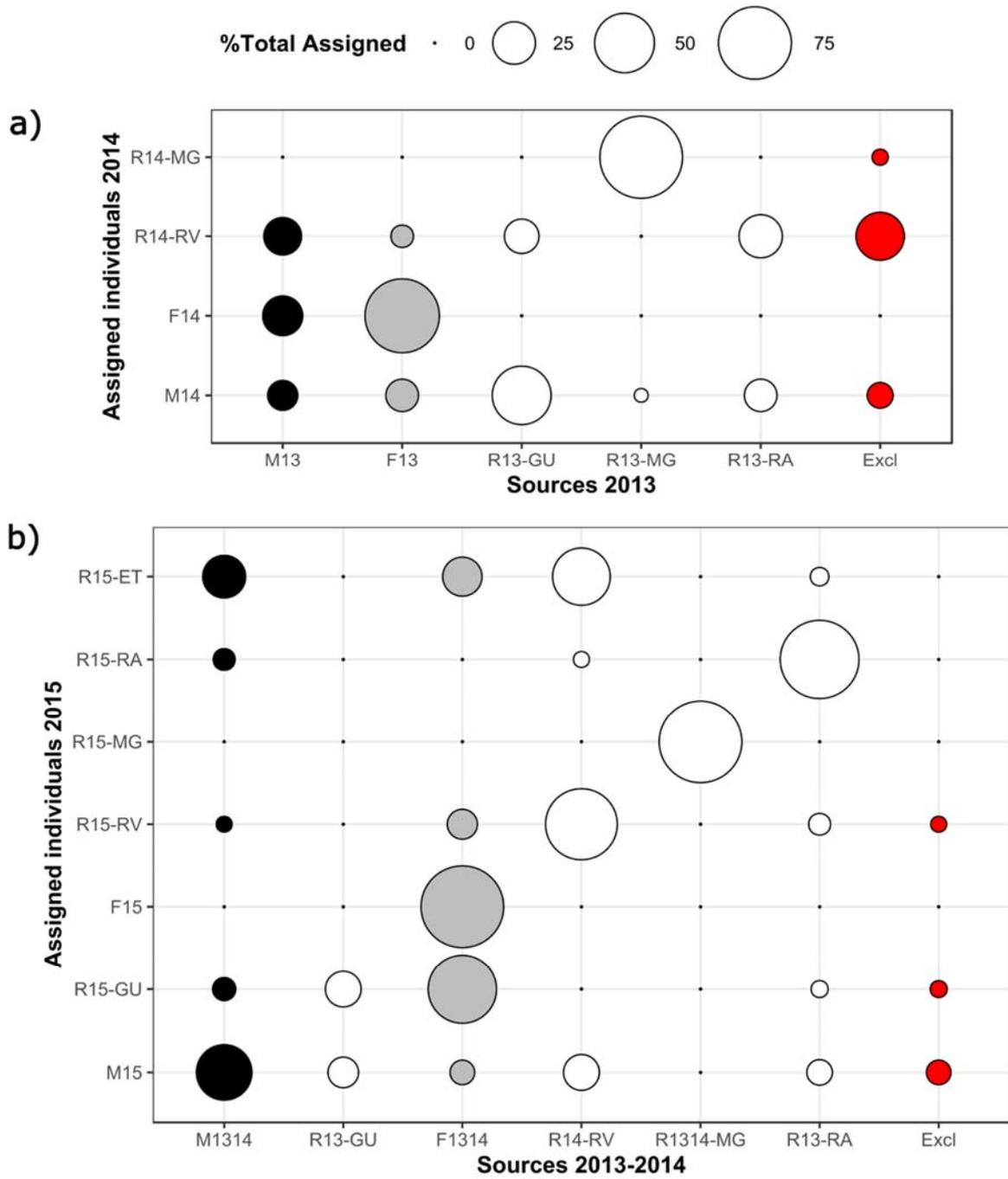
747



748

749

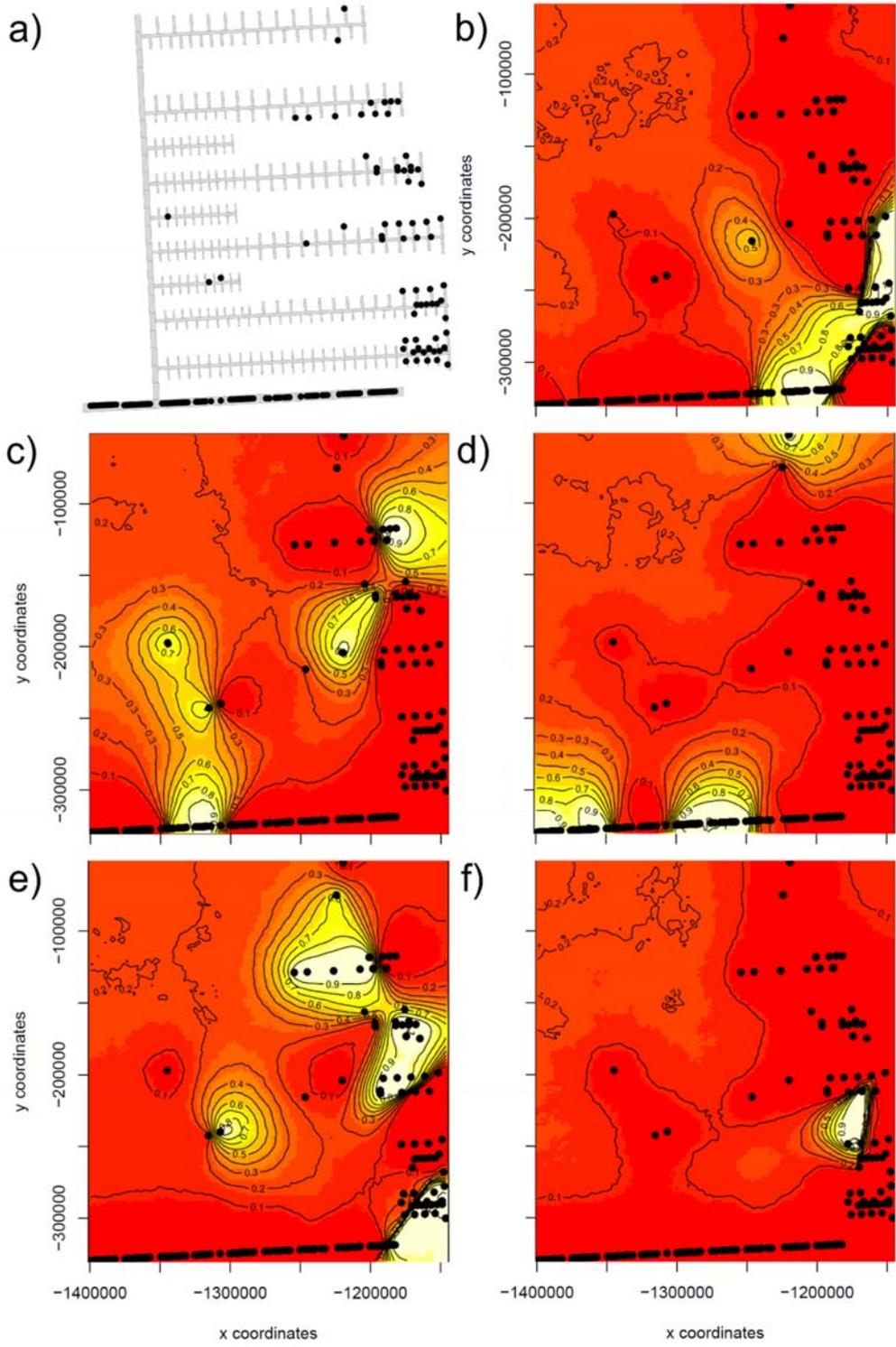
750 **Figure 5**



751

752

753



756

757