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## **Investigating the quality of European silver eels by quantifying contaminants and parasite infestation in a French Mediterranean lagoon complex**

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## **Abstract**

Coastal lagoons are diverse habitats with significant ecological gradients, which provide crucial ecosystem services but face threats from human activities such as invasive species and pollution. Among the species inhabiting the lagoons, the critically endangered European eel (*Anguilla anguilla*) is an emblematic species strongly impacted by contamination and parasitism. Several indicators were developed to assess the quality of eel at a large geographic scale. Most indicators are based on the concentration of individual pollutant and/or abundance of parasites separately without considering individual variations. This study assessed the quality of 59 eels captured at three different sites inside a Mediterranean lagoon complex (the Camargue, South of France), by integrating multiple degradation factors (POPs, TEs, and *A. crassus* infestation) and considering individual eel characteristics (length, age, growth rate, and sex). Using multivariate TOPSIS analysis including these degradation factors, this study found that eel quality decreased with age but did not significantly vary between sites. When focusing on each degradation factor, *A. crassus* infestation rates were lower in older eels, independently to the site, however, the POPs and TEs contaminations were lower in the Grandes Cabanes site compared to the Vaccarès and Fumemorte sites even if larger and younger eels were more contaminated by POPs. These findings reveal the fine-scale spatial variability in eel quality, with TOPSIS analysis providing a robust method to rank and score scenarios. This approach enhances the understanding of habitat degradation sources affecting eel contamination and parasitic infestation, supporting more effective strategies for sustainable habitat management.

## **Keywords**

*Anguilla anguilla* – *Anguillicola crassus* – Contamination – Spawner quality – Mediterranean lagoon – Trace elements – Persistent Organic Pollutants

## Introduction

At the interface between land and sea, coastal lagoons exhibit diverse aquatic habitats with significant ecological gradients ranging from freshwater to hypersaline conditions (Kjerfve 1994; Pérez-Ruzafa et al. 2011). They support a rich variety of bird, fish, and invertebrate species, many of which are commercially valuable (Pérez-Ruzafa and Marcos 2012), especially in the Mediterranean region (Grillo and Venora 2011; Kara and Quignard 2018). In addition to biodiversity, coastal lagoons offer crucial ecosystem services such as shoreline protection, flood control, and waste assimilation, driven by their strategic coastal positioning and unique ecological features (Barbier et al. 2011; Costanza et al. 2011; Newton et al. 2018). However, these ecosystems face growing threats from human activities resulting in habitat degradation and loss (Kjerfve 1994; Aliaume et al. 2007; Velasco et al. 2018). Apart from habitat loss, the main threats include overexploitation of resources, introducing and spreading invasive species (Kennish 2002; Newton et al. 2014) and pollution inputs (e.g., nutrient enrichment, organic carbon loading, and chemical contaminants such as persistent organic pollutants and metallic trace elements). Identifying sources of anthropogenic degradation will contribute to the development of sustainable management strategies to preserve these habitats.

The Rhône River delta, known as the Camargue, is one of the largest and most biodiverse Mediterranean lagoon complexes. In the nineteenth century, the construction of extensive dikes to control flooding enabled large-scale agricultural and industrial development, resulting in significant habitat loss (Blondel et al. 2019). The shift to intensive agriculture, particularly rice and market gardening, has been detrimental to biodiversity, notably causing a decline in bird populations due to habitat transformation and pesticide use (Galewski and Devictor 2016). Studies have shown that birds (Berny et al. 2002), turtles (Burkart et al. 2021) and fish (Roche et al. 2002; Oliveira Ribeiro et al. 2008) in the Camargue are exposed to a large variety of pollutants such as persistent organic pollutants (POPs). Additionally, human activities have facilitated the introduction of invasive species, which now represent a significant portion of the region's biodiversity and compete with native species (e.g., Rosecchi et al. 1997; Marchessaux et al. 2020). Thus, the Camargue has undergone significant changes in land cover, land use, and water management in recent decades (Mathevet et al. 2002; Galewski and Devictor 2016) with largely unknown consequences for species communities and ecosystem functioning (Oliver et al. 2015; Laverty et al. 2017; Fraixedas et al. 2019).

The European eel (*Anguilla anguilla*) is one of the most common fish in Mediterranean lagoons (Pérez-Ruzafa et al. 2007, 2011; ICES 2015; Kara and Quignard 2018). This critically endangered species is also an important fishery resource, particularly in the Camargue, where fishers specialise in catching it (Blondel et al. 2019; Pike et al. 2020). This long-lived semelparous species spawns in the North Atlantic Convergence zone (Tesch 2003; Righton et al. 2016), and its larvae migrate to the European and North African coasts where they occupy a wide variety of growing habitats such as rivers, lagoons and estuaries (Tesch 2003; Daverat et al. 2006). The growth phase of yellow eels lasts from three to more than 30 years (Acou et al. 2003; ICES 2022; Rohtla et al. 2023), depending on several biotic and abiotic factors. Yellow eels are predominantly sedentary (Daverat and Tomás 2006; Panfili et al. 2022) and have an opportunistic feeding behaviour, consuming mainly benthic invertebrates and fishes (Bouchereau et al. 2006, 2009; Denis et al. 2022). At the end of this stage, yellow eels develop in silver eels and migrate back to their spawning grounds (ICES 2022). Silver eels do not feed during this migration and the energetic fuel used for migration and gamete maturation relies mainly on their important lipid

stores accumulated during their growth phase (van den Thillart et al. 2007). Silver eels are particularly susceptible to toxic effects of chemical pollution from various lipophilic bio-accumulative contaminants which became bioavailable due to remobilization of lipid reserves during the migration and gamete maturation (Freese et al. 2019). The contaminants comprise trace elements (TEs) (Maes et al. 2008; Romero et al. 2020), polycyclic aromatic hydrocarbons (PAHs), chlorinated and brominated flame retardants (FRs), polychlorinated biphenyls (PCBs), and polychlorinated dibenzo-p-dioxins and furans (PCDD/F) (Oliveira Ribeiro et al. 2005; Tapie et al. 2011; Szlinder-Richert et al. 2014; Sühling et al. 2014; Bourillon et al. 2020). Chemical pollution may be a key factor in explaining the decline of European eels (Drouineau et al. 2018; ICES 2022). Indeed, pollutants have been shown to be highly toxic to aquatic organisms such as fish, with effects ranging from reproductive problems to mortality (Louiz et al. 2009; Beckvar and Lotufo 2011). The use of eels to assess aquatic environmental conditions is advantageous due to their ubiquitous presence, extensive life-history data and ease of identification. In particular, this species reflects different trophic levels and long-term environmental stress, providing valuable insights into ecosystem health.

In addition to chemical pollution, the swim-bladder nematode parasite *Anguillicola crassus* has been proposed as a serious threat for the European eel population. Introduced from Asia in the 1980s, *A. crassus* quickly infested *Anguilla* eel species in different geographical regions of the world (Dupont and Petter 1988; Lefebvre et al. 2013). The presence of the parasite in the Camargue was documented in the early 2000s and has been extensively studied in this lagoon complex (Lefebvre and Crivelli 2004, 2012; Lefebvre et al. 2013). The parasites consume energy through sanguivorous feeding and induce mechanical damage to the swim-bladder wall, potentially affecting the spawning migration of European eels (Höglund et al. 1992; Palstra et al. 2007).

The quality of eels has been assessed using a variety of methods, including the assessment of contamination by TEs (Maes et al. 2005), the quantification of TEs and POPs (Belpaire and Goemans 2007), and the examination of infestation by *A. crassus* (Lefebvre et al. 2002a). In 2015, the Working Group on Eel (WGEEL) established a standardised and harmonised protocol, the eel “patho-index”, to assess the quality of European eels (ICES 2015). The patho-index includes contamination data for TEs, POPs and infestation data for *A. crassus* and facilitates comparisons of eel habitats across their distribution range, revealing large-scale variability of environmental conditions. Several studies have adapted these indicators to their data to evaluate the quality of eels and the environment in Mediterranean lagoons (Amilhat et al. 2014; Capoccioni et al. 2020; Romero et al. 2020; Martínez-Gómez et al. 2023). Recently, Bourillon et al. (2020) also developed another indicator based on comprehensive levels of TEs, POPs, *A. crassus* and lipid content to compare the quality of eels in different habitats, including a Mediterranean lagoon. The different indicators are usually estimated based on contaminant concentrations from one tissue (muscle or liver), although previous studies have shown differences in TEs concentrations between muscle and liver samples (Baraj et al. 2009; Amilhat et al. 2014). Significant variation in eel life history traits (e.g., body length, age, or growth rate) has been observed both between and within habitats (Acou et al. 2003; Melià et al. 2006a; Daverat et al. 2012; Teichert et al. 2023). The quality of eels may be influenced by their individual characteristics, with younger, lighter and faster growing individuals potentially less affected by environmental contaminants than older, larger and slower growing individuals.

However, previous studies have predominantly focused on habitat-level comparisons and single-sex analyses, overlooking the variability in biometric traits within and between sites. Furthermore, the complexity of

the eel life cycle, coupled with the difficulty of accurately quantifying contaminants in large numbers of individuals, has often led to the use of indices that focus on a single sex of eel. For instance, in the study by Bourillon et al. (2020), males were excluded due to a lack of knowledge on their lipid requirements for migration and gonadal development. However, lagoon environments may be important for male production (Melià et al. 2006b; Amilhat et al. 2008). It would therefore be relevant to study the quality of eels according to the individual criteria such as length, age, growth rate for both sexes.

The aims of this study were (i) to compare available ecotoxicological data for eel tissues and sediments to assess the extent to which eels can be valuable indicators of environmental quality, (ii) to position the quality of eels from the Camargue region in comparison to other eel growth habitats and particularly to other Mediterranean lagoons, and finally (iii) to quantify the quality of each individual based on the combination of contaminant quantification and parasite infestation, and to identify sources of habitat degradation for both male and female silver eels at a fine spatial scale within a Mediterranean lagoon complex. In this study, the quality of eels is defined in terms of their level of pollutant concentration (POPs and TEs) and the severity of parasitic infestation by *A. crassus* with eel of the best quality exhibiting the lowest pollutant concentration and infestation.

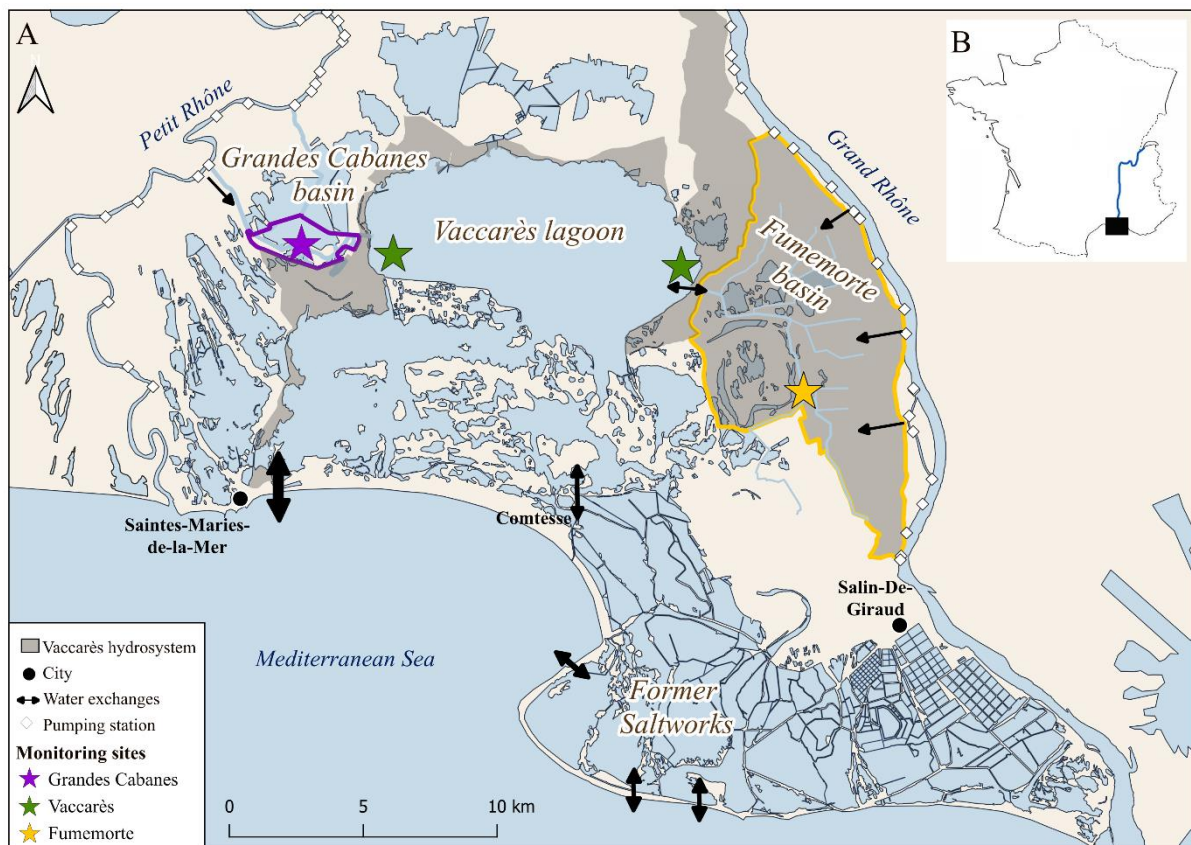
## **Materials & Methods**

### Study area

Located in the Rhône Delta (southern France), the Camargue is a vast wetland (approximately 1 780 km<sup>2</sup>) composed of marshes, lagoons, canals and lands with different geomorphological, physical and chemical compositions. This complex hydrosystem is composed of a variety of aquatic environments, either hypersaline, brackish or freshwater, providing habitats for eel settlement and growth (Melià et al. 2006a; Bevacqua et al. 2019). This highly anthropised environment is made up of many lagoons, drainage canals (i.e., anthropic channels) mainly used for agriculture which are disconnected from the sea and ponds (i.e., “poldered” areas) where the water is pumped from and into the Rhône River. Some other drainage canals are directly connected to the main lagoon known as the Vaccarès lagoon, such as the Fumemorte canal which is the main freshwater supply to the lagoon (Chauvelon 1998). Run-off water, whether from agricultural or industrial areas, flows into the canals and lagoons, contaminating these environments with various pollutants (Hemery et al. 2022).

To investigate the quality of eels in the Camargue, we sampled three sites corresponding to a “poldered” marsh (i.e., the Grandes Cabanes site), a drainage canal (i.e., the Fumemorte canal) and the Vaccarès lagoon (Fig. 1). The Grandes Cabanes site is made up of interconnected marshes and canals and benefits from a direct gravity water supply from the Petit Rhône River. The main objective of this 4.7 km<sup>2</sup> site is the management and the conservation of biodiversity. The use of pesticides was stopped at the end of the 2000s and hunting is one of the only human activities, taking place in autumn for a limited number of participants. Water from the site is drained by gravity through two slice gates into a drainage canal, from which it is pumped back into the river. These “poldered” environments can act as traps for eels, as they can easily colonise such a site as juveniles (i.e., glass eels or elvers), but once they reach the silver stage, they can be blocked in the system, preventing them from reaching the sea. The freshwater Fumemorte canal is the main drainage canal for a 68 km<sup>2</sup> catchment area with a

high proportion of intensive agricultural land and is fed into the Grand Rhône through several pumping stations. Connected to the Vaccarès lagoon, brackish to marine waters can flow up the canal depending on water levels and freshwater supply. The salt dam between the canal and the Vaccarès lagoon, which was used to limit the increase in salinity and was no longer effective, was removed in 2012 and eels can now move freely between the two systems. The Vaccarès lagoon is a brackish lagoon of about 65 km<sup>2</sup> which salinity varies mainly as a function of direct rainfall, freshwater inflows from the alluvial deposits in the catchment area and several agricultural drainage canals, and the opening of the two controlled seawater inlets: the 13 sluice gates at Saintes-Maries-de-la-Mer and the 4 openable sluice gates in the canal of the Comtesse (Fig. 1). Other more or less temporary connections between the ponds and the sea are possible at the former saltworks (Fig. 1).



**Fig. 1:** A- Map of the study area (this colour code is used throughout the work to distinguish between sites), B- France map with the Rhône River in blue and the black rectangle represents the study area.

### Sample collection

At the three sites, 163 eels were sampled from October to December 2021 using fyke nets (6 mm mesh size). Due to the difficulty of catching eels in the Vaccarès lagoon, we completed our own samples with eels caught by professional fishers within the same lagoon. These eels were visually separated in yellow and silver eels (presence of the lateral line with well-developed neuromasts, colour contrast between ventral and dorsal surface and ocular hypertrophy) and considered as females if their length was greater than 450 mm (Acou et al. 2005). From these eels, 59 were randomly selected (10-11 females and 9-10 males per site, Tables 1 and S1 and Fig. S1) and euthanised using ethically appropriate procedures (i.e., the animal is first anaesthetised in a bath of benzocaine

at 50 mg.L<sup>-1</sup> and then euthanised in a bath of 250 mg.L<sup>-1</sup> for 30 min). Their total length (mm), weight (g), eye horizontal and vertical diameters (mm) and pectoral fin length (mm) were measured. These data were used to confirm the stage of the eels according to the index developed by Durif et al. (2005). These 59 eels were analysed in the laboratory for age determination, *Anguillicola crassus* infestation, and concentrations of trace elements (TEs) and of persistent organic pollutants (POPs).

#### Sample preparation

Eels were necropsied immediately after euthanasia. Eel heads, entire swim-bladders and approximately 2 g of liver were removed for otolith extraction, *A. crassus* and TEs analyses. From the anus, the tail of the eel was cut into two sections; approximately 5 g of the first (just after the anus) and second sections were removed, frozen, freeze-dried and grounded for POPs and TEs analyses, respectively (Table S1). All samples were stored at -20°C until analysis.

#### Age determination

Sagittal otoliths were extracted from the head of each eel and cleaned with distilled water. Otoliths from young eels were examined in toto in immersive oil under stereomicroscope. For older eels (> 5 years) or when readings were unclear, the otoliths were embedded in epoxy resin and grounded (following WKAREA recommendations, ICES 2009). After treatment with an EDTA solution, otoliths were stained with toluidine blue to improve visualisation of annual rings. Age determination was conducted by counting the winter rings by an expert operator (ICES 2009).

#### Growth rate

For each individual, a mean continental growth rate ( $\lambda$ ) was calculated:  $\lambda = \frac{L_T - L_0}{A}$  where  $L_T$  was the total length,  $L_0$  was the length of glass eels at arrival in continental habitats and  $A$  was the age (years) of the individuals.  $L_0$  was defined at 66 mm as observed for glass eels entering the Camargue ecosystem (Lambremon et al. 2021).

#### Sediment analysis

Several compounds such as PCBs, PAHs and metallic TEs were quantified in the sediments of the three sites from 2012 to 2018. The “Société Nationale de Protection de la Nature” (SNPN) analysed these elements in the Vaccarès lagoon at 10 sites from 2012 to 2015 and in the Fumemorte canal at a single site in 2012 and 2014 (Cheiron et al. 2013, 2015, 2016b, a). In the Grandes Cabanes site, sediment analyses were carried out at three sites in 2018 (Messineo et al. 2018). To compare contaminants measured in sediments and eels, this study only presents elements measured in both matrices (Table 2).

#### Trace Elements analysis

For the 59 silver eels, 14 TEs (Ag, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, V, and Zn) were measured in liver and muscle tissues. Total Hg analyses were performed on dried tissue aliquots ranging between 5 and 20 mg by atomic absorption spectrophotometry with an Advanced Mercury Analyser (ALTEC® AMA 254). The analytical accuracy and reproducibility were assessed using blanks and TORT-3 lobster hepatopancreas (NRC,



Canada) as certified reference material (CRM) at the beginning and during the analytical session. Recovery for TORT-3 was  $98.7 \pm 1.4$  % (n=15). The limit of detection (LOD) of the AMA was 0.1 ng.

The other elements were analysed by ICP using an Agilent Technologies 5800 VDV ICP-OES and using a Thermo Fisher Scientific XSeries II ICP-MS. Aliquots ranging between 50 and 250 mg were digested with 6 mL 67 % HNO<sub>3</sub> and 2 mL 37 % HCl (Fisher Scientific, trace element grade). Samples were digested overnight at room temperature and then submitted to heating in a Milestone microwave (30 min with constant increasing temperature up to 120 °C, then 15 min at this maximal temperature). Each digested sample was made up to 50 mL with milli-Q quality water. Blanks and CRMs [Dogfish Liver DOLT-5 (NRCC) and TORT-3 (NRCC)] were included in the analytical batch and analysed in the same way as the samples. Recoveries range from 85 to 106 %.

### Lipids and POPs analyses

Muscle lipid content and POPs concentrations were performed according to the methods described by Malarvannan et al. (2014). The muscle lipid content (%) was obtained gravimetrically on an aliquot of the extract which was used for the POPs measurement. Lipids were expressed in percentage of fresh weight of the sampled muscle. The targeted POPs were the PCB congeners (IUPAC numbers: 28/31, 52, 95, 99, 101, 105, 118, 128, 138, 146, 149, 153, 156, 170, 171, 174, 177, 180, 183, 187, 194, 196/203, 199, 206 and 209), dichlorodiphenyltrichloroethane (*p,p'*-DDT) and metabolites (*p,p*-DDD and *p,p*-DDE), chlordane and metabolites (trans-chlordane (TC), cis-chlordane (CC), oxychlordane (OxC), cis-nonachlor (CN) and trans-nonachlor (TN)), hexachlorobenzene (HCB), hexachlorocyclohexanes ( $\alpha$ -,  $\beta$ - and  $\gamma$ -HCHs), PBDEs (BDE 28, 47, 99, 100, 153, 154 and 183), MeO-PBDEs (6-MeO-BDE47 and 2'-MeO-BDE68) and DP (syn-DP and anti-DP).

Briefly, a homogenised sample of approximately 0.3 g pooled eel muscle was weighed in a 15 mL polypropylene Falcon tube, mixed with anhydrous Na<sub>2</sub>SO<sub>4</sub> and spiked with internal standards (PCB 143, <sup>13</sup>C-HCB,  $\epsilon$ -HCH and BDE 77) and extracted twice by vortex and sonication with 6 mL hexane/dichloromethane (1:1, v/v) and cleaned up on 6 g acidified silica (44%). Extracts were eluted with 20 mL hexane and 15 mL dichloromethane. The cleaned extract was concentrated to approximately 2 mL using a rotary-evaporator and further to near dryness under a gentle nitrogen stream and re-dissolved in 100  $\mu$ L recovery standard, CB-207. OCPs, PCBs and PBDEs were quantified by gas chromatography coupled to mass spectrometry operated either in electron capture negative chemical ionization (GC-ECNI-MS) or electron ionization (GC-EI-MS) depending on the analytes' sensitivity (details are provided in Belpaire et al. 2011; Malarvannan et al. 2014).

### Quality assurance and quality control

Procedural blanks were analysed simultaneously with every batch of seven samples to check for interferences or contamination from the solvent and glassware. For the few compounds measurable procedural blanks (PCB 101, PCB 153, PCB 138, PCB 180, HCB, BDE 99), these values were low (< 0.1 ng) and consistent (relative standard deviation (RSD) < 30 %). The procedural blanks were consistent (RSD < 30 %), and therefore the mean value was calculated for each compound and subtracted from those of the samples. The limits of quantification (LOQ) for POPs were calculated as three times the standard deviation of the mean for the blank measurements. For descriptive statistics, values of TEs and POPs below the LOQ were replaced by LOQ/2 (medium bound). The analytical procedure was validated using a standard reference material (SRM) 1945 (NIST,

organics in whale blubber), with less than 20 % deviations from the certified values. The concentration of POPs was expressed as  $\text{ng.g}^{-1}$  wet weight (ww), that of lipids as percentage in muscle, and that of TEs in  $\mu\text{g.g}^{-1}$  dry weight (dw).

Abbreviations are expressed as follows: PBDEs as the sum of 7 BDE congeners, Sum PCBs as the sum of 7 indicator PCB congeners (PCB 28/31, 52, 101, 118, 138, 153 and 180), Sum DDTs as the sum of 3 compounds, HCHs as the sum of 3 isomers, CHLs as the sum of 5 metabolites. These POPs can be classified into 2 sub-categories: those of industrial origin (i.e., PCBs and PBDEs) and those of agricultural origin (i.e., HCHs, HCB, CHLs, DDTs).

### Parasitological analysis

The swim-bladder of each eel was examined under a stereomicroscope for the abundance of *A. crassus* (total number of adults, larval stages and necrotic per eel). The Swim-bladder Degenerative Index (SDI) represents the alterations of the swim-bladder (i.e., opacity, the presence of pigmentation/exudate and thickness, each graded as 0, 1, or 2 according to Lefebvre et al. (2002a)) and ranges from 0 (intact) to 6 (strongly damaged). SDI values  $\geq 4$  are considered indicative of a severely damaged swim-bladder (Lefebvre et al. 2002a). Parasite counts and SDI scores were determined with separate examination by two observers.

### Data analysis

Biometric data were compared between the three sites and between males and females in each site. Because the data were not normally distributed and showed large variance, nonparametric Wilcoxon or Kruskal-Wallis tests were used to compare length, weight, age, lipid content, growth rate and *A. crassus* infestation (i.e., abundance and SDI) between sites.

For each eel, the Eel Quality Classes (EQC) proposed by Belpaire and Goemans (2007) and adjusted by ICES (2015) was estimated. The EQC establish four categories of eels quality (i.e., not deviating, slightly deviating, deviating or strongly deviating) according to the concentrations of contaminants (expressed in  $\text{ng.g}^{-1}$  ww of muscle tissue for TEs and POPs, such as Sum PCBs, but Sum PCBs\* is in  $\text{ng.g}^{-1}$  lipid, and Cu and Zn in  $\mu\text{g.g}^{-1}$  of ww) compared to the thresholds given by Belpaire and Goemans (2007) and their level of infestation by *A. crassus* derived from the SDI compared to the threshold given by ICES (2015). These standardised categories allow the comparison between the three studied sites and with other sites all over Europe (Belpaire and Goemans 2007). The EQC for each of the 27 contaminants (As, Cd, Cr, Cu, Hg, Ni, Pb, Se, Zn, PCB 28/31, PCB 52, PCB 101, PCB 105, PCB 118, PCB 138, PCB 153, PCB 156, PCB 180, Sum PCBs, Sum PCBs\*,  $\alpha$ -HCH,  $\gamma$ -HCH, HCB, pp-DDD, pp-DDE, pp-DDT, Sum DDTs) and the SDI were compared between our three sampling sites using a chi-2 test on the number of individuals in each EQC.

To have a more precise evaluation of each eel quality, a multi-variate analysis was carried out, considering both contaminants and parasitological data. Based on a scoring process, the Technique for Order of Preference by Similarity to Ideal Solution (TOPSIS) ranks the alternatives (which are each individual eel quality) according to their relative distance from the ideal positive and negative solutions, which represent the conditions obtained when the variable have extreme values (Hwang and Yoon 1981). In the present case, the alternatives have been ranked as a function of three criteria: POPs (with two sub-categories: Sum PCBs and PBDEs, and pesticides with HCB, HCHs, Sum DDTs, CHLs), TEs (with both liver and muscles results) and *A. crassus* infestation (with the

abundance of *A. crassus* and SDI). Therefore, the positive ideal solution corresponds to the alternative where the eel contains the lowest level of contamination (i.e., POPs and TEs) and the least *A. crassus* infestation (i.e., abundance of *A. crassus* and SDI). This case reflects the healthiest environment for eel growth within our sites corresponding to the higher score in TOPSIS analysis. Alternatively, the negative ideal solution (lowest TOPSIS score) corresponds to an alternative in which the individual contains the highest levels of contamination by POPs, TEs and *A. crassus* infestation. Prior to the TOPSIS analysis, a vector normalisation procedure was performed to standardise the criteria to a common scale and comparable units (Hwang and Yoon 1981; Zavadskas et al. 2006). Equal weight was assigned to each criterion as no assumption was formulated on their relative importance in the decision process. Therefore, the three criteria have the same weight between them, and within each criterion, the variables all have the same weight (Table S1). Using TOPSIS analyses, an overall score was used to rank the quality of eels by integrating POPs, TEs and *A. crassus* infestation. Furthermore, in order to better understand the sources of degradation in the quality of eels in each of the sites studied, four specific TOPSIS analyses were carried out, three focusing on each criterion separately and one considering the three criteria together (Table S1).

To compare the quality of eels from our three sampling sites, the individual TOPSIS scores were compared using a Bayesian framework. As the individual length, age and/or growth rate have also been documented to correlate with contaminant concentration (Belpaire et al. 2008) and with the risk of *A. crassus* infestation (Lefebvre et al. 2002a; Faliex et al. 2022), these variables were also included into the models.

One eel from the Vaccarès site, which was considered as an outlier in the TOPSIS analysis focused on POPs (see Results), was excluded from the modelling. We modelled the logit of the TOPSIS score, which was between 0 and 1, and we centred and reduced the explanatory variables (i.e., length, age and growth rate).

The overall model including the effects of length (L), age (A) and growth rate ( $\lambda$ ) is defined as follows:

$$\text{logit}(\text{score}_i) \sim N(\mu_i, \sigma)$$

$$\mu_i = \alpha_s + \beta \times L_i + \gamma \times A_i + \delta \times \lambda_i$$

$\alpha_s \sim N(0,100)$ ,  $\beta \sim N(0,100)$ ,  $\gamma \sim N(0,100)$ ,  $\delta \sim N(0,100)$  and  $\sigma \sim \chi(2)$  where  $i$  corresponds to the number of individuals and  $s$  refers to the sites (i.e., Grandes Cabanes, Fumemorte and Vaccarès).

Among the fifteen possible models, we selected the one with posterior convergence (i.e., R-hat < 1.1 and n.eff > 60 000) and the lowest Widely Applicable Information Criterion (WAIC) (Watanabe 2010, 2021, Gelman et al. 2014). When several models had a difference of WAIC less than 2 compared to the lowest WAIC, we selected the most parsimonious model (Burnham and Anderson 2002).

All statistical analyses were performed in the R environment (R Core Team 2018, version 4.4.1), using the MCDM package (Blanca and Ceballos 2016) for TOPSIS analyses and nimble package for the linear regression (de Valpine et al. 2017).

## Results

Silver eels from the Fumemorte site were the oldest (mean  $\pm$  standard deviation;  $10.7 \pm 2.2$  yr for female and  $7.3 \pm 1.3$  yr for male, Table 1) and had the lowest growth rate ( $65 \pm 16$  mm.yr<sup>-1</sup> for female and  $45 \pm 8$  mm.yr<sup>-1</sup> for male) (Kruskal-Wallis test,  $p < 0.04$ ). Eels from the Grandes Cabanes site had a higher SDI ( $3.1 \pm 1.0$  for female and  $3.3 \pm 1.3$  for male, Table 1) (Kruskal-Wallis test,  $p < 0.04$ ). The length, weight, lipid content and *A. crassus* abundance were not significantly different among sites (Kruskal-Wallis test,  $p > 0.08$ ). Considering only the female silver eels, those caught at the Fumemorte site were older, larger and had a lower growth rate than the other sites (Table 1, Kruskal-Wallis test,  $p < 0.05$ ). The female silver eels from the Vaccarès site had a higher lipid percentage ( $24.6 \pm 2.8$  %) (Kruskal-Wallis test,  $p < 0.02$ ). No significant differences were observed between sites for the weight, *A. crassus* abundance and SDI (Kruskal-Wallis test,  $p > 0.05$ ). Male silver eels had a higher SDI ( $3.3 \pm 1.3$ ) and were younger ( $3.9 \pm 0.4$  yr) at the Grandes Cabanes site (Kruskal-Wallis test,  $p < 0.04$ ), whereas they were lighter ( $99.3 \pm 6.8$  g) and had a lower growth rate ( $45 \pm 8$  mm.yr<sup>-1</sup>) at the Fumemorte site (Kruskal-Wallis test,  $p < 0.03$ ). Their length, lipid content and *A. crassus* infestation did not differ between sites (Kruskal-Wallis test,  $p > 0.05$ ) (Table 1).

For each site, the level of parasite infestation (*A. crassus* abundance and SDI) was not significantly different between the two sexes (Wilcoxon test,  $p \geq 0.057$ ). In the Vaccarès lagoon, no significant differences in age and lipid content between males and females was observed (Wilcoxon test,  $p \geq 0.4$ ). The growth rate of males and females was not significantly different in the Gande Cabanes site (Wilcoxon test,  $p = 0.06$ ). Otherwise, males were shorter, lighter, younger, had a slower growth rate and a higher lipid content than females (Wilcoxon test,  $p \leq 0.02$ ). In our study, information on sex was strongly correlated with body length, with all males measuring less than 455 mm and all females measuring more than 550 mm (Table 1). Therefore, we will focus on length rather than sex for further analyses that include biometric information.

Table 1: Biometric information expressed as mean  $\pm$  SD (min-max) of the 59 sampled silver eels.

	Female silver eels (FV)			Male silver eels (MII)			
	Grandes Cabanes (N=11)	Fumemorte (N=10)	Vaccarès (N=10)	Grandes Cabanes (N=8)	Fumemorte (N=10)	Vaccarès (N=10)	
Length (mm)	687 $\pm$ 36 (643-750)	734 $\pm$ 53 (635-798)	675 $\pm$ 60 (588-739)	415 $\pm$ 29 (374-453)	388 $\pm$ 16 (370-408)	408 $\pm$ 28 (363-450)	
Weight (g)	609.0 $\pm$ 104.3 (518.6-834.6)	689.6 $\pm$ 140.5 (482.1-914.9)	591.5 $\pm$ 151.0 (394.7-809.8)	119.7 $\pm$ 20.0 (95-149.1)	99.3 $\pm$ 6.8 (89.1-110.5)	123.2 $\pm$ 26.5 (90.4-171.4)	
Age (year)	6.6 $\pm$ 2.9 (5-15)	10.7 $\pm$ 2.2 (8-15)	5.3 $\pm$ 1.9 (3-8)	3.9 $\pm$ 0.4 (3-4)	7.3 $\pm$ 1.3 (6-10)	6.3 $\pm$ 3.1 (2-11)	
Growth rate (mm.yr <sup>-1</sup> )	104 $\pm$ 23 (44-130)	65 $\pm$ 16 (44-92)	128 $\pm$ 43 (84-208)	91 $\pm$ 13 (77-117)	45 $\pm$ 8 (31-57)	76 $\pm$ 57 (33-180)	
Lipid content (%)	20.4 $\pm$ 2.9 (15.8-24.7)	20.5 $\pm$ 3.9 (15.8-28.9)	24.6 $\pm$ 2.8 (19.4-28.4)	24.7 $\pm$ 3.7 (20.8-29.5)	25.3 $\pm$ 4.1 (18.6-32.4)	24.1 $\pm$ 2.7 (20.7-28.6)	
<i>A. crassus</i>	Abundance (number)	13.6 $\pm$ 14.3 (0-39)	14.2 $\pm$ 16.0 (0-46)	3.4 $\pm$ 5.3 (0-17)	3.1 $\pm$ 2.9 (0-7)	3.0 $\pm$ 2.5 (0-7)	3.2 $\pm$ 4.3 (0-14)
	SDI	3.1 $\pm$ 1.0 (2-5)	2.7 $\pm$ 1.1 (1-5)	2.6 $\pm$ 1.1 (0-4)	3.3 $\pm$ 1.3 (2-5)	1.9 $\pm$ 0.7 (1-3)	2.0 $\pm$ 1.0 (1-4)

Comparison of the data from sediment samples and eels (males and females grouped together) provided insight into the complementarity of these matrices (Table 2). The detection frequency (df) of TEs was higher in sediments

than in eels (except for Cd, Cu and Hg, which showed a low detection frequency). Conversely, PCBs were rarely detected in sediment samples, whereas they were significantly present in eel muscle tissues (Table 2). However, it is important to note that we did not have the same number of samples for each site and matrix. In addition, the detection limit varied depending on the matrix studied and had also improved over time.

Table 2: Mean  $\pm$  standard deviation (SD) of concentrations of metallic TEs ( $\mu\text{g}\cdot\text{g}^{-1}$  of dw) and PCBs in the tissues (liver and muscle) of the silver eels (males and females grouped together) ( $\text{ng}\cdot\text{g}^{-1}$  of ww) and in the sediment samples ( $\text{ng}\cdot\text{g}^{-1}$  of dw). The detection frequency (df) is the percentage of detected samples on the total number of samples (N). The limit of quantification (LOQ) of each contaminant in each matrix is specified.

Variable	EEL									SEDIMENT								
	Matrix	LOQ	Grandes Cabanes (N=19)		Fumemorte (N=20)		Vaccarès (N=20)		Grandes Cabanes (N=3)			Fumemorte (N=2)			Vaccarès (N=16)			
			df	Mean $\pm$ SD	df	Mean $\pm$ SD	df	Mean $\pm$ SD	LOQ	df	Mean $\pm$ SD	LOQ	df	Mean $\pm$ SD	LOQ	df	Mean $\pm$ SD	
<b>Trace Elements</b>																		
As	Liver	0.1	53	0.2 $\pm$ 0.3	75	0.5 $\pm$ 0.4	95	1.3 $\pm$ 0.9	NA	100	10.9 $\pm$ 1.3	NA	100	8.4 $\pm$ 1.5	NA	100	8.2 $\pm$ 2.4	
	Muscle	0.1	16	0.1 $\pm$ 0.1	60	0.3 $\pm$ 0.2	95	0.7 $\pm$ 0.6										
Cd	Liver	0.01	100	0.09 $\pm$ 0.1	100	0.33 $\pm$ 0.16	100	0.17 $\pm$ 0.08	0.4	33	0.4 $\pm$ 0.3	0.6	0	< LOQ	0.5	0	< LOQ	
	Muscle	0.01	0	< LOQ	5	0.01 $\pm$ 0.01	5	0.01 $\pm$ 0.01										
Co	Liver	0.1	95	1.2 $\pm$ 1.2	100	2.1 $\pm$ 1.5	100	0.6 $\pm$ 0.2	NA	100	20.9 $\pm$ 2.8	NA	100	19.4 $\pm$ 1.5	NA	100	14.6 $\pm$ 4.5	
	Muscle	0.1	47	0.1 $\pm$ 0.1	60	0.2 $\pm$ 0.1	70	0.2 $\pm$ 0.2										
Cu	Liver	0.01	100	86.15 $\pm$ 60.24	100	75.04 $\pm$ 34.38	100	105.48 $\pm$ 63.15	NA	100	23.6 $\pm$ 6.6	NA	100	17.7 $\pm$ 1.4	10	38	8.6 $\pm$ 5.0	
	Muscle	0.01	100	2.19 $\pm$ 0.25	100	2.33 $\pm$ 0.28	100	2.38 $\pm$ 0.40										
Hg	Liver	0.01	100	0.53 $\pm$ 0.28	100	0.42 $\pm$ 0.22	100	0.42 $\pm$ 0.34	0.1	33	0.1 $\pm$ 0.0	NA	100	0.04 $\pm$ 0.01	0.3	88	0.05 $\pm$ 0.02	
	Muscle	0.01	100	0.39 $\pm$ 0.22	100	0.62 $\pm$ 0.32	100	0.48 $\pm$ 0.33										
Ni	Liver	0.04	89	0.67 $\pm$ 0.73	100	1.28 $\pm$ 1.01	100	0.15 $\pm$ 0.08	NA	100	26.5 $\pm$ 3.9	NA	100	22.8 $\pm$ 0.3	NA	100	18.0 $\pm$ 5.3	
	Muscle	0.04	47	0.05 $\pm$ 0.03	55	0.08 $\pm$ 0.16	40	0.04 $\pm$ 0.03										
Pb	Liver	0.01	100	0.28 $\pm$ 0.1	100	0.34 $\pm$ 0.21	100	0.26 $\pm$ 0.14	NA	100	19.4 $\pm$ 4.8	NA	100	13.5 $\pm$ 0.5	NA	100	10.8 $\pm$ 3.7	
	Muscle	0.01	74	0.02 $\pm$ 0.01	80	0.02 $\pm$ 0.02	70	0.02 $\pm$ 0.02										
Zn	Liver	0.01	100	217.42 $\pm$ 103.19	100	234.1 $\pm$ 89.92	100	255.00 $\pm$ 91.90	NA	100	69.8 $\pm$ 18.3	NA	100	54.4 $\pm$ 0.1	NA	100	38.1 $\pm$ 12.8	
	Muscle	0.01	100	64.47 $\pm$ 40.77	100	56.25 $\pm$ 12.3	100	59.90 $\pm$ 10.54										
<b>PCBs</b>																		
PCB 28	Muscle	0.5	11	0.3 $\pm$ 0.2	70	1.6 $\pm$ 1.6	35	0.6 $\pm$ 0.6	1	0	< LOQ	NA	100	0.1 $\pm$ 0	0.1	0	< LOQ	
PCB 52	Muscle	0.5	89	1.6 $\pm$ 1.0	100	2.1 $\pm$ 1.7	85	1.6 $\pm$ 1.0	1	0	< LOQ	0.1	0	< LOQ	0.1	0	< LOQ	
PCB 101	Muscle	0.1	100	2.8 $\pm$ 1.5	95	4.9 $\pm$ 4.2	100	2.3 $\pm$ 1.1	1	33	0.7 $\pm$ 0.3	0.2	0	< LOQ	0.2	0	< LOQ	
PCB 118	Muscle	0.1	100	2.1 $\pm$ 0.8	100	4.8 $\pm$ 4.0	100	2.9 $\pm$ 1.1	1	33	0.7 $\pm$ 0.4	0.2	0	< LOQ	0.2	0	< LOQ	
PCB 138	Muscle	0.1	100	4.6 $\pm$ 3.1	100	8.4 $\pm$ 4.2	100	5.3 $\pm$ 2.7	1	67	2.1 $\pm$ 1.8	NA	100	0.22 $\pm$ 0.02	0.1	19	0.08 $\pm$ 0.06	
PCB 153	Muscle	0.1	100	9.6 $\pm$ 5.8	100	17.0 $\pm$ 7	100	11.9 $\pm$ 4.0	NA	100	2.8 $\pm$ 1.2	NA	100	0.33 $\pm$ 0.01	0.1	38	0.12 $\pm$ 0.13	
PCB 180	Muscle	0.1	100	3.8 $\pm$ 2.5	100	5.7 $\pm$ 2.9	100	3.2 $\pm$ 1.8	1	33	1.2 $\pm$ 1.2	NA	100	0.18 $\pm$ 0.02	0.1	12	0.07 $\pm$ 0.06	

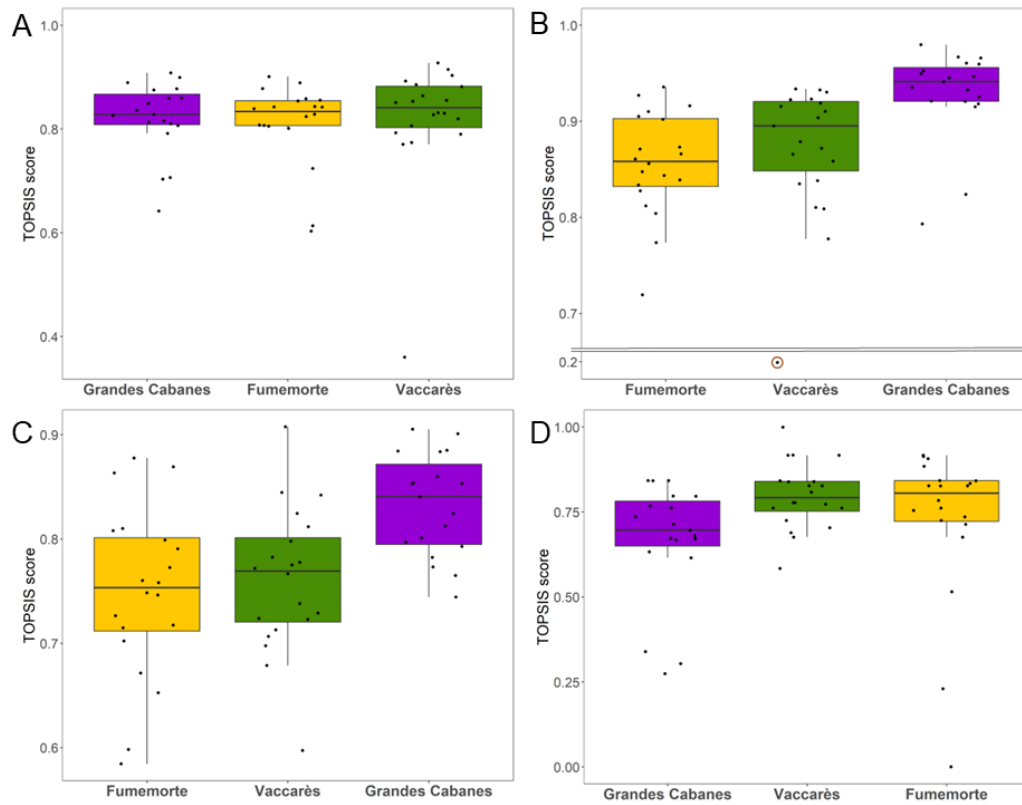
The EQC of the eels caught in the three sites were mostly not deviating or slightly deviating according to Belpaire and Goemans (2007) and ICES (2015), except for a few contaminants (i.e., As, Cd, Hg, Ni, PCB 28/31, PCB 52,

PCB 101,  $\alpha$ -HCH, pp-DDT, pp-DDE and Sum DDTs) and the SDI (Fig. 2). The proportions of the different EQC classes differed between sites for 17 of the 27 contaminants analysed and for the SDI (chi-2 test,  $p \leq 0.01$ ). Eels from the Grandes Cabanes site were less contaminated with POPs and TEs than those from the Vaccarès site, which were less contaminated than those from the Fumemorte site. Silver eels from the Grandes Cabanes site had a higher SDI (Fig. 2).



**Fig. 2:** Eel Quality Classes (EQC) distribution of silver eels (males and females grouped together) from Grandes Cabanes, Fumemorte and Vaccarès sites. Twenty-eight parameters were considered in  $\text{ng}\cdot\text{g}^{-1}$  ww, with Sum PCBs corresponding to the sum of the 7 PCBs (PCB 28/31, 52, 101, 118, 138, 153 and 180) in  $\text{ng}\cdot\text{g}^{-1}$  ww (except Sum PCBs\* was the sum of the 7 PCBs in  $\text{ng}\cdot\text{g}^{-1}$  lipid weight; Cu, Zn were in  $\mu\text{g}\cdot\text{g}^{-1}$  ww and SDI without unit).

The overall TOPSIS score (i.e., including POPs, TEs and *A. crassus*) decreased significantly with eel age but did not differ between sites (Table 3, Table S2, Fig. 3-A). The TOPSIS score for POPs contamination increased in larger eels and younger eels and was slightly higher at the Grandes Cabanes site, meaning that smaller and older eels were more contaminated (Table 3, Fig. 3-B). The TOPSIS score for TEs contamination was higher in the Grandes Cabanes site, indicating that eels from that site were less contaminated (Table 3, Fig. 3-C), but was not influenced by other variables. The TOPSIS score for *A. crassus* infestation, was lower for the oldest eels (Fig. 3-D, Table 3-D).



**Fig. 3:** TOPSIS score for A- all the criteria, B- only on POPs, C- only on TEs and D- only on *A. crassus* infestation (the boxplots are ordered by increasing median, i.e., the site with the lowest score is on the left and the site with the highest score is on the right). The circle highlights an outlier from the POPs analysis (which was removed for further analyses).



Table 3: Results of the selected linear model for each TOPSIS analysis (with the median values of each posterior and its 95 % credible interval).

Model	Parameter	2.50 %	50 %	97.50 %
<b>POPs + TEs + <i>A. crassus</i></b>				
logit(score) ~ site + age	$\alpha_{Grandes\ Cabanes}$	1.28	1.46	1.64
	$\alpha_{Fumemorte}$	1.53	1.72	1.91
	$\alpha_{Vaccarès}$	1.48	1.66	1.84
	$\gamma$	-0.38	-0.27	-0.15
	$\sigma$	0.32	0.38	0.46
<b>POPs</b>				
logit(score) ~ site + length + age	$\alpha_{Grandes\ Cabanes}$	2.35	2.57	2.78
	$\alpha_{Fumemorte}$	1.86	2.09	2.31
	$\alpha_{Vaccarès}$	1.77	1.98	2.19
	$\beta$	0.01	0.14	0.27
	$\gamma$	-0.47	-0.32	-0.17
	$\sigma$	0.37	0.44	0.55
<b>TEs</b>				
logit(score) ~ site	$\alpha_{Grandes\ Cabanes}$	1.45	1.64	1.83
	$\alpha_{Fumemorte}$	0.95	1.14	1.33
	$\alpha_{Vaccarès}$	0.99	1.19	1.38
	$\sigma$	0.35	0.42	0.51
<b><i>A. crassus</i></b>				
logit(score) ~ age	$\gamma$	-2.33	-1.2	-0.07
	$\sigma$	3.66	4.34	5.26

## Discussion

The present study provides information on the quality of silver eels in a Mediterranean lagoon complex heavily impacted by human activity. It also provides an opportunity to combine information on contaminants (POPs and TEs) and parasitic infestation (*A. crassus*), together or separately, to better understand the local causes of eel quality deterioration.

### Complementarity of sediment and eel contaminations

The study of contaminants in habitat sediments and in eels provides information on the complementarity of these samples. Although TEs were well detected in sediments (except for Cd, Cu and Hg), analyses of eel samples revealed the presence of contaminants that were not detected in sediments, such as PCBs. These low detections of PCBs in sediments have already been highlighted in Flanders (Belpaire et al. 2011). Our results therefore confirm other studies (de Boer and Hagel 1994; Belpaire et al. 2011; Bettinetti et al. 2011) that support the benefits of using eels to monitor the status and trends of PCBs and other lipophilic compounds in aquatic environments, due to their strong bioaccumulation potential. However, to quantify contaminants in eels, the current methodology requires the sacrifice of specimens. Given the critically endangered status of eels (Pike et al. 2020), it is imperative to develop non-lethal techniques for contaminant analysis. Moreover, our observations revealed

variability in detection limits across different matrices (Table 2), with a notable improvement in detection sensitivity over time. To aid in the conservation of this declining species, it is also essential to refine existing contaminant quantification techniques in sediments and eels and to consider the LOQs in contamination assessments.

A study of both sediment and eel tissue samples can provide further insight into the environmental contamination. Although the majority of eels exhibit sedentary behaviour during their growth phase, some display more diverse behaviours, such as occasional migration (Daverat and Tomás 2006; Panfili et al. 2012; Teichert et al. 2023). This implies that the contaminants present in the eels may originate from an environmental context different from that in which they were caught. However, in the present study, it is assumed that the eels were captured in their growth environment due to the configuration of the study system. In the Grandes Cabanes basin, which was a "poldered" area at the time of sampling, eels from the Petit Rhône River were able to colonise, mostly as juveniles (glass eels and elvers), through a gravity feed. The silver eels produced in this basin can then be trapped there or in the drainage canal that pumps water back to the Petit Rhône River. The Vaccarès lagoon, the largest aquatic habitat in the Camargue, has a higher density of eels than the surrounding canals (Hoste, unpublished data). The high density suggests that the eels caught in the Vaccarès site grew up in the lagoon, but the Vaccarès lagoon may also act as a receptacle for silver eels migrating to the sea from peripheral habitats, such as the Fumemorte canal. Integrating data from two distinct sampling sites within this expansive environment yields a more comprehensive understanding of the open ecosystem. The Fumemorte canal is the main freshwater inflow to the Vaccarès lagoon. It is permanently connected to the Vaccarès lagoon and therefore eels from the Fumemorte canal can move freely between the canal and the lagoon during their growth depending on their behaviour and how sedentary they are (Daverat and Tomás 2006; Panfili et al. 2012; Teichert et al. 2023). However, based on mark recapture data (Panfili et al. 2012; Hoste, unpublished data), eels from the Fumemorte site demonstrated a high residency strategy. Therefore, it is likely that most of the eels captured in the Fumemorte site spend most of their growth period at this site, where they initiated their silvering and seaward migration. This hypothesis can be corroborated by a comparison of the analysis of POPs in the sediment and in the eels. Indeed, these contaminants were more frequently detected in the Fumemorte sediments than in other habitats. Concomitantly, the lowest TOPSIS scores for POPs (i.e., higher contamination) were generally observed in eels caught in the Fumemorte canal (except for the outlier in the Vaccarès lagoon).

#### Quality of eel reflecting habitat contamination levels in the Camargue ecosystem

The EQC results indicate that the eel sampled at the three sites exhibited minimal deviation from the baseline, except for a few contaminants (i.e., As, Cd, Hg, Ni, PCB 28/31, PCB 52, PCB 101,  $\alpha$ -HCH, pp-DDT, pp-DDE and Sum DDTs) and the SDI. This result indicates that the Camargue lagoon is generally less contaminated than the sites in Belgium (rivers, channels and lakes) used to define the quality classes of the EQC (Belpaire and Goemans 2007; Belpaire et al. 2011; Malarvannan et al. 2014). While other studies have also examined contaminant levels in eels, this discussion is confined to findings from Mediterranean lagoons, with a particular focus on silver eels.

A comparative analysis of the pollution levels in various Mediterranean lagoons in Italy, Spain, and France reveals that the silver eels from the Camargue lagoon exhibit considerably elevated levels of contamination, particularly in regard to POPs, Cu, and Zn (Ferrante et al. 2010; Quadroni et al. 2013; Amilhat et al. 2014;

Capoccioni et al. 2020; Martínez-Gómez et al. 2023). The silver eels sampled in the Tevere lagoon exhibited higher concentrations of PCBs, HCBs, HCHs, PBDEs and CHLs than those in this study (Quadroni et al. 2013). Eels in the Camargue therefore have higher concentrations of POPs than in other similar environments, particularly PCBs and PBDEs (Capoccioni et al. 2020). With regard to other TEs, namely Cd, Cr, Hg, Pb and Se, the contamination levels in the Camargue study sites were found to be comparable to those observed in other lagoons (e.g., Bettinetti et al. 2011; Quadroni et al. 2013; Capoccioni et al. 2020), except in Spain where lower concentrations were noted (Romero et al. 2020; Salvat-Leal et al. 2024). The SDI scores were found to be comparable to those previously reported by Amilhat et al. (2014) for other French lagoons. With regard to DDT concentrations, a significant divergence from the EQC was observed, particularly in the case of pp-DDT, with markedly disparate levels across the various sites. The levels of sum DDTs in some lagoons in southwestern France were found to be higher or lower than those observed in the Camargue. Although the levels of DDT metabolites were anomalously high in comparison to the EQC and four Spanish Mediterranean lagoons (Pérez-Vegas et al. 2023), they were consistent with those found in other French Mediterranean lagoons (Amilhat et al. 2014). As previously outlined by Amilhat et al. (2014), the shallow depth, the limited connection with the sea, and strong wind events could contribute to the resuspension of the contaminants accumulated in the sediments. The concentrations of Sum DDTs exhibited considerable variation across the Camargue, with the lowest values observed in eels from the Grandes Cabanes. The presence of agricultural runoff in the Vaccarès lagoon and the Fumemorte canal may also contribute to this variability. These findings underscore the necessity for a more comprehensive understanding of the contamination sources affecting eel quality, even at the level of fine spatial scales. Furthermore, variations in the anthropogenic sources of contaminants across Mediterranean lagoons have been identified, underlining the need to implement specific environmental management strategies and actions to protect these ecosystems (Pérez-Vegas et al. 2023).

Fluctuations in contamination of eels in the Camargue also appear to occur on a temporary basis, making it challenging to ascertain a general trend (Roche 2000; Oliveira Ribeiro et al. 2005). Furthermore, the quantification methods have been improved over time, enabling more precise detection limits of compounds (e.g., the detection limit for PCBs is  $0.1 \text{ ng}\cdot\text{g}^{-1}$  of ww in the present study compared to  $0.8 \text{ ng}\cdot\text{g}^{-1}$  of ww in Roche (2000) study). The contamination levels of POPs were previously examined by Roche (2000). The aforementioned study demonstrated concentrations of HCB and PCBs that were 1.2 to 13 times higher and a concentration of  $\gamma$ -HCH that was 32 to 101 times higher at the Fumemorte and Vaccarès sites compared to the findings of the present study. Conversely, a 3 to 6-fold increase in pp-DDE in eels' muscles was demonstrated between 1996 and 1997 (Roche 2000) and 2021 (our study) in the same locations. This result was unexpected, given that the use of DDT was prohibited in France in the 1970s. Although PBDEs were not investigated by Roche (2000), one study showed that PBDE deposition in the lower reaches of the river was markedly high during the 2000s, subsequently declining over time (Liber et al. 2019). In any case, the results of this study confirm the presence of PBDEs in eels. The concentrations of TEs exhibited minimal variation over time (Oliveira Ribeiro et al. 2005). The level of Ni was observed to decrease over time, irrespective of the tissue sampled, whereas an increase in the level of Mn was observed. However, a comparison of contamination trends between different tissues (e.g., muscle and liver) reveals that they exhibit differences in their respective temporal patterns. The observed differences in POPs and TEs in eel tissues may be attributed to modifications of agricultural practices, which represent a significant source of contamination of both POPs and TEs. Moreover, certain TEs are naturally present in the environment in varying concentrations, contingent on the geology of the region. For example, at the level of the Rhône River, researchers

have demonstrated a reduction in the concentrations of Cd, Cu and Zn since the 1970-1980s (Dendievel et al. 2020). Furthermore, Dendievel et al. (2020) emphasised the necessity of distinguishing between the origin of TEs (i.e. natural or anthropogenic) and considering the ecotoxicological mixture risk of metals with POPs.

#### Determination of eel quality based on the integration of various criteria

Previous studies of eel quality have concentrated on habitat rather than individual biometric characteristics (e.g. length, age and growth rate), providing data for large-scale site comparisons and focusing on a single sex. However, it should be noted that eels' biometric characteristics can vary between sites and within the same site (Acou et al. 2003; Melià et al. 2006a; Daverat et al. 2012; Teichert et al. 2023). Therefore, these factors should be taken into account when studying eel quality. Some previous studies have focused on a single sex, for example females (Bourillon et al. 2020), in order to limit the influence of individual biometric characteristics on POPs, TEs and *A. crassus* infestation. The present study analysed both male and female silver eels using a multivariate TOPSIS analysis, incorporating POPs, TEs and *A. crassus* infestation. The findings demonstrate that, despite the influence of individual eel characteristics (such as age or length), differences in eel quality can fluctuate even at a fine scale of the Camargue ecosystem. Consequently, this study emphasised the necessity of sampling diverse habitats to more accurately reflect the quality of eels in a heterogeneous environment such as the Camargue.

The smaller and older eels exhibited lower TOPSIS scores for POPs, which may be indicative of reduced quality, particularly among those caught in the Fumemorte canal and the Vaccarès lagoon. This result is consistent with the findings of certain studies that have identified negative and moderate correlations between the concentrations of specific POPs, such as PCBs, and the size of eels (Roche 2000; Martínez-Gómez et al. 2023). This could be explained by a dilution effect during growth and could be linked to alterations in energy metabolism. In the present case, the small and aged eels were mainly males. No difference in lipid levels was found between males and females at two of the three sites (Fumemorte and Grandes Cabanes). This suggests that older males may be more susceptible to POPs contamination. These results highlighted the importance of further studying male silver eels, which are abundant in many Mediterranean lagoons (Amilhat et al. 2008).

Beyond individual variability, eels in the Grandes Cabanes site had lower POPs and TEs contamination compared to eels in the Fumemorte and Vaccarès sites. The Vaccarès lagoon is subject to a multitude of anthropogenic disturbances, including effluents from cultivated areas where substantial volumes of water from the Rhône River are employed for irrigation purposes. The Rhône sediment is known to be polluted by a broad range of compounds, including POPs and other pesticides (Miège et al. 2012; Liber et al. 2019; Dendievel et al. 2020). In addition to the contamination of the Rhône River, several chemical compounds (e.g., Cu) are also used in locally cultivated areas within the Camargue (Comoretto et al. 2007, 2008). The water from the cultivated areas is drained into the Vaccarès lagoon via a network of drainage canals, including the Fumemorte canal, which may contribute to the higher levels of POPs and TEs contamination observed at that site. Given that the Fumemorte canal represents the primary source of freshwater input into the Vaccarès lagoon, it can be reasonably inferred that this canal also serves as the primary conduit for pollutants into the lagoon. It is therefore unsurprising that the TOPSIS scores of eels from the Vaccarès and Fumemorte sites did not significantly differ in the present study. Interestingly, the eels from the Grandes Cabanes site exhibited lower contamination levels with POPs and TEs compared to other eels. As the water used to fill the polder in the Grandes Cabanes area also originates from the Rhône River, the influence of the imported pollution from the Rhône River cannot be the sole explanation for the lower TOPSIS

score of the Fumemorte and Vaccarès sites (i.e., higher contamination). The local source of contaminants, such as water used to irrigate crops, therefore appears to be significant.

The lack of distinction in the TOPSIS score for *A. crassus* analysis between the eels from the three sites is a notable finding. Indeed, *A. crassus* larvae typically exhibit reduced survival in brackish and saline waters relative to freshwater (Kirk et al. 2000). This reduces the probability of eel infestation when salinity increases (Lefebvre and Crivelli 2012). In light of the aforementioned considerations, it would be reasonable to hypothesise that the TOPSIS score for *A. crassus* analysis should be lower at the Grandes Cabanes and Fumemorte sites, which are predominantly influenced by freshwater, than at the brackish Vaccarès lagoon. This was not the case, as only eel age was found to influence the TOPSIS score for the *A. crassus* analysis. Nevertheless, eels from the Grandes Cabanes site exhibit the highest SDI, thereby substantiating the significant prevalence of the parasite in this particular habitat. The global absence of difference in the TOPSIS scores for *A. crassus* infestation between sites in the Vaccarès hydrosystem is consistent with the observations of Lefebvre et al. (2002b). These authors demonstrated, based on an extensive sampling of over 10,000 eels, that the abundance of *A. crassus* was primarily influenced by eel size, sampling month and year, while the sampling site exerted only a minor influence. Those results indicate that the factors influencing eel infestation by *A. crassus* are complex and may depend on contrasting individual and environmental factors. For example, previous studies have proposed that the contamination of eels with POPs may impair their immune system, thereby increasing their susceptibility to infectious diseases and parasites (Robinet and Feunteun 2002; Lawrence and Elliott 2003).

To our knowledge, this is the first time that a TOPSIS analysis has been used to assess individual eel quality. The utilisation of a TOPSIS analysis enables the integration of a multitude of information sources to calculate a score for the individual's quality. In this study, the same weight was assigned to the three criteria (i.e., TEs, POPs and *A. crassus* infestation), in accordance with the approach adopted by Bourillon et al. (2020), whereby contamination by TEs and POPs was given the same weight as the infestation by *A. crassus*. The decision to assign equal weight to TEs, POPs and *A. crassus* and to each parameter measured within these criteria, was driven by the absence of data elucidating the relative influence of each criterion/parameter. However, an alternative approach would have been to weight the variables according to their importance for contamination. For example, it may be assumed that compounds with a "strongly deviating" class obtained from the EQC (Belpaire and Goemans 2007) would be more harmful to eels and therefore increase their weight in the TOPSIS analysis. Furthermore, Polak-Juszczak and Robak (2015) emphasised the detrimental impact of Hg when its concentration in eel muscle exceeds 0.50-1.20  $\mu\text{g}\cdot\text{g}^{-1}$  ww. Hg could have been assigned a greater weight than the other TEs, particularly when its concentration reached or exceeded 0.50-1.20  $\mu\text{g}\cdot\text{g}^{-1}$  ww. According to the Great Lakes Water Quality Agreement (GLWQA 1987), the concentration of PCBs in fish tissues is to be maintained at or below 100  $\text{ng}\cdot\text{g}^{-1}$  ww. Our findings revealed that a number of eels from the Fumemorte and Grandes Cabanes sites exhibited PCB concentrations that exceeded this threshold. Consequently, it may be necessary to accord greater attention to these elevated PCB concentrations in future analyses. The TOPSIS analysis could be used to assign greater emphasis to specific compounds, such as PCBs, prioritizing the recognition that the quality of eels with high PCB concentrations may be lower than those with elevated levels of other POPs.

Overall, the multivariate TOPSIS analysis enables to refine our understanding of the quality of the eels, even at the intra-site scale, and provides insights into their environment. The TOPSIS analysis revealed that,

although there was no significant difference in the total TOPSIS scores between the eels caught in different sites, the contamination of the eels by POPs and TEs differed with sites. These analyses allowed a more comprehensive understanding and comparison of the various degradation sources observed in the sampled sites within a lagoon complex. In particular, the Camargue exhibited a markedly elevated level of contamination by a range of agricultural (Cu, HCHs, and DDTs) and industrial (PCBs) compounds, when compared to other Mediterranean lagoons. These findings underscore the necessity for management strategies aimed at reducing agricultural and industrial pollution. Restoring ecological connectivity between diverse habitats within the Camargue ecosystems may facilitate the movement of eels between freshwater and brackish/marine habitats, thereby potentially reducing their susceptibility to infestation by *A. crassus* (Marohn et al. 2013).

## **Conclusion**

Certain pollutants have been shown to have high toxicity on aquatic organism such as fish. Indeed, the presence of contaminants has been demonstrated to be associated with reproductive disorders in fish, while the presence of parasites has been shown to affect swimming performance. It is therefore imperative to investigate the combination of contaminants and parasitological analyses. In the present study, the TOPSIS score was employed to facilitate the consideration of all variables without the necessity for prior information. It is challenging to ascertain the impact of each contaminant on eel biology and in situ experiments are required. It is important to note that even if the effects of a single contaminant can be experimentally identified, it is not possible to ascertain how it may act in the presence of other elements, depending on the environmental conditions. Indeed, the combination of several compounds may result in a synergistic effect, which is commonly referred to as a "cocktail effect". Given the complexity of understanding the impact of pollutants in nature, it is strongly recommended to limit any chemical inputs, especially those that have already been clearly identified as hazardous to nature. Furthermore, it is essential to consider the location-specific contexts during quality analyses, as certain contaminants may be locally authorised on a derogatory basis, as is the case in the Camargue with the benzobicyclon, an herbicide commonly used in agriculture, particularly in rice crops, and which has been proven to be toxic to aquatic organisms (Ministère de l'Agriculture et de l'Alimentation 2021).

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### **Statements & Declarations**

The authors state that they have no known financial interests or personal relationships that could have influenced the work reported in this paper.

### **Ethical Approval**

The treatment of animals is in accordance with the laws, guidelines and animal welfare policies of the CE71 Ethics Committee. The Tour du Valat and its staff are authorised to carry out experiments on wild animals (French authorisation number A 13 200 01).

### **Consent to Participate**

Not applicable

### **Consent to Publish**

Not applicable

### **Author Contributions**

Conceptualisation: A. Hoste and D. Nicolas. Methodology: A. Hoste, R. Lagarde, and C. Bouchard. Fieldwork and sample preparation: A. Hoste, K. Verbrugghe, D. Nicolas, and C. Tetrel. Laboratory analysis: E. Amilhat, P. Bustamante, A. Covaci, E. Faliex, G. Poma, H. Vey Payre. Data curation: A. Hoste, E. Migne, and C. Tetrel. Formal analyses: A. Hoste. Writing – original draft: A. Hoste. Writing – review and editing: all authors. All authors read and approved the final manuscript.

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### **Competing Interests**

The authors have no relevant financial or non-financial interests to disclose.

### **Data Availability statement**

Not applicable



## **Supplementary Information**

This file contains additional information on Methods and Results for

**Investigating the quality of European silver eels by quantifying contaminants and  
parasite infestation in a French Mediterranean lagoon complex**

By Hoste A. et al.

Table S1: Weight assigned to each criterion for the different TOPSIS analysis.

Element	Support	Analyse	POPs + TEs + A. <i>crassus</i>	POPs	TEs	A. <i>crassus</i>
Ag	Liver	TEs	1/104	X	1/26	X
As			1/104	X	1/26	X
Cd			1/104	X	1/26	X
Co			1/104	X	1/26	X
Cr			1/104	X	1/26	X
Cu			1/104	X	1/26	X
Fe			1/104	X	1/26	X
Hg			1/104	X	1/26	X
Mn			1/104	X	1/26	X
Ni			1/104	X	1/26	X
Pb			1/104	X	1/26	X
Se			1/104	X	1/26	X
Zn			1/104	X	1/26	X
Ag			Muscle	1/104	X	1/26
As	1/104			X	1/26	X
Cd	1/104			X	1/26	X
Co	1/104			X	1/26	X
Cr	1/104			X	1/26	X
Cu	1/104			X	1/26	X
Fe	1/104			X	1/26	X
Hg	1/104			X	1/26	X
Mn	1/104			X	1/26	X
Ni	1/104			X	1/26	X
Pb	1/104			X	1/26	X
Se	1/104			X	1/26	X
Zn	1/104			X	1/26	X
Sum PCBs	Muscle	POPs: Industrial origin		1/16	1/8	X
Sum of the 18 other PCBs			1/16	1/8	X	X
PBDEs			1/8	1/4	X	X
HCB		POPs: agricultural origin	1/16	1/8	X	X
HCHs			1/16	1/8	X	X
Sum DDTs			1/16	1/8	X	X
CHLs			1/16	1/8	X	X
Abundance of A. <i>crassus</i>	Swim- bladder	Parasitology	1/8	X	X	1/2
SDI			1/8	X	X	1/2

Table S2: Information of the WAIC comparison between the fifteen models for each TOPSIS analysis.

Model	WAIC			
	POPs + TEs + <i>A. crassus</i>	POPs	TEs	<i>A. crassus</i>
logit(score) ~ site	73.19	89.58	<b>67.35</b>	353.47
logit(score) ~ length	226.71	263.87	206.69	353.45
logit(score) ~ age	226.49	262.71	206.73	<b>350.66</b>
logit(score) ~ growth	227.19	262.78	206.39	352.4
logit(score) ~ site + length	67.98	91.44	69.43	353.42
logit(score) ~ site + age	<b>56.71</b>	79.29	69.26	351.53
logit(score) ~ site + growth	70.78	81.06	66.97	354.43
logit(score) ~ length + age	228.31	264.48	208.69	351.65
logit(score) ~ length + growth	227.93	264.71	208.49	348.99
logit(score) ~ age + growth	228.66	264.44	208.57	353.13
logit(score) ~ site + length + age	57.07	<b>76.83</b>	71.21	353.13
logit(score) ~ site + length + growth	54.71	82.6	68.44	351.53
logit(score) ~ site + age + growth	58.89	78.72	69.8	354.46
logit(score) ~ length + age + growth	230.02	266.55	210.57	350.17
logit(score) ~ site + length + age + growth	56.48	78.93	70.27	352.79

Fig. S1: Comparison between sub-sampled eels for quality analysis and eels collected during the same period, A- for the body length, B- for weight and C- for age (N represented the number of sampled individuals).

