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European eels and heavy metals from the Mar Menor lagoon (SE Spain)

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ABSTRACT

Heavy metal pollution is related to the fall in European eel (*Anguilla anguilla*) populations. The Mar Menor lagoon (SE Spain) is home to an endangered population of this species, which is still caught for human consumption. The presence of Pb, Cd and Hg in the livers and muscles and the Se:Hg ratio in muscle of 150 eels from this lagoon were determined. Pb concentrations were higher than those reported from other populations in the world, while Cd and Hg concentrations in the tissues analysed were lower. In terms of food safety, Se concentrations play an important role in sequestering Hg in eels from this lagoon.

1. Introduction

Albeit in different ways, the Mar Menor lagoon and the European eel (*Anguilla anguilla*) are both threatened. This coastal lagoon is regarded as one of the most ecologically rich ecosystems in the Mediterranean Basin. It is included in the Ramsar Convention on Wetlands, and is a Special Protected Area of Mediterranean Interest, a Special Protected Area under the European Union (EU) Wild Birds Directive, and a Site of Community Importance as part of the Natura 2000 Network (EU Habitats Directive). Despite these figures of protection, the Mar Menor is under huge anthropogenic pressure linked to (i) hydrological change, (ii) mining activity, (iii) agrochemical runoff from intensive agriculture in its watershed, and (iv) contaminants of emerging concern (Jiménez-Martínez et al., 2016). Although the mining activity in the nearby Cartagena-La Unión area ceased decades ago, the discharge of metal-enriched waste continues to be the most important source of heavy metal input into the lagoon. As an area of arid climate, when torrential rains fall the mining waste remaining in upland areas runs into the Mar Menor along the normally dry gullies. High metal and metalloid contents in the Mar Menor have been reported from sediments (Conesa and Jiménez-Cárceles, 2007; Albaladejo et al., 2009; María-Cervantes et al., 2009; Serrano et al., 2019), along with bioaccumulation in macrophytes (Sanchiz et al., 2000, 2001; Serrano et al., 2019), certain filter feeders and invertebrates (Albaladejo et al., 2009), and fish (De León et al., 1982; Marín-Guirao et al., 2008). The lagoon is also vulnerable to eutrophication and in recent years there has been a proliferation of phytoplankton, affecting benthic primary producers and leading to oxygen depletion (Gimenez-Casalduero et al., 2017). Recent environmental incidents have provoked massive mortality in many fish and crustacean species.

European eels are likewise endangered. These migratory fish conduct an extraordinary 5000–6000-km journey to the Sargasso Sea in the North Atlantic Ocean to spawn, from where their larvae travel all the way back to Europe. However, in recent decades a drastic decline in the number of juvenile eels in European waters has been reported (ICES/EIFAC, 2003). The situation of eel stocks is so alarming that the species is now listed as Critically Endangered on the IUCN's Red List (Jacoby and Gollock, 2014) and, through European Eel Regulation EC No 1100/2007 (Council Regulation, 2007) implemented with the help of the Eel Management Plans, the European Union has established measures for restoring eel stocks. Although a number of policies have been put into practice since the entry into force of this regulation, European eel recruitment remains low throughout its geographical range and its stock status remains critical (ICES, 2018). A combination of different causes such as climate change, overfishing, habitat degradation and the poor quality of spawners (due to pollution and disease) are thought to be responsible for this decline. It has also been suggested that the bioaccumulation of chemical substances including heavy metals may be having an important impact on eel physiology. Pollutants can disturb the immune, reproduction, nervous and endocrine systems, thereby

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negatively affecting cellular and organ functions at individual and even population levels (see review in Geeraerts and Belpaire, 2010). In 2007, a European Eel Quality Database was set up to gather information on contaminants in eels from all over Europe (ICES, 2007), and aims to provide a comprehensive overview of the quality of European eel populations, data that will be essential for eel management.

Thus, in light of the above, the levels of lead (Pb), cadmium (Cd) and mercury (Hg), all known to be harmful heavy metals, in European eels from the Mar Menor lagoon were investigated. On the other hand, the dietary selenium (Se) status is highly related to Hg toxicity (Ralston and Raymond, 2010), and the antagonist effect of Se on Hg is well known since many years ago. The molar ratio Se:Hg may provide an accurate index of risk from fish consumption, and the Selenium Health Benefit Value (HBV_{Se}, Ralston et al., 2016) has been recently considered as a good instrument to better understand of the Se available that remains after its interaction with Hg. Thus, due to the importance of factors affecting public health, information regarding Se concentrations, Se:Hg ratio and the HBV_{Se} were also analysed.

2. Material and methods

2.1. Studied area

The Mar Menor is the largest saltwater lake in Europe (135 km^2). Located in the south-east of the Iberian Peninsula (37°38′ N, 0°42′ W), it is separated from the Mediterranean Sea by a 24-km sand bar known as La Manga, through which water exchange with the sea takes place via natural openings. It is permanently hypersaline (41–47 g L^{-1}) and water temperatures oscillate approximately between 10 °C and 31 °C (mean annual temperature 18 °C).

2.2. Sample collection

Yellow eels were caught by local fishermen using traditional gear in October 2015–February 2017. After being anesthetized and then euthanized with a lethal dose of tricaine methanesulfonate (MS222) at *100 mg L^{-1}*, the total length to the nearest millimetre and the total weight to the nearest gram were obtained. Eels were then dissected to obtain portions (0.2–0.5 g) of liver and muscle, which were then stored at −20 °C until processed. Specific and consistent sections of muscle were taken from 4 to 5 cm behind the anal cavity, and the livers were weighted.

All fish were handled in accordance with EU regulations concerning the protection of experimental animals (Directive 2010/63/UE). Protocols were approved by the Ethics Committee of the University of Murcia) (permit number: No A13161101). All efforts were made to minimize animal handling and stress.

2.3. Sex and development stage determination

Gonads of eels ranging from 26 to 80.4 cm of total length were weighted before they were fixed in Bouin's fixative, embedded in paraffin and slices of 5 μm thick were cut and stained with Hemotoxiline-Eosine following routine methods. Sex and developmental stage were assessed by histological examination of gonad tissue (Colombo and Grandidr, 1996; Geffroy et al., 2013; Mazzeo et al., 2016).

2.4. Body condition

Scaled Mass Index (SMI) was calculated following Peig and Green (2009). This index adjusts the mass of all specimens to that which they would have at length L_0, following the formula

$$SMI = Mi\,(L_0/Li)^{bsma}$$

The process is the next: 1) it was realized a standardized major axis (SMA) regression on ln-transformed total length (L) and weight (W) data, $\ln W = \ln a + b_{ols} \ln L$, where b_{ols} = slope of the ordinary least squares regression of body mass against total length and a is the intercept of the regression; 2) the scaled index is calculated for each specimen applying the formula $SMIi = M_i\,(Lo/Li)^{bSMA}$, where Mi and Li are the raw data for the i individual; b_{sma} (the scaling exponent) is the slope of the standardized major axis (SMA) regression on ln-transformed total length and weight data, and is calculated as $b_{SMA} = b_{ols}/r$, where r = Pearson's correlation coefficient of SMA regression, and L_0 = mean length of the sample.

2.5. Age determination

Otoliths were processed following ICES (2009) guidelines and described by Mayo-Hernández et al. (2015). Otoliths were removed, cleaned, dried and the sagittal otoliths stored. Due to the fact that the majority of the eels were of 5 years old, it was possible to read “in toto”, that is by clearing all the otolith by immersion in glycerine, to increased light penetration, and examined under a stereoscopic microscope (40× magnification, Olympus BX41) with reflected light against a dark background. Those readings that did not agree were repeated and if the disagree continued, the otolith was rejected.

Following convention, the reference age date was set as 1 January and so eels were age 0 in their year of arrival in continental waters. Age was determined by two independent readers counting the otolith winter rings (ICES, 2009) in the left ear.

2.6. Metal analysis

To determine Pb, Cd and Se content, liver and muscle samples were analysed using inductively coupled plasma optical emission spectrometry (ICP-OES, ICAP 6500 Duo, Thermo Scientific, with One Fast System). Previously, the samples were digested in special Teflon reaction tubes with trace mineral-grade nitric acid and hydrogen peroxide (69 and 33%, respectively, Suprapure, Merck) and heated for 20 min at 220 °C in a microwave digestion system (UltraClave-Microwave Milestone®). Finally, the samples were diluted to 10 ml with double deionised water (MilliQ). Two readings were taken for every sample; the concentration values used in the analyses were the mean of the two readings. To check for possible contamination, one blank sample for every eleven samples was also analysed.

Multi-element calibration standards (SCP Science, in 4% nitric acid) were prepared with specific concentrations of these elements and intermediate patterns of each were prepared. The calibration device was established per batch, with a minimum of three points for every lot. Each run started with the calibration standards, continued with samples and intermediate patterns, and finished with the series with intermediate patterns (10% variation coefficient). The wavelengths were 220.353 nm (Pb), 214.438 nm (Cd) and 196.090–203.985 (Se), and the recovery rates for reference materials (Standard Reference Material L577b) were 98.47% (Pb), 98.12% (Cd) and 103.93% (Se).

To determine the total Hg content, samples were analysed using an atomic absorption spectrometer AMA254 Advanced Mercury Analyzer (Leco) without sample pre-treatment or sample pre-concentration. The recovery rate for reference materials (Mercury ICP Standard 1000 mg L^{-1} Hg, Merck) was 98.87%.

Inorganic element concentrations were expressed in micrograms per gram in wet weight (μg g^{-1} ww). The detection limits (DL) were 0.001 μg g^{-1} (Pb, Cd and Se) and 0.003 μg g^{-1} (Hg).

2.7. Data analysis

The software R 3.4.4 (R Core Team 2018) was used to analyse the data. For biometric data, age and inorganic element concentrations, geometric medians and standard errors were obtained. In the chemical analysis, data below the DL were expressed as half of this figure (0.0005 μg g^{-1} for Pb, Cd and Se, and 0.0015 μg g^{-1} for Hg) in the statistical analysis. The Kolmogorov-Smirnov test was used to evaluate the data distribution, and Mann-Whitney U, Kruskal-Wallis and Spearman tests were used as nonparametric statistical methods. The significance level for all tests was set as 0.05.

To facilitate the understanding of the biological accumulation, an Individual Mean Bioaccumulation Index (IMBI) was calculated (Maes et al., 2005):

$$IMBI = \left[\sum_{i=1}^{n}\left(\frac{Ci}{Cimax}\right)\right]/n$$

where Ci = the individual metal concentration of heavy metal i, $Cimax$ = the maximum observed concentration of heavy metal i, and n = number of analysed metals. IMBI values were between 0 and 1.

In terms of food safety, the Se:Hg molar ratios and the Selenium Health Benefit Value (HBV_{Se}, Ralston et al., 2016) in muscle tissue were calculated:

$$HBV_{Se} = ([Se - Hg]/Se) \times (Se + Hg)$$

where Se and Hg are molar concentrations of these elements (μmole kg^{-1}). A positive value of HBV_{Se} and a molar ratio of Se:Hg greater than one are considered healthy (Ralston et al., 2016; Melgar et al., 2019).

3. Results

The biometric data, age and sex ratio of sampled European eels are shown in Table 1. The percentage of the samples with values above the instrumental detection limit (DL) are above 90% except Cd in muscle (36%) and Hg in liver (86.7%). The detected concentrations of Pb, Cd, Hg and Se in livers and muscles are given in Table 2. The order to element concentrations were Se > Pb > Hg > Cd (muscle) and Se > Pb > Cd > Hg (liver), and statistical differences among elements in both tissues were detected. Significant higher concentrations of Pb, Cd and Se were detected in livers, although the highest Hg concentrations was found in muscles.

Correlations between elements were low (or non-existent) in each tissue (Table 3). Cd and Se were the elements with more inter-tissue correlations, and the highest relationship was found between Cd and Pb in liver ($r = 0.468, p < .01$). The tissue with less correlations between elements was the muscle. Biometric data were correlated with Hg and Se concentrations in both tissues, while Pb concentration only was correlated with total length and weight in muscle, and Cd was not correlated with these biometric data. The eel's total length showed a moderate-to-high correlation with Hg and Se concentrations in muscle and liver respectively, while weight showed a moderate-to-hight correlation with Se concentration in liver. Regarding age, the highest correlation was detected with Se concentration in liver, while moderate and low correlations were observed between this parameter and Hg in muscle and liver, respectively. No correlation between age and Cd tissue concentrations was detected. Biometric measures had moderate-to-high positive inter-correlations and with age ($p < .01$, Table 3). For each element, the correlation between tissues was positive and significant ($p < .01$) (Spearman correlation coefficient of 0.309, 0.411, 0.661 and 0.272 for Pb, Cd, Hg and Se, respectively).

IMBI geometric means (Pb, Cd and Hg) were 0.127 ± 0.007 (range 0.011–0.632) and 0.075 ± 0.007 (range 0.017–0.394) in liver and muscle, respectively. A positive correlation ($r = 0.214$, $p < .01$) between IMBI in liver and age was observed. There was a positive relationship between tissues ($r = 0.397$, $p < .01$). Statistical differences between tissues were found.

In muscles, the Se and Hg molar concentrations (μmole kg^{-1}) were 3.838 ± 0.240 and 0.041 ± 0.006, respectively, while the Se:Hg ratio was 92.630 ± 18.999. Only 6.0% of eels had negative HBV_{Se} values, the geometric mean of the remaining samples being 5.776 ± 0.219.

Table 1
Descriptive statistics of biometric data and age (geometric mean, standard error and range) and sex ratio (number and percentage) in European eels from the Mar Menor lagoon (Spain).

Age (years)	3.4 ± 0.1 (1 − 10)
Weight (g)	256.9 ± 17.0 (32.0–1024.0)
Total length (mm)	546.0 ± 9.2 (296.0–804.0)
SMI	0.23 ± 0.01 (0.05–0.65)
Sex ratio	Female: 130 (86.7)
	Male: 2 (1.3)
	Intersex: 1 (0.7)
	Undetermined: 3 (2)
	Not recorded 14 (9.3)

SMI: Scaled mass index.

Table 2
Concentrations of Pb, Cd, Hg and Se (geometric mean ± Standard error, minimum and maximum, μg g^{-1} wet weight) in livers and muscles of European eels from the Mar Menor lagoon (Spain).

	Muscle	Liver
Pb	0.093 ± 0.016 (nd-1.434)	1.500 ± 0.100 (nd-7.976)
Cd	0.002 ± 0.001 (nd-0.047)	0.039 ± 0.005 (nd-0.458)
Hg	0.008 ± 0.001 (nd-0.177)	0.006 ± 0.001 (nd-0.155)
Se	0.303 ± 0.019 (nd-1.544)	4.999 ± 0.339 (nd-18.168)

nd = not detected.

4. Discussion

Recent studies have confirmed the presence of heavy metals such as Pb, Cd and Hg in the sediment and other fish (gobids) and invertebrate species of the Mar Menor lagoon (María-Cervantes et al., 2009; Tsakovski et al., 2009).

In 1982, De León et al. reported high concentrations of Pb and Cd in the muscles of several fish species including European eels (2.5 for Pb and 1.1 μg g^{-1} for Cd, wet weight) in the Mar Menor lagoon. In recent decades, many studies have also assessed the presence of heavy metals in eels from European marine, brackish and freshwater environments (Table 4). Data on contaminant levels in these studies have been recorded in the context of stock restoration, the Water Framework Directive, human health issues and consumer protection. Generally speaking, eels from this study had higher Pb, lower Hg and similar Cd concentrations in their livers and muscles than eels from other European environments.

Although the order of heavy metal concentrations in a particular tissue reported in previous studies does not show a defined pattern, a predominance of Hg concentrations in muscle can be observed (Table 4). In this tissue, Pb concentrations were higher than those reported for Cd in eels from several Spanish environments (Sánchez et al., 1998; Bordajandi et al., 2003; Usero et al., 2003; Ureña et al., 2007) and as well as in other European ecosystems (e.g. Genç and Yilmaz, 2017 in Turkey). Regarding liver, the order observed in the present study is in line with those reported in Spain (Usero et al., 2003) and Turkey (Genç and Yilmaz, 2017), while other studies showed higher (Linde et al., 2001, in Spain; Yildiz et al., 2010 in Turkey) or similar (Has-Schön et al., 2006 and 2008) Cd concentration than those reported for Pb. Regarding the bioaccumulation of a particular heavy metal in different tissues, most of the previous studies (Table 4) described higher Pb and Cd concentrations in liver than those found in muscle. This trend is also observed for Hg bioaccumulation, although a higher Hg concentration in muscle compared to liver has been reported in three works (Has-Schön et al., 2006 and 2008; Eira et al., 2009). In Spain, only from two locations (from an oil station on the river Ferrerias and from Pb–Zn mines on the river Urumea) has metal pollution ever been reported with similar (Linde et al., 2004) or higher Pb (Sánchez et al., 1998)

Table 3
Spearman correlations between element concentrations (muscle/liver) and biometric parameters of European eels from the Mar Menor lagoon (Spain).

	Cd	Hg	Se	Total length	Weight	SMI	Age
Pb	0.218**/0.468**	−0.061/0.085	−0.028/0.323**	−0.208*/0.114	−0.189*/0.093	−0.101/−0.002	−0.128/0.217**
Cd		−0.113/0.167*	0.161*/0.162*	−0.093/−0.037	−0.061/−0.065	−0.130/−0.079	−0.014/0.126
Hg			0.238**/0.380**	0.575**/0.338**	0.575**/0.276**	0.459**/0.359**	0.446**/0.183*
Se				0.209*/0.784**	0.200*/0.778**	0.242**/0.646**	0.121/0.688**
Total Length					0.962**	0.577**	0.790**
Weight						0.606**	0.830**
SMI							0.444**

* Significant correlation at 0.05 level.
** Significant correlation at 0.01 level.

concentrations to those found in this study (only four samples were analysed from the river Urumea). Higher Pb concentrations have been reported in eel muscles from La Camargue (France), a Biosphere Reserve (Oliveira Ribeiro et al., 2005) and Köyceğiz Lak (Turkey) (Genç and Yilmaz, 2017), the latter an area under intense pressure from tourism-related activities. Muscle Pb concentrations in other fish species from the Mar Menor have been analysed (De León et al., 1982; Benedicto et al., 2007; Marín-Guirao et al., 2008; Sánchez-Bassols, 2008), most of which were higher than those found in the present study.

A significant but low negative relationship between muscle Pb concentrations and biometric data was detected; on the other hand, a positive relationship between age and liver concentrations was noted (Table 3). The relationship between total length and trace element concentrations (existence or type of relationship) has been reported to vary according to tissue, trace element and species (e.g. Al-Yousuf et al., 2000; Ayas and Köşker, 2018; Canli and Atli, 2003; Szefer et al., 2003; Yeltekin and Oğuz, 2018; Yi and Zhang, 2012). However, specifically, the decrease in Pb concentrations in muscles with an increase in fish total length and/or weight has been identified in species such as *Mola mola* (Baptista et al., 2019) and *Atherina hepsetus* (Canli and Atli, 2003). In contrast to our results, Farkas et al. (2000) reported an increase in Pb concentrations in livers with eel weight. According Baptista et al. (2019), several biological and ecological factors, such as growth rate or an ontogenetic shift in dietary preferences could explain the lack of a general pattern in the elemental body dynamics of trace elements. These latter authors report that whole body growth could be accompanied by an increase in liver volume, which would explain the non-observed changes in liver metal concentrations in *M. mola* in relation to total length. In our study, although a close correlation between body and hepatic weight was detected ($r = 0.947$), no relationship between liver Pb concentrations and total length nor total weight in eels from Mar Menor lagoon could be identified.

Concentrations of Cd in muscles were intermediate compared to previously reported data (Table 4); liver concentrations, however, were lower than those reported in the majority of studies. Cd concentrations in the muscles of eels from the Mar Menor were found to be lower than those previously reported in eels and other species from this site (De León et al., 1982; Benedicto et al., 2007) but similar to levels found by other studies (Marín-Guirao et al., 2008; Sánchez-Bassols, 2008). Cd as a pollutant is included on the priority list of hazardous substances (ATSDR, 2014; US EPA, 2014). Although Cd emissions into the environment were high for many years, they seem to have decreased since 1980 due to increased regulation and the implementation of efficient at-source capture and recycling techniques (Cullen and Maldonado, 2013). According to Marín-Guirao et al. (2007), only 14% of Cd in the Mar Menor penetrates in particulate form due to 'salinity shock', compared to 98% of Pb. Although the dissolved Cd is rapidly eliminated from the water column at neutral pH and high salinity levels (Marín-Guirao et al., 2007), particulate forms (Pb) may be transported further and may accumulate in high concentrations in the sediments over a wider area. This could explain the differences between the amounts of these metals found in eel tissues in our study.

No correlation was observed between hepatic or muscular Cd concentrations and biometric data in eels from the Mar Menor. To date, positive (Esteve et al., 2012; Farkas et al., 2000) and negative (Amilhat et al., 2014; Noël et al., 2013; Ureña et al., 2007) correlations, as well as no correlation (Barak and Mason, 1990; Batty, 1996; Genç and Yilmaz, 2017; Maes et al., 2005; Rudovica and Bartkevics, 2015; Tabouret et al., 2011), have been reported in eels from other ecosystems without following any specific pattern.

On the other hand, a moderate relationship between Cd and Pb concentrations in eel liver tissue was found (Table 3). A common source of both elements could be inferred since these elements were the main metals produced by the mining activities that once took place near the lagoon (De León et al., 1982; Marín-Guirao et al., 2005).

Hg pollution is a very important problem in marine environments (Gworek et al., 2016). The results of this study showed lower Hg concentrations than those reported in many Mediterranean fish species (Llull et al., 2017) and lower than those previously reported in eels from a number of European locations (Table 4), but similar concentrations to those reported by Usero et al. (2003) in eels from the Atlantic coast of Spain. The visceral distribution observed in this study agreed with that previously reported in fish, that is, higher in muscles than in livers (Golovanova, 2008).

Hg biomagnification is known to occur in marine and freshwater food chain webs (Campbell et al., 2003). A moderately close relationship between muscle Hg concentrations and both age and biometric data was observed, being this correlation lower in the liver (Table 3). According Ourgaud et al. (2018), Hg accumulation depends on several factors such as its levels in the trophic chain, and fish age and total length. Eels are predators and scavengers, and their diets change with total length and age; throughout their lifetimes they feed on a wide range of organisms, from insect larvae, amphipods and isopods, to fish (Arias and Drake, 1985), although the trophic divergence associated with anatomic dimorphism could also affect the accumulation of pollutants (De Meyer et al., 2018). However, our results (relationship between total length and Hg hepatic concentrations) agree with those reported by Esteve et al. (2012) in eels from La Albufera (Valencia, Spain).

On the other hand, the relationship between Hg and Cd in livers was low, while there was no correlation with Pb in either livers or muscles (Table 3). Contradictory results regarding the relationship between salinity and mercury bioaccumulation in biota exist in the literature (Wang and Wang, 2010; Dutton and Fisher, 2011; Fry and Chumchal, 2012; Reinhart et al., 2018). The high salinity of the studied ecosystem could explain our data.

Bervoets and Blust (2003) suggest that individual bioaccumulation levels could provide a good estimate of the environmental quality of the sediment and a measure of fish health condition. In fact, the IMBI has been used by several authors of studies of European eels, who have reported contradictory results. Maes et al. (2005) reported a negative correlation between the IMBI (muscle) and condition index, while Esteve et al. (2012) observed an increase in IMBI (liver) with total

Table 4
Pb, Cd, Hg and Se concentrations in European eel tissue from different countries and locations.

Location	n	Biometric data			Age	Gender
		Total length (cm)	Weight (g)	K		
Camargue (F)	15/15	17 to 57+	*ns*	*ns*	1–6	*ns*
River Turia (S)	14	*ns*	*ns*	*ns*	*ns*	*ns*
River Gediz (T)	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
North-west Mediterranean coast (F)	4 to 27	35.0 to 38.8$^{\text{Ħ}}$	67.4 to 96.2$^{\text{Ħ}}$	0.16 to 0.17	2.6 to 3.9$^{\text{Ħ}}$	Male
Atlantic coasts (S)	1*	*ns*	*ns*	*ns*	*ns*	*ns*
Rivers Ferrerias and Raíces (S)	58	*ns*	*ns*	*ns*	*ns*	*ns*
Camargue (F)	26 to 27	39.5 to 66.0$^{\text{Ħ}}$	116.35 to 558.36$^{\text{Ħ}}$	*ns*	*ns*	*ns*
River Ferrerias (S)	20	*ns*	*ns*	*ns*	*ns*	*ns*
Albufera Lake (S)	49	41.633 ± 12.428	173.177 ± 173.876	0.106 ± 0.021	*ns*	Male and female
Latvia lakes (L)	5 to 11	55 to 95$^{\text{Ħ}}$	519 to 1580$^{\text{Ħ}}$	0.15 to 0.18	*ns*	Female
Albufera lake (S)	12/12	44.3/38.5	133/121	0.15/0.21	*ns*	*ns*
Flanders (B)	20 to 33	*ns*	*ns*	*ns*	*ns*	*ns*
Ría de Aveiro (P)	3	*ns*	*ns*	*ns*	*ns*	*ns*
Lesina Lagoon (I)	104	30.1–41.5	55.0–131.5	*ns*	*ns*	*ns*
East Anglia (E)	2 to 113	36.6 to 67.0$^{\text{Ħ}}$	*ns*	*ns*	*ns*	*ns*
Ría de Aveiro (P)	40	*ns*	*ns*	*ns*	*ns*	*ns*
Flanders (B)	1410–2809	41.79 ± 9.28	153.46 ± 152.69	*ns*	*ns*	*ns*
Rivers in France (F)	53	58.1 ± 13.9	441 ± 264	0.22 ± 0.17	*ns*	*ns*
River Urumea (S)	3/4	*ns*	*ns*	*ns*	*ns*	*ns*
Koryaany Reservoir (CR)	10	61.6	343	*ns*	*ns*	*ns*
Adour Estuary (F)	20 to 51	31.8 to 43$^{\text{Ħ}}$	70.4 to 153$^{\text{Ħ}}$	*ns*	7 to 8$^{\text{Ħ}}$	*ns*
Adour Estuary (F)	7 to 15	43.2 ± 12.2	180.1 ± 163.8	*ns*	*ns*	*ns*
Gironde (F)	$10^{L}/20^{M}$	62.1 ± 2.2	527 ± 46	*ns*	7 to 15	Female
River Neretva (C)	12	*ns*	*ns*	*ns*	Older	*ns*
Lake Svitava (B&H)	10	*ns*	*ns*	*ns*	5 to 7	*ns*
River Tiber (I)	4/4	*ns*	*ns*	*ns*	4–5	*ns*
North Luxembourg	2 to 9	*ns*	721 to 976$^{\text{Ħ}}$	*ns*	*ns*	*ns*
East Anglia (E)	51/51	40.1/52.5	127.7/310.5	*ns*	12.8/13.6	Male and female
Estuaries and coastal lagoons (P)	4 to 26	39.6 ± 7.7	116 ± 64	*ns*	*ns*	*ns*
Köyceğiz Lake (T)	76	48.06 ± 12.31	192.24 ± 106.51	0.20 ± 0.16	*ns*	*ns*
Mar Menor (S) Our study	150	54.6 ± 0.9$^{\text{¥}}$	256.9 ± 17.0$^{\text{¥}}$	0.161 ± 0.038	1–10	Male and female

(*continued on next page*)

Table 4 (*continued*)

Location	Pb		Cd		Hg		Se		Ref
	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	
Camargue (F)	*na*	0.04[£]/0.04[£]	*na*	0.02[¥]/0.01[¥]	*na*	0.22[¥]/0.23[¥]	*na*	*na*	1
River Turia (S)	0.1018	*na*	0.0049	*na*	*na*	*na*	*na*	*na*	2
River Gediz (T)	0.0032 ± 0.003	0.9610 ± 0.002	1.2067 ± 1.278	2.071 ± 0.0060	*na*	*na*	*na*	*na*	3
North-west Mediterranean coast (F)	*na*	*na*	0.005 to 0.067[H]	0.18 to 3.00[H]	*na*	*na*	*na*	*na*	4
Atlantic coasts (S)	0.03 to 0.09[H]	0.40 to 0.60[H]	0.015 to 0.050[H]	0.12 to 0.48[H]	0.010 to 0.023[H]	0.011 to 0.023[H]	*na*	*na*	5
Rivers Ferrerias and Raíces (S)	0.001 to 0.108[H]	0.14 to 1.925[H]	0.006 to 0.067[H]	0.462 to 1.416[H]	0.155 to 0.533[H]	0.168 to 0.485[H]	*na*	*na*	6
Camargue (F)	0.21 to 0.79[H,Ш]	*nd* to 0.64[H,Ш]	*nd*	0.13 to 0.44[H,Ш]	0.16 to 0.61[H,Ш]	0.32 to 0.76[H,Ш]	*na*	*na*	7
River Ferrerias (S)	*na*	0.54 to 1.42[H]	*na*	1.49 to 2.62[H]	*na*	*na*	*na*	*na*	8
Albufera Lake (S)	*na*	0.119 ± 0.094	*na*	0.064 ± 0.066	*na*	0.104 ± 0.100	*na*	4.571 ± 2.909	9
Latvia lakes (L)	0.0192 to 0.047[H]	*na*	0.0051 to 0.011[H]	*na*	0.13 to 0.36[H]	*na*	0.32 to 0.46[H]	*na*	10
Albufera lake (S)	0.02–0.30	0.02–0.44	< 0.02	0.03–3.80	0.02–0.24	0.07–0.33	*na*	*na*	11
Flanders (B)	0.038 to 0.053[H]	*na*	0.002 to 0.019[H]	*na*	0.094 to 0.174[H]	*na*	0.329 to 1.023[H]	*na*	12
Ría de Aveiro (P)	0.044 to 0.078[H]	*na*	0.009 to 0.042[H]	*na*	*na*	*na*	*na*	*na*	13
Lesina Lagoon (I)	*na*	*na*	0.03 ± 0.01	*na*	0.18 ± 0.04	*na*	*na*	*na*	14
East Anglia (E)	0.03 to 0.08[H]	0.26 to 0.80[H]	0.02 to 0.08[¤]	0.06 to 0.47[H]	0.13 to 0.39[H]	0.07 to 0.59[H]	*na*	*na*	15
Ría de Aveiro (P)	0.023	0.188	0.003	0.058	0.138	0.084	*na*	*na*	16
Flanders (B)	0.081 ± 0.172	*na*	0.016 ± 0.062	*na*	0.117 ± 0.099	*na*	0.754 ± 0.500	*na*	17
Rivers in France (F)	0.024 ± 0.031	*na*	0.011 ± 0.017	*na*	0.199 ± 0.122	*na*	*na*	*na*	18
River Urumea (S)	< 3/4.5[Ш]	2.3/4.9[Ш]	0.3/ < 0.3[Ш]	0.9/9.1[Ш]	*na*	*na*	*na*	*na*	19
Koryaany Reservoir (CR)	*na*	*na*	*na*	*na*	0.162–0.827	0.175–1.430	*na*	*na*	20
Adour Estuary (F)	0.004 to 0.014[H]	*na*	0.001 to 0.004[H]	*na*	0.179 to 0.307[H]	*na*	*na*	*na*	21
Adour Estuary (F)	*na*	*na*	*na*	*na*	0.18 to 0.31[H]	*na*	*na*	*na*	22
Gironde (F)	*na*	*na*	< 0.02	1.5 ± 0.2	0.17 ± 0.02	0.36 ± 0.06	*na*	*na*	23
River Neretva (C)	0.112 ± 0.028	0.128 ± 0.012	0.027 ± 0.007	0.139 ± 0.012	0.114 ± 0.009	0.081 ± 0.03	*na*	*na*	24
Lake Svitava (B&H)	0.123 ± 0.004	0.21 ± 0.006	0.02 ± 0.003	0.274 ± 0.007	0.159 ± 0.004	0.072 ± 0.004	*na*	*na*	25
River Tiber (I)	0.065/0.090[Ш]	*na*	0.00047/0.00086[Ш]	*na*	0.23/0.24[Ш]	*na*	*na*	*na*	26
North Luxembourg	0.034 to 0.050	*na*	0.021 to 0.064[H]	*na*	0.159 to 0.317[H]	*na*	*na*	*na*	27
East Anglia (E)	*na*	*na*	*na*	*na*	0.104/0.255	*na*	*na*	*na*	28
Estuaries and coastal lagoons (P)	*na*	0.15 to 0.87[HШ]	*na*	nd to 0.667[HШ]	*na*	0.06 to 1.16[HШ]	*na*	*na*	29
Köyceğiz Lake (T)	1.07 ± 0.11[Ш]	1.30 ± 0.14[Ш]	0.22 ± 0.03[Ш]	0.24 ± 0.02[Ш]	0.14 ± 0.03[Ш]	0.16 ± 0.04[Ш]	*na*	*na*	30
Mar Menor (S)	0.093 ± 0.016[¥]	1.500 ± 0.100[¥]	0.002 ± 0.001[¥]	0.039 ± 0.005[¥]	0.008 ± 0.001[¥]	0.006 ± 0.001[¥]	0.303 ± 0.019[¥]	4.999 ± 0.339[¥]	–
Our study	(0.299 ± 0.051[¥§])	(4.402 ± 0.293[¥§])	(0.006 ± 0.003[¥§])	(0.114 ± 0.015[¥§])	(0.026 ± 0.003[¥§])	(0.018 ± 0.003[¥§])	(0.974 ± 0.061[¥§])	(14.669 ± 0.995[¥§])	

The data are presented as mean ± SD, except [£]median, [¥]geometric mean and [H]range of means. For elements, the concentrations are presented as μg g^{-1}, wet weight, except [Ш]dry weight. K: Fulton condition index (100*weight/length^3). [L]liver, [M]muscle. *One pool (10 fish); *nd* = not detected; *na* = not analysed; *ns* = data not shown. [§]For dry weight estimated (our study).

Country: F = France; S = Spain; T = Turkey; L = Latvia; P = Portugal; B = Belgium; I = Italy; E = England; CR = Czech Republic; C = Croatia; B&H = Bosnia and Herzegovina; H = Hungary.

References: (1) Batty, 1996; (2) Bordajandi et al., 2003; (3) Yildiz et al., 2010; (4) Amilhat et al., 2014; (5) Usero et al., 2003; (6) Linde et al., 2004; (7) Oliveira Ribeiro et al., 2005; (8) Linde et al., 2001; (9) Esteve et al., 2012; (10) Rudovica and Bartkevics, 2015; (11) Ureña et al., 2007; (12) Maes et al., 2005; (13) Pérez-Cid et al., 2001; (14) Storelli et al., 2007; (15) Barak and Mason, 1990; (16) Eira et al., 2009; (17) Maes et al., 2008; (18) Noël et al., 2013; (19) Sánchez et al., 1998; (20) Palikova and Baruš, 2003; (21) Tabouret et al., 2011; (22) Arleny et al., 2007; (23) Durrieu et al., 2005; (24) Has-Schön et al., 2006; (25) Has-Schön et al., 2008; (26) Mancini et al., 2005; (27) Boscher et al., 2010; (28) Edwards et al., 1999; (29) Neto et al., 2011; (30) Genç and Yilmaz, 2017.

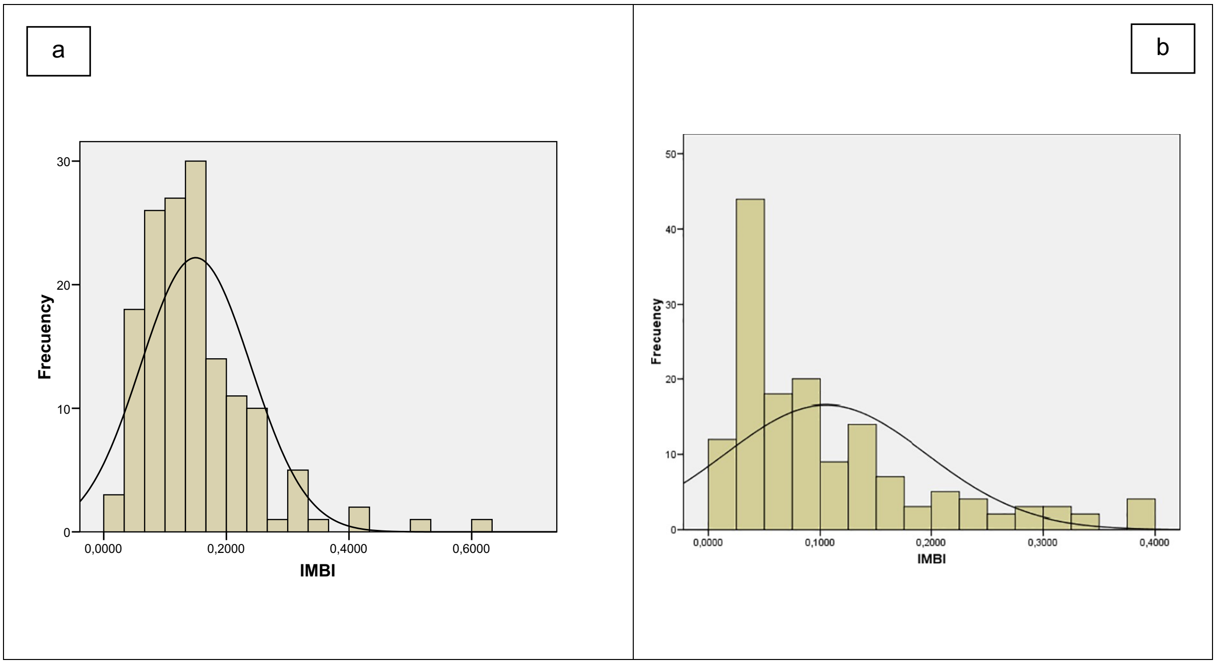

Fig. 1. Level of heavy metal pollution (IMBI) in *Anguilla anguilla* from Mar Menor lagoon: a) Liver; b) Muscle.

length. On the other hand, Genç and Yilmaz (2017) reported higher IMBI values in *A. anguilla* livers than in other species. In the present study, no relationship was found between the IMBI and condition index nor between IMBI and total length, although a low correlation in the IMBI (liver)/age was detected. The bimodal distribution of the IMBI values reported by Maes et al. (2005) was not detected (Fig. 1).

According ICES (2015), data from contaminant analysis could be extremely useful for international assessment and inclusion in an international database for food safety. In the Mar Menor lagoon, eels are fished for human consumption. Although these are not consumed locally, eels are marketed on other Spanish areas. In terms of human safety, the maximum safe level for Pb in fish flesh according to European legislation is 0.3 μg g^{-1} ww (Commission Regulation (EU) 2015/1005). In this study, 20 specimens (13.3%) exceeded this concentration. The eel weight without viscera is equivalent to 89.3–94.7% of the total eel weight, so the consumption of one of these specimens supposes an intake of until 575 μg of Pb. Regarding EFSA (2010), Pb is related with cardiovascular effects, nephrotoxicity and developmental neurotoxicity, so Pb in the Mar Menor could be a risk to human safety by consumption of the eels. On the other hand, the protective role of Se against Pb is well known (Rastogi et al., 1976), but in the present study no correlation between both elements in muscle was detected (Table 3). The maximum permitted Cd level in eel flesh is 0.050 μg g^{-1} (ww) (Commission Regulation (EU) N° 488/2014). In the Mar Menor in this study, the maximum Cd concentration in eel flesh was 0.047 μg g^{-1}; indeed, only 54 fish (36.0%) had Cd above the detection limit (0.001 μg g^{-1}). Finally, muscular Hg concentrations were low (nd-0.177 μg g^{-1}, ww) in all sampled specimens and were below the maximum permitted by the legislation (Commission Regulation (EU) N° 629/2008). Thus, a priori, there is no food risk of Cd and Hg contamination when consuming eels from this place, but the risk by Pb should be taken into account in the species management plans.

The natural antagonistic role of Se:Hg is well known (Ralston and Raymond, 2010). In eels from the Mar Menor, tissue concentrations of Se were higher than those of Cd, Pb and Hg (Table 2). In three of the four previously published works reporting Se concentrations in European eels (Maes et al., 2005; Esteve et al., 2012; Rudovica and Bartkevics, 2015), hepatic and muscular Se concentrations were similar to those found in this study. Se concentrations in livers were closely correlated to both biometric data and age (Table 3); however, the correlation between Se and Hg concentrations was low. The geometric mean of the ratio Se:Hg was higher than that reported recently in other fish species (Johnson et al., 2018; Melgar et al., 2019), even though nine of the specimens from the Mar Menor had ratios lower than 1:1 (which are regarded as insufficient to reduce the absorption of Hg in humans, Ganther et al., 1972). On the other hand, an HBV_{Se} value above zero helps protect human health (Ralston et al., 2016). In the eels sampled from the Mar Menor, 141 specimens had a positive HBV_{Se} value whose geometric median (5.776 ± 0.219) was lower than that reported in tuna (Kaneko and Ralston, 2007; Ralston et al., 2016; Ruelas-Inzunza et al., 2018; Melgar et al., 2019) but higher than for sharks, swordfish, pilot whales (Kaneko and Ralston, 2007) and freshwater fish such as bluegill, crappie and largemouth bass (Johnson et al., 2018). The nine specimens with negative HBV_{Se} values were all eels with Se:Hg ratios < 1:1. Nevertheless, five of these nine eels were specimens with Se and Hg concentrations below the DL and so they cannot be regarded as specimens with negative protection against Hg.

In conclusion, Pb concentrations in eels from the Mar Menor were low compared to eels captured several decades ago in the same ecosystem but higher than those reported from other parts of the world. Although Cd and Hg concentrations in the analysed tissues were low, the IMBI values highlight the need for further monitoring of heavy metals in European eels from the Mar Menor. The heavy metal of most concern for human food safety is Pb; the Se content in the eels from the Mar Menor confirms this element's ability to sequester Hg.

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CRediT authorship contribution statement

Diego Romero: Conceptualization, Data curation, Formal analysis, Writing - original draft, Writing - review & editing. **Elena Barcala:**Data curation, Formal analysis, Investigation, Writing - review & editing. **Emilio María-Dolores:**Data curation, Writing -

review & editing. **Pilar Muñoz:**Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Validation, Writing - review & editing.

Declaration of competing interest

None.

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