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Cadmium and lead in European eels from the river Segura (southeast Iberian Peninsula)

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ABSTRACT

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European eel (*Anguilla anguilla*) populations have suffered a sharp range retraction (90–99%) over the past 40 years. However, in recent years this fish has been detected again in the river Segura (Spain), in both the main river and in channels such as the irrigation canals and ditches that drain the Huerta de Murcia. Even though the water quality of these habitats has improved, there are no data on heavy metal concentrations in eels from these areas. Therefore, in this study Pb and Cd concentrations were measured in the blood, liver and muscles of eels from three different habitats: the river (n=23), canals (n=13) and drainage ditches (n=9). Lead was found in more specimens than Cd, while both Pb and Cd concentrations were low, being similar or even lower than in non-polluted zones. Although significant statistical differences were found in liver concentrations between river and canal eels, there was no overall pattern to the findings for these two elements in eel body dynamics.

KEY WORDS: blood, European eel, heavy metals, liver, muscle, river Segura.

RESUMEN

Cadmio y plomo en anguila europea del río Segura (Sureste de la Península Ibérica).

*Las poblaciones de anguila europea (*Anguilla anguilla*) han sufrido una fuerte disminución (90-99%) en los últimos 40 años. Sin embargo, en los últimos años se ha vuelto a detectar en el río Segura (España), tanto en el río principal como en cauces, canales de riego y acequias que drenan la Huerta de Murcia. Aunque la calidad del agua de estos hábitats ha mejorado, no hay datos sobre las concentraciones de metales pesados en las anguilas de estas zonas. Por tanto, en este estudio se midieron las concentraciones de plomo (Pb) y cadmio (Cd) en la sangre, el hígado y los músculos de anguilas de tres hábitats diferentes: río (n=23), canales (n=13) y acequias (n=9). El Pb se encontró en más ejemplares que el Cd, mientras que tanto las concentraciones de Pb como de Cd fueron bajas, siendo similares o incluso inferiores a las de las zonas no contaminadas. Aunque se encontraron diferencias estadísticas significativas en las concentraciones hepáticas entre las anguilas de río y de canal, no hubo un patrón general en los hallazgos para estos dos elementos en la dinámica corporal de la anguila.*

PALABRAS CLAVE: *anguila europea, hígado, metales pesados, músculo, río Segura.*

INTRODUCTION

The European eel (*Anguilla anguilla*) is a catadromous fish with a unique life cycle that involves an extraordinary migration of over 5000 km. Eels spawn in the Sargasso Sea (northern Atlantic Ocean) and their larvae are then transported by ocean currents to the European continent when they undergo a metamorphosis and develop into the so-called 'glass eels'. They migrate up coastal streams and estuaries, first reaching the somatic growth stage, characterized by its yellow pigmentation (the 'yellow stage'), before undergoing a final metamorphosis and becoming fully grown 'silver eels'. At this point they are ready to conduct their reproductive migration back to the Sargasso Sea as they are now physiologically adapted for saltwater (Pannetier et al., 2016). Nevertheless, all these stages are under threat since European eel populations have seriously declined over the past 40 years (ICES, 2022) and this fish is now classified as Critically Endangered on the IUCN threatened species Red List (Pike et al., 2020). Possible causes include changes in climate and oceanic currents, overfishing, the presence of barriers to migration (e.g. turbines and dykes), pathogens (especially by *Anguillicola crassus*, herpesvirus and rhabdovirus) and a fall in water quality due mainly to the presence of pollutants (ICES, 2007; Geeraerts & Belpaire, 2010).

Given their life cycle, European eels are especially vulnerable to a range of contaminants and pollutants (Geeraerts & Belpaire, 2010). In general, the accumulation of pollutants can have a delayed impact since contaminants may be remobilized during the eel's reproductive migration and interfere with their nervous, locomotive, immune and endocrine systems (Sühring et al., 2005; Geeraerts & Belpaire, 2010; Freese et al., 2016), gonad maturation (Pierron et al., 2008, 2009; Baillon et al., 2015), locomotion (Van Ginneken et al., 2009), and cause toxicity events in embryos (Palstra et al., 2006). During the yellow stage, they are voracious predators and live in close proximity to the sediments. Thus, many contaminants including some heavy metals can potentially accumulate in their tissues in very high concentrations, most notably in the liver and muscles, depending on the contamination profile of the area they inhabit (Belpaire & Goemans, 2007; Geeraerts & Belpaire, 2010). Previous studies have suggested that eels accumulate larger quantities of metals than other fish linked to their high body lipid content (Barak & Mason, 1990; Collings et al., 1996; Pérez Cid et al., 2001; Usero et al., 2004; Durrieu et al., 2005).

The river Segura (SE Spain) is characterized by both frequent drought and sudden intense rainfall and flooding caused by the region's great climatic seasonality. By the 1990s, the river Segura had become one of the most polluted rivers in Europe due to the density of the local human popu-

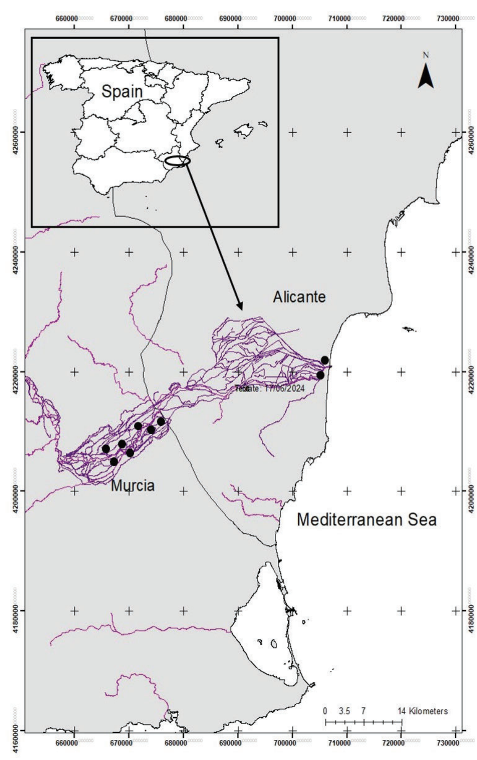

Figure 1. Location of the sampling areas around the river Segura and its network of irrigation canals. *Localización de las zonas de muestreo en el entono del río Segura y sus canales de irrigación.*

lation and the food processing industry (Ródenas & Albacete, 2014). Nevertheless, the quality of this river's waters has improved in recent years and native species such as the European eel have returned (Verdiell Cubedo & Parrondo Celdrán, 2018a, 2018b). Cadmium and lead have been reported in agricultural soils in the area and in crabs from this river (Mico Llopis et al., 2006; Salvat-Leal et al., 2020). However, the river waters broad tracts of agricultural land (Huerta de Murcia), within which there are numerous canals (irrigation channels) and drainage ditches inhabited by several fish species including the European eel. Therefore, the concentrations of lead and cadmium in eels could vary among the different habitats, so it is important to evaluate the concentrations of these heavy metals in eels and so provide further data on their future spawner quality for submission to the European Eel Quality Database (ICES, 2007), a key tool in eel management. Thus, we investigated the levels of Pb and Cd in the blood, liver and muscles of European eels from three different habitats (river, canals and ditches), within the bounds of the river Segura system.

MATERIAL AND METHODS

Sample collection

A total of 45 eels were caught in the river Segura and the network of channels (Fig. 1) using traditional fishing gear (fyke nets) in December 2017–April 2019. All fish were handled in accordance with EU regulations concerning the protection of experimental animals (European Union, 2010). All efforts were made to minimize animal handling and stress. The licence for the handling of the specimens was granted by the Directorate General for Natural Environment of the Autonomous Community of the Region of Murcia (Resolution auf/2018/0010).

After being anesthetized and then euthanized with a lethal dose of tricaine methane sulfonate (MS222) at 100 mg/L, the total length to the nearest millimetre and the total weight to the nearest gram were measured. Blood samples were obtained with an insulin syringe; whilst a portion (0.2–0.5 g) of liver and muscle (4–5 cm from behind the anal cavity) was removed. All the samples were stored at −20 °C until processed.

Metal analysis

Blood and tissue samples were pretreated as per Romero et al. (2020): 0.1–0.5 g of sample was submitted to acid digestion using trace mineral grade HNO_3 (69%) and H_2O_2 (33%) in a microwave digestion system (UltraClave-Microwave Milestone) at 220°C for 20 min. Samples were diluted with 10 mL of double deionized water (Milli-Q) and Cd and Pb concentrations were determined using an Inductively Coupled Plasma Optical Emission Spectrophotometer (ICP-OES, ICAP 6500 Duo Thermo Scientific, with One Fast System). All concentrations are expressed as microgram per gram wet weight (ww). Detection limits (DL) were established at 0.001 µg/g (ww). The certified reference material was ERM-CE278k.

Data analysis

SPSS version 24 software (IBM® SPSS® Statistics, www.ibm.com) was used to analyze the data. Minimums and maximums were derived for biometric data and geometric means and standard errors for metal concentrations. In the chemical analysis, data below the DL were expressed as half of this figure (0.0005 µg/g) following the middle-bound approach (GEMs/Food-WHO, 1995; Sand et al., 2013; Ostry et al., 2015). The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to evaluate the data distribution; the Mann-Whitney U, Kruskal-Wallis and Spearman tests were used as nonparametric statistical methods. The significance level for all tests was set at 0.05.

Table 1. Biometric data of European eels from river Segura. *Datos biométricos de las anguilas del río Segura*.

Habitat	Weight (g)	Total length (cm)
River (n=23)	46.9–796.0	30.3–710.0
Canal (n=13)	120.0–789.0	40.4–736.0
Drainage ditches (n=9)	77.0–516.0	49.2–625.0
Whole (45)	46.9–796.0	30.3–736.0

RESULTS

The biometric data of the sampled European eels are shown in Table 1. The detected concentrations of Pb and Cd and the percentage of the samples with values above the instrumental DL are shown in Table 2.

The tissue with highest metal concentrations was the liver, followed by blood and then by muscle. For blood and tissues, Pb concentrations were higher than those found for Cd. For blood and muscle, no significant statistical differences were found between the three habitats, whilst for liver, differences were found between the Cd and Pb concentrations from the river and canal eels, with specimens from the river having higher concentrations of both metals.

There was a negative relationship for the whole

Table 2. Cadmium and lead concentrations in blood, liver and muscle of European eels from the river Segura and percentage of the samples above the detection limit (DL). *Concentraciones de cadmio y plomo en sangre, hígado y músculo de anguila europea del río Segura y porcentaje de muestras por encima del límite de detección (LD).*

		n	%>DL (Cd/Pb)	Cd	Pb
Whole population	Blood	44	50/100	0.002 ± 0.010 (nd-0.428)	0.074 ± 0.034 (0.015-1.208)
	Liver	40	95/100	0.025 ± 0.023 (nd-0.922)	0.164 ± 0.176 (0.035-7.166)
	Muscle	40	10/85	0.001 ± 0.001 (nd-0.039)	0.012 ± 0.004 (nd-0.102)
River	Blood	22	54.5/100	0.002 ± 0.007 (nd-0.157)	0.089 ± 0.038 (0.015-0.711)
	Liver	22	100/100	0.037 ± 0.040 (0.008-0.922)	0.216 ± 0.317 (0.035-7.166)
	Muscle	23	0/91.3	nd	0.014 ± 0.005 (nd-0.091)
Canal	Blood	13	38.5/100	0.002 ± 0.033 (nd-0.428)	0.068 ± 0.096 (0.015-1.208)
	Liver	12	83.3/100	0.010 ± 0.004 (nd-0.051)	0.105 ± 0.014 (0.061-0.213)
	Muscle	11	18.2/72.7	0.001 ± 0.004 (nd-0.039)	0.007 ± 0.008 (nd-0.074)
Drainage ditches	Blood	9	55.6/100	0.003 ± 0.009 (nd-0.081)	0.055 ± 0.014 (0.019-0.135)
	Liver	6	100/100	0.035 ± 0.027 (0.007-0.171)	0.149 ± 0.021 (0.072-0.210)
	Muscle	6	33.3/83.3	0.001 ± 0.001 (nd-0.006)	0.020 ± 0.014 (nd-0.102)

nd = not detected; DL = detection limit; data = µg/g, wet weight, geometric mean ± standard error, minimum and maximum.

population between eel weight and Pb concentrations in blood and liver (r=-0.349 and r=-0.416, respectively, $p<0.05$). However, this relationship was only found in the blood of eels from the river (r=-0.583, $p<0.01$). A positive relationship was found between Cd and Pb in blood and liver in the whole population (r=0.805 and r=0.537, respectively, $p<0.01$) and in eels from the river (r=0.783 and r=0.577, respectively, $p<0.01$). There was a negative relationship between the total length of eels and Pb concentrations in blood of the whole population and in eels from the river (r=-0.546 and r=-0.697, respectively, $p<0.01$). In specimens from the river there were other negative correlations: r=-0.491 ($p<0.05$) for the total length/Cd concentrations in liver, and r=-0.755 and r=-0.846 ($p<0.01$) for weight and total length with Cd concentrations in the blood.

Finally, additional relationships were detected (i) between eel weight and Cd concentrations in the liver of specimens from the canals and drainage ditches (r=0.758 and r=0.886, respectively, $p<0.05$); (ii) between biometric measures (weight and total length) and Cd concentrations in blood (r=0.900, $p<0.05$) and between both metals in the blood of specimens from the drainage ditches (r=0.900, $p<0.05$); and (iii) between total length and Pb concentrations in the muscles of eels from drainage ditches (r=0.900, $p<0.05$). In each of these cases, the numbers of eels used for the correlations was 5–10.

DISCUSSION

Although the European eel was once an abundant species in the river Segura and its irrigation network (Mas, 1986), in the final decades of the twentieth century this fish all but disappeared from these habitats (Torralva et al., 2005) due, among other causes, to pollution in this river basin. Nevertheless, improved water quality has brought a population of the European eel back to this river and its irrigation channels (Verdiell Cubedo & Parrondo Celdrán, 2018a, 2018b), although to the best of our knowledge heavy metal concentration in eels from this area remained unknown.

Lead and Cd are two heavy metals that are present in all aquatic ecosystems, so their presence in eel blood and tissues of eels was to be expected. However, differences between these two metals were found. Firstly, Pb was present in 95.2% of the samples but Cd in only 51.6% and, secondly, Pb concentrations were higher than Cd concentrations. These results agree with several other author's findings in eels from other areas (see Romero et al., 2020). Nevertheless, Cd and Pb levels in liver and muscles were lower than those detected by other authors in other Spanish rivers, coasts, and lagoons (Table 3), although, somewhat surprisingly, Cd concentrations were similar to those detected by a previous study of eels from the Mar Menor lagoon (Romero et al., 2020). The hepatic concentrations of both metals were higher in eels from the river than from the canals ($p<0.05$). The river is where eels from the Sargasso Sea arrive first and, as they grow, they migrate into the irrigation canals and then into the drainage ditches. This latter habitat drains water from the fields and in doing so may be exposed to local metal contamination, which in turn could explain the differences recorded in hepatic bioaccumulation.

Lead levels were higher than Cd levels in blood and tissues, which agrees with the majority of previous studies reviewed (Barak & Mason, 1990; Sánchez et al., 1998; Bordajandi et al., 2003; Maes et al., 2005, 2008; Mancini et al., 2005; Has-Schön et al., 2006; Noël et al., 2013), but not with Linde et al. (2001, 2004), Ureña et al. (2007), Boscher et al. (2010) and Yildiz et al. (2010). On the other hand, element levels in livers were higher than those found in blood and muscles, which is consistent with data reported by several other authors (Eira et al., 2009; Yildiz et al., 2010; Genç & Yilmaz, 2017; Romero et al., 2020), which could be due to the physiological response of the liver, being the body's main detoxication organ.

Blood has a great importance in ecotoxicology studies since it provides information on recent exposure to environmental pollutants such as heavy metals. Nevertheless, few studies have measured Pb and Cd concentrations in eel blood (Zimmermann et al., 1999; Ureña et al., 2007). Lead concentrations in the blood of eels from the river Segura and its canals and drainage ditches (no statistical differences between them) were intermediate between those reported from farmed

Table 3. Lead and Cd concentrations in European eels from Spain. *Concentraciones de plomo y cadmio en anguila europea de España.*

Reference	n-specimens (n-places)	Location	Cd	Pb				
			Blood	Muscle	Liver	Blood	Muscle	Liver
Linde et al., 1996	12 (3)	Rivers Piles and Pigueña	*na*	*na*	*nd*-2.45$^{ш\,\delta}$	*na*	*na*	*nd*-5.69$^{ш\,\delta}$
Sánchez et al., 1998	7 (2)	River Urrumea	*na*	<0.3/0.3^{ш}	9.1/0.9^{ш}	*na*	4.5/<3^{ш}	4.9/2.3^{ш}
Linde et al., 2001	20 (2)	River Ferreiras	*na*	*na*	1.49 to 2.62^{Ħ}	*na*	*na*	0.54 to 1.42^{Ħ}
Linde et al., 2004	58 (4)	Rivers Ferreiras and Raices	*na*	0.006 to 0.067^{Ħ}	0.462 to 1.416^{Ħ}	*na*	0.001 to 0.108^{Ħ}	0.14 to 1.925^{Ħ}
Usero et al., 2003	4 (4)	Odiel Estuary and Bay of Cádiz (Saltmarsh)	*na*	0.015 to 0.050^{Ħ}	0.12 to 0.48^{Ħ}	*na*	0.03 to 0.09^{Ħ}	0.40 to 0.60^{Ħ}
Ureña et al., 2007	12 (2)	Valencia Albufera Lake	*na*	<0.02$^{\delta}$	0.03-3.80$^{\delta}$	*na*	0.02-0.30$^{\delta}$	0.02-0.44$^{\delta}$
Esteve et al., 2012	49 (1)	Valencia Albufera Lake	*na*	*na*	0.064±0.066$^{¥}$	*na*	*na*	0.119±0.094$^{¥}$
Romero et al., 2020	150 (1)	Mar Menor Lagoon	*na*	0.002 ± 0.001$^{£}$ (*nd*-0.047)$^{\delta}$	0.039 ± 0.005$^{£}$ (*nd*-0.458)$^{\delta}$	*na*	0.093 ± 0.016$^{£}$ (*nd*-1.434)$^{\delta}$	1.500 ± 0.100$^{£}$ (*nd*-7.976)$^{\delta}$
This study	45 (10)	River Segura, drainage ditches and canals	0.002 ± 0.010	0.001 ± 0.001	0.025 ± 0.023	0.074 ± 0.034	0.012 ± 0.004	0.164 ± 0.176

na, not analyzed; *nd*, not detected. Data: µg/g wet weight, except ^{ш} dry weight; $^{\delta}$ range, ^{Ħ} range of means, $^{¥}$ mean ± standard deviation, $^{£}$ geometric mean ± standard error.

(0.037±0.09 ng/µl) and wild (0.111±0.080 ng/µl) eels from the Albufera de Valencia lagoon (Spain) (Ureña et al., 2007). In work on Suckers (Catostomidae sps.) from streams in the USA, Pb blood concentrations were about 0.4–0.6 and 5–15 µg/g dry weight (dw) in reference and polluted areas, respectively (Schmitt et al., 1984, 1993, 2002; Dwyer et al., 1988); common carp (*Cyprinus carpio*), channel catfish (*Ictalurus punctatus*) and largemouth bass (*Micropterus salmoides*) from reference sites had 0.02-0.29 µg/g dw Pb blood concentrations (Brumbaugh et al., 2005). When converting the results from these other studies to wet weight (considering a moist percentage of 84–86%), we noted that the Pb concentrations in the eel blood from the river Segura were similar to those described for fish in non-polluted areas. In addition, the Cd levels in blood (all three habitats) were like those reported by Brumbaugh et al. (2005) and Schmitt et al. (1993) at reference sites. Blood samples give information on elements at the exact time of sample collection (Vermeulen et al., 2009), so our results are relevant as a reference for the current eel population of this river and its recolonization.

Worthy of additional consideration is the lack of homogeneity in the correlations detected in our study (results from the three different habitats vary according to size). According to several authors (Baptista et al., 2019), biological and ecological factors could explain this lack of any general pattern in the elemental body dynamics of trace elements. In fact, the decrease in elemental concentrations in fish tissue with greater length and/or weight has been reported for Pb concentrations in species such as *Atherina hepsetus* (Canli & Atli, 2003), *Mola mola* (Baptista et al., 2019) and, recently, the European eel (Romero et al., 2020), and for Cd concentrations in eel tissues (Ureña et al., 2007; Noël et al., 2013; Amilhat et al., 2014). Conversely, an increase in Pb and Cd concentrations with eel weight has been reported by Farkas et al. (2000). Obviously, these differences between the three habitats affect the results for the population as a whole and so makes it necessary to specify the origin of samples when discussing overall results from these types of habitats. However, the strong positive correlation between Cd and Pb concentrations in blood from all of the habitats, would seem to indicate that there was a common anthropogenic source for both these metals.

European eels are considered to be good bio-monitoring sentinels for freshwater systems (Linde et al., 1996; Esteve et al., 2012). In other species such as the blue crab (*Callinectes sapidus*) commonly used in biomonitoring in river systems, Cd and Pb concentrations were higher than those found in eels: 2.4–35.7 times higher for Cd and 8.5 for Pb (liver vs. hepatopancreas), 3–6.4 times higher for Cd and 4.5–100.7 for Pb (muscle) (Genç & Yilmaz, 2017; Salvat-Leal et al., 2020) (but not for Pb concentrations in hepatopancreas; 0.113 µg/g, Salvat-Leal et al., 2020). Despite this, Genç and Yilmaz (2017) have described the European eel as the species that generally has the highest mean individual bioaccumulation index in livers.

In terms of food safety, in some countries European eels are one of the most popular fish for human consumption (e.g. Turkey, Genç & Yilmaz, 2017), which underscores the importance of determining the levels of hazardous metals in foods that could entail a risk for human health. However, both metals cause damage to various tissues of eels (Nunes et al., 2014), which is often overlooked in favor of food safety. In UE this fish is protected, and fishing is prohibited in some places, such as Segura River basin, although they are still caught and consumed in closed ecosystems such as the Mar Menor lagoon (Murcia, Spain) (Romero et al., 2020). According to European legislation, the maximum safe level for Pb in fish muscle is 0.3 µg/g ww and for Cd 0.050 µg/g ww (European Union, 2023). In none of the eels from the three studied habitats did the levels of these two heavy metals exceed these thresholds and so the consumption of these specimens does not represent a health risk for humans.

In conclusion, eels from the river Segura do not seem to be endangered due to Pb and Cd pollution. Nevertheless, it is necessary to consider the different zones through which eels migrate because, even though they are fluvial habitats, the environmental characteristics of each habitat differ, thereby influencing the eels' ecology within these and the fitness derived from developing there. The information obtained in this study should be added to the European Eel Quality Database and therefore could be useful for species management in this ecosystem.

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