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METAL CONCENTRATIONS IN THE LIVER OF THE EUROPEAN EEL, *ANGUILLA ANGUILLA*, IN ESTUARIES AND COASTAL LAGOONS FROM PORTUGAL

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EUROPEAN EEL
ANGUILLA ANGUILLA
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ABSTRACT. – The concentration of Cd, Cu, Hg, Pb and Zn in the liver of the European eel (*Anguilla anguilla*) was determined to evaluate the contamination burden of this species in Portuguese brackish water systems (Aveiro lagoon, Óbidos lagoon, Tagus estuary, Santo André lagoon and Mira estuary) and relate it to anthropogenic pressures within those ecosystems. The highest levels of most metals were found in eels from the Tagus estuary, in opposition to specimens from Santo André lagoon, which exhibited the lowest values. These results confirmed that eels from the most impacted areas show higher metal concentrations in their liver. Although little variability in metal load was observed within each brackish water system, some heterogeneity in contamination profiles among sampling stations was detected, demonstrating the efficacy of using the European eel as a sentinel species to monitor metals contamination at both large and small spatial scales.

INTRODUCTION

The European eel, *Anguilla anguilla* (L., 1758), is a widely distributed species that colonises almost all types of waters across Europe and northern Africa (Moriarty & Dekker 1997). Since the early 1980s, the population has been drastically declining in all its distribution area (Feunteun 2002) and its stock is considered outside safe biological limits (ICES 2010). Presently, the species is listed as endangered (Appendix II, CITES 2007) and is subject to an EU Council Regulation (EC) No. 1100/2007 establishing measures for the recovery and protection of the stock.

Among the management measures that aim at the recovery of *A. anguilla*, special focus is being put on contamination by metals and their negative impacts on spawners quality (Robinet & Feunteun 2002, Maes *et al.* 2005, Belpaire & Goemans 2007a). These contaminants may affect the endocrine system and disable the normal development of gonads as well as interact with embryonic development, hatching and growth of larvae (for details, see Geeraerts & Belpaire 2010). It is during their continental phase that eels are considered extremely prone to bioaccumulation of metals as a result of several ecological and physiological features: long life span, high fat content, benthic and relatively sedentary behaviour, high trophic level, resistance to water degradation and absence of annual reproductive cycles along with associated changes in lipids metabolism (Bruslé 1990, Belpaire & Goemans 2007a). Assessing the contamination load of the European eel could be a valuable tool for environmental monitoring as it is believed to faithfully characterise contamination pressure for a variety of harmful substances in both local and international scales (Belpaire & Goemans 2007a). This implies that besides contributing to establish measures to protect this species under the requirements of the EU eel recovery plan, monitoring *A. anguilla* contamination burden could also be useful to assess the chemical status of the environment in a wider perspective, such as the Water Framework Directive (Belpaire & Goemans 2007a, b, Belpaire *et al.* 2008) and the Marine Strategy Framework Directive.

Estuaries and coastal lagoons are particularly vulnerable ecosystems that receive considerable urban and industrial effluent discharges from adjacent areas as well as inputs from agricultural runoff yielding substantial quantities of anthropogenic pollutants that include several metals (Ribeiro *et al.* 2005, Has-Schön *et al.* 2006). Due to the presence of impassable dams in water courses and subsequent changes in the European eel natural distribution patterns, the abundance of eels is relatively higher in these brackish waters (*e.g.*, Domingos *et al.* 2006, Costa *et al.* 2008), which being extremely vulnerable to contamination can additionally contribute to producing poor quality reproducers.

Despite this species high economic value and vulnerability to contamination, studies evaluating metal concentrations in eel's tissues are scarce and occasional in Portugal (*e.g.*, Cid *et al.* 2001, Eira *et al.* 2009, Neto *et al.* 2011). Hence, the present work aims to assess metals (Cd, Cu, Hg, Pb and Zn) contamination in *A. anguilla* from several Portuguese brackish water systems and indirectly provide some evidence about their spawners quality. In addition, special emphasis is given to evaluate this species potential usefulness in fingerprinting environmental contamination pressures as well as the spatial scale (inter and intra-system) at which differences in the concentration of those hazardous substances can be detected.

MATERIAL AND METHODS

Study area: Five brackish water systems (Aveiro lagoon, Óbidos lagoon, Tagus estuary, Santo André lagoon and Mira estuary) along the Atlantic Portuguese coast were selected considering both their ecological importance for *A. anguilla* and their distinct anthropogenic pressures (Fig. 1).

Location and number of sampling stations was established based on the need to cover each system and the species distribution range. The distance between adjacent sampling sites varied between 0.6 km and 15.8 km. Their hydrologic and geomorphologic characteristics as well as the potential sources of contamination that might affect metal levels are described in Table I. According to the information obtained from national institutions, for each contamination source and per sampling station a rank of contamination was attributed: 0 - absent, 1 - low, 2 - moderate, and 3 - high (Table I). The sum of all ranks is considered the potential degree of contamination (PDC) per station.

Fish sampling and laboratory procedures: Eels were obtained from local fishermen during February and March 2008. Fyke nets and long-lines were used as capture techniques according to the local geomorphology. Only yellow-phase eels were used in metals analysis considering both their extreme importance for environmental monitoring and their aptitude to incorporate these chemicals in their tissues. As defined in ICES (2010), three criteria were used to discriminate between yellow and silver eels: eye diameter, state of lateral line (presence of black corpuscles) and body colour contrast. The aim was to collect five yellow eels per site, ranging in length between 35 and 45 cm (Neto *et al.* 2011), but due to limited catches a broader length range was used. Eels were transported alive to the laboratory, where they were killed by a benzocaine overdose and frozen (-20 ºC) until analysis.

After defrosting, total length (to the nearest mm) and total weight (to the nearest g) of five fishes from each sampling station were recorded. Their digestive tracts were removed and their liver collected, weighed (to the nearest 0.01 g) and preserved (-80 ºC) for lyophilization (Telstar Cryodos), which was performed during 48 hours to guarantee total removal of the water and to avoid loss of volatile elements. Liver tissue was chosen because it is generally recommended as the best organ to provide information on exposure to the selected metals (*e.g.*, Has-Schön *et al.* 2006, Belpaire & Goemans 2007b, Neto et al. 2011). The possible risks to human health from consumption of this species were not under discussion as this was not an objective of the present work. Freeze-dried livers were then weighed (to the nearest 0.01 g), macerated and stored under controlled humidity conditions until analysis.

Metal analysis: To determine total Cd, Cu, Pb and Zn concentrations, a sample of freeze-dried homogenised hepatic tissue (0.050-0.200 g) was digested by adding 2 mL of a HNO_3/$HClO_4$ mixture (9:1,v/v) in Teflon reactors heated at 110 °C for 2 hours (Julshmamn *et al.* 1982). After cooling, extracts were filtered through Whatman 42 filters and diluted to 10 mL with deionised water. Metal concentration analyses were performed by inductively coupled plasma mass spectrometry (ICP-MS) using a Termo X Series. The assessment of total Hg concentrations was carried out by pyrolysis atomic absorption spectrometry (AAS) with gold amalgamation using an Advanced Mercury Analyser (AMA) LECO 254 (Válega *et al.* 2006). To evaluate the accuracy and precision of the analytical methodology, certified material TORT2 (lobster hepatopancreas) replicates were run in parallel with the biological samples. Procedure blanks were also performed for quality assurance purposes.

Data analysis: Because it was not possible to catch all individuals within the target size range (35-45 cm), differences in eel's length either inter and intra brackish water systems were explored by the non-parametric Kruskal-Wallis test followed by STP (Simultaneous Test Procedure) a posteriori tests (Siegel & Castellan 1988).

In order to evaluate not only each metal concentration but also eel's global contamination load considering all elements analysed, the individual mean (multi-metal) bioaccumulation index (IMBI) was calculated according to Maes *et al.* (2005). Briefly, this index consists in dividing the individual concentration of each metal by the maximum observed concentration (standardizing) and averaging over the number of metals in study (Maes *et al.* 2005).

Differences in the concentrations of each element and IMBI values among eels from the five brackish water systems were investigated by the non-parametric Kruskal-Wallis test followed by STP a posteriori tests. In addition, to better assess the distribution patterns of heavy metal concentrations in eels among systems, a Principal Component Analysis (PCA) (ter Braak & Šmilauer 2002) was performed.

To evaluate if global contamination on eels considering all sampling stations reflected the potential sources of contaminants (Table I) the relationship between IMBI and PDC values was assessed through the Spearman correlation coefficient (Siegel and Castellan, 1988). A PCA analysis was also carried

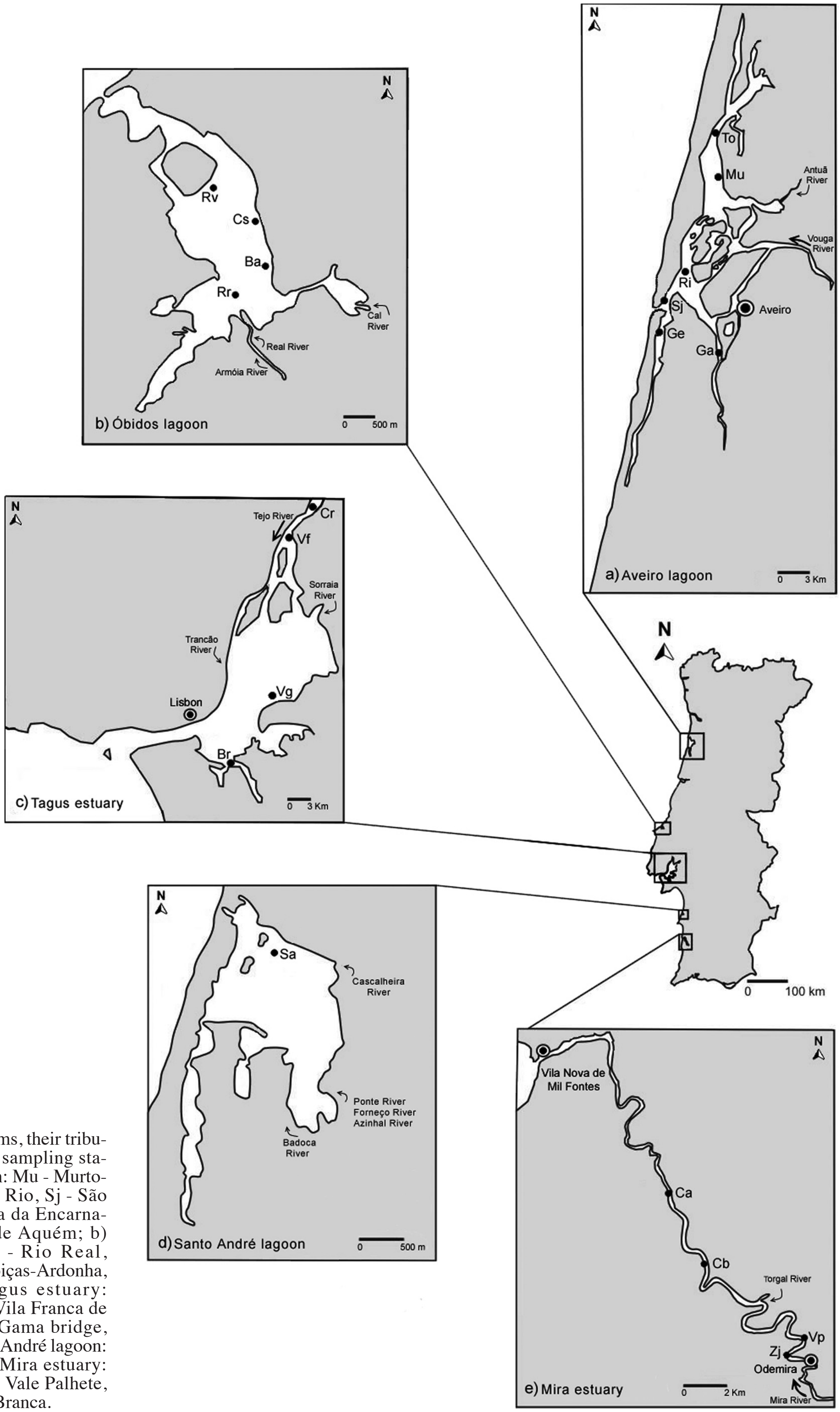

Fig. 1. – Studied systems, their tributaries and location of sampling stations: a) Aveiro lagoon: Mu - Murtosa, To - Torreira, Ri - Rio, Sj - São Jacinto, Ge - Gafanha da Encarnação, Ga - Gafanha de Aquém; b) Óbidos lagoon: Rr - Rio Real, Cs - Casinhas, Ba - Boiças-Ardonha, Rv - Rivais; c) Tagus estuary: Cr - Carregado, Vf - Vila Franca de Xira, Vg - Vasco da Gama bridge, Br - Barreiro; d) Santo André lagoon: Sa - Santo André; e) Mira estuary: Zj - Zambujeiro, Vp - Vale Palhete, Cb - Cuba, Ca - Casa Branca.

Table I. – Characterisation of the hydrology and geomorphology of the studied brackish water systems and potential sources of contamination in each sampling station.

System	Hydrology and geomorphology					Sampling stations	Potential sources of contamination					
	Area (km^2)	Mean depth (m)	Mean residence time (days)	Sea communication	Principal tributaries		Domestic	Industries and/or ports	Traffic	Agriculture	Aquaculture	Husbandry
Aveiro lagoon	74	2	20	Permanent through an artificial canal (350 m width)	Vouga; Antuã	Torreira (To)	0	0	1	0	0	1
						Murtosa (Mu)	0	0	0	1	0	1
						Rio (Ri)	3	2	0	0	1	0
						São Jacinto (Sj)	2	3	2	1	0	0
						Gafanha da Encarnação (Ge)	1	1	1	0	1	0
						Gafanha de Aquém (Ga)	1	0	1	0	1	1
Óbidos lagoon	7	2	7	Permanent through a narrow inlet (150 m width)	Real; Arnóia; Cal	Rio Real (Rr)	0	0	0	3	0	1
						Boiças-Ardonha (Ba)	1	1	0	1	0	0
						Casinhas (Cs)	1	0	0	0	0	0
						Rivais (Rv)	1	0	0	2	0	1
Tagus estuary	320	10	30	Permanent (2500 m width)	Tagus; Trancão; Sorraia	Carregado (Cr)	1	1	2	2	0	0
						Vila Franca de Xira (Vf)	2	1	1	2	0	0
						Vasco da Gama bridge (Vg)	1	1	3	0	0	0
						Barreiro (Br)	3	3	2	0	0	0
Santo André lagoon	2,5	2	na	Periodic opening; It is isolated from sea by a dune system	Cascalheira; Ponte; Forneço; Azinhal; Badoca	Santo André (Sa)	0	0	0	0	0	1
Mira estuary	3	4	15	Permanent (400 m width)	Mira; Torgal	Zambujeiro (Zj)	1	0	1	0	0	0
						Vale Palhete (Vp)	0	0	0	0	0	1
						Cuba (Cu)	0	0	0	2	1	0
						Casa Branca (Cb)	0	0	0	1	0	0

Information obtained from the following institutions: National Water Institute (INAG), National Statistics Institute (INE), Water Supply and Wastewater National Inventory (INSAAR), National Ports Association (APP), National Fisheries Institute (IPIMAR); na - not applicable; 0 - absent, 1 - low, 2 - moderate, and 3 - high degree of contamination.

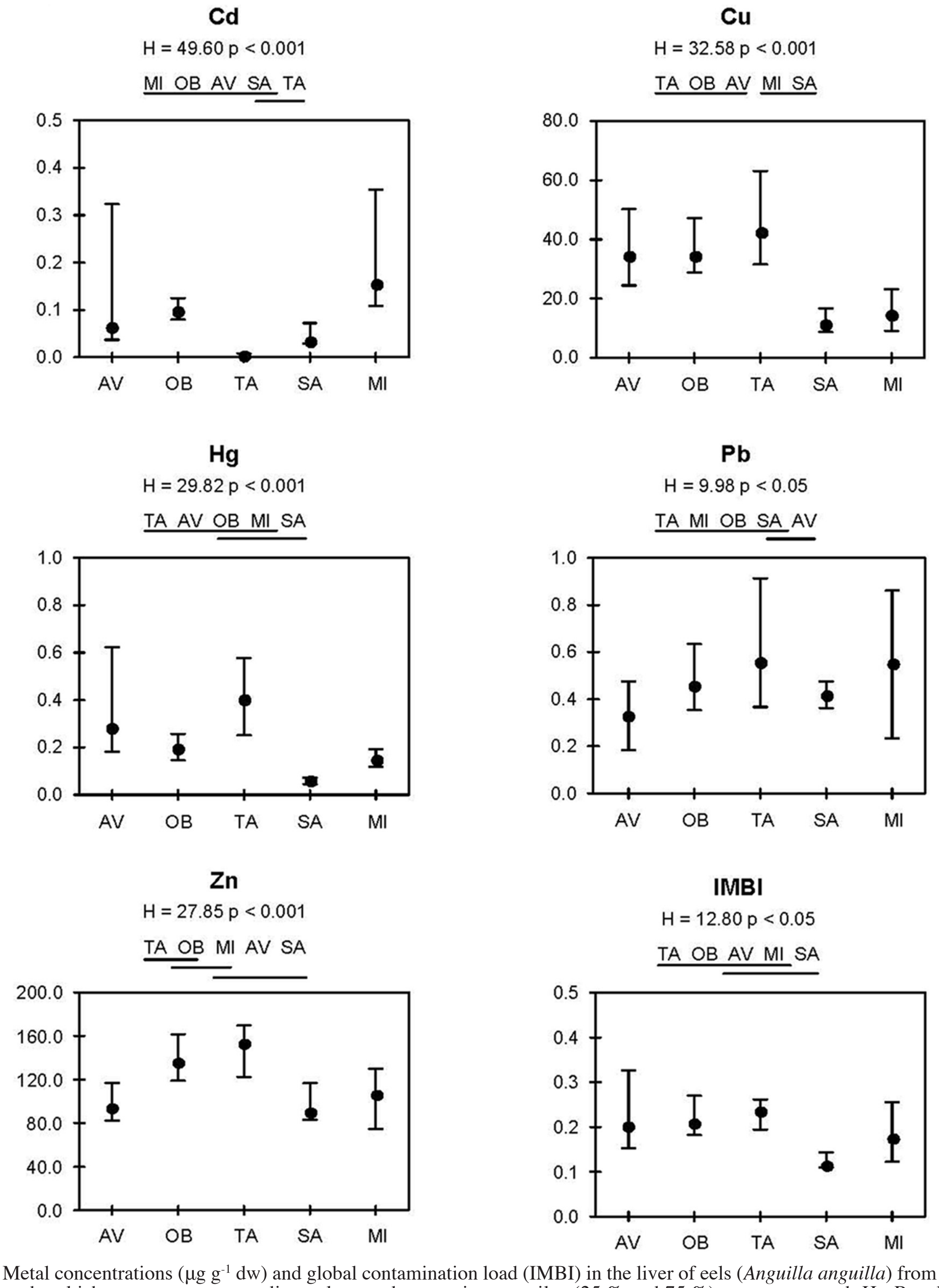

Fig. 2. – Metal concentrations (µg g^{-1} dw) and global contamination load (IMBI) in the liver of eels (*Anguilla anguilla*) from different Portuguese brackish water systems; median values and respective quartiles (25 % and 75 %) are represented; H - Results of the Kruskal-Wallis tests (df = 4; N = 90), rules join the non-significant subsets; AV - Aveiro lagoon; OB - Óbidos lagoon; TA - Tagus estuary; SA - Santo André lagoon; MI - Mira estuary.

out to evaluate the heterogeneity of metal concentrations in eels among stations.

Kruskal-Wallis and Spearman correlation tests were carried out using the Statistica 9.0 software package. PCA's analyses were performed using CANOCO (version 4.5) (ter Braak & Šmilauer 2002). For all tests, a significance level of $p < 0.05$ was considered.

RESULTS

A total of 90 yellow eels was analysed for heavy metal concentrations. Total length of eels ranged from 260 mm to 531 mm (mean ± SD: 396 ± 77 mm) and total weight ranged from 27 g to 310 g (mean ± SD: 116 ± 64 g). Eels from Mira estuary and Santo André lagoon were significantly smaller than those from the other systems ($H = 55.05$, $df = 4$, $N = 90$, $p < 0.001$). No significant differences were found in eel's length between sampling stations within each system (for all, $p > 0.05$).

Inter-systems variation

The contamination of eels collected in the five brackish water systems revealed a significant heterogeneity according to their origin (Figs 2 and 3). Individuals from the Tagus estuary displayed the highest contamination levels, except for Cd which was superior in the Mira estuary. In this latter system eels also exhibited high Pb content. Although slightly inferior, eels from Óbidos lagoon showed levels similar to the ones observed in the Tagus estuary for the majority of metals. The concentrations of Cu and Hg in eels from the Aveiro lagoon were also similar to the most contaminated system. In general, the lowest contamination levels were recorded in eels from Santo André lagoon. The variation in IMBI values confirmed the general pattern observed for each element individually and showed that Santo André lagoon was significantly different from Tagus estuary and Óbidos lagoon (Fig. 2).

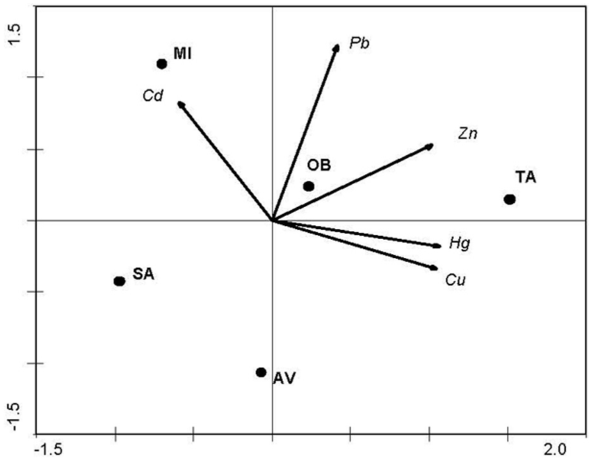

Fig. 3. – Ordination diagram of Principal Component Analysis (PCA) based on median metal concentrations (µg g^{-1} dw) in the liver of eels (*Anguilla anguilla*) from different Portuguese brackish water systems; brackish water systems are represented by circles; median metal concentrations are represented by arrows; the first two ordination axes account for 84.4 % of the total variance; AV - Aveiro lagoon; OB - Óbidos lagoon; TA - Tagus estuary; SA - Santo André lagoon; MI - Mira estuary.

Intra-system variation

Metal concentrations in all sampling stations are summarised in Table II. A significant positive correlation was found between IMBI and PDC values per station ($r_S = 0.79$; $N = 19$, $p < 0.01$).

The PCA used to evaluate the heterogeneity of contamination in the liver of eels within systems showed that, in general, metal concentrations were relatively close inside the water bodies studied: Tagus estuary stations presented always high concentrations, Óbidos lagoon stations medium-high concentrations and Mira estuary and Santo André lagoon low concentrations (Fig. 4). This pattern of similarity was not observed in the Aveiro lagoon because eels from Rio (Ri), S. Jacinto (Sj) and Gafanha de Aquém (Ga) clearly showed higher metal levels than those from Gafanha da Encarnação (Ge), Torreira (To) and Murtosa (Mu). Another exception was the slightly lower contamination load of eels from Vasco da Gama bridge (Vg) comparatively to the other stations within the Tagus estuary (Fig. 4) as well as the surprisingly high lead levels in Cuba (Cb) station from Mira estuary not only within this system but also from a countrywide perspective, as shown in Table II.

Eels from the most impacted sampling sites were also differently affected by the studied metals (Table II and Fig. 4). Whereas eels from Rio (Ri) from Aveiro lagoon exhibited higher concentrations of Cu, Pb and Zn, the specimens from S. Jacinto (Sj) and Gafanha de Aquém (Ga), despite located in the same system, were particularly contaminated by Cd and Hg. In the Tagus estuary, some heterogeneity was also found, namely because individuals from Carregado (Cr) and Vila Franca de Xira (Vf) showed high levels for all metals, whereas eels from Barreiro (Br) were particularly contaminated by Cu, Pb and Zn.

DISCUSSION

Comparison of data from the present study with those from the literature is difficult to make not only due to differences in methodologies used by each author (*e.g.*, fish size, analysed tissue, pooled/individual analysis or metals determination procedure), but also because each area has its specific environmental background (*e.g.*, salinity, pH or sediment characteristics) that might affect metals bioavailability. Nevertheless, information from a number of selected papers was used to compare the contamination levels of Portuguese eels with those reported elsewhere in Europe. As shown in Table III, individuals in the present study displayed, in general, lower concentrations, implying that eels from Portuguese brackish water systems are less exposed to metals contamination than their European conspecifics. This was particularly obvious in relation to Cd, which levels were considerably low when compared with those found in eels from polluted areas in England

Table II. – Median metal concentrations (μg g^{-1} dw) in the liver of eels (*Anguilla anguilla*) at each sampling station.

System	Sampling station	Cd	Cu	Hg	Pb	Zn
Aveiro lagoon	Torreira (To)	0.054 (0.044-0.055)	38.2 (34.7-43.1)	0.12 (0.11-0.15)	0.15 (0.13-0.32)	107 (99-120)
	Murtosa (Mu)	0.056 (0.043-0.079)	30.0 (27.5-43.8)	0.22 (0.17-0.26)	0.40 (0.36-0.45)	94 (93-113)
	Rio (Ri)	0.397 (0.324-0.531)	**65.6** (58.3-74.3)	0.50 (0.43-0.51)	0.54 (0.40-1.02)	117 (111-124)
	São Jacinto (Sj)	**0.667** (0.050-0.894)	17.2 (16.5-27.2)	**1.16** (0.19-2.53)	0.18 (0.18-0.21)	70 (64-72)
	Gafanha da Encarnação (Ge)	0.033 (0.006-0.119)	34.8 (26.1-50.2)	0.21 (0.21-0.22)	0.17 (0.11-0.19)	93 (82-115)
	Gafanha de Aquém (Ga)	0.037 (0.028-0.053)	24.5 (17.954-33.989)	**0.80** (0.29- 1.11)	0.35 (0.26-0.48)	83 (82-88)
Óbidos lagoon	Rio Real (Rr)	0.085 (0.081-0.132)	38.6 (27.6-48.5)	0.17 (0.14-0.19)	0.65 (0.43-0.73)	128 (122-163)
	Boiças-Ardonha (Ba)	0.086 (0.078-0.113)	30.8 (29.5-42.1)	0.27 (0.25-0.30)	0.45 (0.44-0.46)	148 (137-167)
	Casinhas (Cs)	0.104 (0.091-0.117)	30.0 (28.1-35.2)	0.21 (0.18-0.26)	0.36 (0.35-0.44)	119 (111-135)
	Rivais (Rv)	0.108 (0.093-0.123)	38.9 (33.2-45.8)	0.19 (0.15-0.26)	0.62 (0.58-0.70)	145 (112-158)
Tagus estuary	Carregado (Cr)	0.007 (0.004-0.008)	25.0 (21.6-38.0)	0.57 (0.45- 0.72)	0.66 (0.54-1.47)	111 (107-138)
	Vila Franca de Xira (Vf)	0.021 (0.009-0.072)	38.9 (31.3-45.8)	0.51 (0.36-0.59)	0.45 (0.35-0.48)	159 (104-164)
	Vasco da Gama bridge (Vg)	bdl	38.7 (36.5-64.9)	0.45 (0.37- 0.46)	0.37 (0.36-0.51)	144 (135-148)
	Barreiro (Br)	bdl	**61.3** (60.7-74.5)	0.15 (0.10-0.20)	0.60 (0.60-0.97)	**175** (161-193)
Santo André lagoon	Santo André (Sa)	0.032 (0.030-0.052)	11.3 (9.3-14.7)	0.06 (0.05-0.07)	0.42 (0.37-0.47)	90 (83-106)
Mira estuary	Zambujeiro (Zj)	0.150 (0.123-0.250)	13.0 (11.0-15.1)	0.15 (0.11-0.24)	0.35 (0.24-0.86)	124 (110-127)
	Vale Palhete (Vp)	0.106 (0.099-0.162)	16.5 (14.2-26.5)	0.13 (0.10-0.15)	0.37 (0.36-0.60)	73 (65-123)
	Cuba (Cb)	0.337 (0.142-0.371)	16.4 (8.3-29.8)	0.13 (0.12-0.15)	**0.87** (0.21-1.30)	124 (86-155)
	Casa Branca (Ca)	0.209 (0.128-0.386)	12.5 (8.7-15.1)	0.17 (0.17-0.19)	0.60 (0.51-0.63)	99 (76-101)

Interquartile range between brackets; particular high values in bold; bdl - below detection limits

(Bird *et al.* 2008), Belgium (van Campenhout *et al.* 2008), France (Durrieu *et al.* 2005) or Spain (Linde *et al.* 2001). The only exception in the described scenario was the high concentration of Hg found in eels from the Aveiro lagoon, which is similar to the values obtained by Durrieu *et al.* (2005) and Ureña *et al.* (2007). This result is consistent with the ones observed by Pereira *et al.* (2008), who reported high sediment Hg concentrations in this system ranging from 0.001 μg g^{-1} to 52 μg g^{-1}.

Several authors (see Robinet & Feunteun 2002, Belpaire & Goemans 2007a, b) have demonstrated that *A. anguilla* is a highly suitable biomonitor for several environmental contaminants. For example, Belpaire *et al.* (2008) confirmed this species usefulness in fingerprinting the chemical status of rivers in Flanders, especially for polychlorinated biphenyls and organochlorine pesticides. In the present study, a similar approach was performed using metals contamination in brackish water systems. The inter-system variation analysis demonstrated that eels from more impacted systems (Table I) tend to show higher metal concentrations in their liver (Figs. 2 and 3), revealing that *A. anguilla* contamination burden reflects the overall anthropogenic pressures of each water body. Despite this evidence, it should be noted that eel's size was significantly different among systems which might have somehow biased the present results. In fact, several authors have reported the influence of this variable in eel´s contamination levels (*e.g.*, Farkas *et al.* 2000, Bel-

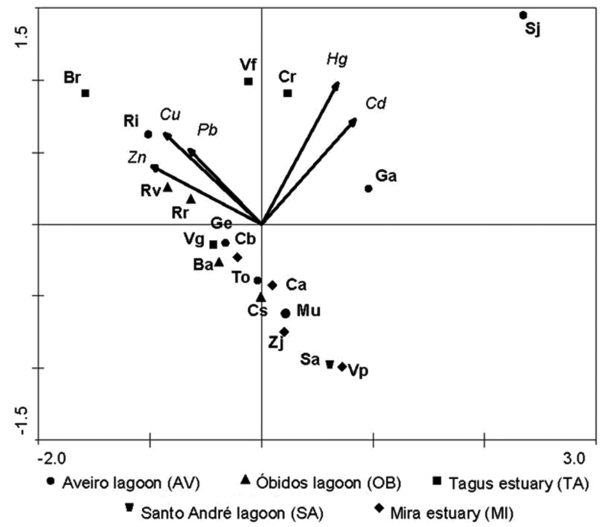

Fig. 4. – Ordination diagram of Principal Component Analysis (PCA) based on median metal concentrations (μg g^{-1} dw) in the liver of eels (*Anguilla anguilla*) at each sampling station from different Portuguese brackish water systems; sampling stations are represented by symbols (see abbreviations in Table I); median metal concentrations are represented by arrows; the first two ordination axes account for 65.5 % of the total variance.

paire *et al.* 2008, Neto *et al.* 2011).

In the Tagus estuary, environmental problems are mostly related to high human population densities (near 2 million inhabitants) and to an inadequate planning regarding industrial development and urbanisation (Vasconcelos *et al.* 2007). For decades, effluents without proper treatment were discharged directly into the estuary, which together with agricultural runoff in the upstream locations played an important role in its present contamination levels (Costa 1999, Caçador *et al.* 2000). In addition, intense commercial navigation and dredging operations occur in this estuary due to an important port located in Lisbon (Vasconcelos *et al.* 2007).

Contamination in Óbidos lagoon is mainly from diffuse sources originated from agriculture and husbandry activities that have an impact in the entire catchment (Carvalho 2006). Nowadays, 80 % of domestic and industrial load enters directly into the coastal zone adjacent to the lagoon through a submersed outfall, but during many years untreated effluents were directly released into the system, which was particularly problematic in Cal River, one of the main tributaries (Carvalho 2006).

Aveiro lagoon provides natural conditions for harbour, navigation and recreation facilities, although intense commercial navigation occurs especially in the mouth of the system where an important port is situated (Dias *et al.* 1999, Vasconcelos *et al.* 2007). A large number of industries is also responsible for discharging their effluents in the area (Dias *et al.* 1999). One of the most concerning cases was the introduction of Hg by a chlor-alkali industry. Despite a crucial change in the production process that decreased metals emission levels, this industry has contributed in the past decades to severe contaminated sediments, which might still be acting as an internal source of this element into the system (*e.g.*, Pato *et al.* 2008). This particular environmental contamination is reflected in the liver of eels from Aveiro lagoon, where the highest levels of Hg in the country were found.

Contrary to all the other brackish water systems, Mira estuary and Santo André lagoon are located near small villages with reduced human activities in and around them (Vasconcelos *et al.* 2007). As a result, eels from these coastal areas exhibited the lowest metal levels. Despite being considered weakly impacted, the Mira estuary has a quite relevant agriculture pressure that contributes to the destruction of saltmarsh areas and intertidal flats (Vasconcelos *et al.* 2007). Therefore, the high Cd concentration found in eels from this system might be caused by an intense use of artificial fertilisers and pesticides, regarded as a potential source of this metal (Has-Schön *et al.* 2006).

An efficient biomonitor should not only reflect the overall anthropogenic pressures that affect a large spatial area but also pinpoint local chemical inputs and variations that might occur between sites (Belpaire & Goemans 2007a, b, Belpaire *et al.* 2008). In the present study, potential sources of contamination affecting each sampling station (Table I) were compared with metal concentrations found in *A. anguilla* (Table III and Fig. 4). This evaluation suggests that eels from the most impacted sites show higher metal concentrations in their liver, which is consistent with this species bioaccumulation propensity. For instance, S. Jacinto (Sj) or Barreiro (Br) specimens, extremely subject to anthropogenic pressures, showed the highest contaminant levels in their liver in opposition with eels from Santo André (Sa) or Vale Palhete (Vp), where negative impacts are reduced. This is also reinforced by the positive correlation between the global contamination load (IMBI) and the potential degree of contamination (PDC) per site.

Intra-system analyses also showed that metal levels in the liver of eels from each site within a system was relatively close, despite the occurrence of some variety in their contamination profile, *i.e.* the element that affected them most. This suggests that eels are affected by different sources of pollution which is in agreement with the study conducted in Flanders by Belpaire *et al.* (2008). In the latter, contamination of eels by lipophilic compounds was more similar within each tributary than between different tributaries, although their contamination profiles along the river proved to be significantly different. The variability in metal concentrations within each Portuguese brackish water body was generally low except for the Aveiro lagoon, where eels from certain sites displayed a considerable higher content in their liver in relation to others. This can be explained both by the variety of contamination sources as well as by the specific geomorphologic features of this system and subsequent effects on

Table III. – Mean metal concentrations (µg g^{-1} dw) in the liver of eels (*Anguilla anguilla*) in several European systems.

Reference	Country	Geographic area	n	Sampling sites	Cd	Cu	Hg	Pb	Zn
Langston *et al.* (2002)	England	Thames estuary	56	11	0.44-1.44	6.3-99.8	nd	nd	122-399
Bird *et al.* (2008)	England	Severn estuary	256	nd	14.30	66.0	nd	nd	174
Van Campenhout *et al.* (2008)*	Belgium	Zuid-Willemsvaart canal	11	nd	≈1.13	≈76.1	nd	≈1.39	≈165
		Beverlo canal	12	nd	≈51.93	≈96.1	nd	≈29.59	≈195
		Weerde lake	11	nd	≈0.85	≈50.7	nd	≈2.52	≈137
		Venepevaart canal	9	nd	≈0.09	≈26.7	nd	≈0.35	≈124
Farkas *et al.* (2000)	Hungary	Balaton lake	29	2	3.22-4.34	55.61-90.52	0.05-0.14	1.41-2.04	197-221
Durrieu *et al.* (2005)*	France	Gironde estuary	20	3	≈8.00	nd	≈1.45	nd	≈230
Ribeiro *et al.* (2005)	France	Vaccares lagoon	27	3	0.13-0.23	59.9-76.5	0.32-0.76	0.38-0.64	201-215
Häs-Schön *et al.* (2006)*	Croatia	Neretva river	12	2	0.54	nd	0.34	0.58	nd
Linde *et al.* (1999)*	Spain	Piqueña river	36	nd	≈2.10	≈37.8	nd	≈2.1	≈117.6
Linde *et al.* (2001)*	Spain	Ferrerias river	40	2	6.26-11.00	48.6-66.9	nd	2.27-5.96	151-197
Linde *et al.* (2004)*	Spain	Raices river	58	2	2.04-3.18	23.4-44.1	0.71-2.04	0.59-0.85	nd
Usero *et al.* (2003)*	Spain	Odiel salt marsh	20	nd	1.39-2.02	98.3-136.5	0.05-0.10	2.10-2.52	155-187
		Cadiz Bay salt marsh	20	nd	0.50	68.9-97.9	0.07-0.08	1.68	134-158
Ureña *et al.* (2007)*	Spain	Albufera lake	12	nd	0.13-1.85	25.6-99.2	0.29-1.39	0.29-1.85	108-214
Genç *et al.* (2008)*	Turkey	Asi river	18	nd	0.21- 0.29	25.2-37.1	1.01- 1.13	0.17-2.02	67-69
Present study	Portugal	Aveiro lagoon	26	6	0.04-0.53	28.2-76.8	0.14-1.43	0.19-1.71	68-120
		Óbidos lagoon	20	4	0.10-0.13	38.1-42.6	0.17-0.25	0.38-0.69	128-142
		Tagus estuary	20	4	0.01-0.04	31.9-65.4	0.20-0.56	0.47-0.90	123-187
		Santo André lagoon	4	1	0.04	13.9	0.16	0.66	100
		Mira estuary	20	4	0.14-0.27	12.6-26.1	0.14-0.17	0.45-0.88	94-136

nd - no data; ≈ - approximately; * - ratio wet-to-dry weight used for transformations = 4.2

metals dispersal. Pato *et al.* (2008) have described that an extensive web of islands and channels makes water circulation inside the lagoon rather difficult, favouring the spreading and trapping of contaminants locally. Eels from Vasco da Gama bridge (Vg) in the Tagus estuary also presented metal levels relatively lower than the other stations from this system. This result can be due to local hydrodynamic processes, which hinder contaminants deposition in sediments and their availability to fishes. In fact, this part of the estuary has slightly lower residence times in comparison with upstream regions or the south-east inner basins (Braunschweig *et al.* 2003) that might promote water exchange and the outflow of substances. The relatively high Pb concentrations found in eels from Cuba (Cb) station was rather surprising. Despite the generalised use of fertilisers in the area (Vasconcelos *et al.* 2007) and the presence of abandoned mines, which might be related to this metal introduction, the causes of this contamination remain unclear.

The most striking case illustrating the variety in contamination profiles of eels was the difference between S. Jacinto (Sj) and Rio (Ri) in the Aveiro lagoon, which despite their proximity and being severely impacted, proved to be affected by different metals. In fact, while S. Jacinto (Sj) pressures are mainly a consequence of the nearby harbour, Rio (Ri) is especially disturbed by industries, namely pulp and paper production. The latter types of anthropogenic impacts are also affecting eels from Barreiro (Br), which might explain the similar contamination profile between these two stations. As mentioned by Belpaire *et al.* (2008), the relatively sedentary behaviour of this species can explain distinct contamination profiles of individuals among adjacent locations. Although recent investigations (Daverat *et al.* 2006) have demonstrated that a considerable fraction of eels collected in estuaries and coastal areas had experienced a freshwater episode, especially in early stages of their life, ecological studies on home range showed that foraging movements of *A. anguilla* are mostly restricted to a few hundred metres (*e.g.*, Laffaille *et al*. 2005). In the present study, eels only appeared to be less discriminating when contamination sources were identical among sites and particularly if there was a diffuse origin, such as in the neighbouring stations of Vila Franca de Xira (Vf) and Carregado (Cr), where specimens showed similar levels for all metals.

In conclusion, *A. anguilla* seems to be an effective bioaccumulator for metals in brackish water systems even under moderate anthropogenic pressures. Eel's usefulness as a sentinel species enables to pinpoint environmental contamination sources at both large (inter-systems) and small (intra-system) spatial scales, which could be a valuable tool for monitoring metals in aquatic systems. This data could be important for improving the environmental conditions of most contaminated locals given that specific action could be taken to eliminate their main pollution sources. Although there is some knowledge on the specific negative impacts that metals have on eels (for details, see Geeraerts & Belpaire 2010), further research is required to evaluate their consequences on this species reproductive success and subsequent implications to the population decline. Nevertheless, because Portuguese eels seem to be less contaminated by these chemicals than their European conspecifics, they might contribute with potentially higher quality spawners for the reproductive stock.

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