Metal levels and accumulation patterns of sea lamprey ammocoetes (*Petromyzon marinus* Linnaeus, 1758) from a tidal freshwater habitat

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Abstract – Ammocoetes of sea lamprey (*Petromyzon marinus* Linnaeus, 1758) from an assumedly pristine tidal freshwater habitat were used for determination of metal levels and accumulation patterns with size. The individuals were collected during the winter of 2011 in river Minho (Iberian Peninsula, Southwest of Europe) and the levels of metals (Cd, Cu, Cr, Fe, Pb, Mn and Zn) in the whole body were analysed by atomic absorption spectrometry. Fe was the metal with highest levels (maximum of 1438 ppm), while Cd and Pb were the lowest accumulated elements (overall means of 0.093 ± 0.065 ppm and 0.259 ± 0.094 ppm, respectively). Larger ammocoetes seemed prone to accumulate Cu, Fe and Mn ($P < 0.05$). Pb and Zn levels also seemed to increase with ammocoetes size; however, statistical differences were not found between size classes ($P > 0.05$).

Key words: Absorption spectroscopy / bioaccumulation / heavy metals / Minho / Miño

Introduction

The sea lamprey (*Petromyzon marinus* Linnaeus, 1758) is widely distributed along North Atlantic and is present in several European rivers from Norway to the Iberian Peninsula and in Mediterranean basins (Hardisty, 1986). The larvae or ammocoetes of sea lamprey remain buried in soft substrates and filter-feed on algae, micro-organisms and organic detritus during a period of 4–7 years, prior to adult marine stage (Almeida and Quintella, 2002; Almeida *et al.*, 2008).

Metals occur naturally worldwide, however, their concentrations occur far higher than natural levels in some ecosystems, mainly due to anthropogenic activities (USDA, 2000; Mason, 2002). Some metals are essential elements (*e.g.* Zn and Cu) for reproduction and growth of living organisms; however, most metals are toxic when in high concentrations. The levels of these elements in sediments, water and aquatic organisms should be monitored in order to study potential changes induced in living beings (Clark, 2002; Mason, 2002). Most toxicological studies with sea lamprey used landlocked populations and aimed at identifying effective lampricides;

however, few works address metal accumulated by anadromous sea lampreys in their native ranges (Holmes and Youson, 1996; Andersen *et al.*, 2010; Pedro *et al.*, 2013).

The absorption of waterborne metals occurs mainly through the skin, viscera and gills of fish and the absorption of metals by filter-feeding organisms is usually higher when compared with other aquatic organisms (Naimo, 1995; Holmes and Youson, 1996). Toxicity studies concluded that ammocoetes of Pacific lamprey *Entosphenus tridentatus* (Richardson, 1836) were relatively insensitive to several chemicals when compared with other aquatic species (Andersen *et al.*, 2010). Therefore, ammocoetes should also be prone to accumulating some metals retained in interstitial waters.

River Minho (or "Miño" in Spanish) works as a natural boundary between Portugal and Spain for 75 km. Tidal freshwater ecosystems occur in the upper estuary of River Minho which are used by a range of species with conservation importance for spawning, refuge and feeding (Sousa *et al.*, 2008). However, these ecosystems are often threatened by several anthropogenic activities (such as farming, industrial activities and navigation), which promote higher concentrations of contaminants (Clark, 2002). The thinner granulometry of such areas is
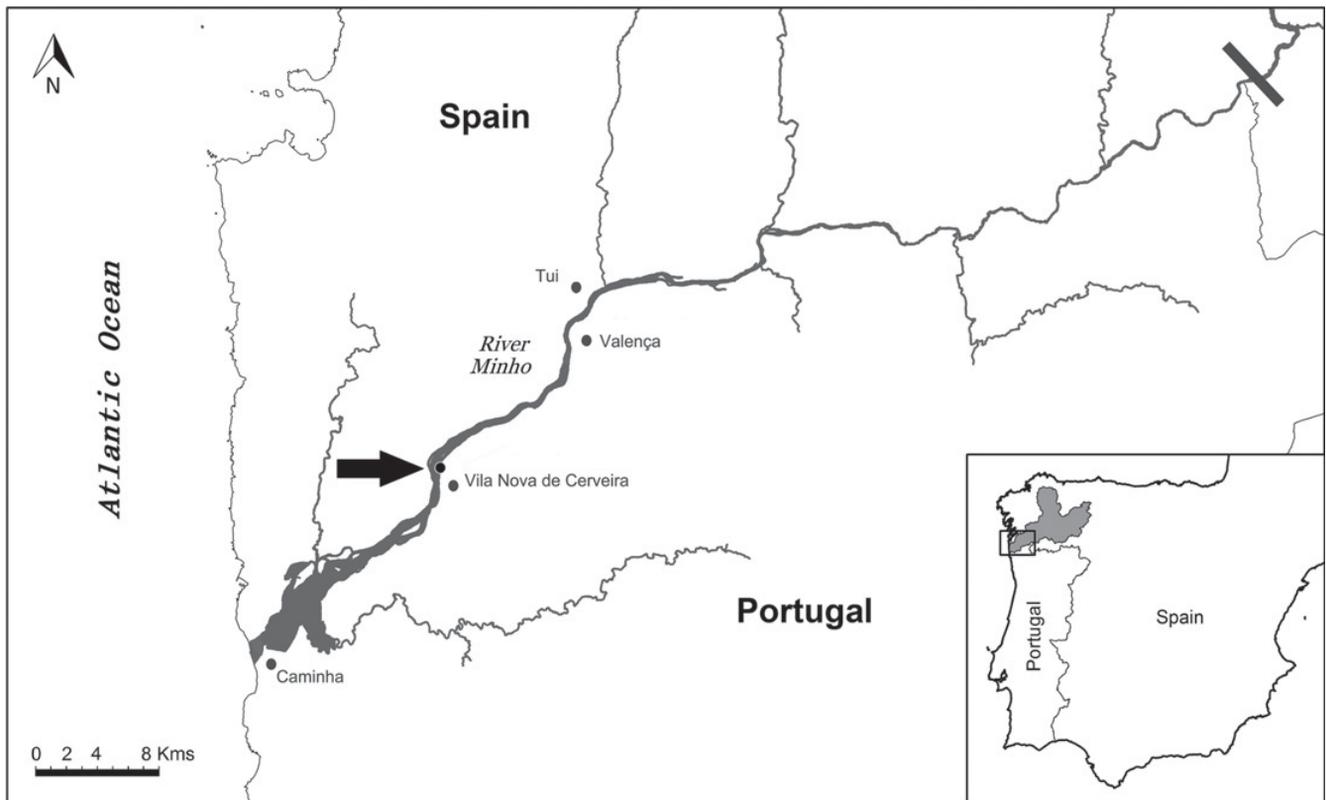

Fig. 1. Study area (arrow). Sea lampreys are restricted to the river section of downstream Frieira dam (bar).

particularly vulnerable to metal accumulation (Reis *et al.*, 2009). Nonetheless, river Minho is still considered a low contaminated area regarding metals and can be used as reference to other areas (Reis *et al.*, 2009).

As far as we know, metal levels of Cd, Cr, Mn, Pb and Zn in ammocoetes of *P. marinus* throughout its native range and metal concentrations in aquatic species of river Minho are still unavailable. Therefore, the objectives of this work were: (i) to determine the levels of metals (Cd, Cr, Cu, Fe, Mn, Pb and Zn) of sea lamprey *P. marinus* ammocoetes from river Minho and (ii) to discuss their accumulation along this stage.

Methods

The river Minho is located in the northwestern corner of the Iberian Peninsula, in SW Europe, flowing westward from the Cantabrian Mountains (north of Spain) to the Atlantic Ocean (Fig. 1). The total area of the Minho watershed is $17\,100\ \mathrm{km^2}$ and the mainstream is 300 km long. The available habitat for native anadromous sea lampreys in river Minho is restricted to the downstream section (approx. 75 km in length), as migrating adults are unable to overcome the first impassable barrier ("Frieira" dam) (Sousa *et al.*, 2012; Araújo *et al.*, 2013).

The sea lampreys were collected in tidal freshwater habitats (41°57.90'N; 8°44.40'W and 41°56.26'N; 8°45.07'W) approx. 14 km upstream river mouth, with dip net (mesh size: 1 mm) in the river Minho from the

Portuguese bank between 3 and 10 February 2011 (overall $n = 145$) during low tide (depth ranging from 0 to 0.50 m). The surface water abiotic variables (temperature, pH, conductivity and dissolved oxygen) were measured prior to sampling using a multi-parametric sea gauge YSI 6820 (Brannum Lane, Yellow Springs, OH). The individuals were transported alive to *Aquamuseu do rio Minho* (Vila Nova de Cerveira, Portugal) and after anaesthetic bath with Tricaine methanesulfonate (MS-222) their length (0.5 mm) and weight were determined (0.01 g). The condition factor K was estimated using the equation: $K = W.L^{-3} \times 100$, where W is the whole body wet weight in g and L total length in cm (Froese, 2006).

Length of time of exposure to environmental metals is reported to influence the amounts of some elements in aquatic organisms (Luoma, 1983; Clark, 2002; Durrieu *et al.*, 2005). Therefore, whole body samples of randomly chosen ammocoetes from three distinct size classes with 35 mm of interval (60–95 mm, 95–130 mm and 130–165 mm; $n = 3$ for each class) which probably correspond to three different age-classes (Holmes and Youson, 1996) were selected to proceed with metal analyses. The determination of metal concentrations followed the method of Reis and Almeida (2008) and Reis *et al.* (2009). The samples were freeze dried (FreeZone Plus 12; Bulk Tray Dryer, Labconco, Kansas City, MO, USA) and homogenized with commercial mills. Triplicates (300 mg/replicate) of each sample were digested in high pression Teflon bombs (model 4782, Parr) at 500 W microwave (NE-1037, Panasonic): two consecutive digestions of 5 min each with

Table 1. Limits of detection and quantification of the analytical methods and the metal concentrations in the certified reference materials. LOD, limit of detection; LOQ, limit of quantification; concentrations are expressed in ppm as mean $\pm$ SD.

Element	Cd	Cr	Cu	Fe	Mn	Pb	Zn
LOD	0.0066	0.016	0.22	16.7	0.67	0.0033	16.7
LOQ	0.0218	0.053	0.73	55.1	2.21	0.0109	55.1
				NRCC DORM-2 ($n = 3$)			
Certified	0.043 ± 0.008	34.7 ± 5.5	2.34 ± 0.16	142 ± 10	3.66 ± 0.34	0.065 ± 0.007	25.6 ± 2.3
Measured	0.050 ± 0.001	29.4 ± 5	2.29 ± 0.04	149 ± 7	3.49 ± 0.55	0.070 ± 0.004	26.9 ± 7.2
				NRCC TORT-2 ($n = 3$)			
Certified	26.7 ± 0.6	0.77 ± 0.15	106 ± 10	105 ± 13	13.6 ± 1.2	0.350 ± 0.130	180 ± 6
Measured	25.2 ± 2.4	0.64 ± 0.02	104 ± 10	103 ± 21	13.3 ± 2.2	0.310 ± 0.050	185 ± 16

5 mL pure nitric acid and one ice cooling bath of at least 2 h between digestions (Reis and Almeida, 2008). Metal levels of each individual were analysed by Atomic Absorption Spectrometry (AAS, SpectrAA 220 FS, Varian), coupled with deuterium lamp for background correction, with flame atomization (FAAS, Marck 7, Varian) and electrothermal atomization (Autosampler 110, Varian), depending on the metal levels of sample and element to be determined. The certified reference materials used to ensure suitability of the analytical method for metal analyses were NRCC Dorm-2 (Dogfish muscle tissue) and NRCC Tort-2 (Lobster hepatopancreas tissue). For each solution, the software Varian was set for a precision lower than 10% between readings with a maximum of four readings per replica to ensure reproducibility of readings and metals were expressed in ppm (in dry weight basis). The limits of detection (LOD) and quantification (LOQ) are shown in Table 1 and were determined using the method of Reis *et al.* (2009).

The existence of statistical differences between size classes was performed after testing for normality (Kolgomorov–Smirnov Test) and for homogeneity of variance (Bartlett's Test) with one–way analysis of variance (ANOVA) followed by Tukey test when significant differences were detected. A $P < 0.05$ was considered significant. "Minitab® Release 14.12.0" software (Minitab Inc.) was used.

Results and discussion

The water temperature at sampling time in river Minho ranged from 8.1 °C to 8.9 °C. The water pH was neutral (7.1 ± 0.17, mean $\pm$ SD) and the dissolved oxygen was 8.6 ± 1.22 mg.L^{-1}. The conductivity results were in accordance with previous works in the same area (75 ± 6.7 µS.cm^{-1}) (Almeida *et al.*, 2008; Reis *et al.*, 2009).

The log transformed *L–W* relationship of ammocoetes was log W (g) $= -5.124 + 2.67$ log L (mm) ($R^2 = 0.98$, $n = 145$). The size of the ammocoetes used in metal determination ranged from 0.79 to 6.89 g in weight (2.80 ± 2.15, mean $\pm$ SD) and from 74 to 165 mm in total length (112 ± 32, mean $\pm$ SD). The condition factor ranged from 0.14 to 0.19.

The patterns of mean metal occurrence in order of increasing concentrations in the ammocoetes were as follows: Cd < Pb < Cr < Cu < Mn < Zn < Fe. This pattern slightly differs from metal levels in sediment samples of river Minho (Cd < Cu < Cr < Pb < Zn < Mn ≪ Fe) (Paiva *et al.*, 1993; Moreno *et al.*, 2005; Lyra, 2007; Mil-Homens *et al.*, 2012).

The concentration of Cd apparently does not change with ammocoete size class ($P = 0.072$, Fig. 2) and the overall levels (ranging from 0.007 to 0.218 ppm) were much lower than those found in muscle of sea lamprey spawners (ranging approx. from 6.5 to 13 ppm) (Pedro *et al.*, 2013) which is in accordance with the literature, as the concentrations of this metal are referred to increase with age of fish as the excretion of this element is difficult (Eisler, 1985; Mason, 2002). The amounts of Cd in the muscle of *Mugil cephalus* (Linnaeus, 1758) living in metal-contaminated ecosystems range from 0.08 to 0.6 ppm (Dural *et al.*, 2007; Ambedkar and Muniyan, 2011) and reach 5.2 ppm in the whole body of benthic flounder *Platichthys flesus* (Linnaeus, 1758) (Eisler, 1985). These values are relatively higher to those found in ammocoetes, which might be a consequence of the low values of this element in river Minho water and sediments (< 0.0005 ppm and ranging from 0.03 to 0.66 ppm, respectively) (Paiva *et al.*, 1993; Lyra, 2007; Santos *et al.*, 2012).

It is assumed that freshwater fish species can regulate Cr over a wide range of environmental conditions and the concentrations of this element tend to increase along the trophic web (Eisler, 1986; Irwin *et al.*, 1997). Aquatic species show a wide range of Cr concentrations in their tissues. Ambedkar and Muniyan (2011) report Cr levels ranging from 0.70 to 1.28 ppm in the muscle of several freshwater species from contaminated areas and Tiller *et al.* (2004) obtained Cr as high as 9.4 ppm in *Oncorhynchus* spp. The concentrations of Cr in ammocoetes ranged from 0.270 to 1.57 ppm and apparently do not seem to change with size ($P = 0.500$). As Cr is one of the metals with lower concentrations in river Minho (mean sediment concentrations ranging from 13.9 to 41.0 ppm) (Lyra, 2007; Moreno *et al.*, 2005) at this time it is difficult to conclude the capacity of ammocoetes to regulate or accumulate Cr. Therefore, study of the response of ammocoetes to environmental levels of Cr is still needed.

Cu and Fe values in ammocoetes of sea lamprey from Minho watershed showed similar ranges to those of sea lampreys ammocoetes from New Brunswick in Canada (Holmes and Youson, 1996). Interspecific differences were
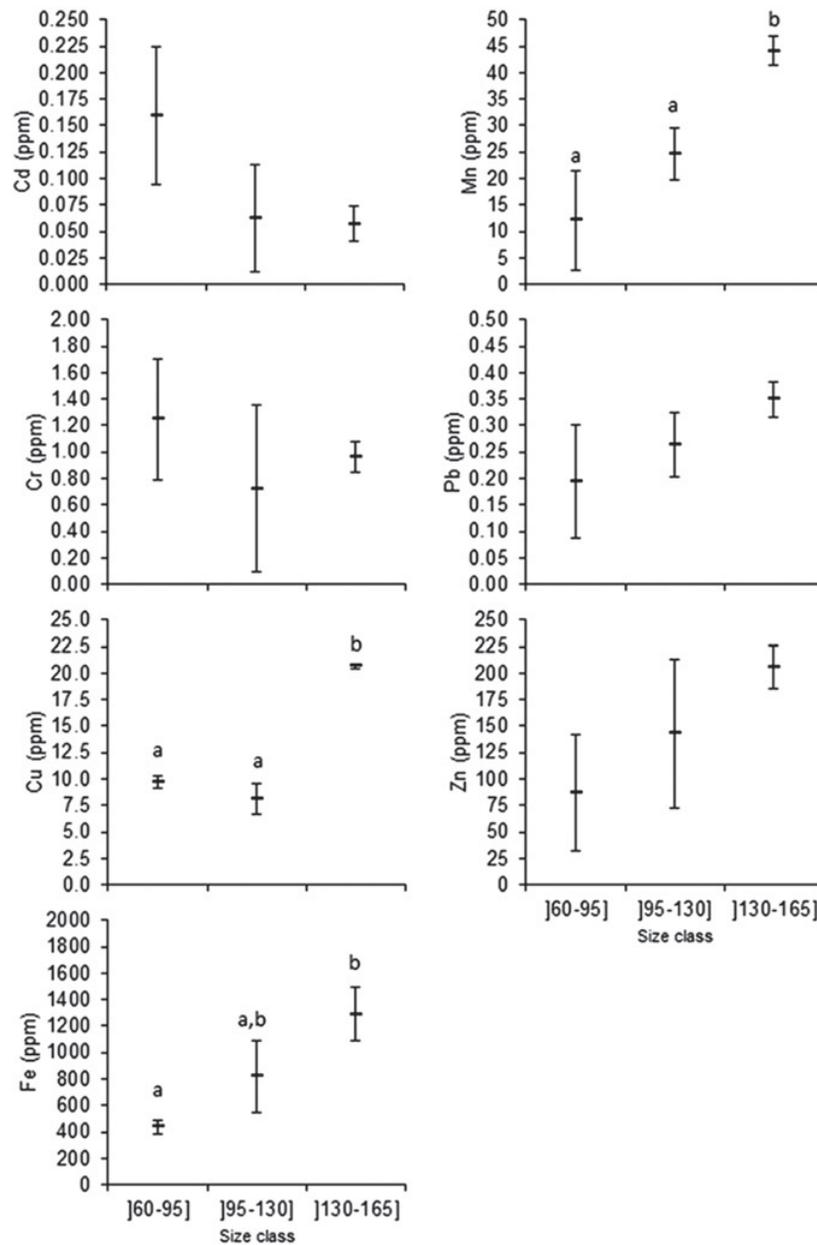
Fig. 2. Concentrations of Cd, Cr, Cu, Fe, Mn, Pb and Zn. Size classes in mm. Bars represent mean $\pm$ SD. Different letters above bars indicate existence of statistical significance between class for each metal ($P < 0.05$).

previously reported in toxicological studies among lamprey species regarding metal accumulation as Fe levels are higher in lamprey *Geotria australis* (Gray, 1851) tissues than in sea lampreys (Macey and Potter, 1986; Sargent and Youson, 1986). The continuous feeding on blood by adult parasitic lampreys leads them to ingest high quantities of Fe and therefore sea lampreys reach Fe concentrations serum as high as 94 500 ppm (Macey and Potter, 1986; Holmes and Youson, 1996; Andersen *et al.*, 1998). Ammocoetes from Minho also seemed to highly accumulate Fe ($F[2,7] = 14.04$, $P = 0.005$), which is reported to correlate with length of time they filter-feed (Tsioros and Youson, 1997). Moreover, Fe is highly abundant in the environment (ranges from 7551 to 34 500 ppm in sediment

samples of Minho) (Moreno *et al.*, 2005; Lyra, 2007) and it was previously reported that Fe accumulation in ammocoetes is site-specific and may reflect different contributions of sediment and waterborne metals (Holmes and Youson, 1996). Cu is less abundant in freshwater ecosystems than Fe (maximum of 36 ppm of Cu in sediment samples of Minho) (Moreno *et al.*, 2005). Nevertheless, ammocoetes seemed to accumulate Cu during the freshwater stage (values ranging from 6.5 to 20.8 ppm, $F[2,7] = 147.36$, $P = 0.000$) and also seem to reach higher values than during the marine stage (spawning sea lamprey muscle concentrations of Cu range from 2.4 to 3.5 ppm) (authors unpublished data; Pedro *et al.*, 2013).

Mn is an essential metal for metabolism of amino acids, lipids and carbohydrates; however, reproductive malfunctions can occur when in high concentrations (Srivastava and Agrawal, 1983). Mn in sediments of river Minho is fairly high (maximum of 302 ppm in river Minho) (Moreno *et al.*, 2005; Reis *et al.*, 2009) and enrichments with this metal might occur in interstitial waters of estuarine habitats (Widerlund and Ingri, 1996; Gaillardet *et al.*, 2011). Ammocoetes live and feed in this layer and seem to accumulate this metal during this stage (values ranging from 5.7 to 46.4 ppm, $F[2,8] = 19.70$; $P = 0.02$).

Once in blood circulation and after intestinal absorption, Pb will be deposited in the liver and skeletal bodies, causing cellular disruption and damages to mitochondria, altering metabolism and replacing lipids and essential metals such as Mn and Zn (Pokras and Kneeland, 2009). A great variability on Pb levels was reported to occur in adult riverine lamprey *Lampetra fluviatilis* (Linnaeus, 1758) (Protasowicki *et al.*, 2011). A relatively wide range of Pb concentrations in ammocoetes of sea lamprey was also obtained (values ranging from 0.129 to 0.374 ppm), which apparently increase with size (Fig. 2). However, such a fact was not statistically confirmed ($P = 0.191$).

Both ammocoetes and adult sea lampreys seem to highly accumulate Zn (overall values ranging from 48.3 to 218.6 ppm in ammocoetes, values ranging from 19 to 33 ppm in adult sea lamprey muscle and from 110 to 137 ppm in gonads; authors unpublished data; Pedro *et al.*, 2013). Ammocoetes might be capable of regulating this metal due to their use in physiological functions related to the regulation of pH, CO_2 transport, protein replication and DNA transcription (Hogstrand and Wood, 1996; Dural *et al.*, 2007). An apparent pattern of accumulation of this metal seemed to occur; however that fact was not statistically confirmed ($P = 0.085$), because a wide range of concentrations were found in ammocoetes. This was similar to previously reported studies with adult sea and riverine lampreys (Protasowicki *et al.*, 2011; Pedro *et al.*, 2013).

Conclusions

The levels of metals in sea lampreys ammocoetes and in other aquatic species from river Minho were scarce until this moment. Our results point out that ammocoetes of sea lamprey *P. marinus* from Minho watershed can accumulate considerable quantities of some elements, but mainly Fe and Zn and might work as input of metals into trophic webs. Low concentrations of Cr and Pb are in accordance with consideration of the Minho watershed as a metal uncontaminated and a metal-pristine area (Reis *et al.*, 2009; Mil-Homens *et al.*, 2012; Santos *et al.*, 2012).

The outstanding accumulation of some metals (such as Fe) and the capacity to detoxify and possibly regulate some elements (Al and possibly Zn) (Holmes and Youson, 1996) might compromise the use of this organism as an indicator of contamination and such possibility should be further studied.

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