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Mercury Distribution in Key Tissues of Caged Fish (*Liza aurata*) along an Environmental Mercury Contamination Gradient

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Abstract—Mercury contamination of aquatic ecosystems became a worldwide environmental problem. Thus, the intense mercury contamination observed at Laranjo basin (Ria de Aveiro, Portugal), was the motivation for the present study. The main goal was to improve the knowledge about mercury accumulation dynamics in key tissues of fish. *Liza aurata* specimens were caged (for 3 days at bottom and surface to evaluate the influence of sediment proximity) within Laranjo basin, at 3 locations differing on their distance to the contamination source. Total mercury (Hg_t) was quantified in three different target tissues—blood, liver and muscle, as well as in water and sediment. Comparative tissues analysis of Hg_t accumulation revealed the following hierarchy: liver > blood > muscle, highlighting liver as the preferential tissue to mercury accumulation. Globally, Hg_t levels ranged from 0.11 μg/g (muscle) to 1.13 μg/g (liver). Liver and blood showed to reflect the environmental contamination status after a short-term exposure (3 days). Analyzing tissue/tissue ratios, it was possible to infer a buffering action of liver, against mercury accumulation and its subsequent toxicity. In addition, blood role in mercury transportation and redistribution was better understood. Finally, the importance of the direct mercury uptake from the water (via gills) was ascertained.

Keywords: mercury, bioaccumulation, fish

INTRODUCTION

Mercury contamination of aquatic ecosystems became a worldwide environmental problem, representing a worrying threat to biota. Mercury is a toxic and hazardous metal that occurs in the aquatic environment due to natural phenomena or anthropogenic activities (Tchounwou *et al*., 2003). This metal has high affinity for suspended particles, which conducts its removal from the water column and accumulation in sediments. Thus, sediments function as deposit and as source of mercury to the pore water and biota (Ramalhosa *et al*., 2001).

Laranjo Basin is an area located in the north-western region of Portugal (Ria de Aveiro), where a well-defined mercury gradient was identified, as a result of

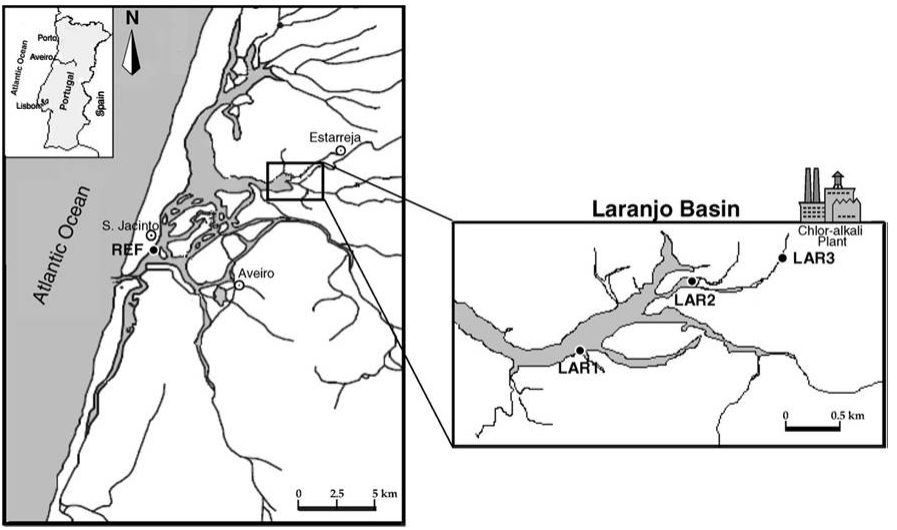

Fig. 1. Map of Ria de Aveiro (Portugal) with locations of fish caging sites (●). The respective ordinates are: reference site (REF) - 40°40′26″ N, 8°43′17″ W; LAR1 - 40°43′24″ N, 8°37′55″ W; LAR2 - 40°43′49″ N, 8°36′53″ W; LAR3 - 40°44′04″ N, 8°36′02″ W.

a chlor-alkali plant discharges (Pereira *et al.*, 1998), and no other important contaminants were detected. Thus, this area offers to researchers a unique opportunity to study mercury accumulation processes in fish under realistic conditions.

The increase of mercury loads to the aquatic environment has resulted in a great accumulation of this metal in fish tissues, affecting adversely the ichthyopopulations. Due to its wide distribution and trophic position, fish are particularly able to reflect aquatic contamination by metals, thus being desirable components of biomonitoring programs. Therefore, from the standpoint of both human and ecosystem health risk assessment, fish emerge as a suitable bioindicator (Mieiro *et al.*, 2009).

Mercury quantifications in tissues are, generally, the best way to understand the dynamics of this contaminant in the fish body, becoming important to assess its distribution and subsequent retention. According to Van der Oost *et al.* (2003), bioaccumulation should be addressed including toxicokinetics, metabolism, and organ-specific bioaccumulation.

While previous fish work almost exclusively focused on mercury accumulation in liver and muscle, the present research brings a new perspective taking into account blood and its role in mercury transportation and distribution, as well as tissue/tissue concentration ratios. Thus, this study conducted at Laranjo basin using *Liza aurata* to improve the knowledge about mercury accumulation dynamics in blood, liver and muscle of *L. aurata*, clarifying the relation between the environmental contamination and the metal uptake.

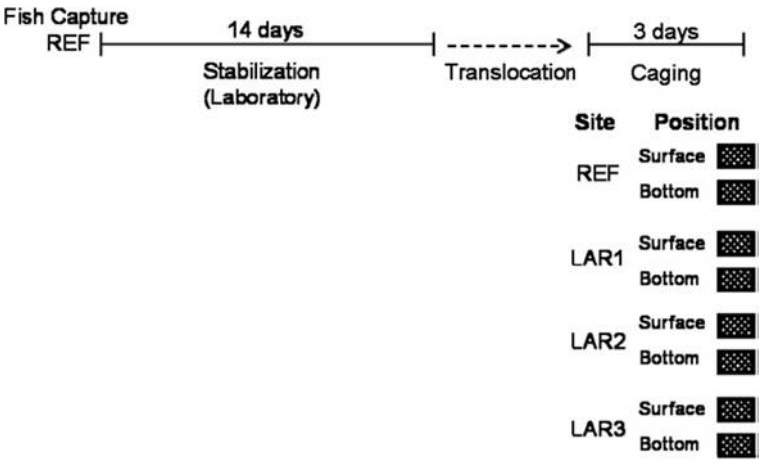

Fig. 2. Schematic representation of the experimental design.

MATERIALS AND METHODS

Laranjo basin, near Estarreja (Fig. 1), is the most contaminated area in the Ria de Aveiro, a lagoon along the north-western coast of Portugal. This area received chlor-alkali plant discharges continuously for five decades, resulting in the generation of a mercury contamination gradient. Nonetheless the industry improved the production process, decreasing the mercury release, high mercury levels are still present in the sediments; its progressive resuspension is responsible for metal exportation and increased availability to organisms (Pereira *et al*., 1997, 1998). S. Jacinto area was selected as a reference site, due to the lagoon entrance proximity (Fig. 1), the distance to the main polluting sources and the low-contamination load (Pacheco *et al*., 2005).

Experimental design

Golden grey mullet (*L. aurata*) is a pelagic species that is in frequent contact with sediments, feeding on small benthic organisms and detritus. Juvenile specimens were used to minimize the interference of variables such as gender and contaminant accumulation. The experimental protocol is depicted in Fig. 2. Fish with an average weight of 13.5 ± 0.9 g and length of 12.1 ± 0.6 cm were caught at the low-contaminated site named REF (S. Jacinto, Figs. 1 and 2), and then transported to the laboratory and allowed to acclimatize for two weeks prior to experimentation. Fish were fed daily with polychaete worms (*Nereis* sp.) collected from a clean area of the Ria de Aveiro. The experiment was conducted by caging groups of 10 mullets (n = 10) in three sites of the Laranjo basin that differed in distance from the metal contamination source (LAR1—the farthest site, LAR2 and LAR3—closest site to the mercury source) for 3 days (Fig. 1). In order to assess the effect of fish position in the water column on mercury uptake, two

cages were placed at each site, one at the surface and the other close to the sediment. Reference groups (REF) were caged in S. Jacinto. During field exposure, fish were kept without any food supply. After exposure, Hg_t content was determined in blood, liver and muscle, as well as in water (dissolved and SPM) and sediment.

At each exposure site, hydrological parameters including temperature, dissolved oxygen, salinity, and pH were measured at the sub-surface and bottom levels during low and high tide conditions. The water column depth was also evaluated and the turbidity was measured using a 20 cm black and white Secchi disc. A 3 L van Dorn bottle was used to collect water samples from the bottom. Sediments samples were collected (±5 cm depth) with a stainless steel shovel, at the same sites for Hg_t analysis.

Mercury analyses

Total mercury (Hg_t) in the water column and in sediment

Water samples were filtered with 0.45 μm Millipore filters. The filtrate was then acidified with HNO_3 (Merck, "mercury-free") to pH < 2 and stored at 4°C until analysis. Suspended particulate material (SPM) collected in the filters was oven dried at 60°C until constant weight. Procedure blanks were always run with samples and its contribution corrected when necessary.

Total dissolved mercury concentrations were measured by Cold Vapour Atomic Fluorescence Spectrometry (CV-AFS; PSA model Merlin 10.023 equipped with a detector PSA model 10.003), using tin(II) chloride as reducing agent, after addition of 500 μL of a saturated solution of potassium persulfate to 50 mL of filtered water and irradiation by a UV lamp (1000 W) for 30 min. Following irradiation, the excess of oxidant was reduced with 37.5 μL of hydroxylamine solution 12% (w/v), prior to analysis (Mucci *et al.*, 1995). The equipment was calibrated every day with acidified (HNO_3 "mercury free") standard solutions prepared from a 1000 mg/L solution (BDH). The detection limit of CV-AFS technique was 0.5 ng/L.

Mercury in SPM was also determined by CV-AFS, after digestion of the filters in glass reactors with 50 mL of a solution 4 mol/L HNO_3, at 60°C for 4 h (Pereira *et al.*, 1995, 1998). Results presented in this study for total mercury concentrations in water column (total water), are always the sum of dissolved and suspended particulate matter metal concentrations, expressed in μg/L, taking in account the mass of SPM and the volume of filtered water.

Sediment samples were oven-dried to constant weight at 60°C homogenized and sieved through a 1 mm sieve, prior to analysis. Samples were analysed by Atomic Absorption Spectrometry (AAS) with thermal decomposition of the sample using the equipment LECO AMA-254 (Advanced Mercury Analyser), with no pre-treatment of samples (Costley *et al.*, 2000). Accuracy was assessed with certified reference materials (CRMs) from the National Research Council of Canada (NRCC). The CRMs used were MESS-3 (0.091 ± 0.009 mg Hg/Kg) and PACS-2 (3.04 ± 0.20 mg Hg/Kg) (both for sediments).

Table 1. Environmental total mercury (Hg_t) concentrations in water and sediment of reference (REF) and contaminated (LAR1, LAR2, LAR3) sites on the caging experiment at Ria de Aveiro.

Site	Tide	Position	Total water Hg (μg/L)	Sediment (ng/mg)
REF	Low	Surface	0.6272	0.001
		Bottom	0.8245	
	High	Surface	0.258	
		Bottom	mv	
LAR1	Low	Surface	0.6833	3.0
		Bottom	0.7542	
	High	Surface	0.6876	
		Bottom	0.4056	
LAR2	Low	Surface	1.5917	7.1
		Bottom	3.3948	
	High	Surface	1.4788	
		Bottom	3.2966	
LAR3	Low	Surface	6.541	36.9
		Bottom	49.5915	
	High	Surface	2.585	
		Bottom	15.2026	

mv = missed value.

Total mercury (Hg_t) in fish tissues

Blood, liver and muscle samples were analyzed by AAS with thermal decomposition of the samples. As performed for the sediment analysis, accuracy was assessed using certified reference materials, namely TORT-2 (0.27 ± 0.06 mg Hg/kg) and DORM-2 (4.64 ± 0.26 mg Hg/kg) (both for biological samples).

RESULTS AND DISCUSSION

Inter-site comparisons were carried out separately for surface or bottom groups. The surface group at LAR1 was not considered due to cage disappearance.

Mercury levels in the environmental

Total mercury concentrations in water column (Table 1) at the reference site and at the three sampling sites at Laranjo basin were always very low (less than 10 μg/L in most of the samples and only with two values higher than 40 μg/L). All the values are less than 1000 μg/L, the permitted value by law for mercury concentrations in water column of aquatic systems.

During high and low tide conditions total mercury concentrations in the water column are of the same order of magnitude. The higher mercury concentrations were found at the bottom almost in all studied stations located in

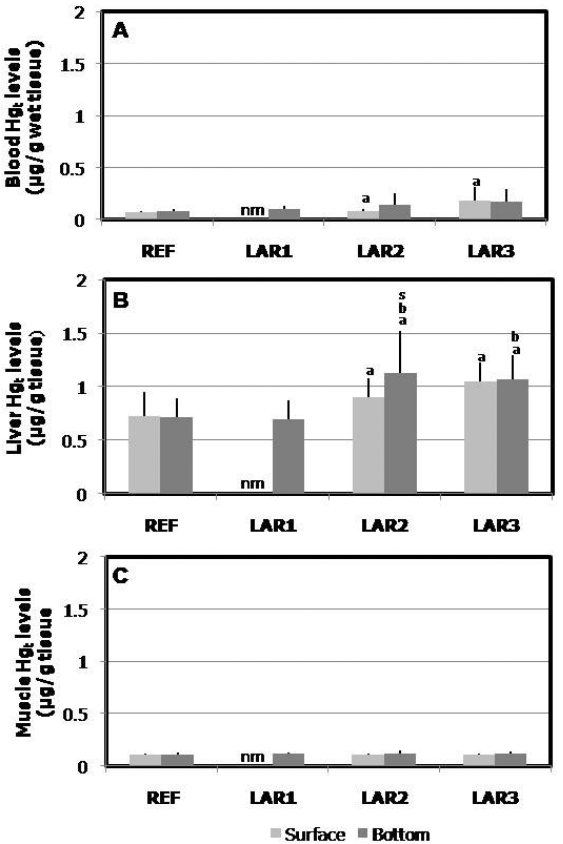

Fig. 3. Total mercury (Hg_t) concentrations in blood (A), liver (B) and muscle (C) of *Liza aurata*, caged during 3 days at different Ria de Aveiro locations, i.e. within a mercury-contaminated area (Laranjo basin-LAR1, LAR2 and LAR3) and a reference site (REF). Letters denote statistically significant differences ($p < 0.05$): (a) vs. REF; (b) vs. LAR1; (s) vs. surface. Error bars represent the standard error. nm = not measured.

the Laranjo basin. The differences observed between mercury concentrations in bottom and surface layers are attenuated as we move farway from the contamination source.

Mercury concentrations in sediments (Table 1) revealed the pronounced human-induced environmental mercury gradient in the lagoon with the highest concentrations found near the contamination source. This way, the results obtained for mercury concentrations in the sediments are mostly related with distance to the industrial source of the metal, with concentrations as high as 37 ng/mg were measured in the most contaminated area, but in the reference site concentrations were very low (0.001 ng/mg).

Table 2. Average of inter-tissue ratios (±SD) of *Liza aurata* caged during 3 days at different Ria de Aveiro locations, i.e. within a mercury-contaminated area (Laranjo Basin—LAR1, LAR2 and LAR3) and a reference site (REF). Letters denote statistically significant differences ($p < 0.05$): (a) vs. REF; (b) vs. LAR1; (c) vs. LAR2.

	Site	Position	Inter-tissue ratio		
			Blood	Liver	Muscle
Tissue/Blood	REF	Surface	—	10.14 ± 2.30	1.60 ± 0.30
		Bottom	—	9.14 ± 4.75	1.34 ± 0.50
	LAR1	Surface	—	nm	nm
		Bottom	—	7.52 ± 2.57	1.24 ± 0.33
	LAR2	Surface	—	12.77 ± 2.66	1.50 ± 0.27
		Bottom	—	8.10 ± 15.76	1.64 ± 1.55
	LAR3	Surface	—	9.66 ± 3.63(c)	0.76 ± 0.42
		Bottom	—	8.60 ± 7.75	1.02 ± 0.63
Tissue/Liver	REF	Surface	0.10 ± 0.02	—	0.15 ± 0.03
		Bottom	0.33 ± 0.68	—	0.16 ± 0.03
	LAR1	Surface	nm	—	nm
		Bottom	0.15 ± 0.05	—	0.17 ± 0.04
	LAR2	Surface	0.08 ± 0.02	—	0.12 ± 0.01
		Bottom	0.12 ± 0.08	—	0.11 ± 0.04(b)
	LAR3	Surface	0.17 ± 0.11(c)	—	0.10 ± 0.01(a)
		Bottom	0.17 ± 0.13	—	0.11 ± 0.04
Tissue/Muscle	REF	Surface	0.64 ± 0.11	6.68 ± 1.34	—
		Bottom	2.07 ± 4.36	6.73 ± 1.72	—
	LAR1	Surface	nm	nm	—
		Bottom	0.86 ± 0.25	6.13 ± 1.09	—
	LAR2	Surface	0.69 ± 0.15	8.33 ± 0.80	—
		Bottom	1.15 ± 0.99	10.30 ± 5.02(b)	—
	LAR3	Surface	1.70 ± 1.07	9.91 ± 1.61(a)	—
		Bottom	1.38 ± 0.86	9.37 ± 2.56	—

Total mercury (Hg_t) levels in fish tissues

Results concerning blood Hg_t levels (Fig. 3A) revealed significant increases in comparison to the REF site only among surface groups, namely at LAR2 and LAR3, corresponding to a two times increment in the last one. Though apparent, the increases detected for LAR2 (2 times) and LAR3 (2.3 times) in bottom groups were not statistically significant. No differences were detected between surface and bottom groups within each site.

Hepatic Hg_t levels were significantly higher in LAR2 and LAR3 compared with the REF site, in both surface and bottom groups (Fig. 3B). Taking into account the bottom groups, LAR2 and LAR3 displayed Hg_t levels significantly higher than LAR1. Differences between surface and bottom groups were only detected at LAR2, pointing out the importance of the sediment proximity on mercury uptake and liver accumulation. It was also confirmed that mercury

released from the sediment can generate layers in water column with different mercury levels. Therefore, both tissues seem to reflect the environmental contamination status (at LAR2 and LAR3 sites) after a short-term exposure (3 days).

On the other hand, muscle Hg_t levels showed no significant differences between LAR and REF sites (Fig. 3C), revealing that longer exposures are needed for the translation of mercury uptake into a significant burden increase in this tissue.

The comparative analysis of the assessed tissues established the following hierarchy: liver > blood > muscle, highlighting liver as the preferential tissue to mercury accumulation. Globally, Hg_t levels ranged from 0.11 μg/g (muscle) to 1.13 μg/g (liver). In order to better understand the toxicokinetics of mercury, tissue-to-tissue Hg_t ratios were calculated (Table 2). The highest ratios were determined for liver/tissue, being the maximum found for liver/blood. Spearman rank correlation (r) analysis was performed and have revealed a significant positive correlation between Hg_t levels in blood and liver ($r = 0.8027$). Moreover, significant differences between ratios were only found when liver/tissue was taken into account, and concerning the most contaminated sites (LAR2 and LAR3). Considering these facts, it was possible to infer a buffering action of liver, protecting the other tissues, namely muscle, against mercury accumulation and the subsequent toxicity.

CONCLUSIONS

The current study demonstrated:

- The importance of the direct mercury uptake from the water (via gills) as an uptake route was ascertained, since the dietary uptake was almost completely restricted by fish caging.
- The analysis of tissue-to-tissue relations provided a new perspective, contributing to the knowledge of mercury toxicokinetics.
- The important role of blood in mercury transportation and redistribution was better understood.

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