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Baseline

Looking at the aquatic contamination through fish eyes – A faithful picture based on metals burden

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ABSTRACT

This study describes for the first time metal accumulation in the eyes of native golden grey mullet (*Liza aurata*) coupled with water/sediment quality assessment. Sampling was performed in the Tagus estuary (Portugal) where a confined area (Barreiro) is severely contaminated by metal/loids. Levels of As, Cu, Pb, Hg and Cd in sediments from Barreiro were one order of magnitude higher than those from the reference site. Data on water column pointed also to a higher availability of Cu, Pb, Cd and Hg (including MeHg) at Barreiro. Accordingly, fish eyes accumulated higher levels of metal/loids at Barreiro than at the reference site. These findings support the use of fish eyes as a target organ in environmental health assessment since they reflect sediment and water contamination. It points also to the importance of evaluate eye changes at structural/functional levels in order to examine in what extent accumulated metals could compromise this perceptive system.

Estuarine habitats are potentially impacted by many anthropogenic pressures, being important sinks of pollutants where metals represent a particular threat for both aquatic wildlife and humans (Mieiro et al., 2009). Since metals tend to concentrate in aquatic organisms being virtually non-degradable, they produce long lasting effects upon the environment even after their major sources had been removed. Some metals like Cu, Zn and Fe control significant metabolic and signaling functions in aquatic organisms. Other metal and metalloids, such as As, Pb, Cd and Hg, are toxic.

Fish play a major ecological role in aquatic food webs due to their function as a carrier of energy from lower to higher trophic levels. Despite their high mobility, fish are considered the most feasible organisms for environmental health assessment (Van der Oost et al., 2003). In fact, several international monitoring protocols and European Directives include measurements in fish species. In particular, mullets (e.g. *Liza aurata*, *Liza saliens*, *Liza ramada* and *Mullus barbatus*) have been recommended as key bioindicators and are extensively used in the assessment of fish health and pollution in aquatic systems (e.g. Zorita et al., 2008; Pereira et al., 2010; Mieiro et al., 2011). Numerous works quantified contaminants in fish organs as a mean to evaluate environmental quality, seeking for causal relationships with animals' health. It has been stated that liver is likely to be the best choice, followed by the kidney (e.g. Amado et al., 2006; Reynders et al., 2008; Mieiro et al., 2011). In fact, these organs play a key role in fish metabolism and it was described that metal sequestration occurs associated with metallothionein synthesis (Viarengo and Nott, 1993). Gills were also demonstrated to be useful in environmental health assessment being able to accumulate different metal levels according to the environment availability (e.g. Pereira et al., 2010; Fernandes et al., 2012). This is partially related with the intense cell division of gills' cells that allows this organ to reflect recent exposures. The previous feature seems to be particularly useful when fish are used as bioindicators due to their mobility. In contrast, it was also reported that the rapid cellular turnover could lead to the loss of trace metals signal and thus be a disadvantage in the environmental monitoring (Dove and Kingsford, 1998).

The eye is a key sensory system in fish due to its role in collecting and focusing images and transforming them into neural signals. Moreover, the eye has a wide surface area in continuous contact with the external medium and thus could be a relevant uptake route of metals and metalloids. It was previously found that large amounts of As were accumulated in the eye of a freshwater fish collected at a contaminated lake (Takatsu et al., 1999). Nevertheless, there are no studies associating environmental data with accumulation levels in fish eyes. In laboratory exposures with zebrafish larvae, it was found that the maximum accumulation of organic mercury was localized in the eye (lens epithelium), followed

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by other organs like brain or gastrointestinal tract (Korbas et al., 2008). This study suggested that the impairment of visual process in fish by mercury may arise from its direct effect on ocular tissue (Korbas et al., 2008). A more detailed study of the same research group showed that methylmercury (MeHg) accumulates in the secondary lens fibers immediately underlying the lens epithelial cells (Korbas et al., 2013). This is in agreement with several studies in mammals (including humans) that reported the accumulation of metals in eyes (e.g. Erie et al., 2005) as well as a visual deterioration associated with Pb and Hg (e.g. Warfvinge and Bruun, 1996; Erie et al., 2005). In fact, the mammals' retinal pigment epithelium is considered a metal-chelating tissue that is capable of binding essential and toxic metals due to their high affinity to melanin in retinal pigment epithelium melanosomes (Erie et al., 2005). As a result of the unique morphology and stability of eye lens over the life of an organism, it has been suggested that lens could potentially offer a historical record of Hg exposures affecting fish throughout its lifetime (Korbas et al., 2008).

Despite the eyes relevance on fish physiology and the previous promising insights, this organ has been underemployed in aquatic environmental health assessment. To fill this knowledge gap an investigative biomonitoring study was carried out in the Tagus estuary (Portugal) where a confined area (Barreiro) is severely contaminated by metal/loids, particularly Hg, Pb and As (Vale et al., 2008). Hence, the present study described for the first time metal/loids accumulation in the eyes of the native golden grey mullet (*Liza aurata*) coupled with water/sediment quality assessment.

The Tagus estuary one of the largest in Europe (320 km^2 total area) is historically contaminated by metal/loids from two major industrial sources (e.g. Canário et al., 2005). High levels of As, Pb, Zn, Cu and Hg have been reported in a confined area (Barreiro), namely in sediments and suspended particles (e.g. Canário et al., 2005; Vale et al., 2008) and organisms (e.g. Caçador et al., 2012). An important Natural Reserve area with low anthropogenic impact is located in the northern part of the Tagus estuary, distancing around 20 km from the Barreiro contaminated hotspot. In previous studies, the Natural Reserve of the estuary was selected as a reference area for comparison proposes.

In winter (December) 2011, a survey was carried out at the Tagus estuary during low-tide and juveniles of the golden grey mullet (*L. aurata*) were collected (n = 10) using a traditional beach-seine net. Two sampling sites were selected taking into account previous environmental quality studies (Fig. 1): Barreiro (BAR) in the most contaminated area and Vale Frades (VF) located in the high protected area of the Tagus Natural Reserve and thus, selected as the reference site. Fish biometrical parameters, such as weight and total length ranged from 140to 210 g and 25 to 35 cm, respectively. Immediately after catching, fish were anesthetized, sacrificed and then eyes were removed, carefully washed with distilled water and frozen (−20 °C) for posterior metal determinations.

Sub-surface water (at 0.2 m depth) was sampled in triplicates to polypropylene bottles for the determination of Cu, Pb, Cd, Hg and MeHg in dissolved fraction of water column. At the same depth, temperature, salinity, pH and dissolved oxygen were measured *in situ* in triplicates with an YSI 650 meter. Surface sediments (approximately 2 cm depth) were also collected in the two sites for metal determinations.

Suspended particulate matter (SPM) was obtained by filtering 250 mL of water through cellulose acetate membranes (0.45 μm) and determined gravimetrically. Copper, Pb and Cd in the collected waters (triplicate samples) were measured using diffusive gradients of thin films (DGT). All DGT holders, Chelex-100 resins and diffusive gels were purchased from DGT Research (Lancaster, UK). The DGT devices were deployed in 2-L polypropylene bottles with unfiltered sampled water and stirred at constant temperature for 48 h. After devices retrieval, resins were eluted by immersion in

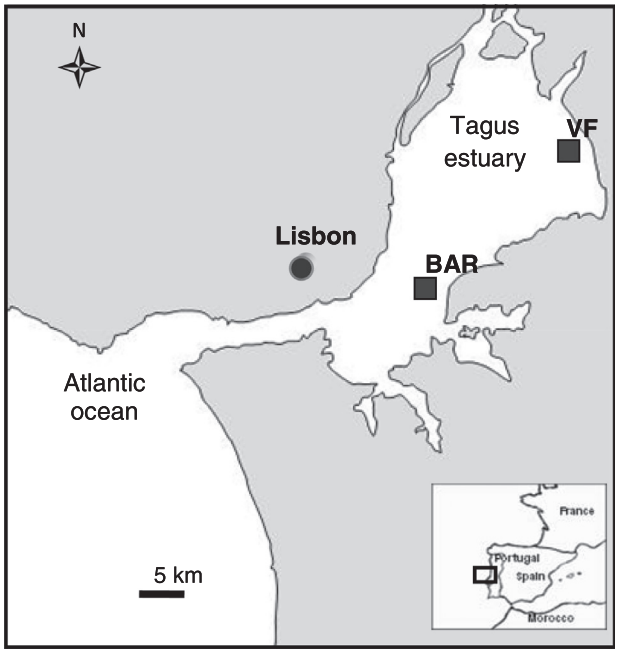

Fig. 1. Location of the sampling sites at the Tagus estuary (Portugal): Vale Frades (VF) (38° 45.186′N, 8° 58.789′W); Barreiro (BAR) (38° 40.272′N, 9° 5.207′W).

5 mL of 1 M HNO_3 (prepared from suprapur nitric acid) at a minimum of 24 h. Eluates were analyzed directly by a quadropole inductively coupled plasma mass spectrometer (ICP-MS) (Thermo Elemental, X-Series). All eluates were analyzed with reagents blanks and an international standard of river water (SLRS-4) used to control eventual contaminations during the analytical procedure and the procedure accuracy, respectively. Water concentrations of Cu, Pb and Cd were calculated according to Zhang and Davison (1999). Total dissolved mercury was determined following U.S.EPA method 1631 (U.S.EPA, 2002). Briefly water samples were preserved by the addition of 0.5% BrCl until analyses (less than one week after collection). The samples were then analyzed by cold-vapor atomic fluorescence spectrometry (CV-AFS) with a PSA model Merlin 10.023 equipped with a detector PSA model 10.003 using $SnCl_2$ reduction. BCR-579 reference material was used to control the accuracy of our procedure.

MeHg in water samples was determined following U.S.EPA method 1630 (U.S.EPA, 2001) by distillation of 50 mL sub-samples, after addition of 1% $C_5H_9NS_2{\cdot}NH_3$ as a complexing agent. Mercury was ethylated with $NaB(C_2H_5)_4$, purged with argon, collected on TenaxTM traps, separated with a GC, thermally desorbed to Hg(0) for detection of MeHg with a Brooks Rand Model III CV-AFS. All batches of samples analyzed for MeHg included at least one method replicate, and at least three analytical replicates of certified reference material (SQC-1238) (Sigma–Aldrich RTC).

Sediment samples (100 mg) were mineralized completely with HF (40%) and Aqua Regia (HCl-36%:HNO_3-60%; 3:1) in closed Teflon bombs (100 °C for 1 h), evaporated to near dryness (DigiPrep Hot-Block – SCP Science), redissolved with 1 mL of doubled-distilled HNO_3 and 5 mL of ultra-pure water, heated for 20 min at 75 °C, heated again for 20 min at 90 °C after ultra-pure water addition (25 mL), and diluted to 50 mL with ultra-pure water. The concentrations of Zn, As, Cu, Pb and Cd were determined by ICP-MS. Sediment samples were analyzed for total Hg by atomic absorption spectrometry (AAS) with thermal decomposition with gold amalgamation, using a mercury analyzer (AMA) LECO 254 (Costley et al., 2000). Reagents blanks and international certified standards of sediments from the National Research Council of Canada