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# Fish eyes and brain as primary targets for mercury accumulation — A new insight on environmental risk assessment

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## HIGHLIGHTS

- The propensity of fish brain, eye wall and lens to Hg accumulation was evaluated.
- Brain, eye wall and lens faithfully reflected water and sediment Hg contamination.
- Fish brain and eyes are key target organs in environmental health assessment.
- MeHg was preferentially accumulated in the three neurosensory structures than iHg.
- Fish lens exhibited a higher Hg retention capacity than brain and eye wall.

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## ABSTRACT

Fish eyes and brain are highly susceptible to environmental Hg exposure but this issue is still scarcely investigated, mainly regarding methylmercury (MeHg) accumulation. Yet, Hg levels in fish lens have not been previously examined under field conditions. Total Hg (tHg), MeHg and inorganic Hg (iHg) levels were assessed in the brain, eye wall and lens of the golden grey mullet (*Liza aurata*) from an Hg contaminated area, both in winter and summer, together with water and sediment levels. Sampling was performed at Aveiro lagoon (Portugal) where a confined area (LAR) is severely contaminated by Hg. Fish brain, eye wall and lens accumulated higher levels of tHg, MeHg and iHg at LAR than the reference site, reflecting faithfully environmental spatial differences. The brain and eye wall responded also to the winter–summer changes found in water and sediment, accumulating higher levels of MeHg (and tHg) in winter. Contrarily, lens was unable to reflect seasonal changes, probably due to its composition and structural stability over time. The three neurosensory structures accumulated preferentially MeHg than iHg (MeHg was higher than 77% of tHg). Lens exhibited a higher retention capacity of MeHg (mean around 1 μg $g^{-1}$ at LAR), accumulating higher levels than the other two tissues. Interestingly, MeHg and iHg levels were significantly correlated for the brain and eye wall but poorly associated within the two analysed eye components. The high levels of MeHg found in the brain, eye wall and lens could compromise their functions and this needs further research.



## 1. Introduction

The nervous system, mainly its sensory organs and pathways, exerts control over a wide array of physiological and behavioural responses, and so, exposure to neurotoxicants has the capacity to affect organism fitness. Mercury (Hg) compounds (including methylmercury — MeHg) have triggered major concerns in terms of environmental and human health. Though Hg is recognised as a pernicious, persistent and ubiquitous contaminant in natural waters, including estuarine and marine environments, the assessment of its potential to induce neuronal and sensory dysfunctions in aquatic animals is an almost unexplored issue.

Fish are key components of the trophic chains, also playing an important role signalling water pollution, once they react with great sensitivity to changes in the aquatic systems (Van der Oost et al.,

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2003; Guilherme et al., 2008; Mieiro et al., 2010). Keeping this in view, numerous works quantified Hg (including MeHg) in fish organs (e.g. liver, gills) as a mean to evaluate environmental quality, trying to establish causal relationships with fish health (e.g. Zorita et al., 2008; Pereira et al., 2010; Mieiro et al., 2011). Generally, the brain and the eyes have been disregarded in those studies. Unexpectedly, total mercury levels in the brain reflected better than metabolic organs (such as the liver and kidney) the concentrations reported in the environment (Mieiro et al., 2009). These authors also found that fish brain can have an important role in biomagnification processes, pointing to its relevance in environmental risk assessment. Recently, fish eyes revealed to faithfully reflect water and sediment contamination by metals (Pereira et al., 2013). However, to the best of our knowledge, no other field studies have correlated metal levels in fish brain or eyes with contamination levels in the environment.

In mammals, the brain has been demonstrated as a primary target for Hg compounds, namely MeHg, and neurological dysfunctions have been widely studied in humans and rodents, being reported the occurrence of intellectual impairments, irritability and fine motor alterations (Aschner et al., 2007; Stringari et al., 2008; Farina et al., 2013). Identical to mammals, fish brain is highly susceptible to environmental Hg exposure, as demonstrated by the few available articles reporting neurodegenerative damage and disturbances on sensory processing (Baatrup et al., 1990) as well as behaviour changes (Berntssen et al., 2003). This is in line with the assertion that both organic and inorganic forms of Hg could be damaging agents to fish central nervous system (Berntssen et al., 2003).

The eye is a key sensory organ that collects and focuses images, transforming them into neural signals. The fish eye has a wide surface area in continuous contact with the external medium and thus could be a relevant uptake route of Hg. Exposure of zebrafish larvae to waterborne MeHg revealed that this metal form was preferentially accumulated in the eyes, specifically in the outer layer of the lens (Korbas et al., 2013). Interestingly, MeHg levels in the lens increased even after exposure, indicating that MeHg is accumulated in this eye component also through redistribution from other tissues (Korbas et al., 2013). These results clearly show that Hg targets the eyes and particularly the lens. The direct MeHg action on eye sensory cells may be partly responsible for visual disturbances (Korbas et al., 2008, 2013). As a result of the unique morphology and stability of eye lens over the organism's life, it has been suggested that lens could potentially offer a historical record of Hg exposures affecting fish throughout its lifetime (Korbas et al., 2008).

The promising results obtained by Korbas et al. (2008, 2010, 2012, 2013) with fish brain and eyes after MeHg exposure and the relevance of these organs on fish physiology pointed to their potential in environmental health assessment. Hence, the present work aimed to study Hg accumulation (including MeHg) in the brain, eye wall and lens in the golden grey mullet (*Liza aurata*) inhabiting an Hg contaminated system, coupled with water and sediment contamination. It was also intended to clarify tissue specificities and the influence of winter–summer variations of environmental conditions on accumulated Hg levels. This is the first study that investigated Hg levels in the eyes (including lens) in wild fish.

## 2. Material and methods

### 2.1. Study area characterization

The Aveiro lagoon (47 km$^2$ of maximum surface area) is a coastal ecosystem located on the northwest coast of Portugal (Fig. 1). It has an inner and enclosed area known as Laranjo basin (a shallow area with 2 km$^2$) that has received Hg effluents from a chloro-alkali plant during around five decades (1950–1994). High levels of Hg are still stored in sediments (Coelho et al., 2005) and could be found in the biota (Guilherme et al., 2008; Mieiro et al., 2010). Due to the absence

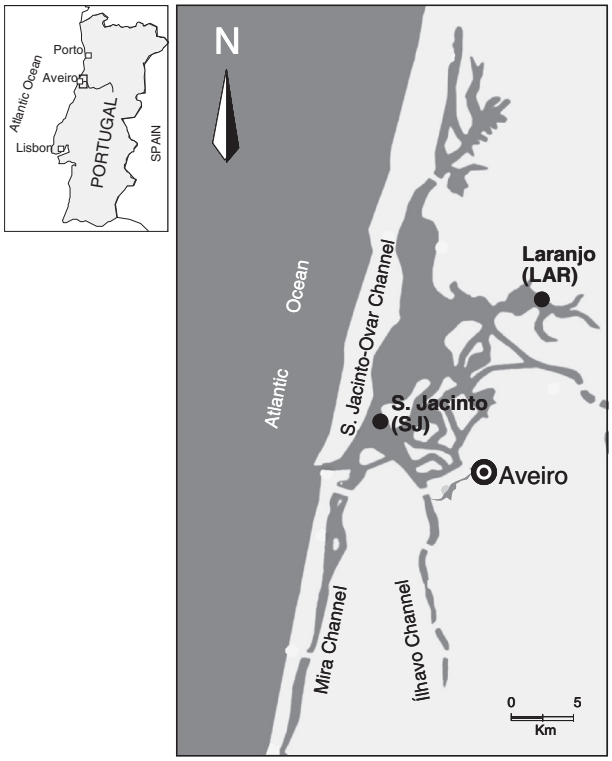


**Fig. 1.** Location of the sampling sites at Aveiro lagoon (Portugal): São Jacinto (SJ) (40°41′00″ N, 8°42′44″ W); Laranjo (LAR) (40°43′28.98″ N, 8°37′35.80″ W).

of other important sources of contaminants, Laranjo basin is considered a "field laboratory", offering a unique opportunity to assess mercury toxicity under realistic conditions (Guilherme et al., 2008). São Jacinto is located near the lagoon entrance, about 10 km from Laranjo basin. In previous studies, São Jacinto area was selected as a reference for comparison proposes, since it was considered unpolluted including in terms of Hg (Guilherme et al., 2008; Mieiro et al., 2010).

### 2.2. Sampling

Two surveys were carried out at Aveiro lagoon, in winter (February 2013) and summer (June 2013), during low-tide, and juveniles of the golden grey mullet (*Liza aurata*) were collected (n = 10) using a traditional beach-seine net. Two sampling sites were selected taking into account previous ecotoxicological studies (Guilherme et al., 2008; Mieiro et al., 2010) (Fig. 1): Laranjo (LAR) in the most contaminated area; São Jacinto (SJ) as the reference site. In winter at LAR and SJ, fish total length was 12.4 ± 0.63 and 11.9 ± 0.15 cm, respectively, while in summer it was 13.6 ± 2.1 and 16.5 ± 2.1 cm, respectively. Immediately after catching, fish were anaesthetized, sacrificed and properly bled, and then the brain and eyes were removed. The eyes were carefully washed with distilled water and gentle rubbing (to remove adherent particles) and dissected for lens removal. The remaining components of the eye, encompassing the eye wall (retina, sclera, cornea, ciliar body, etc.), chambers' content (vitreous and aqueous humours) and other small structures (hereafter collectively called "eye wall", to simplify) were also stored. In the field, the brain, eye wall and lens were instantly frozen in liquid nitrogen. In the laboratory, samples were preserved at −80 °C until further processing for Hg determinations.

Sub-surface water (at 0.2 m depth) was sampled in triplicates to polypropylene bottles for the determination of total Hg (tHg) and MeHg in the dissolved fraction of water column. At the same depth, temperature, salinity and dissolved oxygen were measured in situ in triplicates with an YSI 650 meter (Yellow Springs, USA). Surface