Marine Environmental Research 113 (2016) 70–79

Contents lists available at ScienceDirect

Marine Environmental Research

journal homepage: www.elsevier.com/locate/marenvrev

Insights into neurosensory toxicity of mercury in fish eyes stemming from tissue burdens, oxidative stress and synaptic transmission profiles

Ricardo Pereira [a], Sofia Guilherme [a], Fátima Brandão [a], Joana Raimundo [b], Maria Ana Santos [a], Mário Pacheco [a], Patrícia Pereira [a, b, *]

[a] Biology Department and CESAM, Aveiro University, 3810-193 Aveiro, Portugal
[b] IPMA – Portuguese Institute for the Sea and Atmosphere, Av. Brasília, 1449-006 Lisbon, Portugal

ARTICLE INFO

Article history:
Received 27 July 2015
Received in revised form
28 October 2015
Accepted 29 October 2015
Available online 9 November 2015

Keywords:
Mercury
Ocular toxicity
Oxidative stress
Neurotransmission
Fish eyes

ABSTRACT

This study aims to contribute to fill a knowledge gap related with Hg effects in fish eyes. As a pioneering strategy, Hg bioaccumulation in eye wall of the wild grey mullet (*Liza aurata*) was assessed, together with oxidative stress and synaptic transmission profiles. This approach was complemented by the characterisation of environmental contamination (both in water and sediment). Sampling was conducted in winter and summer in two sites of a Portuguese coastal lagoon (Aveiro lagoon): Largo do Laranjo (LAR) – located in an Hg contaminated/confined area; São Jacinto (SJ) – closer to the lagoon inlet and selected as reference site. Levels of total Hg (tHg), inorganic Hg (iHg) and methylmercury (MeHg) in eye wall were higher at LAR than SJ, both in winter and summer, reflecting the environmental contamination patterns. Moreover, fish caught at LAR in winter showed a significant decrease of catalase and superoxide dismutase activities, in line with the occurrence of peroxidative damage. A different spatial pattern was recorded in summer, being characterised by the increment of glutathione peroxidase and glutathione reductase activities at LAR, as well as total glutathione content, preventing the occurrence of lipid peroxidation. Also in summer, a significant decrease of acetylcholinesterase activity was recorded in fish eyes at LAR, pointed out Hg as an anticholinergic agent. Besides Hg, water salinity had probably an indirect effect on spatial and winter-summer variation patterns of AChE. Current data pointed out that Hg (in iHg and MeHg forms) could exert ocular toxicity both by the promotion of oxidative stress and by the interference with neurotransmission processes.

1. Introduction

Mercury (Hg) has triggered major environmental and human health concerns. This element is present in aquatic environments in organic (mainly methylmercury – MeHg) and inorganic forms, and both can be bioaccumulated by fish, exerting toxicity at different levels. Some of the reported Hg effects in fish are inhibition of hepatic biotransformation enzymes [Guilherme et al., 2008a], oxidative stress in brain [Berntessen et al., 2003], genotoxicity in blood [Guilherme et al., 2008b] and reproductive alterations [Crump and Trudeau, 2009]. Still, there is a knowledge gap on Hg effects at the neurosensory structures, particularly the eyes. Overall, this is a quite relevant scientific issue since most species of fish depends greatly on vision as a source of information. The vision mediates a large range of fish behaviours, from feeding to orientation and schooling [Blaxter and Hallers-Tjabbes, 1992]. Thus, functional disruption of fish eyes due to Hg accumulation could influence fish fitness and survival by reducing or changing the sensory information reaching the brain.

Only a few studies provided evidences on the propensity of fish eyes towards Hg accumulation. It was demonstrated that Hg may target the eyes under environmental exposures [Pereira et al., 2013, 2014] and that MeHg is preferentially accumulated in the eyes in comparison with other key organs such as liver [Korbas et al., 2012, 2013]. A recent innovative study showed that MeHg accumulates in the secondary lens fibers immediately underlying the lens epithelial cells [Korbas et al., 2013]. Abundant Hg deposits were also found in the photoreceptor layer, as well as in the inner and outer nuclear layers [Mela et al., 2010]. This is in agreement with several

* Corresponding author.
E-mail address: pkpereira@ua.pt (P. Pereira).

http://dx.doi.org/10.1016/j.marenvres.2015.10.015
0141-1136/

studies performed 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 to Hg [e.g. Warfvinge and Bruun, 1996; Erie et al., 2005] and other contaminants [Carvalho and Tillitt, 2004]. In fact, mammalian retinal pigment epithelium is considered a metal-chelating tissue that is capable of binding essential and toxic metals in melanosomes due to their high affinity to melanin [Erie et al., 2005].

Though scarce, the available literature pointed out that the impairment of visual processes in fish might arise from Hg direct effect on ocular tissue [Korbas et al., 2008]. Tanan and co-authors (2006) showed electrophysiological anomalous responses on horizontal cells of eyes due to MeHg, while Bonci et al. (2006) found loss of immunoreactivity in specific eye cells after MeHg exposure. The mechanisms that underlie such functional alterations of fish eyes are still poorly understood. In this context, the involvement of oxidative stress is worthy of attention since it has been widely accepted as one of the mechanism of Hg toxicity in other fish organs [Ercal et al., 2001; Shanker and Aschner, 2003; Farina et al., 2013]. Mercury is highly reactive with sulphydryl groups of proteins, forming covalent bonds with reduced glutathione (GSH) and cysteine residues of proteins. GSH serves as the major cytosolic antioxidant, helping to scavenge reactive oxygen species (ROS), namely via the enzymatic activity of glutathione peroxidase (GPX), playing a key role in maintaining the intracellular redox state. Moreover, GSH directly binds to MeHg acting as an endogenous ligand, and the conjugate formed contributes to MeHg efflux from the cells [Ballatori and Clarkson, 1982]. Thus, it has been noted that MeHg promotes a decrease in intracellular GSH levels, which is considered one of its cytotoxic effects [Choi et al., 1996]. Additionally, the inhibition of antioxidant enzymes has been referred as a relevant mechanism involved in oxidative stress due to Hg [Roos et al., 2009]. In this view, it is quite plausible to hypothesize the occurrence of oxidative stress as a central event of functional disruption in fish eyes. Oxidative stress was already identified as a chief process in human's visual deterioration [Spector, 1995; Tezel, 2006], which strongly supports the current hypothesis.

Alterations on retinal synapses are on the basis of a number of visual disturbances in mammals, such as delayed vision and glaucoma [Weber et al., 2008]. Despite the similarities of electrical synaptic transmission in vertebrates, no studies are available on the compromise of fish vision due to changes of retinal synaptic function. Acetylcholinesterase (AChE) is a paradigmatic endpoint of neurotransmission since the acetylcholine (ACh)-AChE system forms an important component of the nervous activity. AChE of several fish tissues seems to be sensitive to Hg exposure that was already proposed as an active anticholinesterase agent [Shaw and Panigrahi, 1990]. Indeed, *in vivo* studies revealed that Hg was on the basis of decreasing activity of AChE in fish brain [Shaw and Panigrahi, 1990] and muscle [Jesus et al., 2013]. Fish eyes were never analysed for AChE after Hg exposure, although this endpoint has been presented as a reliable indicator of visual degeneration in mouse [Bytyqi et al., 2004].

To the best of our knowledge, oxidative stress and AChE in fish eyes due to Hg accumulation was not previously described. Hence, the current work was designed to clarify the occurrence of oxidative stress and AChE activity inhibition in the eyes of the golden grey mullet (*Liza aurata*) after Hg environmental exposure (Aveiro lagoon, Portugal). For this purpose, an integrative approach was established combining iHg and MeHg external levels of exposure, iHg and MeHg accumulation, oxidative stress endpoints (enzymatic and non-enzymatic antioxidants, as well as peroxidative damage) and AChE activity in fish eyes. This approach was replicated in two contrasting seasons (winter vs. summer) in order to cover distinct environmental conditions and availability of both Hg counterparts.

2. Material and methods

2.1. Study area characterization

The Aveiro lagoon is a coastal ecosystem located on the northwest coast of Portugal (Fig. 1) with a maximum surface area of 47 km^2. It has an inner and enclosed area known as Laranjo basin (a shallow area with 2 km^2) that received Hg effluents from a chlor-alkali plant during more than four 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., 2008a; Mieiro et al., 2010; Pereira et al., 2013]. Due to the absence of other important sources of contaminants, Largo do Laranjo is considered a "field laboratory", offering a unique opportunity to assess Hg toxicity under realistic conditions [Guilherme et al., 2008a; Mieiro et al., 2010; Pereira et al., 2014]. São Jacinto is located near the lagoon entrance, about 10 km from Largo do Laranjo. In previous studies, São Jacinto area was selected as a reference for comparison purposes, since it was considered unpolluted including in terms of Hg [Guilherme et al., 2008a; Mieiro et al., 2010]. Surface sediments of both areas were surveyed for other trace elements besides Hg [unpublished data] and PAH compounds [Pacheco et al., 2005]. A low contamination level was found being also identical at both areas.

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 (*L. aurata*) were collected

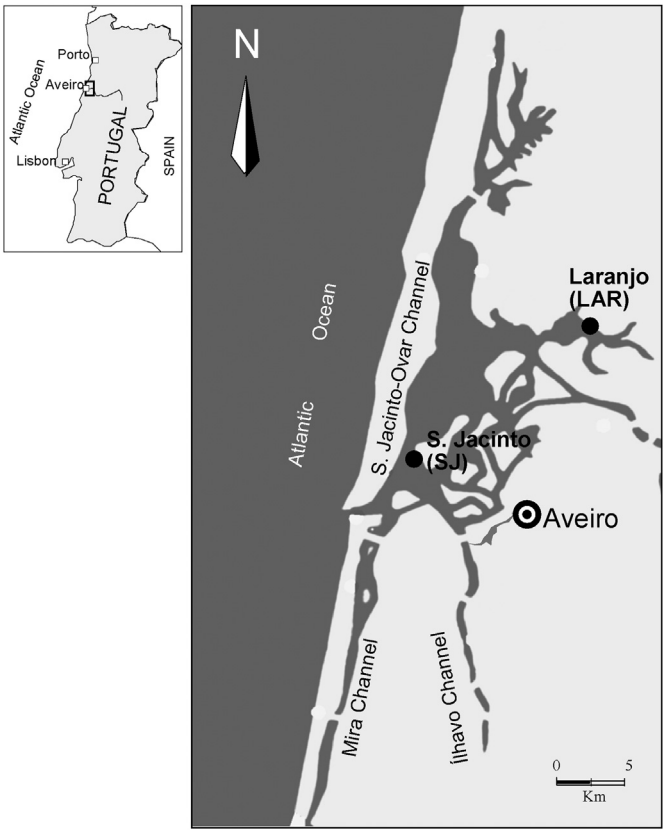

Fig. 1. Location of the sampling sites at Aveiro lagoon (Portugal): São Jacinto (SJ) (40°41′00″ N, 8°42′44″ W); Laranjo (LAR) (40°43′28.98″ N, 8°37′35.80″ W).