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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

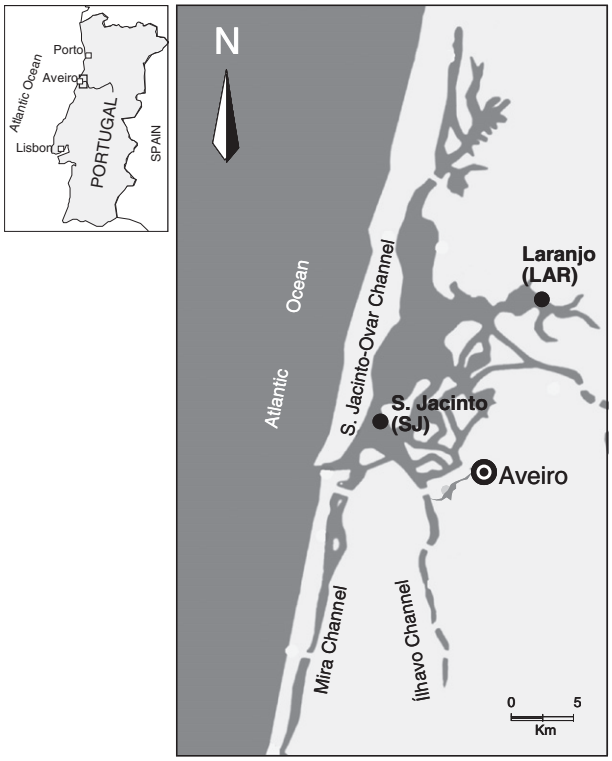

Fig. 1. Location of the sampling sites at Aveiro lagoon (Portugal): São Jacinto (SJ) (40°41′00″ N, 8°42′44″ W); Laranjo (LAR) (40°43′28.98″ N, 8°37′35.80″ W).

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

sediments (approximately 2 cm depth) were collected in the two sites for tHg and MeHg determinations.

2.3. Analytical procedures

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 analysed by cold-vapour 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 the procedure.

Methylmercury 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 Tenax™ 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 analysed 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 were analysed for tHg by atomic absorption spectrometry (AAS) with thermal decomposition following gold amalgamation in an Hg analyser (AMA) LECO 254 (Costley et al., 2000). Methylmercury was determined in dry sediments by alkaline digestion (KOH/MeOH), organic extraction with dichloromethane (DCM) preconcentration in aqueous sulphide solution, back-extraction into DCM and quantification by GC-AFS in a Agilent Chromatograph coupled with a pyroliser unit and a PSA fluorescence detector (Canário et al., 2004). Recoveries and the possible MeHg artefact formation were evaluated by spiking several samples with Hg(II) and MeHg standard solutions with different concentrations. Recoveries varied between 97 and 103% and no artefact MeHg formation was observed during our procedure. Precision of Hg analysis, expressed as relative standard deviation (RSD) of 4 replicate samples, was less than 4% ($p < 0.05$). Certified reference materials (MESS-2, IAEA-405 and BCR-580) were used to ensure the accuracy of the procedure. Levels of tHg and MeHg obtained in the reference materials were consistent within the ranges of certified values.

The brain, eye wall and lens samples were firstly lyophilised and homogenised. Samples were then analysed for tHg as previously described for sediment. For MeHg analysis a modified Westöö (1967) and Armstrong et al. (1999) methodology was used. Briefly, approximately 2 mL of Milli-Q water and 3 mL of KOH (6 M) solution were added to 200 mg of dried sample. The mixture was shaken for 2 h and then 3 mL of HCl (6 M) and 4 mL of a $KBr/CuSO_4$ (3:1) solution were added. After 10 min of shaking, 5 mL of DCM was then added, the mixture centrifuged and finally the organic phase separated. A slight sulphide solution (≈0.06 mM) was used to extract MeHg from the organic phase and then MeHg was back extracted to DCM. Methylmercury in DCM was quantified by GC-AFS using the chromatographic equipment described above. Again, the possible MeHg artefact formation was evaluated by spiking several samples with Hg(II) and MeHg standard solutions of different concentrations. Recoveries varied between 92 and 103% and no artefact MeHg formation was observed. For all the analysis, precision expressed as the relative standard deviation of 3 replicate samples, was less than 2% ($p < 0.05$). Certified reference materials (DORM-3, DOLT-4) were used to ensure the accuracy of the procedures.

A crude estimation of the total inorganic mercury (iHg) concentrations in the brain, eye wall and lens was done by subtracting tHg levels by the corresponding MeHg concentrations. For this estimation it was assumed that MeHg is the only organic mercury compound that is bioaccumulated in fish (Zhang and Adeloju, 2012).

2.4. Data analysis

Statistical software (Statistica 6.0) was used for statistical analyses. All data were first tested for normality (Shapiro–Wilk test) and homogeneity of variance (Levene's test) to meet statistical demands. Two-way ANOVA was performed to compare sampling sites or seasons and to test the influence of the combined effect of site and season on tHg and MeHg accumulation levels. The Tukey test was applied for post-hoc comparison. Differences between means were considered significant when $p < 0.05$. The following correlations were statistically tested using Spearman correlation analysis – the brain vs. eye wall, the brain vs. lens and the eye wall vs. lens – for tHg and MeHg concentrations. Correlations were considered significant for $p < 0.05$.

3. Results

3.1. Water and sediment characteristics

Water temperature was higher at LAR and SJ in summer than in winter, while salinity was lower at LAR than SJ, particularly in winter (Table 1). Dissolved oxygen was around 100% but undersaturation was recorded at LAR in summer. LAR presented higher levels of total dissolved Hg and MeHg in water than SJ, being these differences accentuated in winter. In this season, an enhanced proportion of Hg was present in the MeHg form relatively to summer at LAR (Table 1).

Surface sediments from LAR exhibited higher levels of tHg and MeHg than SJ both in winter and summer (Table 2). Seasonal differences were recorded for tHg and MeHg in sediments, showing levels one order of magnitude higher in winter than in summer at LAR. The percentage of Hg in the MeHg form was maxima at LAR in summer.

3.2. Mercury levels in the brain, eye wall and lens

The brain of fish from LAR showed significantly higher accumulation of tHg, MeHg and iHg than SJ in both seasons (Fig. 2). No statistical differences were found between sites for the percentage of MeHg with respect to tHg in summer and winter. Differences between the two surveys were found for tHg and MeHg with higher levels in winter than in summer at LAR. The percentage of MeHg was significantly higher in winter than in summer in both sampling sites, while no seasonal differences were found for iHg.

The inter-site differences found in the eye wall for tHg, MeHg and iHg were similar to those described for the brain, with significantly higher levels being recorded at LAR than SJ (Fig. 3). The percentage of

Table 1
Water temperature (T), salinity, dissolved oxygen (DO), total dissolved Hg, dissolved methylmercury (MeHg) and the percentage of MeHg with respect to total mercury. Winter and summer data, measured at low-tide, are presented for São Jacinto (SJ) and Laranjo (LAR) at Aveiro lagoon. Mean and associated standard deviations are presented. n.d. not determined.

Season	Site	T (°C)	Salinity	DO (%)	Total Hg (ng L^{-1})	MeHg (ng L^{-1})	MeHg (%)
Winter	SJ	13 ± 0.15	31 ± 0.13	94 ± 0.50	<0.1	<0.01	n.d.
	LAR	12 ± 0.00	4.9 ± 0.01	87 ± 0.84	4.4 ± 0.90	1.0 ± 0.24	23 ± 2.4
Summer	SJ	18 ± 0.12	33 ± 0.11	102 ± 1.9	1.0 ± 0.02	0.016 ± 0.007	1.4 ± 0.79
	LAR	18 ± 0.05	21 ± 0.07	65 ± 0.50	1.5 ± 0.77	0.040 ± 0.008	3.0 ± 0.83

Table 2
Total Hg, methylmercury (MeHg) and the percentage of MeHg with respect to total Hg in surface sediment. Winter and summer data are presented for São Jacinto (SJ) and Laranjo (LAR) at Aveiro lagoon. Mean and associated standard deviations are presented.

Season	Site	Total Hg ($\mu g\ g^{-1}$)	MeHg ($\mu g\ g^{-1}$)	MeHg (%)
Winter	SJ	0.021 ± 0.010	0.00005	0.38
	LAR	2.9 ± 0.37	0.029	0.95
Summer	SJ	0.025 ± 0.005	0.0001 ± 0.00002	0.44 ± 0.04
	LAR	0.44 ± 0.25	0.008 ± 0.003	1.9 ± 0.42

MeHg exhibited the same spatial variation trend. Eye wall showed significantly higher levels of tHg, MeHg and its percentage in winter than in summer at LAR. MeHg percentage was also significantly higher in winter than in summer in the eye wall from SJ. No seasonal differences were found for iHg at both sites.

Lens of fish from LAR presented significantly higher levels of tHg, MeHg and iHg than SJ, both in winter and summer (Fig. 4), while no spatial differences were recorded for the percentage of MeHg. Levels of tHg, MeHg (or its percentage) and iHg in lens were similar in winter and summer.

Lens accumulated higher levels of tHg, followed by the brain and the eye wall with means ranging from 0.25 to 1.09 $\mu g\ g^{-1}$, 0.11–0.61 $\mu g\ g^{-1}$ and 0.05–0.30 $\mu g\ g^{-1}$, respectively. A similar trend was recorded for MeHg levels: 0.25–1.0 $\mu g\ g^{-1}$ in the lens, 0.09–0.53 $\mu g\ g^{-1}$ in the brain and 0.04–0.28 $\mu g\ g^{-1}$ in the eye wall. Lens presented also the highest proportion of Hg in the MeHg form (≥96%), followed by the eye wall (≥83%) and brain (≥77%). On the contrary, the brain showed slightly higher mean values of iHg (0.01–0.07 $\mu g\ g^{-1}$) than the eye wall and lens (0.01–0.03 $\mu g\ g^{-1}$ and 0.01–0.04 $\mu g\ g^{-1}$, respectively).

Significant interactions between site and season were recorded for tHg and MeHg in the brain and eye wall (Table 3) but no significant interactions were obtained for the percentage of MeHg and iHg. No significant interactions were found between site and season for tHg, MeHg (including its percentage) and iHg in the lens.

3.3. Relationships between MeHg and inorganic Hg in the brain, eye wall and lens and correlations between tissues

Fig. 5 presents the relationships between MeHg and iHg levels ($\mu g\ g^{-1}$) for the brain, eye wall and lens. A significant, positive and linear relationship ($r^2 = 0.64$) was obtained for the brain, while no significant associations were found for the eye wall and lens.

All the analysed tissues were significantly correlated for MeHg, whereas iHg was only correlated in the case of the brain vs. eye wall (Fig. 6). Stronger correlations were obtained for MeHg levels in the brain vs. eye wall ($r^2 = 0.93$), whereas significant but poorer relationships were found in the brain vs. lens ($r^2 = 0.50$) and in the eye wall vs. lens ($r^2 = 0.45$).

4. Discussion

4.1. Mercury levels in the brain, eye wall and lens and association with environmental availability

LAR exhibited a higher availability of total dissolved Hg (tHg) and MeHg than SJ in both seasons, which is in line with spatial differences previously observed (Mieiro et al., 2011). Differences between the two sampling sites regarding Hg in water were accentuated in winter relatively to summer (44 and 100 times higher for tHg and MeHg in winter, respectively while values only doubled in summer). This is probably due to the higher re-suspension of Hg enriched sediments at Laranjo basin in winter. In fact, sediments from LAR exhibited also higher levels of Hg in winter than in summer (tHg was almost 7 times higher in winter than in summer, while MeHg enhanced 4 times). The spatial contamination trend recorded in water was also found in the sediments with higher

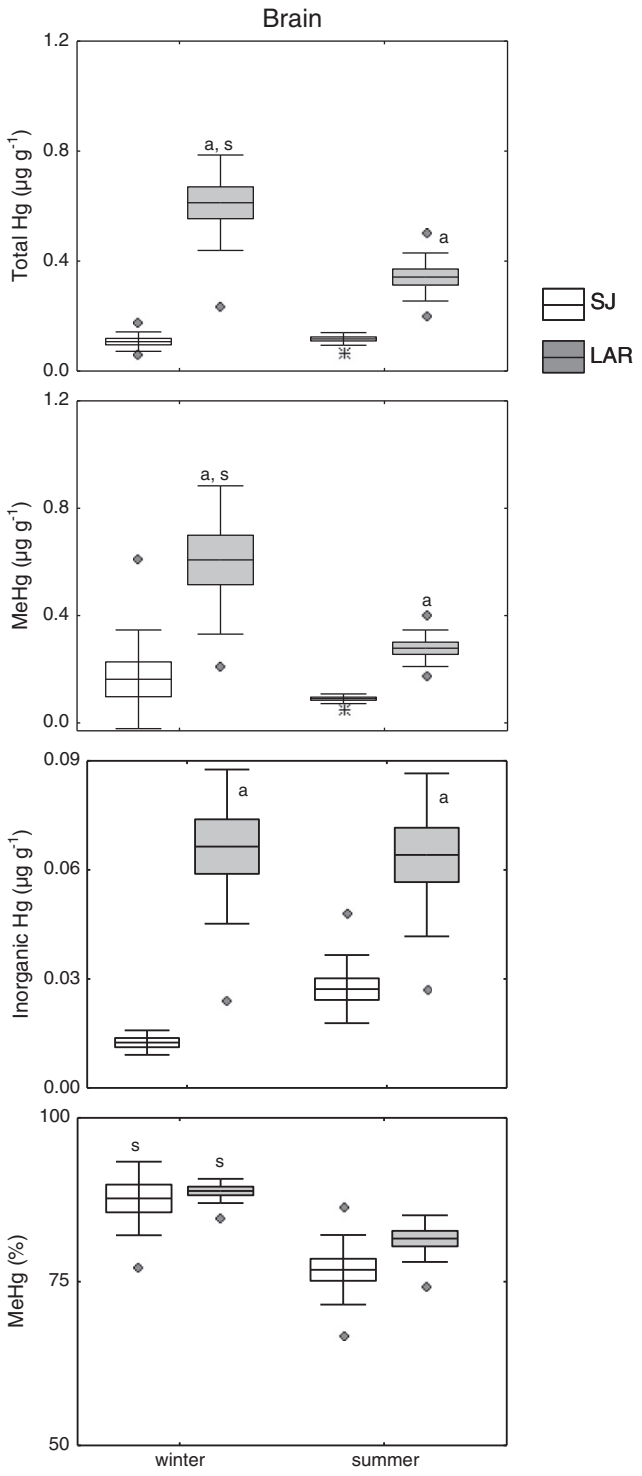

Fig. 2. Total Hg, MeHg, inorganic Hg ($\mu g\ g^{-1}$, dry weight) and % of MeHg (in relation with total Hg) in the brain of *L. aurata* captured in winter and summer in Laranjo (LAR) and São Jacinto (SJ) at Aveiro lagoon. Mean, standard deviation, standard error, outliers (●) and extreme values (※) are presented. *a* indicates significant differences between sites (within the same season) and *s* denotes seasonal significant differences (within the same site).

levels of tHg and MeHg recorded at LAR than SJ. It was previously documented that LAR sediments are heavily contaminated by Hg and the depth variation reflects the industrial discharge evolution during the last decades (Ramalhosa et al., 2001).

The brain of fish from the contaminated area (LAR) accumulated higher levels of MeHg and iHg (and consequently tHg) than at the reference site, clearly evidencing a mercury exposure. MeHg in the brain

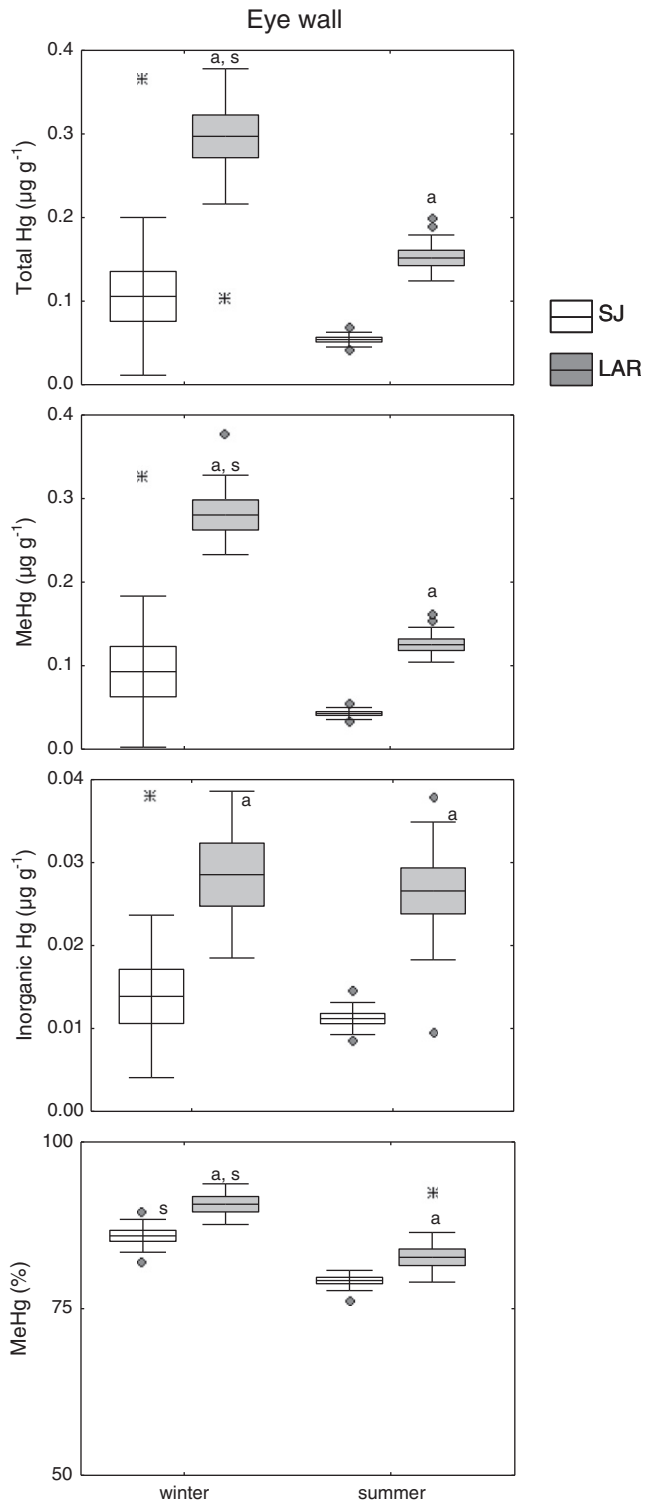

Fig. 3. Total Hg, MeHg, inorganic Hg (µg g^{-1}, dry weight) and % of MeHg (in relation with total Hg) in the eye wall of *L. aurata* captured in winter and summer in Laranjo (LAR) and São Jacinto (SJ) at Aveiro lagoon. Mean, standard deviation, standard error, outliers (●) and extreme values (※) are presented. *a* indicates significant differences between sites (within the same season) and *s* denotes seasonal significant differences (within the same site).

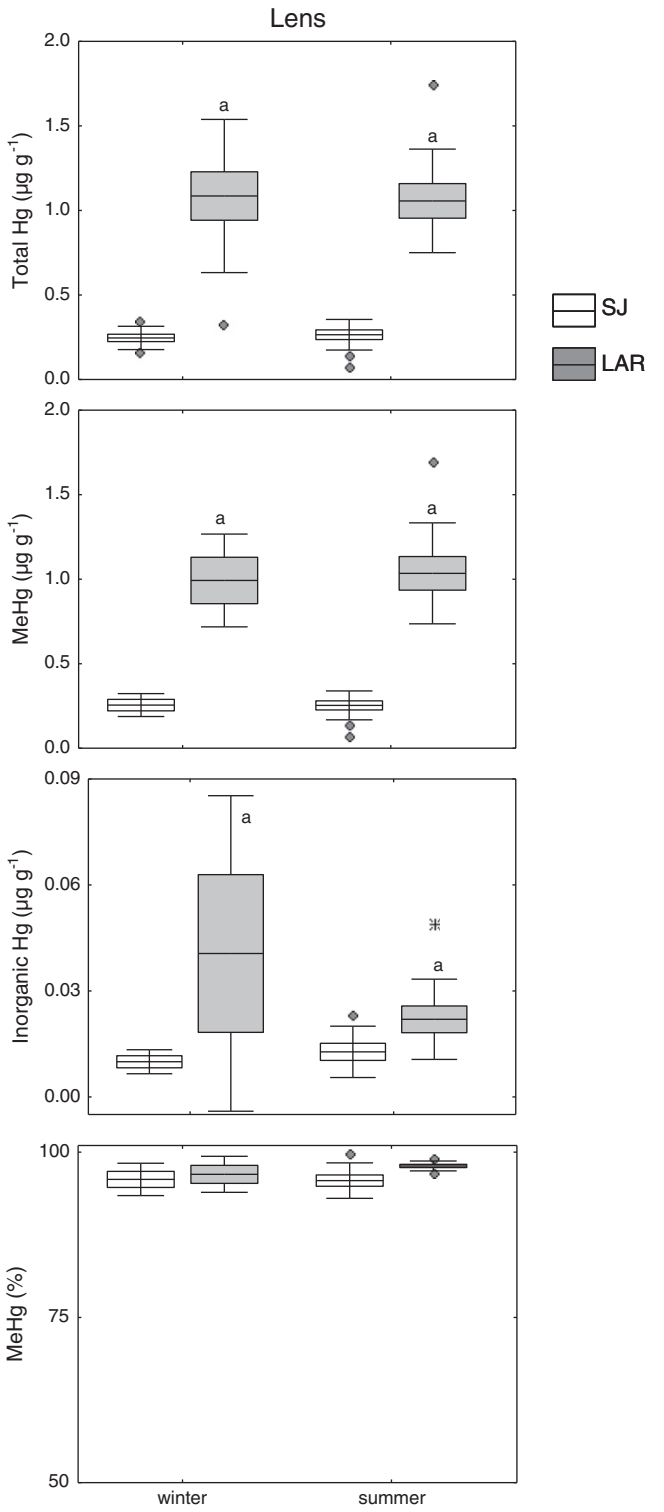

Fig. 4. Total Hg, MeHg, inorganic Hg (µg g^{-1}, dry weight) and % of MeHg (in relation with total Hg) in the lens of *L. aurata* captured in winter and summer in Laranjo (LAR) and São Jacinto (SJ) at Aveiro lagoon. Mean, standard deviation, standard error, outliers (●) and extreme values (※) are presented. *a* indicates significant differences between sites (within the same season).

(consistent with tHg) reflected inter-site differences recorded in water and sediment. These results are in line with previous findings of Mieiro et al. (2009) who demonstrated that *L. aurata* brain was able to reflect environmental variations of Hg in a field study performed at Aveiro lagoon. That work only determined tHg in *L. aurata* brain while the current one contributed with identical conclusions for MeHg, one of the most toxic forms of Hg. Lipid solubility of Hg compounds promotes its accumulation in lipid-rich compartments such as the brain (Guzzi and Porta, 2008). Additionally, MeHg (and specifically the thiol-bound MeHg) is similar enough to endogenous substrates for the active transporters

Table 3
Two-way ANOVA analysis testing the effect of site, season and interaction (site × season) on the concentrations of total Hg, MeHg, percentage of MeHg and inorganic Hg in the brain, eye wall and lens of *L. aurata*. The F and p values are given for each variable. n.s. — not significant.

Organ	Dependent variable	Site		Season		Site × season	
		F	p	F	p	F	p
Brain	Total Hg	128.12	<0.001	16.28	<0.001	18.83	<0.001
	MeHg	32.28	<0.001	12.99	<0.001	5.31	<0.05
	% MeHg	3.84	n.s.	36.32	<0.001	1.44	n.s.
	Inorganic Hg	64.37	<0.001	1.20	n.s.	2.25	n.s.
Eye wall	Total Hg	48.81	<0.001	22.71	<0.001	5.13	<0.05
	MeHg	58.55	<0.001	33.95	<0.001	8.90	<0.05
	% MeHg	19.10	<0.001	60.75	<0.001	0.44	n.s.
	Inorganic Hg	30.77	<0.001	0.73	n.s.	0.017	n.s.
Lens	Total Hg	83.53	<0.001	0.0031	n.s.	0.072	n.s.
	MeHg	73.13	<0.001	0.051	n.s.	0.062	n.s.
	% MeHg	2.62	n.s.	0.35	n.s.	0.62	n.s.
	Inorganic Hg	6.48	<0.05	1.02	n.s.	1.87	n.s.

regulating the cellular uptake and efflux of molecules, promoting its higher accumulation in tissues (Korbas et al., 2012). Mercury accumulation in mammals' brain is well reported but it is scarcely documented in fish (Rouleau et al., 1999; Berntssen et al., 2003; Mieiro et al., 2010, 2011), particularly in what concerns to the mechanisms by which Hg

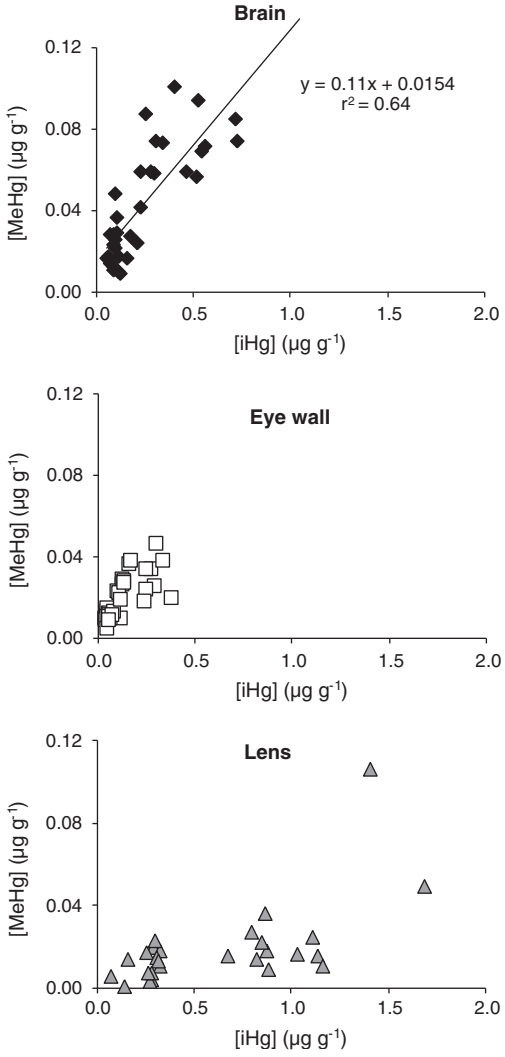

Fig. 5. Relationships between concentrations of MeHg and inorganic Hg (iHg) (µg g^{-1}, dry weight) for the brain, eye wall and lens of *L. aurata* captured in Aveiro lagoon. Winter and summer data are plotted together.

reaches the brain. It has been demonstrated that MeHg passes through the blood–brain barrier (BBB) and reaches either cellular or nuclear components, causing irreversible damage to the nervous system in human (Zheng et al., 2003) and fish (Boening, 2000). Contrarily, Rouleau et al. (1999) found that fish BBB is impervious to iHg in plasma, suggesting that waterborne iHg is taken up by water-exposed receptor cells of sensory nerves and subsequently transferred toward the brain by axonal transport.

Fish eyes are in permanent and direct contact with dissolved metals and those linked with re-suspended sediment particles. In fact, the significantly higher accumulation of MeHg and iHg (and likewise tHg) in fish eye wall (all ocular components except lens) measured at LAR in winter and summer indicated an enhanced uptake. Inorganic Hg and MeHg levels in the eye wall were around 2 to 3 times higher at LAR than SJ in both seasons. These results are in agreement with a previous study that found higher levels of trace elements in fish eyes from a contaminated area of the Tagus estuary (Portugal) (Pereira et al., 2013). Fish lens from LAR also exhibited an enhanced accumulation of MeHg and iHg (in line with tHg) in relation to fish from Korbas et al. (2013) investigated the uptake and accumulation of MeHg in zebrafish larvae and found the highest levels in the secondary lens fibers underlying the lens epithelium. It was also reported that MeHg targets photoreceptors which are directly involved in visual perception (Korbas et al., 2013).

The brain and eye wall of *L. aurata* accumulated higher levels of MeHg (and tHg) in winter than in summer at LAR. This is in agreement with the higher environmental availability of Hg (including in the MeHg form) in winter relatively to summer. Besides that, the influence of water salinity on Hg accumulation should be considered since values at LAR were 4-fold lower in winter than those recorded in summer. It was previously reported that tHg accumulation in crabs from Aveiro lagoon was favoured by low salinity (Pereira et al., 2006), consistent with the current data on fish. Mercury is able to form strong inorganic complexes with chloride at saline and oxygen-rich waters (Conaway et al., 2003). In line, current data revealed a higher percentage of dissolved MeHg at LAR in winter (when salinity was lower) than in summer. Since MeHg counterparts are highly accumulated in fish comparing to chloride Hg forms (Korbas et al., 2012), water salinity could indirectly influence the accumulated Hg levels. Elevated water temperature can also increase metal accumulation associated with the higher metabolism of ectothermic organisms (Sokolova and Lannig, 2008). Current data revealed a higher accumulation in winter relatively to summer but only at LAR. This suggests a minor role of temperature on Hg accumulation. Despite the higher winter availability of MeHg (and tHg) in water and sediment from LAR, lens did not reveal that seasonal variation as occurred for the brain and eye wall. Lens presented levels of MeHg and its percentage, as well as iHg, identical in winter and summer both at SJ and LAR.

4.2. Tissue-specific accumulation — considerations on mercury toxicokinetics

The three neurosensory structures accumulated Hg preferentially in the MeHg form. A minimum percentage of MeHg was found in the brain (77%), while in the lens compartment more than 96% of tHg was in the MeHg form. In fact, the estimated levels of iHg were very low, suggesting a preferential uptake of the MeHg counterpart, as previously documented for invertebrates (e.g. Tsui and Wang, 2004; Raimundo et al., 2010) and fish (e.g. Burger and Gochfeld, 2006). It was previously demonstrated that MeHg load in fish brain was around 200 times higher than for iHg (Oliveira Ribeiro et al., 2000). Current levels pointed to a lower difference in the accumulation of both Hg chemical forms in *L. aurata* brain, with levels of MeHg being only 3 to 13 times higher than iHg. MeHg is an Hg form very stable in the brain, even if it can eventually change to HgSe (Korbas et al., 2013).

To the best of our knowledge, this is the first quantitative study reporting MeHg and iHg levels in fish eyes (with the separation of lens) and data pointed to unprecedented conclusions in wild fish. Lens

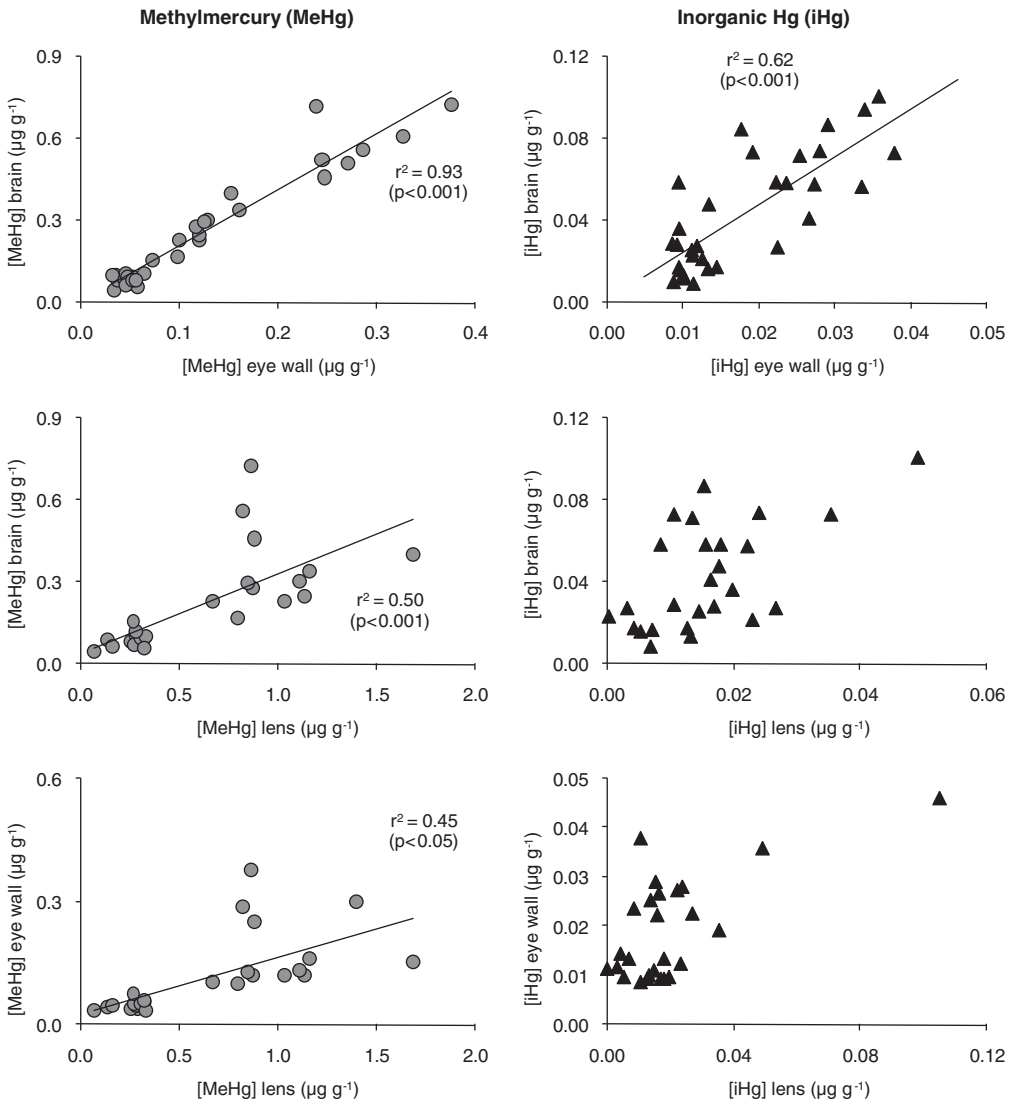

Fig. 6. Relationships between the brain and eye wall, the brain and lens, as well as the eye wall and lens for MeHg and inorganic Hg (iHg) concentrations (µg g^{-1}, dry weight) in *L. aurata* captured at Aveiro lagoon. Winter and summer data are plotted together.

exhibited the highest accumulated levels of MeHg (and likewise tHg) followed by the brain and eye wall. MeHg levels in the lens doubled relatively to the brain whereas in relation to eye wall were 4 to 6 times higher. A higher proportion of Hg in the MeHg form was also detected in the lens relatively to the brain or eye wall of *L. aurata*. In agreement, Korbas et al. (2013) found higher levels of MeHg in the secondary lens fibres (directly underlying the lens epithelium) relatively to the brain and optic nerve in zebrafish larvae. This is probably related with a higher proportion of protein material in the lens comparatively to the brain and eye wall, since the particular affinity of MeHg to thiol groups of proteins has been demonstrated (Leaner and Mason, 2004). Lens is the site of particularly high protein production (named as crystallins) and deposition (Korbas et al., 2010).

Lower accumulation of MeHg in the brain relatively to the other tissues was accompanied by slightly higher iHg levels, particularly at LAR (ranges of iHg mean values were: 0.064–0.066 µg g^{-1}, 0.027–0.029 µg g^{-1} and 0.022–0.041 µg g^{-1}, for the brain, eye wall and lens, respectively). This could be partially related with a demethylation capacity since it was described that some of the MeHg in the brain may be converted into iHg (Ercal et al., 2001). The relationship found between MeHg and iHg accumulation in the brain ($r^2 = 0.64$) (not found in the other tissues) is in line with the association between the two Hg forms, reinforcing the hypothesis of demethylation activity in the brain. Whether the conversion of MeHg into iHg plays a neuroprotective role is a matter that requires further investigation. On one hand, it has been demonstrated that iHg could be excreted 3-fold faster than MeHg in fish (Trudel and Rasmussen, 1997). On the other hand, iHg showed to be more potent than MeHg in inhibiting glutamine synthetase activity in cortical astrocytes (Allen et al., 2001). In the light of the findings of Allen et al. (2001), the iHg levels currently found in *L. aurata* brain are not negligible in terms of neurotoxic hazard. Inorganic Hg accumulated in *L. aurata* brain in a minimum of 11% (mean value in relation with MeHg) while the values estimated by Ercal et al. (2001) were lower, ranging between 3 and 6%. Thus, demethylation should not be assumed as the only mechanism promoting iHg accumulation in this organ. It should be also considered the axonal transport of iHg and/or its (re)distribution from other tissues including the blood.

Levels of MeHg and iHg found in the brain, eye wall and lens are also influenced by organs' anatomic location that determines the exposure route and distribution. The transport of MeHg via blood both to the brain and the eye wall is likely to occur. In fact, both tissues are protected by the epithelial barriers (blood–brain and blood–retinal barriers), which strictly regulate the transport of molecules from the bloodstream. Thiol-bound MeHg (specifically MeHg cysteineate, the most dominant form of MeHg in vivo) can penetrate these barriers (Korbas et al., 2013). The similar pathways of MeHg for the brain and eye wall

could explain the high correlations found between both tissues for its accumulation. On the contrary, iHg was only weekly correlated in the case of the brain vs. eye wall, confirming its lower mobility in fish body. The different toxicokinetics of MeHg and iHg was corroborated by the absence of statistical correlations between the two chemical forms in the eye wall and lens.

Liza aurata lens exhibited considerably higher levels of MeHg than the other ocular tissues, as aforementioned. The high affinity of MeHg for lens in relation with the rest of eye is probably explained by their distinct tissue structure and composition. It is not known at the present why the eye lens accumulates such high levels of MeHg but it could be partially related with its major protein nature, because there is a high affinity of MeHg to thiol groups of proteins (Leaner and Mason, 2004). Considering that eyes are in contact with water and suspended particles, Hg can target lens after its direct uptake by the eyes' surface. However, poorer correlations were found between the eye wall and lens for MeHg (comparatively with the brain vs. eye and the brain vs. lens) and no associations were found between the eye wall and lens for iHg. Additionally, it should be hypothesised that Hg may reach the eyes by axonal transport (via optic nerve), similarly to what was proposed to the brain (Rouleau et al., 1999), and then translocated to the lens. It was previously demonstrated that MeHg levels continue to increase in the lens after fish removal from the exposure solutions due to redistribution from other organs like the liver and brain (Korbas et al., 2010).

The lens is an avascular structure surrounded by an elastic collagen coat (Lemire et al., 2010). In adult fish like in mammals, the lens is separated from the eye wall by the aqueous and vitreous humours, being linked only by a ligament/muscle connection. Keeping in view this pseudo-isolation of lens from the remaining eye compartments, their high accumulation ability could be regarded as a physiological defence mechanism of fish against environmental exposure to Hg, namely MeHg. This could be particularly important in the protection of more vulnerable eye components and ultimately of fish vision. Despite that, considering the high accumulated levels of MeHg in lens and its elevated toxicity, it should be pondered that fish could develop ocular anomalies in lens, such as cataracts or opacity, as previously reported in humans exposed to Hg (Lemire et al., 2010).

4.3. Insight on environmental risk assessment

The selection of the best tissue to signal environmental spatial differences is difficult on the basis of current data. In fact, all the three tissues (the brain, eye wall and lens) were able to detect the higher contamination at LAR than SJ. Moreover, environmental health assessment should be performed in distinct temporal periods due to changes on availability of contaminants and organisms' physiology. From the seasonal point of view, lens is the least responsive tissue. Lens did not mirror the higher environmental levels of total dissolved Hg and MeHg in winter at LAR, while the brain and eye wall signallized those winter–summer differences.

The distinct spatial and temporal patterns of MeHg and iHg accumulation in lens could be indicative that this compartment is able to reflect marked environmental differences like those imposed by different locations, whereas subtle temporal changes could not be identified by this eye component. This is probably due to lens composition and structural constancy over time, without cell or non-cellular component turnover. Thus, metal load is cumulative along time, reflecting a sequence of exposures. Taking into account fish mobility, this constraint becomes more relevant, probably avoiding that lens could reflect recent exposures. On the contrary, lens could offer a historical record of Hg exposures affecting fish through its lifetime due to its unique stability (Dove and Kingsford, 1998; Korbas et al., 2008).

From the analytical perspective, all the three analysed tissues displayed relatively high Hg levels (mainly MeHg) and a sufficient amount of mass for analysis. The brain and eyes are interesting due to the possibility of Hg determinations in different compartments (like lens in the eyes or different brain areas) and the quantification of distinct organic counterparts (e.g. MeHg).

5. Conclusions

Results of this work provided these main findings:

- Fish eyes and brain are primary target organs in environmental health assessment since they faithfully reflect water and sediment Hg contamination. The brain, eye wall and lens accumulated Hg preferentially in the MeHg form. Thus, it is important to evaluate changes in these organs/tissues at structural and functional levels in order to examine in what extent accumulated Hg could compromise neurosensory processes.
- Within fish eyes, current data revealed unprecedented conclusions for lens under field research. Lens exhibited higher MeHg accumulation than the brain and the remaining eyes' components.
- The MeHg levels were highly related between the brain and eye wall pointing out similarities on the distribution from bloodstream, as well as on the storage. On the contrary, MeHg levels were poorly associated within eyes' components, presumably due to the distinct chemical nature of lens and the remaining eye structures.
- All the three tissues were able to distinguish contrasting spatial differences but only the brain and eye wall reflected winter–summer changes, probably due to the high retention capacity of lens. This information on tissue-specificities could be very useful for environmental health assessment and subsequent management and policy.

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