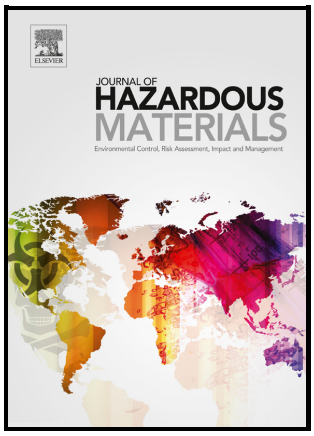

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Ecophysiological effects of mercury bioaccumulation and biochemical stress in the deep-water mesopredator *Etmopterus spinax* (Elasmobranchii; Etmopteridae)

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Abstract

Mercury (Hg) is a non-essential metal that can have toxic effects on the fitness of organisms and tends to bioaccumulate with age and to biomagnify in higher trophic levels. Few studies have assessed oxidative stress and neurotoxicity in deep-water sharks. This study evaluated early ontogenetic changes and physiological effects (antioxidant defences, oxidative damage, aerobic metabolism and neurotransmission functions) of Hg accumulation in the white muscle and brain tissues of the velvet belly lantern shark *Etmopterus spinax* from the southern Iberian coast (NE Atlantic). Results suggested that the low mercury concentrations observed may induce acute effects in *E. spinax* before they reach sexual maturity. We found different Hg concentrations in *E. spinax*: [Hg] males > [Hg] females; [Hg] muscle > [Hg] brain. Females appeared to have higher redox capability translated into higher activities and levels of antioxidant defences than males. However, higher levels of oxidative damage were also observed in females. Whilst the mechanisms underlying these effects remain unknown, these results suggest differences in mercury accumulation between tissues and sex, and potentially deleterious effects on oxidative stress status and neurophysiology of *E. spinax*, potentially impairing swimming performance and reproduction, which could subsequently impact on the health of both individuals and population.

Keywords:

Bioconcentration, Elasmobranchii, Metal contamination, Biochemical responses, Deep sea

1. Introduction

The health status and viability of marine ecosystems and their biota are impacted by the presence of persistent marine pollutants, with mercury (Hg)one of the most persistent and toxic metals occurring in marine and coastal systems. Hg is continuously mobilised, deposited, and re-mobilised between the earth-ocean-atmospheric system [1,2], with both natural and anthropogenic inputs. The natural sources of Hg (e.g. volcanic eruptions and forest fires) slowly free this element, whereas human activities (e.g. the burning of fossil fuels and mining) accelerate the rates of Hg emissions [2]. A significant proportion of the global Hg is stored in the oceans, with the coastal areas of the northern hemisphere (including the North Atlantic, Mediterranean Sea and North Pacific) being reported as the areas of highest Hg emission and re-emission compared to the southern hemisphere, and is mainly due to anthropogenic activities [3].

Conservation measures to protect elasmobranchs (sharks, skates and rays) are required due to fishing pressure, with declining populations often reported [4,5], although other anthropogenic stressors such as habitat loss and environmental degradation may also affect population health [6,7]. Many elasmobranchs are long-lived, meso- to top predators, with an important role in marine food webs and community dynamics [8], and also have large, lipid-rich livers and, in the case of demersal species, slow metabolic rates. These factors may increase their susceptibility to contamination through both bioaccumulation and biomagnification [9,10]. Consequently, there has been ongoing interest in the Hg concentration in elasmobranchs [7]. Despite the range of published studies on Hg concentrations in elasmobranchs (e.g. [9,11–13]), Hg levels in different tissues have not been fully characterised across the wider diversity of shark species, trophic positions and geographical distribution, and knowledge on the potential effects of Hg accumulation on the health of sharks, both at an individual and population level, remain limited [14]. Furthermore, due to the complexity of biogeochemical cycles in the marine environment, particularly in relation to mercury, there is a need to better understand the extent of Hg bioaccumulation and toxic potential to marine fish populations.

Diet is considered the main pathway of Hg bioaccumulation and biomagnification into biota, with marine fish and fish-derived products among the main contributors to human intake of Hg [1,15,16]. This assimilation occurs after bacterial mediated methylation of Hg both in sediments and the water column; thus, more than 90 % of total Hg in fish muscle can be in the more toxic organic form, methylmercury ($HgCH_3$ or MeHg) [2,17]. MeHg is highly lipophilic being readily absorbed into the body of aquatic organisms, mainly via the digestive system. This is of major

concern because it is also known that the elimination rates of MeHg are very slow, leading to the bioconcentration of Hg in organisms' cells [18]. The presence of Hg inside a cell can disrupt its normal functions and cause deleterious effects, even at low doses [19]. Fishery products provide more than 20 % of the world's per capita animal protein intake, and are a rich source of high-quality protein and several important elements [20]. Due to the great affinity and strong bonding of methylmercury to proteins, the processing methods for fish meat do not reduce its levels [1,21]. For all these reasons, Hg has been the third-ranking hazard substance in the priority list of the US Agency for Toxic Substances and Disease Registry for decades [22].

The velvet belly lanternshark *Etmopterus spinax* (L.) is a small (typically < 41 cm total length) deep-water shark that occurs in the eastern Atlantic Ocean, ranging from Iceland and Norway to the Gulf of Guinea, including the Mediterranean Sea and the Azores [23]. Along the southern Iberian slopes, *E. spinax* is a common bycatch species in crustacean bottom trawl fisheries, where it is usually discarded [24,25]. In this area, it typically feeds on decapod crustaceans, including euphausiids and shrimps (e.g. *Pasiphaea* spp.), with teleost fishes and cephalopods of secondary importance [26]. Whilst *E. spinax* caught off the southern Portuguese coast are of limited commercial value, this species may play an important role in the ecosystem. Due to their life history traits, this species is vulnerable to fishing pressure [25,27], and other extrinsic pressures, such as marine pollution. In this context, a better understanding of their functional ecology and physiology is needed, particularly in relation to anthropogenic pressures [28].

We assessed the bioaccumulation and neurotoxic effects of mercury in *E. spinax* to improve the current understanding of Hg bioaccumulation and its neurotoxic effects in mesopredatory sharks. Muscle tissue was selected, as it is one of the main sites for Hg accumulation, but also essential to shark movement and behaviour. Brain tissue can also bioaccumulate Hg, transported by thiol-containing molecules in the blood, which could directly impair neurophysiological processes. Hg concentration was quantified in both tissues, and biochemical biomarkers related to oxidative stress, energy metabolism and neurotoxicity were assessed. The objectives of this study were to: 1) quantify the concentrations of total Hg in muscle and brain tissues of *E. spinax*; 2) assess the oxidative stress status and aerobic energy metabolism in muscle tissue; and 3) evaluate the neurotoxic effects, oxidative damage and aerobic metabolism in the brain.

2. Material and Methods

2.1 Study organisms

Specimens of *E. spinax* were collected as bycatch from crustacean bottom trawl fisheries in the summer of 2016, along the southern Portuguese slope (depths = 450 – 550 m). Total length

(cm), weight (g) and sex were recorded. Tissue samples (white muscle and brain) were taken from 24 individuals to examine sexual and ontogenetic differences in Hg bioaccumulation. The specimens examined were all dead bycatch from commercial fishing and all subsequent work undertaken was in accordance with national rules and the European Directive 2010/63/EU of the European Parliament and the European Union Council on the protection of animals used for scientific purposes.

2.2 Quantification of mercury concentration

Hg concentrations in muscle and brain tissue were measured by atomic absorption using the Advanced Mercury Analyzer AMA 254 (Altec, Czech Republic). The analytical procedure was validated using DORM-3 (fish protein) and TORT-2 (lobster hepatopancreas), two biological reference materials for trace metals certified by the National Research Council of Canada, at the beginning and end of each set, thereby ensuring the accuracy of the method [29].

2.3 Sample preparation for oxidative stress and cellular energy allocation analysis

All biomarker determinations were performed spectrophotometrically in micro-assays set up in 96 well flat-bottom plates at 25 ºC [30], with the Microplate reader MultiSkan Spectrum (Thermo Fisher Scientific, USA). Fish tissue samples were individually homogenised by sonication (pulsed mode of 10 % for 30 s, 250 Sonifier, Branson Ultrasonics) on ice using ultra-pure water: 1600 µl for muscle, 1000 µl for brain.

From the muscle tissue homogenates, one aliquot was used for electron transport system (ETS) activity assessment, one aliquot containing 4 % butylated hydroxytoluene (BHT) in methanol was used for the determination of lipid peroxidation (LPO), and the remaining homogenate was diluted 1:1 in 0.2 M K-phosphate buffer (pH 7.4) and centrifuged for 20 min at 10,000 g (4 ºC). Aliquots of the post-mitochondrial supernatant (PMS) were divided into microtubes for posterior analysis of protein, catalase (CAT) activity, glutathione S-transferase (GST) activity and total glutathione (tGSH) levels.

From the brain tissue homogenates, one aliquot was used for measuring ETS activity; another aliquot containing 4 % BHT in methanol was used for the determination of LPO, and the last aliquot was diluted 1:1 in 0.2 M K-phosphate buffer, pH 7.4, and centrifuged for 20 min at 10,000 g (4 ºC) for subsequent analysis of acetylcholinesterase (AChE) activity and protein. All aliquots were kept at –80 ºC until they were analysed.

The protein concentration of PMS was determined according to the Bradford method [31], using bovine γ-globulin as standard. Acetylcholinesterase (AChE) activity was measured by following Ellman's method [32] adapted to a microplate [33]. Catalase (CAT) activity was determined by measuring the decomposition of the substrate H_2O_2 at 240 nm [34]. Glutathione-S-transferase

(GST) activity was determined following the conjugation of GSH with 1-chloro-2,4-dinitrobenzene (CDNB) at 340 nm [35]. Total glutathione (TG) content was determined at 412 nm using a recycling reaction of reduced glutathione (GSH) with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) in the presence of glutathione reductase (GR) excess [36,37]. TG content was calculated as the rate of TNB^{2-} formation with an extinction coefficient of DTNB chromophore formed, $\varepsilon = 14.1 \times 103 M^{-1} cm^{-1}$ [37,38]. Endogenous lipid peroxidation (LPO) was established by measuring thiobarbituric acid-reactive substances (TBARS) at 535 nm [39].

The method described by De Coen & Janssen [40] adjusted for microplate [41] was used to assess energy consumption (Ec – measured as ETS activity). ETS was measured as the rate of INT (Iodonitrotetrazolium) reduction in the presence of the nonionic detergent Triton X-100, with the absorbance read kinetically at 490 nm. The cellular oxygen consumption rate was calculated based on the stoichiometrical relationship in which for 2 µmol of formazan formed, 1 µmol of oxygen is consumed. Energy consumption (Ec) was estimated by the conversion to energetic values using the specific oxyenthalpic equivalent for an average lipid, protein and carbohydrate mixture of 480 kJ mol^{-1} O_2 [42].

2.4 Statistical analysis

Normality of data was evaluated the using Shapiro-Wilk test. Hg accumulated in male tissues was square root transformed to meet normality. Values for biochemical biomarkers in male muscle were ln transformed to meet the normality criteria. A Student's *t* test was used to investigate differences in Hg accumulation between female and male sharks. Significant variations of Hg concentrations with length and biochemical biomarkers of *E. spinax* were defined by Pearson correlation test. The significance level was set at 0.05, and calculations were performed using GraphPad Prism version 7.00 for Windows (GraphPad Software, La Jolla, California, USA).

Structural Equation Modeling (SEM) was used to investigate mechanistic pathways that may explain the direct effects and highlight the correlations [43] between sexes, Hg concentration in each tissue, and the biochemical responses measured. SEM allows the data to be interpreted through a refined hypothesised model to evaluate the effects of sex on Hg bioaccumulation and its relationship with biochemical responses measured in each tissue. The values of each variable were normalised to a common metric to obtain path coefficients (partial multiple regression coefficients) for the comparison of the relative importance of each effect. All response variables were previously normalised so that their responses had equal weight in the analysis. The model's exogenous variable for sexwas set as 0 (male) or 1 (female). The fit of the path model was validated using three goodness-of-fit measures: the $\chi 2$ test value for test of the similarity between the observed and the predicted covariance matrices; the combination of the

comparative fit index (CFI, value ≥0.95) with the standardised root-mean-squared residual (SRMR, value ≤0.08) as indicative of a good fit [44]. Preliminary SEM models were designed and tested, being the final model selected based on a likelihood ratio test ($p < 0.05$) and the goodness of fit values described above. Standardised coefficients < 0.1 were not represented in the final SEM graphics. SEM analyses were performed with RStudio software 1.2.5042 version, from The R Foundation for Statistical Computing, using the "lavaan" [45].

3. Results

3.1 Mercury accumulation by tissue and influence of sex and size

The mean size of juveniles were similar for both sexes, 21.4 ± 4.5 cm total length and 38.3 ± 29.3 g total weight for females, and 21.5 ± 3.7 cm total length and 38.2 ± 16.7 g total weight for males. As expected, total length and weight of sharks was positively correlated (Pearson's r = 0.963, R^2= 0.928, $p < 0.0001$, $n = 24$).

The Hg concentrations in the brain tissue were similar in both females (0.11 ± 0.05 mg/kg wet weight) and males (0.12 ± 0.07 mg/kg ww). In contrast, mean Hg concentrations in the muscle of females (0.55 ± 0.13 mg/kg ww) and males (0.72 ± 0.19 mg/kg ww) were significantly different (*t* test, $t = 2.193$, df = 16, $p = 0.043$, Fig. 1a,b).Therefore, subsequent analyses were undertaken for female and male sharks independently.

There was no correlation between Hg concentration and length for either white muscle (Pearson r = -0.439, R^2 = 0.193, $p = 0.153$, $n = 12$) or brain (Pearson's r = -0.076, R^2 = 0.006, $p = 0.796$, $n = 14$) tissues, when considering all data. Nevertheless, Hg concentrations in the brain was positively correlated with Hg concentrations in white muscle (Pearson's r = 0.675, R^2 = 0.455, p = 0.016, n =12). In males, marginally significant correlations between length and Hg concentration were found in muscle (Pearson's r = -0.737, R^2 = 0.543, $p = 0.094$, $n = 6$) and brain tissue (Pearson's r = -0.593, R^2 = 0.352, $p = 0.071$, $n = 10$).

3.2 Biochemical responses and its correlation with shark physiology and Hg burden

The SEM model for the analysis of the relationships between Hg concentration in muscle tissue and biomarkers provided a good fit to the data: $\chi^2 = 5.200$, $df = 8$, $p = 0.726$; GFI = 1.000 and SRMR = 0.077. The model explained all measured variables (R^2: 0.107 – 0.679, Fig. 2). Figure 3 illustrates the values obtained for biochemical biomarkers measured in muscle tissue according to sex and total length.

The high antioxidant capability observed for female *E. spinax* was due mainly to an increased activity of CAT (Fig. 2,3a). Positive correlations between CAT and GST activities (standardized coefficient (r) = 0.857, Fig. 2) and GST and tGSH levels (r = 0.519, Fig. 2) were observed.

Nevertheless, this upregulation of antioxidant defences was not enough to cope with levels of reactive oxygen species (ROS), since a significant level of LPO was observed in female muscle (r = 0.685, Fig. 2), concomitantly with Hg content in this tissue (r = 0.718, Fig. 2).

The SEM model for muscle tissue also highlighted a significantly higher Hg concentration in the muscle of male sharks (r = -0.481, Fig. 2). In addition, a significant increase in the aerobic production of energy, as assessed by ETS activity, was also observed in males (r = -0.496, Fig. 2).

The SEM model for the relationships between Hg concentration in brain tissue and biochemical parameters by sex also resulted in a good fit to the data (χ^2 = 7.896, df = 4, p = 0.095; GFI = 0.799 and SRMR = 0.079). Measured variables were also correlated, with R^2 varying from 0.108 to 0.440 (Fig. 4), with the measured values for the biochemical biomarkers in brain tissue shown in relation to sex and shark length (Fig. 5).

The redistribution of Hg accumulated in the muscle tissue to other tissues, such as the brain, is corroborated by the significant positive coefficient (r = 0.596) obtained in the SEM model (Fig. 4). Neurotoxic effects of Hg were observed through a decreased activity of brain AChE (r = -0.297, Fig. 4) associated with increased Hg concentrations. Moreover, the level of Hg in the brain (r = 0.395, Fig. 4) and the activity of AChE (r = 0.549) significantly contributed to high levels of LPO in the brain of sharks. Once again, this oxidative damage was higher in females (r = 0.228, Fig. 4) than in males, although without the same extent that was previously observed for muscle tissue. In contrast to what was observed for muscle tissue, aerobic energy production (ETS activity) in the brain was significantly higher in female sharks (r = 0.528, Fig. 4).

4. Discussion

Mercury is a natural and ubiquitous contaminant in the marine environment, although elevated levels have been associated with anthropogenic activities, past and present. Mercury is known to bioaccumulate and biomagnify in marine predators, including sharks [11,46]. Recently, the novel technology available to quantify this pollutant has highlighted that the Hg concentrations in the ecosystems are higher than previously known [2]. Thus, further information is required to monitor Hg cycles and availability, as well as to understand the physiological and biochemical alterations that accumulation of this non-essential metal means to the health status of marine organisms.

The specimens of *E. spinax* analysed in this study were 16 – 32 cm in total length and, given a length at birth of 8.3-11.3 cm [47] and a length-at-maturity of 25.4 cm (male) and 30.9 cm (female; [48]) were primarily immature individuals. Whilst this species has no commercial value in Portuguese fisheries, and therefore is neither consumed directly (as seafood) nor

indirectly (e.g., pharmaceutical uses) by humans, it can be noted that the Hg concentrations in the muscle of all specimens were below the 1 mg/kg wet weight adopted by the European Union as a safe value for human consumption of all shark species [49]. Nevertheless, the maximum observed concentration, at 0.96 mg/kg (a 20.7 cm male specimen), was close to this threshold.

Results from this study showed that *E. spinax* from the southern Portuguese coast have mean Hg values in muscle tissue (0.61 ± 0.17 mg/kg ww) that were slightly lower than observed in some other shark species from the North Atlantic. For example, mean concentrations in great lanternshark *Etmopterus princeps* (57.9 cm mean total length) and Portuguese dogfish *Centroscymnus coelolepis* (102.4 cm mean total length), both deep-water species, have been reported as 1.72 and 4.96 mg/kg ww, respectively [12], with Hg concentrations of the blue shark *Prionace glauca* (112 to 167 cm total length) being 1.36 ± 0.83 mg/kg ww [9]. Earlier studies from Mid Atlantic Ridge reported Hg concentrations of 0.04 – 0.18 mg/ kg dry weight (dw) in the liver of *E. spinax* [50] and 1.0 – 3.6 μg/g (ww) in the muscle of *E. pinceps* (33 – 54 cm total length) [51].

Hg bioaccumulation in fish is known to be determined by several abiotic (e.g., water pH, salinity, temperature, or organic matter available) and biotic factors (e.g., fish size and age, sex, trophic level, metabolic rate) [18]. Sexual differences in Hg bioaccumulation have been suggested to occur due to differences in endogenous factors such as energetic requirements, size, maturity, or the maternal transfer of Hg to embryos [7,52]. In the current study, male *E. spinax* were observed to have a higher Hg concentration than females, and such sexual differences have been reported in other elasmobranchs. For example, the Hg concentrations in the muscle tissue of large (>100 cm) male common smooth-hound *Mustelus mustelus* have been found to be greater than in large (> 125 cm) females [53], and this was suggested to be related to a slower growth rate of males leading to a greater concentration effect. Similarly, adult male silky shark *Carcharhinus falciformis* were observed to have higher Hg concentrations than females, whichwas suggested to be due to the maturation of males [54].

The results of the present study did not indicate a significant correlation between length and Hg concentration, although positive relationships have been reported in a range of other sharks [55], skates [56] and teleost fish [57]. Whilst recognizing the limited number of large specimens in the present study, it may also be noted that no correlation between length and Hg concentration has also been observed in *Mustelus mustelus*[53].

Given that the Hg concentrations of early free-living *E. spinax* had slightly higher Hg concentrations than larger juveniles, future studies could usefully quantify the Hg concentrations in the muscle and liver of gravid females, and the yolk sac and muscle of developing embryos, to further understand the potential maternal transfer of Hg.

The paradigm of Hg bioaccumulation with growth suggests that a positive correlation between Hg concentrations in muscle tissue and body size in fish can be expected, as Hg has high bioaccumulation potential, but very low detoxification rates [20]. However, the samples used in the present study were limited to specimens taken as bycatch of bottom trawls fishing for crustaceans, and such gears are likely to be less effective for sampling larger lantern sharks [58].

Whilst the longevity of a species can also influence the bioaccumulation of Hg, ontogenetic changes in diet and foraging habitats may contribute to Hg biomagnification [13]. For instance, *E. spinax* feeds on decapod crustaceans, cephalopods and teleost fish, with the former being more important in the diet of juveniles [26,59]. Whilst contemporary data on the Hg concentrations of potential prey species are lacking for the study area, the concentrations of total Hg reported from the predominant prey species of juvenile *E. spinax* have been reported to be in the range of 0.10-0.49 µg/g dry weight (the euphausiid *Meganyctiphanes norvegica*) and 0.16-2.23 µg/g dry weight (the shrimp *Pasiphaea sivado*), but these data were from Mediterranean waters [60].

Additionally, Hg concentrations in juveniles may be influenced by changes in metabolism and the development of reproductive organs and gamete production, leading to the mobilisation and use of significant amounts of lipids. In terms of the reproductive modes of sharks, the transfer of Hg from females to their offspring has been demonstrated in the placentally-viviparous species Atlantic sharpnose shark *Rhizoprionodon terraenovae* [14], although the potential transfer of mercury through the yolk sacs of aplacentally viviparous species is less well understood [11,52]. Prenatal exposure to Hg has been shown to cause biochemical alterations in mammalian offspring, leading to impaired development of their antioxidant systems and neurotoxic effects [15].

Due to the previously mentioned characteristics and behaviour of Hg, and known impacts on health, Hg is a contaminant that is a particularly good candidate model for global studies of pollution levels in oceans, effective monitoring of biota health status, and seafood safety. Whilst there only is a small number of studies examining the Hg concentrations in the deep-water ichthyofauna of the North-east Atlantic and Mediterranean [9,14,46,61], these studies considered varied locations, timing and species. Consequently, more standardised monitoring could be considered to better understand the longer-term trends in the contaminant levels in deep-sea environments, especially with regards to more persistent contaminants, such as Hg.

Oxidative stress was detected in muscle tissue, and such results were expected since it has been widely observed that Hg exposure causes an increased production of reactive oxygen species (ROS) (reviewed in [15,19]). Thus, changes in enzymatic (such as CAT and GST) and non-

enzymatic (such as tGSH) antioxidants have been used as biomarkers of oxidative stress, as they play a vital role in regulating these free radicals in the cells to counteract oxidative damage. The SEM model highlighted the positive relationships between the studied antioxidant defences, CAT – GST – tGSH, corroborating their complementary action in the cells. A positive correlation between CAT and GST activities has also been observed in the liver tissue of juvenile *P. glauca* [9]. Among antioxidant defences, GSH is the most abundant intracellular low molecular weight thiol compound in animal tissues that, helped by the activity of enzymes such as GST, conjugates and detoxifies most xenobiotics and other ROS-damaged molecules in the cell. Although we could not observe a clear pattern in our data, the Hg affinity for GSH usually leads to the formation of the excretable GS-$HgCH_3$ complex and has been indicated as one primary mechanism of Hg detoxification from the cell [15]. Therefore, Hg excretion with consequent decreases in GSH that is present intracellularly plus the direct interaction of Hg in combination with nucleophilic protein groups have been suggested as major paths of Hg-induced oxidative stress [15].

Aerobic energy production in the mitochondria through the electron transport system (ETS) activity is vital to cell function playing a role in the generation of ROS species as well as the production of energy to feed antioxidant defenses [62]. The SEM model allowed us to observe the negative direct effects of Hg concentration in ETS activity and the consequent contribution to oxidative damage. Previous evidence of Hg interaction and disruption of ETS activity was demonstrated by an increase in ROS formation [15,63].

The results for Hg levels in brain tissue (0.12 ± 0.06 mg/kg ww) were similar to the mean values of 0.14 mg Hg/ kg reported in the related *Etmopterus princeps* [12] and also *R. terraenovae*, 0.19 ± 0.22 mg/kg ww [14]. Few studies have evaluated the potential of Hg to bioaccumulate in the brain tissue of sharks and to what extent it may lead to neurophysiological impacts [14,64]. Our results are in agreement with the positive correlation between total Hg concentration in brain and muscle tissue observed in *R. terraenovae* [14], with Hg concentration in muscle being higher than in brain tissue, as previously reported for both *R. terraenovae* [14] and, *E. princeps* [12]. The various neurotoxic effects, and corresponding thresholds of Hg concentration in wildlife [65] are:were neurobehavioural changes (> 0.1 mg/kg ww), neuropathological signs and neurochemical changes (> 0.4 mg/kg ww); clinical symptoms (> 6.75 mg/kg ww). Thus, the results of this study suggest that most of the studied *E. spinax* individuals could be subject to a degree of neurobehavioural changes.

One possible pathway for Hg entrance in brain tissue was previously described in mammals, consists of the binding of Hg to thiol-containing molecules in the blood, allowing the active transport of Hg by the amino acid transporters through the blood-brain barrier [15]. Hg in its

organic MeHg form is recognised as a highly noxious neurotoxin for humans and other animals. Impairment of intracellular calcium and glutamate homeostasis allied with oxidative stress were pointed out as primary mechanisms of action in MeHg-induced neurotoxicity [15]. Our results indicate that oxidative damage, measured as LPO levels, in *E. spinax* brain tissue as a consequence of a high Hg concentration in the brain and decreased AChE activity. The inhibition of AChE activity with increased Hg levels could be anticipated, since cholinesterases have thiol groups (sulfhydryl) for which Hg has been shown to link covalently, and so inhibiting the enzymatic activity in brain cells [15]. Despite the diversity of sensory and communication systems in fish, particularly the diversity of brain morphology, neurological effects of mercury accumulation in their brains were suggested to be mainly related to the higher accumulation in the forebrain section, therefore impairing the sensory function, the autonomic and neuroendocrine responses to stress, together with other behavioural changes [14,66]. This tendency to Hg bioaccumulation in the forebrain of marine animals has been attributable to the highest number of thiol groups found in the cells of this region [65].

5. Conclusions

Results of this study indicate that pre-adult *E. spinax* have Hg concentrations ≤ 1 mg/kg. However, the lower detoxification rates of Hg and the ontogenetic change of feeding habitats are expected to increase Hg levels in adults. Furthermore, differences in the Hg concentration in relation to gender were observed, with higher redox capability and higher oxidative damage observed in females. These results suggest that even low Hg concentrations can have acute effects in *E. spinax*, exposing individuals to oxidative stress and neurophysiological disorders. Behavioural impairment, caused by mercury neurotoxicity resulting in impaired muscular function and disorientation, could compromise species behaviours, such as the ability to locate prey. Additionally, the higher energetic demands to compensate for the oxidative stress may impact on reproductive or locomotory performance and, consequently, decrease the fitness of individuals in the population.

Conflict of interest

The authors declare that they have no conflict of interest.

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Authors contributions

Conceptualization: ACMR, CG and RV; Methodology and investigation: ACMR, CG, RV, DG; Formal analysis and original draft writing: ACMR and CG; Writing – review and editing: All authors; Resources and funding acquisition: JMSG, AMVMS.

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Fig. 1. Mercury concentration (mg/kg) in brain and white muscle (a) female and (b) male of *E. spinax*. Length-at-birth (L_{birth}), length-at-matutiry (L_{mat}) and maximum length (L_{max}) [23] are indicated.

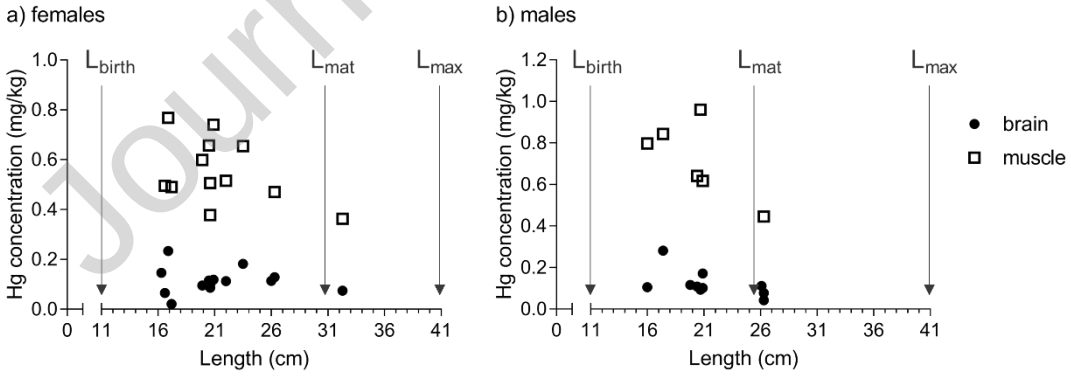


Fig. 2. SEM graphical representation of Hg concentration in muscle of *E. spinax* and its relationship with sex, biochemical biomarkers related with oxidative stress (CAT: catalase activity; GST: glutathione S- transferase activity; tGSH: total glutathione levels; LPO: lipid peroxidation) and aerobic metabolism (ETS: electron transport system activity). The arrow width is proportional to the strength of the standardised path coefficient. * $p < 0.05$.

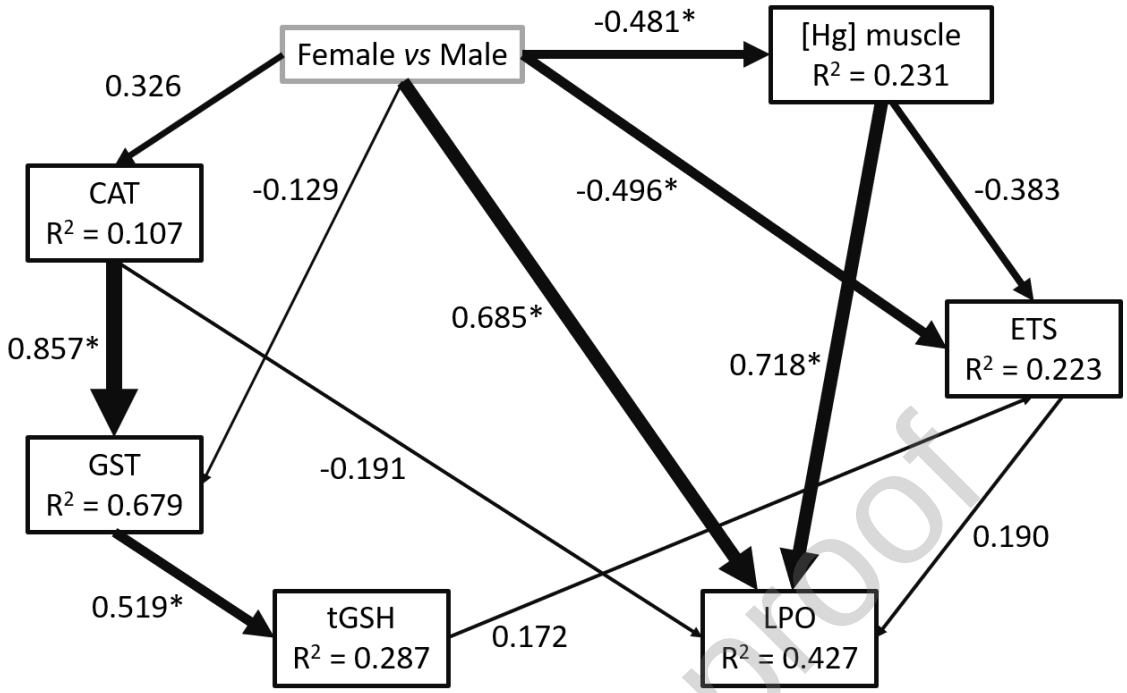


Fig. 3. Biochemical biomarkers assessed in the muscle in relation to total length (cm) of female (n = 12; open symbol) and male (n = 6; filled symbol) *E. spinax*. Biochemical biomarkers are (a) catalase (CAT) activity; (b) glutathione S-transferase (GST) activity; (c) total glutathione (tGSH) levels; (d) lipid peroxidation (LPO) levels; and (e) electron transport system (ETS) activity.

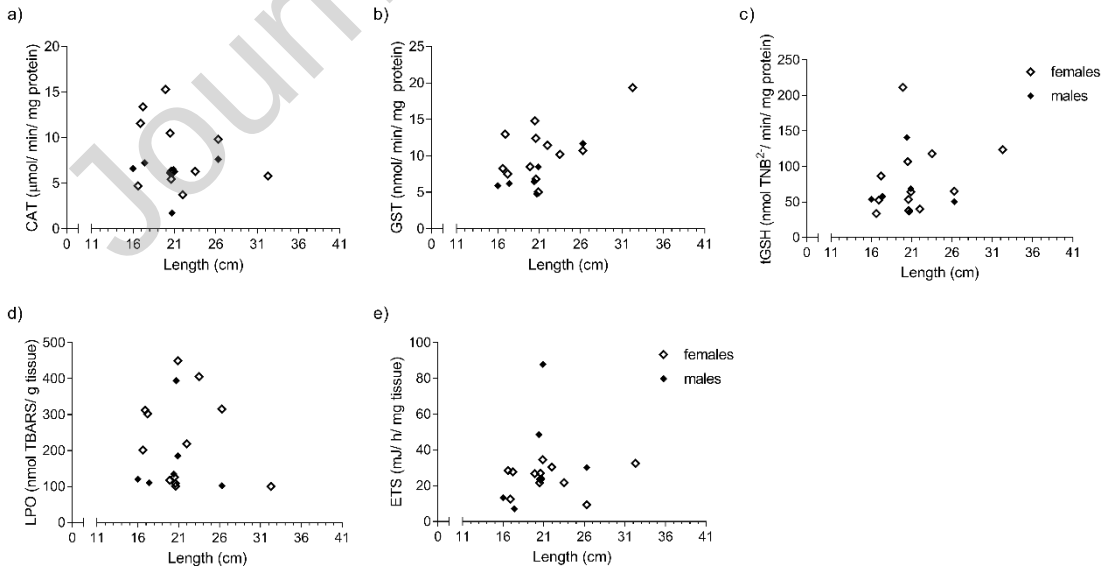


Fig 4.Fig. 4. SEM graphical representation of Hg concentration in the brain of *E. spinax* and its relationship with Hg concentration in muscle, sex, biochemical biomarkers related with neurotoxicity (AChE – acetylcholinesterase activity), oxidative damage (LPO – lipid peroxidation) and aerobic metabolism (ETS – electron transport system activity). The arrow width is proportional to the strength of the standardised path coefficient. * $p < 0.05$.

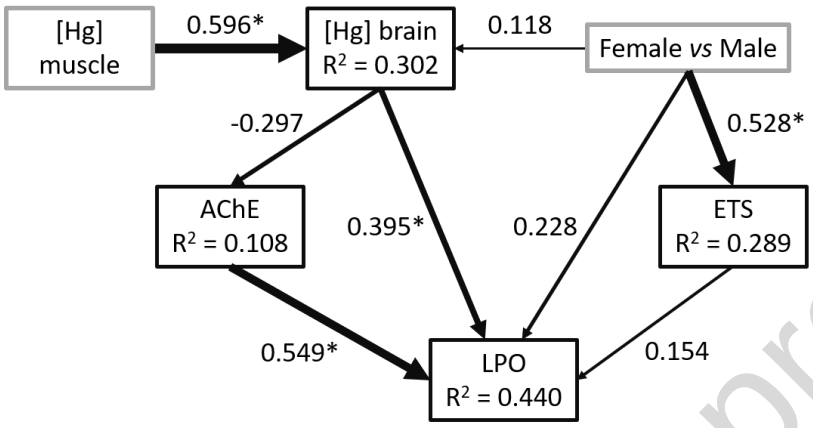
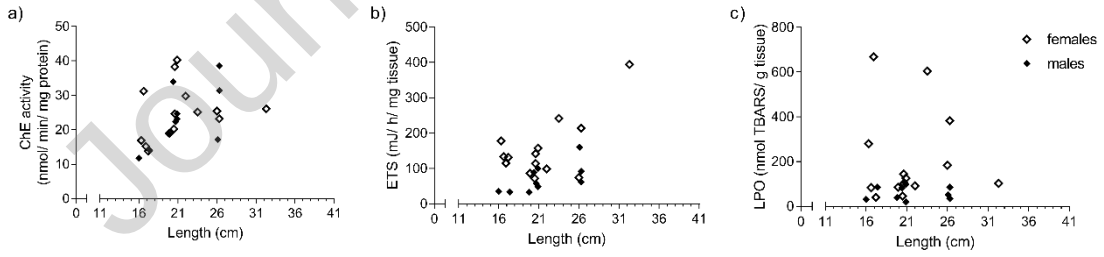


Fig. 5. Biochemical biomarkers assessed in the brain in relation to total length (cm) of female (n = 14; open symbol) and male (n = 10; filled symbol) *E. spinax*. Biochemical biomarkers are (a) cholinesterase activity (ChE, nmol/ min/ mg protein); electron transport system activity (ETS, mJ/ h/ mg tissue) and lipid peroxidation (LPO, nmol TBARS/ g tissue).



CRediT authorship contribution statement

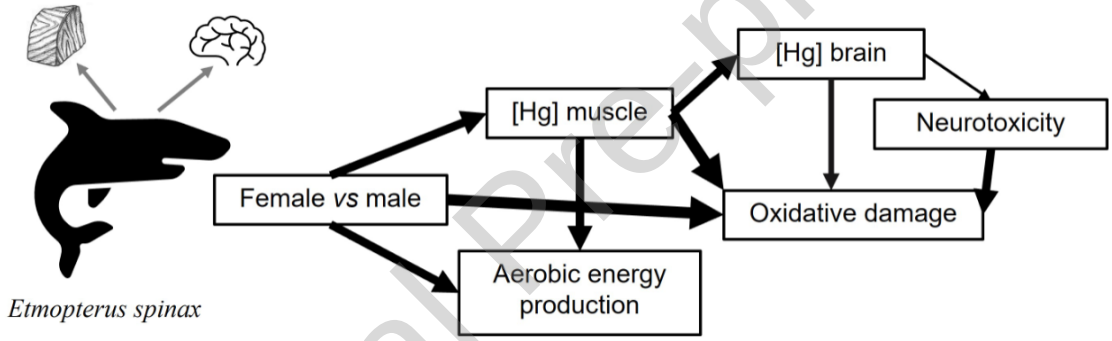
Conceptualization: ACMR, CG and RV; Methodology and investigation: ACMR, CG, RV, DG; Formal analysis and original draft writing: ACMR and CG; Writing – review and editing: All authors; Resources and funding acquisition: JMSG, AMVMS.

Declaration of Competing Interest

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Graphical abstract



Highlights

- Knowledge is still needed on oxidative stress and neurotoxicity in deep-water sharks.
- Physiological effects of Hg accumulation in the muscle and brain tissues were assessed.
- Hg accumulation in *E. spinax*: [Hg] males > [Hg] females; [Hg] muscle > [Hg] brain.
- Females showed higher antioxidant capability and oxidative damage levels than males.
- Low [Hg] can induce oxidative stress and neurophysiological disorders in *E. spinax*.