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ORIGINAL PAPER

Total and Organic Mercury in Fish from Different Geographical Areas in the North Atlantic Ocean and Health Risk Assessment

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Abstract
Fish consumption is considered the main route of exposure to mercury for the human population. In this sense, this study analysed the total and organic mercury content present in the muscle tissue of 38 fish species caught in different geographical areas of the North Atlantic Ocean (included in FAO fishing areas 27 and 31). The potential risk to human health through fish consumption was assessed using the maximum limit for mercury intake of 1.3 μg methylmercury Kg body weight^{-1} week^{-1}. The results show that carnivorous species presented higher mercury content (range 0.03–0.88 μg g^{-1}) when compared to omnivorous (range 0.003–0.19 μg g^{-1}) in all sampling sites. Furthermore, demersal fish exhibited higher mercury levels (range 0.01–0.88 μg g^{-1}) than the pelagic species (range 0.003–0.38 μg g^{-1}) did. From the 38 species analysed only *Zeus faber* presented mercury levels (0.68 ± 0.07 μg g^{-1}) above the maximum limit (0.5 μg g^{-1}) established for human consumption. On the other hand, mercury intake can be higher than the recommended due to the consumption of 13 species from fishing area 27 (Azores archipelago and Northwest Portuguese coast) and one species (*Cynoscion nebulosus*) from fishing area 31 (Southeast Mexican coast). However, only the consumption of *Zeus faber* and *Aphanopus carbo* from the fishing area 27 is discouraged. Thus, the fish consumption per capita in a specific area can exceed the limits of mercury ingestion, even when the mercury content in the fish is below the recommended for consumption.

Keywords Mercury bioaccumulation · Organic mercury · Commercial fish species · Estimated daily intake · Target hazard quotient

Introduction

The marine fisheries sector plays both an important economic and social role in the world (Teh and Sumaila 2013). Global total marine catches increased from 81.2 million tonnes in 2017 to 84.4 million tonnes in 2018, with 21.33 million tonnes being caught in the Atlantic Ocean. For statistical purposes, 19 major marine fishing areas have been created covering all adjacent oceans and seas. The Atlantic Ocean was thus divided into 6 major fishing areas: area 21 (Atlantic, Northwest), area 27 (Atlantic, Northeast), area 31 (Atlantic, Western Central), area 34 (Atlantic, Eastern Central), area 41 (Atlantic, Southwest) and area 47 (Atlantic, Southeast). According to FAO (2020), the contribution of each of these fishing areas to the total caught in the Atlantic Ocean in 2018 ranged between 7 and 44%, with fishing area 27 being the largest contributor to the total caught in the Atlantic Ocean.

Fish consumption has been well-known for its benefits to human health due to the presence of a variety of essential nutrients, such as omega-3 polyunsaturated fatty acids (n-3 PUFAs), protein, iodine, selenium, vitamin D, and other essential elements (Marrugo-Negrete et al. 2020; WHO 2003). For example, the long-chain omega-3 polyunsaturated fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) prevent heart diseases (Innes and Calder 2018), improve neurological functions in childhood (Koletzko et al. 2010), reduce cholesterol levels (Mason et al. 2016) and contribute to development and fetal growth (Marrugo-Negrete et al. 2020). Unfortunately, fish

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consumption is also considered as the major pathway of mercury (Hg) exposure in humans (Moriarty et al. 2020). Due to its high toxicity, even in small amounts (Chakraborty 2017), certain fish species' consumption can pose a potential risk to consumers' health. This is particularly important in vulnerable groups such as fetuses and newborns (Burger and Gochfeld 2009) since Hg can pass through the placenta and cross the blood–brain barrier. Consequently, Hg can have harmful effects, especially on the brain of the developing fetus (Pugach and Clarkson 2009).

Hg is predominantly found in the inorganic form (Lee et al. 2020a); however, this inorganic Hg can be transformed into an organic form (e.g. methylmercury – MeHg) through a bacterial process (Vieira et al. 2017). MeHg can bioaccumulate and biomagnify in the aquatic trophic chain, from plankton to the largest predatory fish (Custódio et al. 2020). In addition, MeHg has a high affinity for sulfhydryl groups in proteins, and for this reason, the Hg contained in the muscle tissue of fish is found mainly as MeHg (Bebianno et al. 2007; Polak-Juszczak 2018).

After ingestion, more than 90% of the MeHg is absorbed by the gastrointestinal tract (Lee et al. 2020b) and then transported to the blood where it may cross the blood–brain barrier (Moriarty et al. 2020). MeHg is a potent neurotoxin that affects particularly the brain and nervous system of human beings (Unoki et al. 2018). MeHg poisoning may cause a neurological disease (Minamata disease) whose clinical manifestation includes dysarthria, postural and action tremor, cognitive impairment, hearing loss and disequilibrium (Jackson 2018). In recognition of the risks associated with exposure to Hg through the diet, the international community has taken action to protect human health (Buck et al. 2019). The European Union and Food and Agriculture Organization (FAO)/World Health Organization (WHO) Joint Expert Committee on Food Additives (JECFA) have determined the maximum permitted level of 0.5 μg g^{-1} Hg (ww) in the muscle of most fish species, and 1 μg g^{-1} Hg (ww) in the muscle of some predatory fish species (Codex Alimentarius Commission 2018; EU 2008). Furthermore, JECFA has established a reference dose (RfD) for MeHg Called provisional tolerable weekly intake (PTWI) of 1.3 μg kg body weight $(bw)^{-1}$ $week^{-1}$ (EFSA Scientific Committee 2015). This RfD estimates the maximum daily exposure value for the human population that is unlikely to have an appreciable risk of harmful effects over a lifetime (Milatou et al. 2020).

This study aims to: (i) evaluate the total [Hg] and organic [Hg] in the muscle of fish species of commercial interest in two different fishing areas in the North Atlantic Ocean; (ii) compare the total [Hg] in the different species according to their trophic level, habits and lifestyle; (iii) estimate the contribution of each species to the OHg intake for human consumers; (iv) evaluate the human health implications of such OHg exposure using the non-carcinogenic target hazard quotient (THQ) and (v) calculate the number of meals that can be taken taking into account the reference values established by JECFA in order to establish recommendations for fish consumption.

Materials and Methods

Fish Sampling and Preparation

A total number of 180 individuals, corresponding to 38 fish species, were obtained through recreational fishing and in local markets in two FAO fishing areas of the North Atlantic Ocean (Fig. 1). Twenty nine of these species were caught in two distinct locations belonging to the FAO fishing area 27, more precisely, 18 from the Azores Archipelago, Portugal (Aa) and the other nine from the Northwest coast of Portugal (NPc). The remaining 11 species were caught on the Southeast Mexican coast (SMc), which belongs to the FAO fishing area 31. Due to the natural species distribution and the characteristics of the coastal areas, it was not possible to collect the same species of fish in all locations. However, all species present in this study are widely consumed by the population of the respective sampling areas.

Fish specimens were identified, measured and weighted. A portion of the dorsal muscle tissue without skin or bones was then collected and stored at −20 ºC, in individual and properly identified polyethylene ziplock bags (Scancell Folien Vertriebs™), for later Hg quantification. Trophic levels, food preferences and lifestyle for each species were assessed electronically at www.fishbase.org (Froese et al. 2019) and presented in Table 1.

Based on the trophic level, the species were divided into two feeding categories (omnivores and carnivores), according to Stergiou and Karpouzi (2002). These authors' classification was based on the eating habits of 146 species in the Mediterranean Sea and considered omnivores those species with a trophic level greater than 2.1 and lower than 3.7 while carnivorous presented trophic levels between 3.7 and 4.5.

According to their lifestyle, fish species were also classified as demersal or pelagic. Demersal fish species are those who live near the sea substrate, and may be dependent on the bottom (benthic) or dwell in the interface between the bottom and the water column (benthopelagic) (Pinho and Menezes 2009). The pelagic fish species are those that spend much of their lives swimming in open water away from the bottom (Castro and Huber 2008).

Total Hg and Organic Hg Quantification

The quantification of total Hg in fish samples was carried out by placing approximately 50 mg of fish tissue in the

Fig. 1 Map of fishing areas 27 and 31 established by FAO showing the sampling areas: Aa – Azores archipelago (**Aa**), Northwest Portuguese coast (**NPc**) and Southeast Mexican coast (**SMc**)

combustion chamber of the Advanced Mercury Analyzer (AMA-254, made by ALTEC and distributed by LECO). This process does not require previous digestion of the sample. The procedure is based on a pyrolysis process of the tissue using a combustion tube heated at 750 °C under an oxygen atmosphere, and the released Hg is trapped in a gold amalgamator and subsequently detected and quantified by atomic absorption spectrometry (Costley et al. 2000). On the other hand, the concentration of OHg (quantified as total organic Hg) was determined according to the method described by Valega et al. (2006). Briefly, the procedure was as follows: 200 to 400 mg of tissue were placed in 50 ml test tubes for centrifugation; 5 ml of a solution of KBr (18%) in H_2SO_4 (5%) and 1 ml of $CuSO_4$ (1 mol L^{-1}) were added to the tubes, which were kept at room temperature for 15 min. After this period, 5 ml of toluene was added. The tubes were then shaken vigorously for 15 min and centrifuged for another 15 min. After centrifugation, 5 ml of $Na_2S_2O_3$ was added to 3 ml of the organic fraction previously separated into clean scintillation vials. Then, the vials were shaken vigorously for 5 min. For organic Hg measurements, 500 µL of the aqueous phase were placed in the combustion chamber of the AMA 254 analyzer.

All Sample analysis were triplicated to check the reproducibility of the results, and three blank analyses (analysis without sample) were performed between samples to verify that Hg was not being accumulated over the samples. In this study, blank readings typically correspond to values <0.02 ng of Hg. Analytical quality for each procedure was checked using the reference material DOLT-5 (Dogfish Liver Reference Material for Trace Metals, National Research Council of Canada). Obtained data (0.41 $\pm$ 0.03 µg g^{-1} of Hg for THg and 0.11 $\pm$ 0.02 µg g^{-1} of Hg for OHg) and reference values (0.44 $\pm$ 0.18 µg g^{-1} of Hg for THg and 0.119 $\pm$ 0.058 µg g^{-1} of Hg for OHg) were not statistically different ($p > 0.05$).

Risk Assessment in Human Population

To evaluate the risk for human health through fish consumption, the Hg estimated weekly intake (EWI) was calculated by the following formula (1) (WHO 2008):

$$EWI = \frac{amount\ of\ fish\ ingested\ \left(gweek^{-1}\right) \times [Hg]\ in\ fish\ ingested\ (\mu gg^{-1})}{Kilogram\ body\ weight(kgbw)} \quad (1)$$

For this calculation, the amount of consumed fish per week was based on the average annual fish consumption per capita (2015–2017) published by FAO in the 2020 edition of The State of World Fisheries and Aquaculture. Thus, for Portugal (Azores archipelago and Northwest Portuguese coast) the average annual consumption per capita is 60 kg

Table 1 Information of fish species regarding sampling site, scientific and common names, trophic level, feeding group, food preferences and lifestyle

Site	Scientific name	Common name	Trophic level	Feeding group	Food preferences	Lifestyle
Aa	*Phycis phycis*	Forkbeard	4.3	Carnivores	Nekton	Demersal
Aa	*Pagellus acarne*	Axillary seabream	3.8	Carnivores	Zoobenthos	Demersal
Aa	*Sphyraena sphyraena*	European barracuda	4	Carnivores	Nekton	Pelagic
Aa	*Pagellus bogaraveo*	Blackspot seabream	4.2	Carnivores	Nekton	Demersal
Aa	*Scomber scombrus*	Atlantic mackerel	3.4	Omnivores	Zooplankton	Pelagic
Aa	*Trachurus trachurus*	Horse mackerel	3.7	Omnivores	Zooplankton	Pelagic
Aa	*Serranus atricauda*	Blacktail comber	4.3	Carnivores	Nekton	Demersal
Aa	*seriola dumerili*	Greater amberjack	4.5	Carnivores	Nekton	Pelagic
Aa	*Pagrus pagrus*	Red porgy	3.9	Carnivores	Nekton	Demersal
Aa	*Zeus faber*	John dory	4.5	Carnivores	Nekton	Demersal
Aa	*Balistes carolinensis*	Grey triggerfish	4.1	Carnivores	Zoobenthos	Demersal
Aa	*Mullus surmuletus*	Surmullet	3.5	Omnivores	Zoobenthos	Demersal
Aa	*Sardina pilchardus*	European pilchard	3.1	Omnivores	Zooplankton	Pelagic
Aa	*Diplodus sargus*	White seabream	3.1	Omnivores	Zoobenthos	Demersal
Aa	*Chelon labrosus*	Thicklip grey mullet	2.6	Herbivores	Plants	Demersal
Aa	*Sparisoma cretense*	Parrotfish	2.9	Omnivores	Zoobenthos	Demersal
Aa	*Pontinus kuhlii*	Offshore rockfish	4.1	Carnivores	Nekton	Demersal
Aa	*Helicolenus dactylopterus*	Blackbelly rosefish	3.5	Carnivores	Zoobenthos	Demersal
NPc	*Trachurus trachurus*	Horse mackerel	3.7	Omnivores	Zooplankton	Pelagic
NPc	*Trachurus picturatus*	Blue jack mackerel	3.3	Omnivores	Zoobenthos	Demersal
NPc	*Sardina pilchardus*	European pilchard	3.1	Omnivores	Zooplankton	Pelagic
NPc	*Merluccius merluccius*	European hake	4.4	Carnivores	Nekton	Demersal
NPc	*Micromesistius poutassou*	Blue whiting	4.1	Carnivores	Zoobenthos	Pelagic
NPc	*Aphanopus carbo*	Black scabbardfish	4.5	Carnivores	Nekton	Demersal
NPc	*Trisopterus luscus*	Pouting	3.7	Omnivores	Zoobenthos	Demersal
NPc	*Scomber scombrus*	Atlantic mackerel	3.6	Omnivores	Zooplankton	Pelagic
NPc	*Sarda sarda*	Atlantic bonito	4.5	Carnivores	Nekton	Pelagic
SMc	*Haemulon plumierii*	White grunt	3.8	Carnivores	Zoobenthos	Demersal
SMc	*Caranx crysos*	Blue runner	4.1	Carnivores	Nekton	Demersal
SMc	*Ocyurus chrysurus*	Yellowtail snapper	4	Carnivores	Nekton	Demersal
SMc	*Archosargus probatocephalus*	Sheepshead	3.5	Omnivores	Zoobenthos	Demersal
SMc	*Lagodon rhomboides*	Pinfish	4.4	Carnivores	Zoobenthos	Demersal
SMc	*Centropomus undecimalis*	Common snook	4.2	Carnivores	Nekton	Demersal
SMc	*Diapterus auratus*	Irish mojarra	2.4	Omnivores	Detritus	Demersal
SMc	*Peprilus paru*	American harvestfish	4.5	Carnivores	Zooplankton	Demersal
SMc	*Umbrina roncador*	Yellowfin drum	3.5	Omnivores	Zoobenthos	Demersal
SMc	*Lachnolaimus maximus*	Hogfish	4.2	Carnivores	Zoobenthos	Demersal
SMc	*Cynoscion nebulosus*	Spotted weakfish	4	Carnivores	Nekton	Demersal

Aa Azores archipelago, *NPc* Northwest Portuguese coast, *SMc* Southeast Mexican coast

whereas for Mexico (Southern Mexican coast) an average of 20 kg was considered (FAO 2020).

The target hazard quotient (THQ) is used to determine the non-carcinogenic risk level due to pollutant exposure (Sarkar et al. 2016). THQ indicates the ratio between exposure to Hg and the JECFA RfD. If the ratio is less than one, it means that the level of exposure is smaller than the RfD, suggesting that a daily exposure at this level is not likely to cause any deleterious effects during the lifetime of a human consumer. In other words, a THQ below one means that the adverse effects are negligible. The THQ is based on the following Eq. (2) (Vieira et al. 2020a):

$$THQ = \frac{EF \times ED \times FIR \times C}{RfD \times ABW \times AT} \times 10^{-3} \quad (2)$$

where EF is the exposure frequency (365 days year^{-1});

ED is the exposure duration (adults, 70 years, equivalent to the average lifetime);

FIR is the food ingestion rate (g person^{-1} day^{-1});

C is the metal concentration in fish (μg g^{-1}, wet weight);

RfD is the oral reference dose (μg g^{-1} day^{-1});

ABW is the average body weight (60 kg);

AT is the averaging exposure time for non-carcinogens (365 days year^{-1} × ED).

Recommendations for fish consumption (choices to avoid, good choices and best choices) were based on the maximum values of fish meals per week without exceeding the RfD established by JECFA can be calculated according to Vieira et al. (2015), using the following formula (3):

$$\text{Meals per week} = \frac{(\text{RfD} \times \text{Bw}) \times 7\text{days}}{(\text{Fish meal size (g)} \times [\text{Hg}])/1000} \quad (3)$$

The size of the fish meal was based on the 2015–2020 Dietary Guidelines for Americans who recommend at least 226.8 g of fish per week based on a 2000 cal diet (Dietary Guidelines Advisory Committee 2015), and a body weight (Bw) of 60 kg based on World Health Organization guidelines (WHO 2008) was used.

Statistical Analysis

The normality of the data was verified using the Shapiro–Wilk test. Since the data were not normally distributed, non-parametric statistical method Kruskal–Wallis test was used to compare total [Hg] of different food preferences from each sampling site and total [Hg] of different feeding groups between sampling sites. Non-parametric statistical method Mann Whitney test was also used to compare the total [Hg] of the feeding groups of each site and total [Hg] present in pelagic species of sampling sites from fishing area 27. Statistical analyses were performed using GraphPad Prism (version 6.01). Statistical significance was defined as $p < 0.05$. [Hg] data are presented as mean value ± standard error value (mean ± SE).

Results and Discussion

Total [Hg] According to Feeding Strategy and Lifestyle

Total [Hg] present in different fish species may be influenced by their eating habits (Ethier et al. 2008), since Hg can be transferred from prey to predators (Vieira et al. 2020b). Considering the feeding groups, it was possible to observe a similar pattern of Hg bioaccumulation in all sampling stations (Fig. 2), with carnivorous species presenting significantly higher total [Hg] ($p < 0.05$) than the omnivorous ones. Total [Hg] in fish caught in the Azores archipelago (Fig. 2a) ranged from 0.003 μg g^{-1} to 0.17 μg g^{-1} in omnivorous species and between 0.03 μg g^{-1} and 0.89 μg g^{-1} in the carnivorous. The variation in [Hg] of species from the Northwest Portuguese coast (Fig. 2b) was 0.01–0.19 μg g^{-1} and 0.04–0.88 μg g^{-1} for omnivorous and carnivorous species, respectively. In the Southeast Mexican coast (Fig. 2c), the [Hg] presented lower values (omnivores: 0.01–0.12 μg g^{-1} and carnivorous: 0.03–0.42 μg g^{-1}). No statistical differences ($p > 0.05$) were found between the total [Hg] in the carnivorous from the different sampling sites. The same result was obtained for omnivorous species; however, in FAO fishing zone 27, omnivorous caught in the Azores archipelago had a significantly lower total [Hg] than the ones caught on the Northwest Portuguese coast.

Increased Hg levels in carnivorous fish species, when compared to omnivorous, have also been observed by Xu et al. (2017) in fish species from two cage-cultured farms in Southern China, by Buck et al. (2019) in 40 different waterbodies from 26 countries and by Custódio et al. (2020) in fish species from Brazil. Fish species from higher trophic levels tend to accumulate greater amounts of Hg in their tissues (Zhang et al. 2020) since this metal is efficiently assimilated by the tissues of aquatic organisms, being subsequently transferred along the trophic chains through diet (Kershaw and Hall 2019).

The differences in the total [Hg] found in omnivorous species from the different sampling sites (Azores archipelago and Northwest Portuguese coast) in FAO fishing area 27 may be related to the fact that the Northwest Portuguese coast sampling site is located in the limit of the Eastern North Atlantic Upwelling System (Sousa et al. 2020). The coastal upwelling environment is biologically rich and plays an important role in many of the world's fisheries. However, these areas can represent a significant source of Hg for marine food chains, since they contribute to the presence of Hg in surface waters (Conaway et al. 2009) through the transfer of Hg from the deepest areas to shallow areas (Silva et al. 2011).

Specifically, omnivorous and carnivorous fish species may have different food preferences (Table 1). Considering the FAO fishing area 27, the species caught in the Azores archipelago show a preference for algae, zooplankton, zoobenthos and nekton. In contrast, the species obtained on the Northwest Portuguese coast prefer zooplankton and nekton. On the other hand, the fish species sampled in the FAO fishing zone 31 preferentially feed on detritus, zooplankton, zoobenthos and nekton.

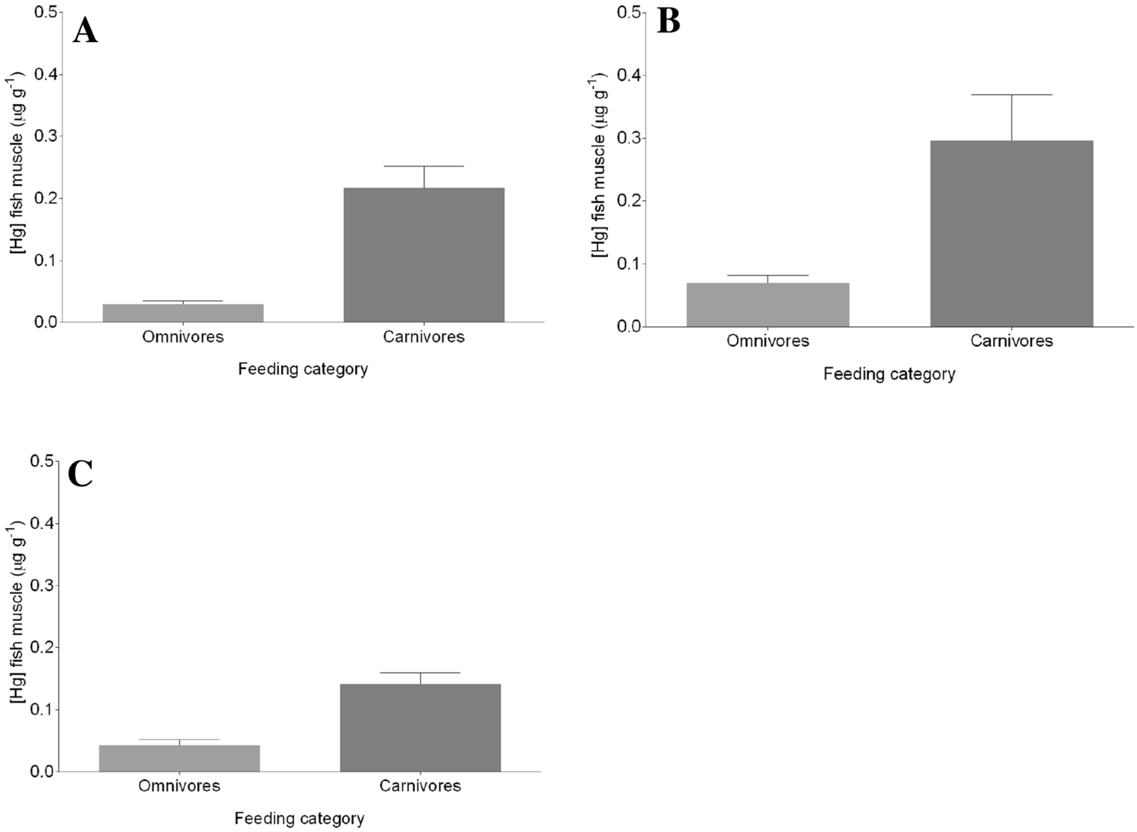

Fig. 2 Total Hg concentration (µg g^{-1}, ww) present in the muscle of carnivores and omnivores fish species from **a** Azores archipelago **b** Northwest Portuguese coast and **c** Southeast Mexican coast

Table 2 Total Hg concentration (µg g^{-1}, ww) present in the muscle of fish species from Azores archipelago, Northwest Portuguese coast and Southeast Mexican coast according to their food preferences

Food preferences	Azores archipelago	Northwest Portuguese coast	Southeast Mexican coast
Plants	0.02 ± 0.003	–	–
Detritus	–	–	0.04 ± 0.02
Zooplankton	0.01 ± 0.002	0.03 ± 0.01	0.04 ± 0.01
Zoobenthos	0.05 ± 0.01	0.13 ± 0.01	0.08 ± 0.02
Nekton	0.22 ± 0.04	0.28 ± 0.06	0.18 ± 0.03

In all sampling sites, fish with the highest total [Hg] were those that had a food preference for nekton (Table 2), which were significantly different ($p < 0.05$) from species with other feeding choices. Food items from higher trophic levels, such as nekton, usually result in greater exposure to Hg, as observed by Azevedo et al. (2019); Wang et al. (2019) and Vieira et al. (2020a). Accordingly, the second highest value of total [Hg] was attributed to species feeding on zoobenthos followed by zooplankton, whereas the detritus- and plant-feeding species presented the lowest Hg values.

Regarding the lifestyle (Fig. 3), distinct scenarios were observed in relation to the average total [Hg] of the fish species caught in the two locations of the FAO fishing area 27. In the Azores archipelago, total [Hg] was 0.06 ± 0.02 µg g^{-1} in pelagic fish and 0.13 ± 0.2 µg g^{-1} in the demersal. On the other hand, in the Northwest Portuguese coast pelagic species presented total [Hg] of 0.07 ± 0.02 µg g^{-1} while in the demersal species [Hg] of 0.26 ± 0.06 µg g^{-1} was detected. Significant differences ($p < 0.05$) were found between the total [Hg] of pelagic species and the total [Hg] of demersal species in both sampling sites. Unfortunately, pelagic fish species were not obtained in the fishing area 31; however, total [Hg] of 0.11 ± 0.01 µg g^{-1} was found in the demersal species.

When comparing the total [Hg] present in pelagic fish from the different sampling sites, species obtained on the Northwest Portuguese coast showed total [Hg] significantly higher ($p < 0.05$) than species caught in the Azores archipelago. Moreover, it was observed that the demersal species from the Northwest Portuguese coast presented a significantly higher total [Hg] when compared to species obtained in the Azores archipelago and Southeast Mexican coast.

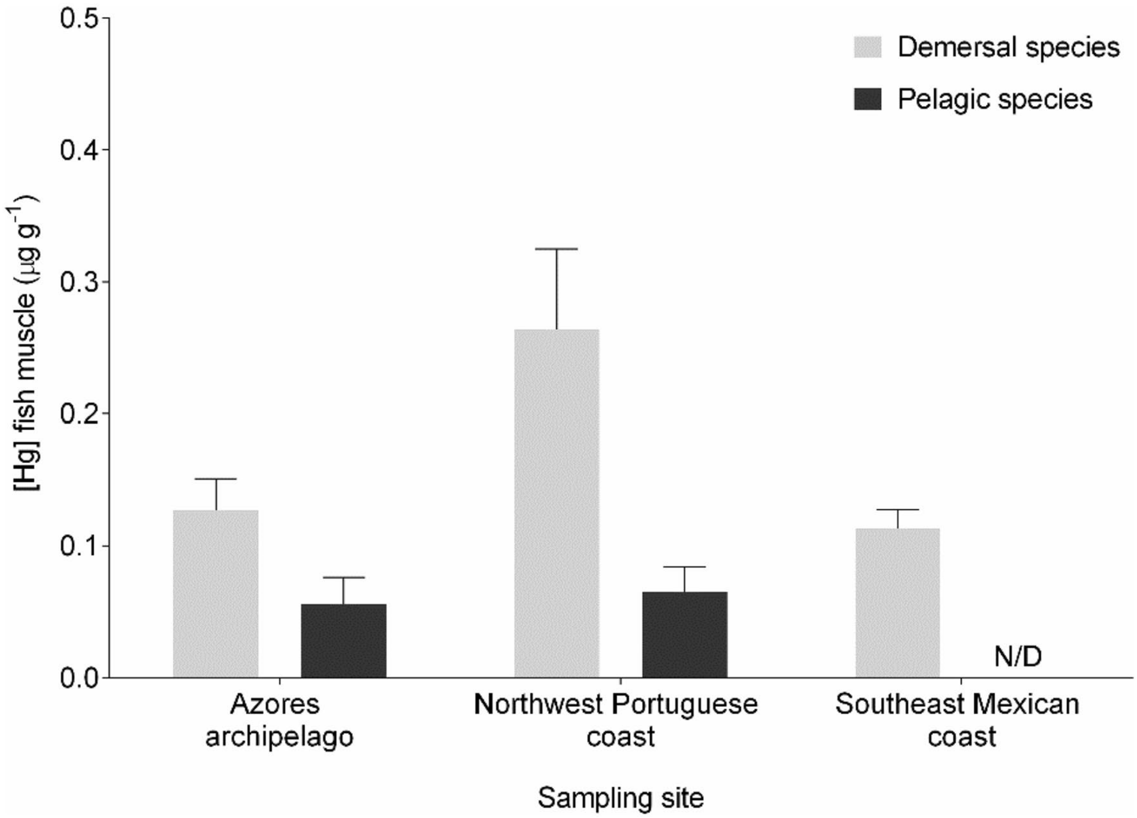

Fig. 3 Total Hg concentration (µg g^{-1}, ww) present in the muscle of Pelagic and Demersal fish species from different sampling sites

Other studies have also reported differences in Hg concentrations between pelagic and demersal species (Azevedo et al. 2019; Sulimanec Grgec et al. 2020; Vieira et al. 2020a). Choy et al. (2009) analysed stomach contents and observed that the prey of fish species that live at greater depths present higher levels of Hg than the prey in shallower waters, suggesting that deeper water species tend to be more exposed to Hg because they feed on species with higher [Hg]. This could be the reason for the differences between pelagic and demersal species found in the Azores archipelago since demersal species live in deeper waters than the pelagic. On the other hand, the great increase in the total [Hg] of pelagic species on the Portuguese coast, may be related to the fact that they were captured in an upwelling area. The main source of Hg in the open ocean is the deepwater column (Choy et al. 2009), and in the upwelling areas the deepwater layers are displaced to the surface, which means that pelagic species in these upwelling areas may be more exposed to Hg.

Interspecific Differences of [Hg]

Considering the FAO fishing area 27, more specifically in the Azores archipelago, the average total [Hg] varied from 0.007 ± 0.001 µg g^{-1} in *Trachurus trachurus* to 0.682 ± 0.07 µg g^{-1} in *Zeus faber* (Fig. 4a). Total [Hg] present in *Mullus surmuletus* (0.06 ± 0.01 µg g^{-1}), *Helicolenus dactylopterus* (0.35 ± 0.05 µg g^{-1}), *Phycis phycis* (0.06 ± 0.01 µg g^{-1}), *Diplodus sargus* (0.11 ± 0.02 µg g^{-1}), *Chelon labrosus* (0.02 ± 0.002 µg g^{-1}) and *Pagellus bogaraveo* (0.06 ± 0.02 µg g^{-1}) are within the [Hg] ranges reported by Andersen and Depledge (1997) (0.03 to 0.07 µg g^{-1}, 0.30 to 0.41 µg g^{-1}, 0.06 to 0.07 µg g^{-1}, 0.03 to 0.17 µg g^{-1}, 0.01 to 0.03 µg g^{-1} and 0.03 to 0.11 µg g^{-1}, for each species respectively). Values are also similar to the total [Hg] reported by Monteiro et al. (1991) for *Helicolenus dactylopterus* (0.29 ± 0.02 µg g^{-1}) and *Pontinus kuhlii* (0.16 ± 0.01 µg g^{-1}). In the Northwest Portuguese coast (Fig. 4b), the species with the lowest [Hg] was *Sardina pilchardus* (0.014 ± 0.002 µg g^{-1}), and the one with the highest [Hg] was *Aphanopus carbo* (0.68 ± 0.07 µg g^{-1}). The total [Hg] observed in *Aphanopus carbo* is consistent with the values reported by Afonso et al. (2008) (0.63 ± 0.27 µg g^{-1}). When comparing the fish species that were common to both sampling stations of FAO fishing area 27, specimens of *Scomber scombrus* and *Trachurus trachurus* from the Northwest Portuguese coast presented significantly higher total [Hg] than those from the Azores archipelago. The difference may be related to the characteristics of the sampling sites (as mentioned above for the pelagic species). This may also be explained

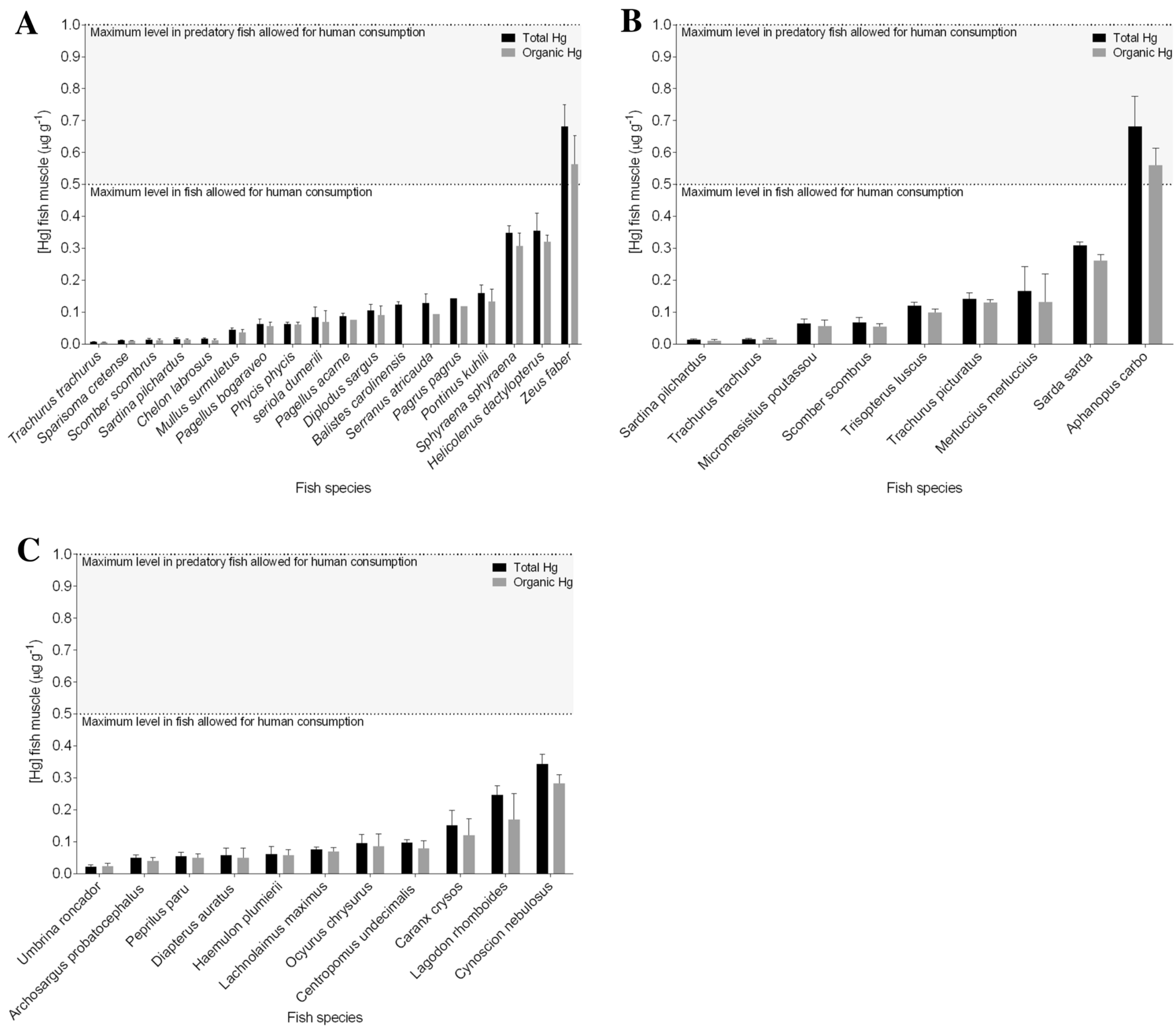

Fig. 4 Total and Organic Hg concentration (μg g^{-1}, ww) present in the muscle of fish species from **a** Azores archipelago **b** Northwest Portuguese coast and **c** Southeast Mexican coast

by biotic factors such as fish length (Backstrom et al. 2020), since the size of the individuals analysed in the Azores archipelago (12.5 ± 0.6 cm for *Trachurus trachurus* and 17.7 ± 0.6 cm for *Scomber scombrus*) were smaller than the individuals sampled in the Northwest Portuguese coast (16.4 ± 0.9 cm for *Trachurus trachurus* and 24.5 ± 0.5 for *Scomber scombrus*). On the other hand, no significant differences ($p > 0.05$) were observed in the [Hg] of *Sardina pilchardus*, although the specimens captured in the Azores archipelago were statistically smaller than the specimens of the Northwest Portuguese coast (12.4 ± 0.9 cm against 16.04 ± 0.4 cm). In the FAO fishing area 31 (Fig. 4c), [Hg] ranged between 0.02 ± 0.005 μg g^{-1} (*Umbrina roncador*) and 0.34 ± 0.03 μg g^{-1} (*Cynoscion nebulosus*). Similar total [Hg] for *Cynoscion nebulosus* (0.33 ± 0.05 μg g^{-1}) have been found by Adams et al. (2010) in specimens collected from South Florida waters (FAO fishing area 31).

Of the 38 fish species analysed in this study, only *Zeus faber* presented [Hg] above the FAO/WHO and EU regulatory limit (0.50 μg g^{-1} ww); all were below the limit (1.0 μg g^{-1} ww) established for predatory species (e.g. bonito (*Sarda sarda*), scabbard fish (*Aphanopus carbo*), seabream (*Pagellus spp.*)] (Codex Alimentarius Commission 2018; EU 2008). Junqué et al. (2018) observed [Hg] above the limit of 0.5 μg g^{-1} ww in *Zeus faber* specimens from the Western Mediterranean Sea. A [Hg] of 0.75 μg g^{-1} was also found by Di Lena et al. (2017) in the Central Adriatic (CA) and Central Tyrrhenian Seas (CT), Italy. On the other hand, although *Aphanopus carbo* presents the highest [Hg] (0.68 ± 0.07 μg g^{-1}), this species belongs to the above-mentioned list of predatory species and, therefore, does not exceed the permitted limit (1 μg g^{-1} ww).

Risk Assessment for the Human Fish Consumers

Most of the Hg present in the fish muscle is found in its organic form (Ferreira da Silva and de Oliveira Lima 2020; Sulimanec Grgec et al. 2020), as observed by the present study, where the majority (> 80%) of Hg present in the muscle tissue of the 38 fish species was organic (Table 3).

According to (FAO 2020), the sampling locations of FAO fishing area 27 have a weekly fish consumption of 1150.1 g, decreasing to 383.5 g in the sampling location located in FAO fishing area 31. This is reflected in the calculated Hg intake through fish consumption (Table 3) which ranged between 0.1 and 10.8 μg MeHg kg bw^{-1} $week^{-1}$ in the Azores archipelago, 0.2 and 10.7 μg MeHg kg bw^{-1} $week^{-1}$ on the Northwest Portuguese coast and from 0.13 to 1.8 μg MeHg kg bw^{-1} $week^{-1}$ on the Southeast Mexican coast.

Table 3 Hg concentration (μg g^{-1}, ww), estimated daily intake (EDI) and the estimated target hazard quotient (THQ) for each fish species from Azores archipelago Northwest Portuguese coast and Southeast Mexican coast

Site	Scientific name	Organic Hg (μg g^{-1})	Organic Hg (%)	EDI	THQ
Azores archipelago	*Phycis phycis*	0.06	98	1.2	0.9
	Pagellus acarne	0.08	87	1.5	1.2
	Sphyraena sphyraena	0.31	89	5.9	4.7
	Pagellus bogaraveo	0.06	89	1.1	0.9
	Scomber scombrus	0.01	87	0.2	0.2
	Trachurus trachurus	0.01	83	0.1	0.1
	Serranus atricauda	0.09	85	1.8	1.4
	seriola dumerili	0.07	84	1.3	1.1
	Pagrus pagrus	0.12	83	2.3	1.8
	Zeus faber	0.56	83	10.8	8.6
	Mullus surmuletus	0.04	84	0.7	0.6
	Sardina pilchardus	0.01	83	0.3	0.2
	Diplodus sargus	0.09	86	1.7	1.4
	Chelon labrosus	0.01	81	0.3	0.2
	Sparisoma cretense	0.01	87	0.2	0.2
	Pontinus kuhlii	0.13	84	2.6	2.0
	Helicolenus dactylopterus	0.32	82	6.1	4.9
Northwest Portuguese coast	*Trachurus trachurus*	0.01	86	0.3	0.2
	Trachurus picturatus	0.13	90	2.4	1.9
	Sardina pilchardus	0.01	86	0.2	0.2
	Merluccius merluccius	0.13	80	2.6	2.0
	Micromesistius poutassou	0.06	89	1.1	0.9
	Aphanopus carbo	0.56	82	10.7	8.5
	Trisopterus luscus	0.10	85	2.0	1.6
	Scomber scombrus	0.05	81	1.0	0.8
	Sarda sarda	0.26	83	5.0	3.9
Southeast Mexican coast	*Haemulon plumierii*	0.10	91	0.7	0.5
	Caranx crysos	0.12	96	0.8	0.6
	Ocyurus chrysurus	0.08	88	0.5	0.4
	Archosargus probatocephalus	0.04	85	0.3	0.2
	Lagodon rhomboides	0.17	92	1.1	0.9
	Centropomus undecimalis	0.08	82	0.5	0.4
	Diapterus auratus	0.05	88	0.3	0.3
	Peprilus paru	0.05	89	0.3	0.3
	Umbrina roncador	0.02	94	0.1	0.1
	Lachnolaimus maximus	0.07	88	0.4	0.4
	Cynoscion nebulosus	0.28	82	1.8	1.4

The consumption of 9 of the 17 fish species caught in the Azores archipelago (*Pagellus acarne*, *Sphyraena sphyraena*, *Serranus atricauda*, *Seriola dumerili*, *Pagrus pagrus*, *Zeus faber*, *Pontinus kuhlii*, *Helicolenus dactylopterus, Diplodus sargus*) leads to an intake of Hg above the levels recommended by the JECFA. On the Northwest Portuguese coast, the consumption of *Trachurus picturatus*, *Merluccius merluccius*, *Trisopterus luscus* and *Sarda sarda* also contributes to a Hg intake exceeding the recommended levels. On the other hand, in the Southeast Mexican coast, only the consumption of one species (*Cynoscion nebulosus*) exceeds the limits. The majority of species that contribute to Hg intake above the reference dose were caught in fishing area 27 due to the fact that the consumption of fish per capita in these sampling areas is three times higher than the fish consumption in the sampling area of the fishing area 31.

THQ values (Table 1) ranged between 0.1 and 8.6 in the Azores archipelago, between 0.2 and 8.5 on the Northwest Portuguese coast and between 0.1 and 1.4 on the Southeast Mexican coast, which means that the consumption of some species analysed in this study can be considered potentially hazardous to human health.

According to the 2015–2020 Dietary Guidelines for Americans, consumption of about 226.7 g per week of fish can provide an average of 250 mg per day of fatty acids (EPA and DHA) (Dietary Guidelines Advisory Committee 2015). Based on this quantity of fish consumed and the reference dose established by JECFA, the maximum number of weekly meals were grouped into three categories (Fig. 5): choices to avoid (less than 1 meal per week), good choices (1 meal per week) and best choices (more than 2 meals per week).

Considering the number of meals per week that a person should have to obtain the recommended amounts of fatty acids, only the consumption of two species (*Zeus faber* and *Aphanopus carbo*) is discouraged. These species were both captured in fishing zone 27 and are the species with the highest Hg levels in this study.

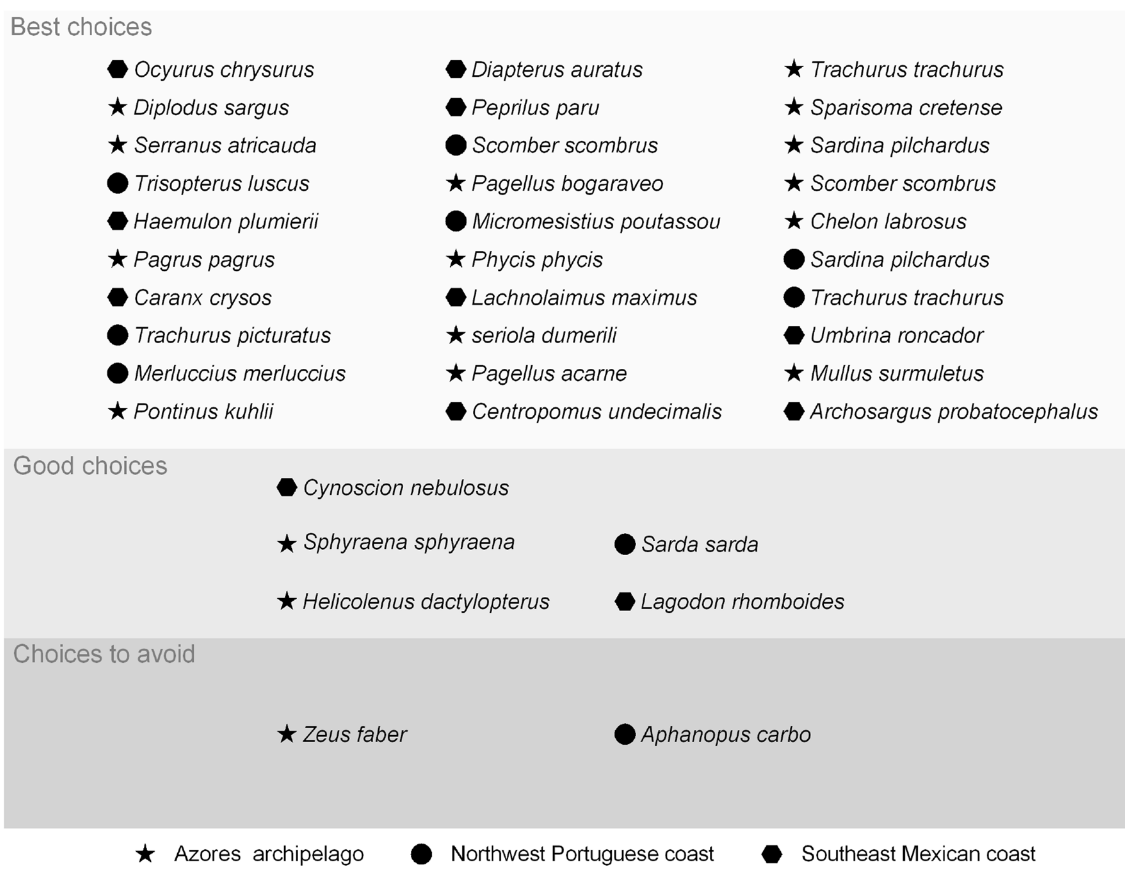

Fig. 5 Recommendations for fish consumption considering the [Hg] present in each fish species and the fish consumption established in the Dietary Guidelines for Americans

Conclusion

Not surprisingly, the total [Hg] present in the carnivorous species is significantly higher than the [Hg] measured in the omnivorous in all sampling sites; however, no significant differences were found between FAO fishing areas. Furthermore, fish species that feed on organisms from higher trophic levels (nekton) exhibit significantly higher total [Hg] than fish species that feed on lower trophic levels suggesting biomagnification trends. Considering the lifestyle of fish species in FAO fishing area 27, it was possible to observe that demersal species contain higher levels of Hg than pelagic species. The present study also demonstrates that only one of the 38 analysed fish species exceeded the permissible limits for fish consumption (*Zeus faber*), and that more than 80% of the THg present in the muscle is in its organic form.

The consumption of nine species of fish caught in the Azores Archipelago, four species from the Northwest Portuguese coast and one species from the Southeast Mexican coast contribute to a Hg intake higher than the recommended by JECFA. This indicates that frequent consumption of these species, without respecting the permitted meals per week, can be considered dangerous for human health. Finally, only the consumption of two species is discouraged *(Zeus faber* and *Aphanopus carbo*), considering the consumption of a portion of 226.7 g per week.

Although the majority of fish species analysed in this study have organic [Hg] below the recommended limit for fish consumption, some of them can be considered potentially dangerous for human health, especially in areas that have a higher consumption of fish per capita.

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Data Availability The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interests (financial or non-financial).

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