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Short abstract (150 words)

Pelagic sharks (blue shark *Prionace glauca* and shortfin mako *Isurus oxyrinchus*) caught by long-line fleets in the NE Atlantic were analysed for total mercury (Hg). Hg concentration in muscle increased with size and weight in both species, but at a higher rate in shortfin mako. Spatial variation was observed only in the blue shark with higher Hg values in the North of the Azorean archipelago. These high-level predators are particularly susceptible to bioaccumulate Hg. However, a significant positive relationship between Hg concentration and trophic level ($\delta^{15}N$) of individuals was observed only in the shortfin mako. Most sharks landed were juveniles which presented Hg concentration lower than the maximum limit allowed by the European Union for marketing. However, concentrations above this threshold were most recorded in blue sharks larger than 250 cm total length and in shortfin makos larger than 190 cm, raising the question of the commercialization of large-sized individuals.

Abstract

Pelagic sharks (blue shark *Prionace glauca* and shortfin mako *Isurus oxyrinchus*) caught by long-line Spanish and Portuguese fleets in the NE Atlantic, were sampled at Vigo fish market (Spain) for total mercury (Hg) analysis. Hg concentration in white muscle increased with size and weight in both species, but at a higher rate in shortfin mako than in the blue shark. No difference was found with sex, year and season. Spatial variation was observed in the blue shark with higher Hg values in the North of the Azorean archipelago, but not in the shortfin mako. These high-level predators are particularly susceptible to bioaccumulate contaminants (Hg) in their tissues (muscle). However, a significant positive relationship between Hg concentration and trophic level ($\delta^{15}N$) of individuals was observed only in the shortfin mako. Most sharks landed were juveniles which presented Hg concentration lower than the maximum limit allowed by the European Union (1 mg kg^{-1} wet weight) for marketing. However, concentrations above this threshold were most recorded in blue sharks larger than 250 cm total length (TL) and in shortfin makos larger than 190 cm TL, raising the question of the commercialization of large-sized individuals.

KEYWORDS: Mercury · Pelagic sharks · Blue shark · Shortfin mako · Long-line fishery · NE Atlantic

1. Introduction

Long-line Spanish and Portuguese fleets which exploit offshore north-eastern Atlantic waters target pelagic sharks, particularly the blue shark *Prionace glauca* (Linnaeus, 1758) and the shortfin mako *Isurus oxyrinchus*, Rafinesque, 1810, along with the swordfish *Xiphias gladius* Linnaeus, 1758 (Torres et al., 2016). For the past 15 years (2001–2015), the mean landings per year of blue shark represented 2 167 tonnes (63% of long-line landings) and 501 tonnes (14%) for the shortfin mako at the fish market of Vigo in Galicia, Spain (Xunta da Galicia, 2008, pers. comm.; ICCAT, 2015). Sharks are essentially sold for human consumption (meat and fin).

The blue shark can reach 380 cm in total length (TL) and could live up to 20 years in the North Atlantic (Skomal and Natanson, 2003). Blue shark females are sexually mature at 220 cm TL (5-

6 years) and males at 180 cm TL (4-5 years) (Moreno, 2004; Compagno et al., 2005). The shortfin mako presents a heavier body at similar size than the blue shark, a longer maximum size (440 cm) and a longer life span (30 years max) (Natanson et al., 2006). Median size and age at maturity would be about 280 cm TL and 7-10 years for females, which present a larger size than males, and 200 cm TL, 5-6 years for males (Moreno, 2004; Barreto et al., 2016). However, information on age and growth of both shark species is conflicting and still a matter of debate due to difficult and random sampling of these predators (Skomal and Natanson, 2003; Barreto et al., 2016). These two shark species are highly mobile predators able to migrate over thousands of kilometers in the north Atlantic Ocean (Kohler et al., 2002). Spanish and Portuguese long-liners catch mostly small individuals therefore juveniles represent the major part of shark landings at Vigo fish market for both species (73% of blue sharks and 94% of shortfin makos) (Biton-Porsmoguer, 2015).

Sharks position as high-level predators in the marine food web (Ferretti et al., 2010) makes them especially susceptible to contain high concentration of contaminants and particularly mercury (Hg) (Storelli et al., 2002), as Hg is known to bioamplify along food webs, increasing with the trophic level of organisms (Harmelin-Vivien et al., 2009, 2012; Lavoie et al., 2013). Trophic level of organisms is routinely estimated by the nitrogen isotopic ratio ($^{15}N/^{14}N$), expressed relative to a standard as $\delta^{15}N$, which tends to increase with the size of individuals and from prey to predator. $\delta^{15}N$ is then used to follow the transfer and accumulation of contaminants like Hg or PCB in organisms and food webs (Cabana and Rasmussen, 1994; Booth and Zeller, 2005; Cossa et al., 2012). Mercury is a highly toxic trace element present in all compartments of the biosphere. It enters marine food webs from natural and anthropogenic sources (Cossa et al., 2009), and foraging pathway is recognized as being the main Hg contamination way (Mathews and Fisher, 2009). Hg is susceptible to impact aquatic ecosystems including commercial species (McKinley and Johnston, 2010), which could result to adverse health effects on humans like toxic effects on the nervous, digestive, cardiovascular and immune systems, and alterations of fetal neurodevelopment (Castoldi et al., 2003; Diez, 2008). As consumption of marine organisms contributes to most Hg intake in humans, a maximum acceptable level in marine products have been laid down by European Commission regulations and set at 1 mg kg^{-1} wet weight (ww) for high-level pelagic predators (European Commission, 2006: Regulation N° 1881/2006). Fisheries Department from Galician region is supposed to apply the European regulation and must control sanitary state for all landed sea products (Law 11/2008, December 3rd 2008; Xunta da Galicia, 2008). But are the sharks landed and commercialized in Galicia fulfil all these requirements?

The main goals of the present study were thus to: (i) measure the total mercury concentration in the muscle of sharks caught in the north-eastern Atlantic Ocean and sold at Vigo fish market, (ii) determine the influence of size, weight, sex, trophic level, zones, season and year on Hg content in these sharks, and (iii) consider the possible implications for the fishery management.

2. Material and methods

2.1. Sampling and stomach content analysis

Sharks were caught by Spanish and Portuguese long-line vessels in the north-eastern Atlantic in five zones (A to F) between the Iberian Peninsula and the Azores archipelago (15º-35ºW and 30º-45ºN), in 2012 and 2013 (Fig. 1). A total of 40 blue shark (*Prionace glauca*) and 48 shortfin mako *(Isurus oxyrinchus*) landed at the fish market of Vigo (Spain) were sampled (Table 1). Blue shark and shortfin mako respectively measured from 74 to 284 cm and from 99 to 219 cm total length (TL). White muscle samples were extracted one centimeter beneath the skin from each individual, put in plastic bags and stored frozen at -20ºC. Once at the laboratory, samples were cleaned with distilled water before freeze-drying, grinding and analyzing for total mercury (Hg) and nitrogen stable isotopes ($\delta^{15}N$). Shark stomachs were extracted and stored at -20°C. After identification and weighing (wet weight, ww) of the prey found in stomach contents, those recently consumed (in good state of conservation) were freeze-dried and analyzed for Hg and $\delta^{15}N$ in the same way as shark muscle samples. Total wet weights of partially digested prey were reconstructed according to the size of their hard pieces (beaks for cephalopods and otoliths or vertebrae for teleost fish) using pre-established relationships (Biton-Porsmoguer, 2015). Reconstructed weight percentages (% ww) of the main prey types found in stomach contents were then determined for both shark species.

2.2. Mercury and stable isotope analyses

Total Hg concentrations were determined with a semi-automated atomic absorption spectrophotometer AMA 254 (Altec Ltd, Prague, Czech Republic) with a detection limit of 0.003 ng/mg, following the procedure described in Cossa et al. (2009). Hg quantification procedure consisted in three automatic sequences: (1) ashing at 550°C of the freeze-dried sample for Hg volatilisation, (2) evolved elemental Hg amalgamation on a gold trap, (3) atomic absorption spectrophotometric measurement of the Hg collected after heating the gold trap at 800°C. The accuracy of measurement was assessed every ten samples using certified reference materials from the National Research Council of Canada (fish muscle tissues DORM-4). Hg concentration level of samples was initially expressed as Hg dry weight (dw) concentration in fish muscle or prey muscle samples. However, as Hg concentrations are expressed in wet weight (ww) in international and European regulations, dry weight concentrations were converted into wet weight concentrations, considering that dw concentration = 5 ww concentration (Cresson et al., 2014). The relevancy of this factor has been corroborated with the samples of this study (personal data).

Stable isotope analyses were performed with a continuous flow mass spectrometer (Delta V Advantage, Thermo Scientific®, Bremen, Germany) coupled to an elemental analyzer (Flash EA 1112 Thermo Scientific®, Milan, Italy). N stable isotope ratios were expressed in the standard δ notation: $\delta^{15}N‰ = [(R_{sample}/R_{standard}) - 1] \times 10^3$, where R is the ratio $^{15}N/^{14}N$ in sample and standard (atmospheric air for nitrogen). Accuracy of measurement was estimated by replicate measurements of internal standard (acetanilide) and was lower than 0.1‰.

2.3. Data analysis

Quantification of Hg concentrations in sharks' diet: The diets of the blue shark and shortfin mako in the sampling area have been previously studied (Biton-Porsmoguer et al., 2013, 2015). The blue shark mainly fed on cephalopods, then teleost fish and rarely cetaceans, while the shortfin mako mainly preyed on teleost fish, then cephalopods, and occasionally cetaceans and chelonians. The importance in weight (% dw) of each prey type was determined for each species and Hg concentration of their diet was quantified with the equation as in Harmelin-Vivien et al. (2012):

$$C_{diet(i)} = \sum[C_{prey(x)} \times W_{prey(x)(i)}]$$

where $C_{diet(i)}$ is the Hg concentration in the diet of the shark species (i), $C_{prey(x)}$ is the Hg concentration of the prey (x), $W_{prey(x)(i)}$ is the percentage in weight of the prey (x) in the (i)th shark species, and $\sum$ the sum of the product for all the (x) prey types. In a similar way, the $\delta^{15}N$ value of the diet of each shark species was determined following the equation:

$$\delta^{15}N_{diet(i)} = \sum[\delta^{15}N_{prey(x)} \times W_{prey(x)(i)}]$$

where $\delta^{15}N_{diet(i)}$ is the $\delta^{15}N$ of the diet of the shark species (i), $\delta^{15}N_{prey(x)}$ is the $\delta^{15}N$ of the prey (x), $W_{prey(x)(i)}$ is the percentage in weight of the prey (x) in the (i)th shark species, and $\sum$ the sum of the product for all the (x) prey types. These calculations represent only estimations as Hg concentrations and $\delta^{15}N$ are measured on prey muscles, while weights include all prey tissues.

Biomagnification factor calculations: A mean biomagnification factor (BMF) was determined for the two sharks in the north-eastern Atlantic following Fisk et al. (2001), based on mean Hg concentration and trophic level of sharks and their diet, using the following equation:

$$BMF_{shark(i)} = [(C_{shark(i)}/C_{diet(i)})/(\delta^{15}N_{shark(i)}/\ \delta^{15}N_{diet(i)})]$$

Where $C_{shark(i)}$ is the Hg concentration in the shark species (i), $C_{diet(i)}$ is the Hg concentration in the diet of the (i) shark species, $\delta^{15}N_{shark(i)}$ is the trophic level of the (i) shark species, and $\delta^{15}N_{diet(i)}$ is the trophic level of the diet of the (i) shark species. This factor is based on the assumption that mercury concentration in a predator depends on those of its prey, corrected for trophic level difference between predator and prey. We replace the trophic level estimation used by Fisk et al. (2001) by $\delta^{15}N$ values, which represents a more accurate estimation of organism trophic position.

Statistical analysis: As the number, size, weight and sex of individuals differed between sampling zones and species, differences were tested by ANCOVA to remove the effects of these parameters and consider only the factor tested. When significant differences were observed Tukey post-hoc tests were performed. Relationships between Hg content and sharks size, weight and $\delta^{15}N$ were tested by Pearson's linear correlation on $\log_{10}$ transformed Hg concentrations to linearize the regression and stabilize the variances. Differences in slope and elevation were tested by appropriate *t*-test. *T*-test was also used for interspecific comparisons of Hg and $\delta^{15}N$ values.

3. Results

3.1. Influence of biological parameters on Hg level

In both species, mercury concentration did not vary with sex for individuals of similar size or weight (ANCOVA, all $p > 0.05$). Thus, sexes were combined for further analyses. Total Hg level in muscle ranged from 0.14 to 1.71 mg kg^{-1} ww in the blue shark and from 0.12 to 2.57 mg kg^{-1} ww in the shortfin mako (Table 2). Mean Hg concentration and mean $\delta^{15}N$ value were significantly higher in the shortfin mako than in the blue shark (t-tests, $t = -2.213$, $p = 0.029$ and $t = -2.848$, $p = 0.006$ respectively) (Table 2). Hg concentration increased significantly with size and weight in both species, as significant linear relationships were observed between log Hg and TL, (cm) and between log Hg and total weight (W kg ww) (log Hg = 0.009 W- 0.555, $R^2 = 0.59$, $p < 0.001$ in blue shark; log Hg = 0.012 W- 0.630, $R^2 = 0.66$, $p < 0.001$ in shortfin mako). Whatever the parameter considered (size or weight), the slope of the regression was significantly higher (*t*-test, $p < 0.05$) for the shortfin mako than the blue shark, suggesting different bioaccumulation capacities between the two species. However, the relationship between Hg concentration and the trophic level of individual, expressed as $\delta^{15}N$ values, was significant only for the shortfin mako (log Hg = 0.169 $\delta^{15}N$ – 2.181, $R^2 = 0.10$, $p = 0.036$), but not for the blue shark (log Hg = -0.069 $\delta^{15}N$ + 0.422, $R^2 = 0.02$, $p = 0.197$).

3.2. Geographical and temporal variations

In blue sharks of similar size, significantly higher mean Hg concentrations (x1.6) were recorded in individuals collected in zone A, than in zone B, D and F, in which blue shark had similar lower concentrations (ANCOVA $F = 3.46$, $p = 0.026$) and weight (ANCOVA $F = 3.37$, $p = 0.029$). Pearson's linear correlations between log Hg and size presented similar slopes in all zones, but a higher intercept value in zone A (Fig. 3), suggesting the influence of environmental differences between zone A in the North of the Azores archipelago and the other zones, rather than a biological difference between individuals. In contrast, no difference of Hg level among zones was observed for the shortfin mako, taken into account differences in size or weight of individuals in the different zones (ANCOVA, $F = 2.46$, $p = 0.086$ for size; $F = 1.49$, $p = 0.242$ for weight). In both species and all zones combined, Hg concentration in muscle did not significantly vary with year (2012 *vs* 2013) nor season (spring *vs* autumn) (ANCOVA, all $p > 0.05$).

3.3. Biomagnification factor and influence of diet

The biomagnification factor BMF takes only into account the last trophic level of the food web analyzed (the predator and its prey). Hg level and $\delta^{15}N$ of the main prey ingested by these two sharks displayed highly variable inter- and intraspecific values (Table 3). The lowest mean Hg concentrations were recorded in pelagic teleost prey fish, such as *Scomber* sp. (0.02 mg kg^{-1} ww) and *Scomberesox saurus* (0.08 mg kg^{-1} ww), and the highest ones in some cephalopods (*Illex* sp., 2.24 mg kg^{-1} ww) and the cetacean *Delphinus delphis* (1.77 mg kg^{-1} ww). The lowest $\delta^{15}N$ value was observed in the teleost fish *Euthynnus alletteratus* (9.7‰) and the highest one in the cephalopod *Ancistroteuthis lichtensteinii* (12.9‰). No significant correlation was found between log Hg and $\delta^{15}N$ in prey, or $\delta^{15}N$ in prey and sharks combined ($R^2 = 0.06$, $p > 0.05$ for both regressions). The blue shark and shortfin mako ingested then prey with various Hg burden, some presenting higher Hg concentration and trophic level than them. The blue shark mainly fed on cephalopods (76% by weight), teleost fish (18%) and cetaceans (0.03%), while the shortfin mako preyed mainly on teleost fish (66% by weight), cephalopods (27%), cetaceans (0.05%) and sea turtles (0.03%). When growing, the blue shark consumed less cephalopods and more teleosts and cetaceans, while the shortfin mako ingest less teleosts and more cetaceans and chelonians. Mean Hg concentration and $\delta^{15}N$ in diet was higher for the blue shark than for shortfin mako, globally and in most size classes (Table 4), while the reverse was observed in shark muscle with generally higher Hg level in shortfin mako muscle than in blue shark muscle. Mean BMF value was low (<1) in the blue shark, but higher than 1 in the shortfin mako (Table 4), suggesting Hg bioamplification from diet to predator in the shortfin mako only. When the different size classes were considered, it was observed that BMF tended to increase with the size of individuals in both species, but not in a linear way. BMF remained always <1 in the blue shark suggesting no Hg bioamplification in this species. In the shortfin mako, no bioamplification was recorded in the smaller individuals (<130 cm TL) with BMF <1, while Hg bioamplification occurred for larger-sized individuals (particularly >200 cm TL) with BMF largely >1. Such results indicated no straightforward relationship between Hg level of prey and predator, but rather suggested differences in metabolism and/or prey consumption rate between the two shark species.

3.4. Mercury in sharks and food safety

The significant exponential relationship evidenced between Hg level in muscle and body size of sharks revealed that some individuals presented higher mean Hg concentration than the European regulatory threshold (1.0 mg kg^{-1} ww) for the commercialization of high-level pelagic predators. Hg level above this value was observed for 4 individuals over 200 cm TL in the blue shark and for 12 individuals over 150 cm TL in the shortfin mako (Fig 4 and 5). We defined (i) a *size range of potential risk* for consumers with individuals between 200-250 cm TL for the blue shark (Fig 4), and 150-190 cm TL for shortfin mako (Fig 5), as such highly contaminated sharks were present but not numerous in this size range, and (ii) a *size at risk* (250 cm TL for the blue shark and 190 cm TL for the shortfin mako) above which most individuals presented higher Hg level than the allowed EU limit.

4. Discussion

4.1. Bioaccumulation of Hg in blue shark and shortfin mako

In both shark species Hg concentration in muscle was positively correlated with size and thus age (Fig. 2), independently of sex, suggesting similar feeding habits in males and females, as observed by Biton-Porsmoguer et al. (2016) with stomach content analysis. Increase of Hg level in organism as they were growing and getting older is a well-known bioaccumulation process in marine organisms, particularly in teleost fish (Cossa et al., 2012; Cresson et al., 2014) and high-level predators like sharks (Storelli et al., 2001, 2002; Branco et al., 2004, 2007; Torres et al., 2017) and cetaceans (André et al., 1991). This age-related increase in Hg content is related to an efficient accumulation of mercury due to the decrease of both detoxification capability and growth rate (Amlund et al., 2007; Dang and Wang, 2012). But many parameters can influence the bioaccumulation of Hg in organism along with age, such as growth rate, reproduction, metabolic activity, Hg concentration in prey and feeding rate (Trudel and Rasmussen, 2006;

Cossa et al., 2012). Diet is recognized as the main pathway of Hg intake in high-level predators (Boening, 2000). Both shark species ingested prey of various Hg content and $\delta^{15}N$ values, some being more contaminated and positioned at a higher trophic level than sharks (Table 3). Hg concentration and $\delta^{15}N$ in diet did not increase with shark size (Table 4). Thus, the hypothesis of an increase in Hg uptake when sharks were growing linked to an increase in Hg content of diet was not supported by our study. Larger sharks did not always seem to feed at a higher more contaminated trophic level than smaller ones, as observed by McMeans et al. (2010) in Arctic sharks. Feeding on larger prey did not imply to feed on more contaminated or higher trophic level organisms. No relationship between Hg concentration and $\delta^{15}N$ was found in the blue shark, as also observed by Torres et al. (2017) south of the Azores and Escobar-Sánchez et al. (2010) at Baja California, or by Rumbold et al. (2014) for coastal sharks in Florida. Conversely, positive relationship between Hg and $\delta^{15}N$ was observed in shortfin mako. This result was also observed in a few shark species analyzed in Australia (Pethybridge et al., 2011) or in the Celtic sea (Domi et al. 2005). Hg is efficiently accumulated in shark tissues with a very slow elimination rate as in teleost fish (Amlund et al., 2007). In addition, the fractionation and turn-over of $\delta^{15}N$ between diet and shark tissue seems to be also slower in sharks than teleost fish (Hussey et al. 2012), suggesting particular metabolic activity related to nitrogen cycling in elasmobranchs. Torres et al. (2017) recorded also similar Hg concentrations and $\delta^{15}N$ values in male and female blue sharks. The absence of influence of sex, season and year on mercury level in both species can be explained by the high mobility of these sharks regardless of sex (Queiroz et al., 2016) and the large spectrum of prey consumed at different seasons during the two years (Biton-Porsmoguer et al., 2013, 2015).

A higher increase in Hg content with size was observed in the shortfin mako compared with blue shark (Fig. 2), suggesting different bioaccumulation processes between the two species (age, diet, activity and/or metabolism). While the blue shark and the shortfin mako are long-lived species (with a higher life span for shortfin mako) (Skomal and Natanson, 2003; Natanson et al., 2006), the age of specimens analyzed in this study ranged likely from 1 to 10 years for both species with a majority of juveniles (87.5%), especially in the shortfin mako (Biton-Porsmoguer, 2015). Shortfin makos were heavier than blue sharks at the same size, invalidating the possibility of a dilution of Hg by growth in the blue shark to explain its lower Hg content. Higher Hg content of diet in shortfin mako was also not supported by our results (Table 4). Thus, the higher

Hg concentration observed in the shortfin mako could not be attributed to an older age, a slower growth rate or a higher Hg content of its prey. Differences in metabolic activity and feeding rate could explain the higher Hg uptake with size observed in shortfin mako compared to blue shark. Shortfin mako belongs to the warm-bodied Lamnidae which present an elevated aerobic metabolism compared to their ectothermic relatives like the blue shark which belongs to the Carcharhinidae (Shadwick and Goldbogen, 2012). The shortfin mako can maintain higher musculature, brain, eyes and viscera temperatures than the surrounding water (1 to 10°C higher than ambient temperature) (Carey, 1982). Therefore, it needs to eat more frequently than the blue shark and accumulates more mercury due to an increased feeding rate. This hypothesis was supported by the lower vacuity index (percentage with an empty stomach) observed in the shortfin mako (36%) compared to blue shark (47%) (Biton-Porsmoguer, 2005).

4.2. Regional differences

Mercury levels recorded in the blue shark in this study (0.14-1.71 mg kg^{-1} ww) were in agreement with values previously reported in different studies performed in the north Atlantic Ocean (0.16-1.84 mg kg^{-1} ww) and Tasmania (SW Pacific Ocean) (0.27-1.20 mg kg^{-1} ww), but not on the south coast of Brazil (0.46-2.40 mg kg^{-1} ww) (De Carvalho et al., 2014) where higher Hg concentrations were recorded (Table 5). This may be related to the high mobility of these sharks, their ability to cover large areas for feeding and their dietary opportunism. However, Branco et al. (2007) record a higher accumulation rate of total mercury with size in the blue shark from the equatorial Atlantic (0.68-2.50 mg kg^{-1} ww) compared with specimens from the Azores archipelago (0.22-1.30 mg kg^{-1} ww). They relate this pattern to higher Hg concentration in prey, as well as differences in quantity and type of food eaten in the Equator. Such a hypothesis was not relevant in our study, as the rate (regression slope) of Hg increase in blue shark with size was similar in all zones (Fig. 3). Higher Hg concentrations in the north of Azores could not be attributed to gender, as no difference with sex was observed. In mid-Atlantic (south of the Azores), Torres et al. (2017) record lower Hg concentrations in the blue shark than we did for individuals of similar size range in the same area (0.33 ± 0.02 and 0.41 ± 0.02 respectively). The higher intercept value of the linear regression for specimens from the north of the Azores suggested rather the influence of environmental conditions with probably higher Hg concentrations in sea water and prey in this zone, but no difference in metabolism, feeding rate or prey types between populations. The Azores archipelago is a volcanic region with naturally high mercury level in sea water and sediment (Guest et al., 1999) that may explain the significantly higher levels of Hg found in the blue sharks of zone A. Moreover, the Azores archipelago constitutes probably a nursery area for the blue shark and juveniles can stay in this area at least for two years (Vandeperre et al., 2014), accumulating thus a higher Hg burden during this time. However, in the absence of Hg concentration analysis in seawater and in a higher number of prey in the different zones, we cannot test this hypothesis.

No difference in Hg concentration among zones was observed for the shortfin mako in this study. This homogeneity was probably due to the high swimming activity of this species, which moves frequently between distant zones (Kohler et al., 2002). The Hg values recorded in the shortfin mako were high (0.12-2.57 mg kg^{-1} ww) and in the range of those observed for this species in the north Atlantic and other oceans (0.15-3.12 mg kg^{-1} ww) (Table 5).

4.3. Biomagnification of Hg in sharks

Mercury, under its organic monomethylmercury form, is one of the few trace metals that biomagnifies along food webs (Gray, 2002). Biomagnification is assumed when BMF factor (concentration in predator/concentration in diet corrected from their respective trophic level) is higher than 1 (Fisk et al., 2001). Biomagnification of Hg was observed only in the shortfin mako, not in the blue shark (Table 4). These two sharks consumed prey with a great variability in both Hg concentration and $\delta^{15}N$ value (Table 3). For the blue shark, Hg content in diet was higher than in shark muscle for all size classes, leading up to a BMF always <1, while slightly increasing with size. For the shortfin mako, higher Hg content in shark muscle compared with diet and BMF values >1 were observed from individuals larger than 130 cm, and more significantly larger than 200 cm. However, BMF calculation should be considered cautiously. First, the reduced number of prey analysed in this study is not sufficient to characterize the global diet of the two species and a broader study should be conducted to conclude. Second, the same mean Hg and $\delta^{15}N$ values of prey groups were used for all size-classes of both sharks. However, as observed by Biton-Porsmoguer et al. (2015), they could prey on different species. Finally, the time lag between ingestion and assimilation of prey was not considered. Stomach content offered a snap shot of shark's diet when caught, while muscle Hg concentration reflected food assimilation over several months. Nonetheless, similar results are reported by Maz-Courrau et al. (2012) at Baja California with Hg biomagnification in the shortfin mako but not in the blue shark. The absence of any Hg biomagnification in blue shark is also observed in the Pacific Ocean of México by Escobar-Sánchez et al. (2011). Difference in BMF factor between the two species, with Hg biomagnification occurring only in the shortfin mako is probably related with their metabolic differences (endothermy in shortfin mako vs ectothermy in blue shark) (Shadwick and Goldbogen, 2012) as discussed above, with a positive relationship between Hg content and $\delta^{15}N$ in the shortfin mako and not in blue shark.

4.4. Food safety and fishery implication

The mean values of mercury level in the muscle of blue shark and shortfin mako analyzed in this study were lower than the upper limit allowed by the European Union in high-level pelagic predators for human consumption (1 mg kg^{-1} ww) (Table 2), as also observed by Torres et al. (2017) for these species. However, Hg concentration exceeded the legal EU standard in larger individuals (Fig. 4 and 5). Above 250 cm TL in *P. glauca* and 190 cm TL in *I. oxyrinchus* most, if not all, individuals exhibited Hg content well above the EU sanitary risk limit, representing *a size at risk* for the consumption of these species. Measuring mercury concentration above European and international regulatory thresholds in shark muscle is regularly reported in the literature, whatever the ecology, geographic distribution or feeding habits of species (Storelli et al., 2002; Branco et al., 2004, 2007; Pethybridge et al., 2010; Escobar-Sánchez et al., 2011; De Carvalho et al., 2014; Cresson et al., 2014; Mc Kinney et al., 2016, and references in Table 5). All authors raise the health issue of shark, and more generally high-level predator, consumption. However, Hg concentration value alone does not seem to be sufficient to evaluate the toxicity of marine organisms for human consumption, but rather the molar ratio Se:Hg, as selenium is known to play a protective role against the toxic effects of Hg and enhance detoxifying mechanisms (Branco et al., 2007; Khan and Wang, 2009). But studies on sharks record low Se:Hg values (Kaneko and Ralston, 2007; Escobar-Sánchez et al., 2011) or no relationship between Se and Hg concentrations (Torres et al., 2017), which leaves unsold the problem of Hg toxicity in larger sharks.

The commercialisation of blue shark (>250 cm TL) and shortfin mako (>190 cm TL) exceeding the *size at risk* is thus an issue for Galician Fishery Authorities in terms of compliance with European legislation. The shark fisheries are not regulated (no quotas and size limits) in Spain. According to our results, in terms of human food safety, we wonder about the possible risk linked to large shark consumption, especially when they are caught between the Azores Archipelago and the Iberian Peninsula. However, limiting large shark consumption may lead to an increase in finning practice (prohibited in the EU in 2003 with application for Spain and Portugal in June 2013). Therefore, maritime control should be enforced for the strict application of the Council Regulation (EC No 1185/2003). Furthermore, some nursery areas, such as the Azores Archipelago (Vandeperre et al., 2014), Gulf of Cadix (Compagno et al., 2005), and north of Galicia (Biton-Porsmoguer, pers. com.), may concentrate big blue shark females exceeding *the size at risk* for mercury level. Within these areas shark fishing should be limited or at least regulated. No information about reproduction activities has been recorded for shortfin mako up to now.

5. Conclusions

Mercury concentrations in muscle of the blue shark *P. glauca* and the shortfin mako *I. oxyrinchus* in north-eastern Atlantic were in the range of values reported in other regions for these widely-distributed sharks. An increase in Hg burden with length and weight was observed in both species, but at a higher rate in the shortfin mako. Bioaccumulation of Hg with individual trophic level ($\delta^{15}N$) and biomagnification from prey to predator was only observed in the shortfin mako, and likely related to its endothermic metabolism and higher feeding activity. The absence of significant difference between sexes, seasons and years in both species could be explained by the high mobility of these sharks and their feeding adaptation to different environmental conditions. Most blue shark and shortfin mako landed and sold at the fish market of Vigo (Spain) exhibited Hg concentration in muscle lower than the maximum limit allowed by the European Union for human consumption (1 mg kg^{-1} ww), as they were juveniles. However, Hg content above this legal threshold was recorded in the majority of adult blue shark larger than 250 cm TL and shortfin mako larger than 190 cm TL. Then, we recommend avoiding the capture and commercialization of individuals exceeding these respective lengths for the two species. In addition, a size range of potential risk was defined (from 200 to 250 cm for the blue shark and from 150 to 190 cm for the shortfin mako) in which some individuals might present Hg concentration above the legal limit. The landing of blue shark and shortfin mako in Vigo during the next decade should assume the implementation of management measures for the sustainable fishing exploitation and the conservation of these species.

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Tables

Table 1. Number of individuals analyzed by sex, season, year and zone for blue shark and shortfin mako. M: Male, F: Female.

Species	**Sex**		**Season**		**Year**		**Zone**					
	M	**F**	**Winter**	**Summer**	**2012**	**2013**	**A**	**B**	**C**	**D**	**E**	**F**
Blue shark	20	20	18	22	19	21	18	11	0	9	0	2
Shortfin mako	26	22	18	30	18	30	9	16	13	0	7	3

Table 2. Mean (SD), (*min* – *max*) total length (TL, cm), total weight (W, kg ww), total Hg concentration (Hg, mg kg^{-1} ww) and $\delta^{15}N$ (‰) of the analyzed blue shark and shortfin mako.

Species	TL (cm)	W (kg ww)	Hg (mg kg^{-1} ww)	$\delta^{15}N$ (‰)
Blue shark	160 (56)	21.0 (21.2)	0.52 (0.35)	11.3 (0.8)
	(*74 – 284*)	(*1.5 – 77.0*)	(*0.14 – 1.71*)	(*10.0 – 13.7*)
Shortfin mako	156 (36)	40.3 (62.7)	0.74 (0.56)	11.8 (0.6)
	(*99 – 219*)	(*7.0 – 76.8*)	(*0.12 – 2.57*)	(*9.6 – 12.9*)

Table 3. Mean (SD) Hg concentration (mg kg^{-1} ww) and trophic level ($\delta^{15}N$) in the various prey of blue shark and shortfin mako. N = number of samples analyzed; unid. = unidentified

Prey type	N	Hg (mg kg^{-1} ww)	N	$\delta^{15}N$ (‰)
Cephalopods				
Ancistroteuthis	1	1.17	3	12.9 (0.1)
lichtensteinii	1	0.23	3	10.7 (0.1)
Gonatus steenstrupi	1	0.20	3	11.3 (0.1)
Illex coindetii	1	2.24	3	11.7 (0.1)
Illex sp.	6	1.24 (0.86)	12	12.1 (0.9)
Cephalopods unid.				
Teleost fish				
Balistes capriscus	1	0.06	3	10.1 (0.1)
Euthynnus alletteratus	2	1.08 (1.07)	6	9.7 (0.3)
Scomberesox saurus	8	0.08 (0.03)	12	11.2 (0.5)
Scomber scombrus	3	0.13 (0.08)	9	11.2 (0.2)
Scomber sp.	2	0.02 (0.001)	6	12.2 (0.6)
Thunnus alalunga	3	1.35 (0.09)	9	11.8 (0.5)
Xiphias gladius	1	1.43	3	12.0 (0.2)
Teleosts unid.	3	0.20 (0.21)	9	11.1 (0.1)
Chelonians				
Caretta caretta	1	0.59	3	9.8 (0.1)
Cetaceans				
Delphinus delphis	2	1.77 (0.36)	6	10.6 (0.5)
Cetaceans unid.	2	0.46 (0.05)	6	10.9 (0.1)

Table 4. Mean Hg concentration (mg kg^{-1} ww) and $\delta^{15}N$ (‰) in the two shark species and their respective diet, and biomagnification factor (BMF) calculated from sharks to their diet in all individuals combined and in the different size classes analyzed (T1-T5), N = number of individuals analyzed.

Species	N	Hg-shark (mg kg^{-1} ww)	$\delta^{15}N$-shark (‰)	Hg-diet (mg kg^{-1} ww)	$\delta^{15}N$-diet (‰)	BMF
Blue shark	**40**	**0.52**	**11.3**	**0.95**	**11.8**	**0.57**
T1 (<100 cm TL)	6	0.25	11.3	1.06	11.8	0.25
T2 (100-129 cm TL)	9	0.36	11.0	1.12	11.9	0.35
T3 (130-159 cm TL)	8	0.37	11.3	1.05	11.8	0.37
T4 (160-200 cm TL)	7	0.62	11.6	0.68	11.5	0.91
T5 (>200 cm TL)	10	0.86	11.3	1.03	10.9	0.80
Shortfin mako	**48**	**0.74**	**11.8**	**0.61**	**11.6**	**1.19**
T2 (100-129 cm TL)*	11	0.28	11.4	0.65	11.1	0.42
T3 (130-159 cm TL)	16	0.75	11.5	0.69	11.5	1.09
T4 (160-200 cm TL)	12	0.54	12.1	0.84	11.3	0.60
T5 (>200 cm TL)	9	1.59	12.0	0.59	10.1	2.26

* The single 99 cm TL mako was included in T2 size class

Table 5. Range of total Hg levels in the blue shark (*Prionace glauca*) and the shortfin mako (*Isurus oxyrinchus*) in the Atlantic and other oceans. * = mean values; TL = total length of individuals (cm); Hg = total Hg concentration in muscle (mg kg^{-1} ww); Ref. = reference of the study cited.

Region	**Blue shark**		**Shortfin mako**		**Ref.**
	TL (cm)	**Hg (mg kg^{-1} ww)**	**TL (cm)**	**Hg (mg kg^{-1} ww)**	
Azores & Iberian Peninsula (NE Atlantic)	79-284	0.14-1.71	100-219	0.12-2.57	(1)
Azores Archipelago (NE Atlantic)	84-239	0.16-1.84	-	-	(2, 3)
South-Eastern Azores (NE Atlantic)	98-223	0.14-0.50	153-216	0.35-1.53	(4)
Canary Islands (NE Atlantic)	216-258	0.16-1.84	-	-	(2)
New England (NW Atlantic)	-	-	199*	2.65*	(5)
North Atlantic	160-274	1.25*	106-285	3.12*	(4)
Equatorial Atlantic	172-265	0.68-2.50	-	-	(6)
South Atlantic	122-274	1.01*	94-262	2.14*	(4)
Brazil (SW Atlantic)	77-137	0.46-2.40	-	-	(7, 8)
Indian Ocean	164-269	1.24*	119-262	2.34*	(4)
South Africa (SW Indian Ocean)	-	-	161-220	2.69*	(9)
Tasmania (SW Pacific Ocean)	89-335	0.27-1.20	-	-	(10)
Baja California (NE Pacific Ocean)	-	-	127*	1.05*	(11)
California (NE Pacific Ocean)	-	-	75-330	0.15-2.90	(12)
Hawaï (Pacific Ocean)	-	-	105-240	0.40-3.10	(12)
Adriatic Sea (Mediterranean)	-	0.38*	-	-	(13)

(1) Present study, (2) Branco et al. (2004), (3) Branco et al. (2007), (4) IEO (2003), (5) Teffer (2014), (6) Torres et al. (2016), (7) Dias et al. (2008), (8) De Carvalho et al. (2014), (9) McKinney et al. (2016), (10) Davenport (1995), (11) Maz-Courrau et al. (2012), (12), Suk et al. (2009), (13) Storelli et al. (2001).

Fig. 1.

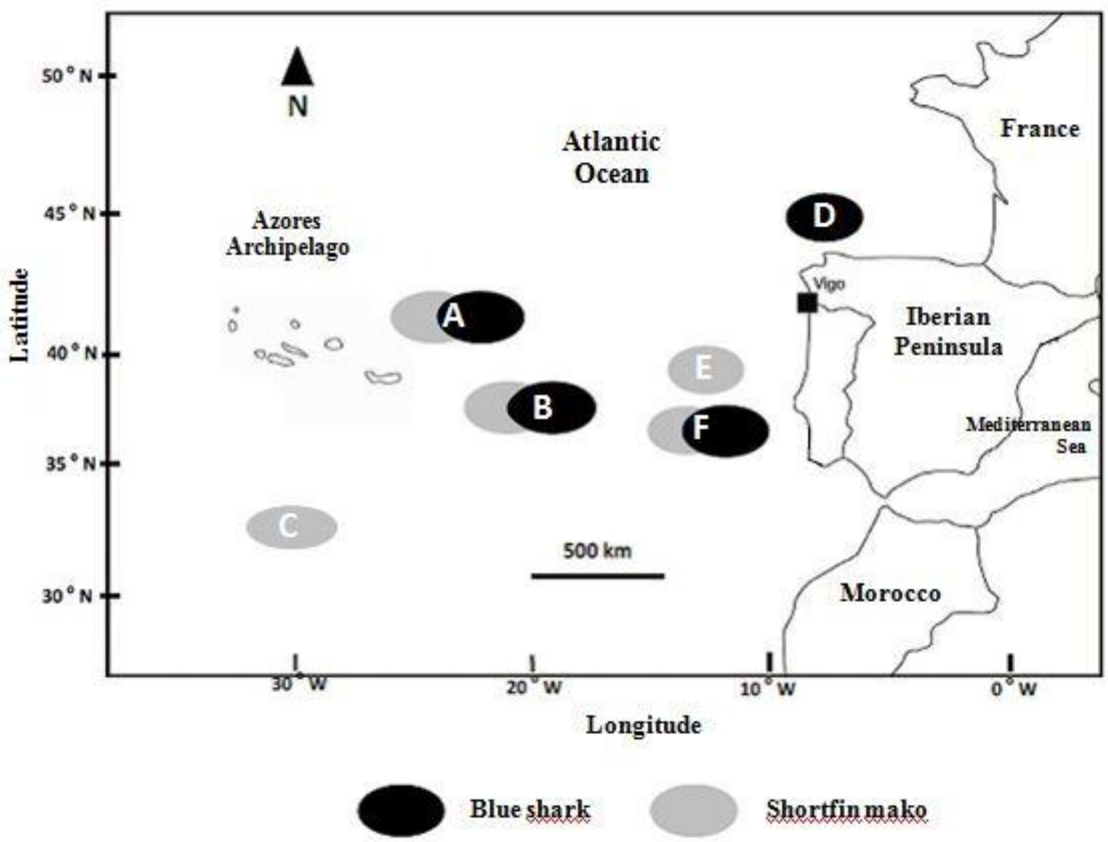

Fig. 1. Map of the 6 sampling areas (A to F) between the Azores Archipelago and the Iberian Peninsula (North-eastern Atlantic Ocean) and two sampled species (the blue shark in black areas and the shortfin mako in grey areas)

Fig. 2.

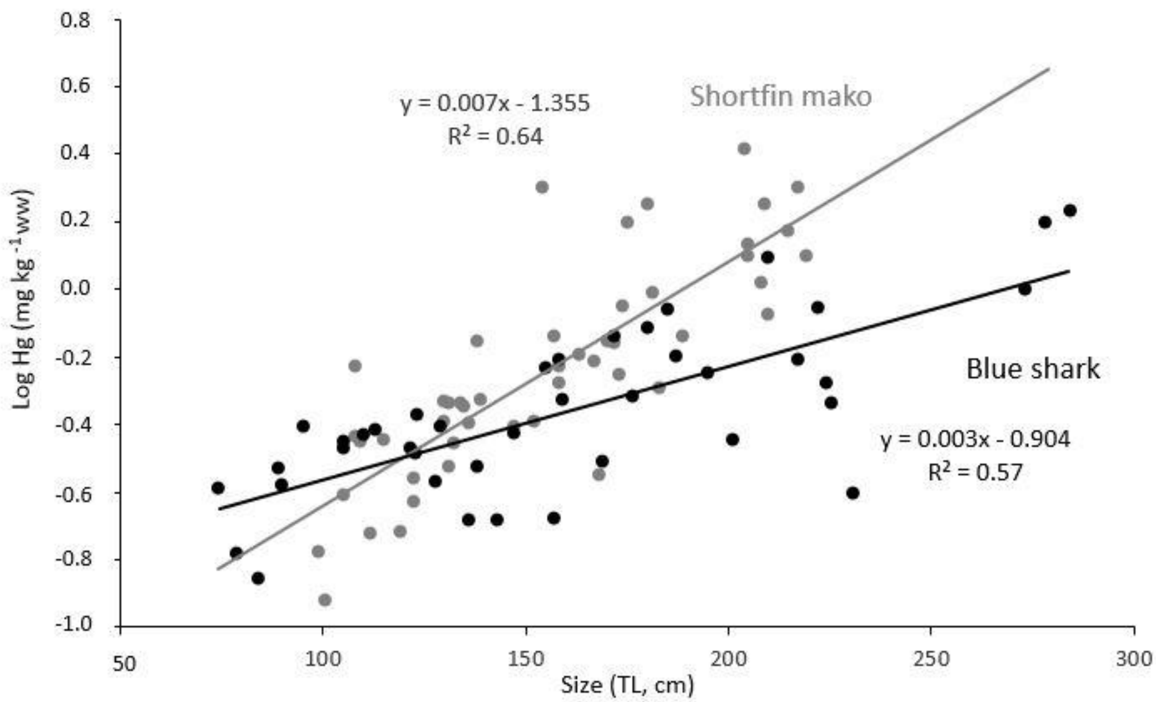

Fig. 2. Correlation between log10 Hg and size in blue shark (N=40, black line and circles) and shortfin mako (N=48, grey line and circles)

Fig. 3.

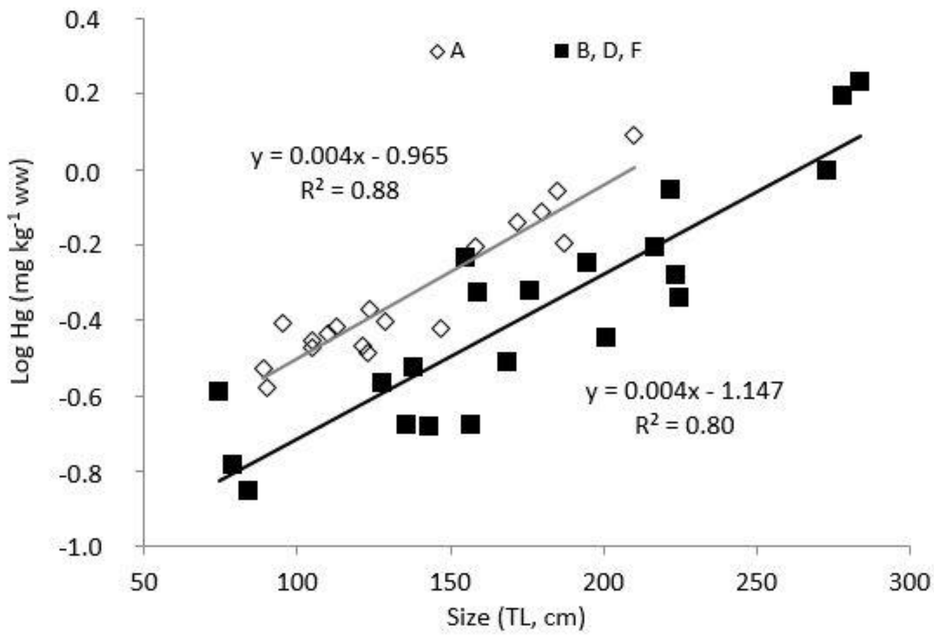

Fig.3. Spatial difference of correlation between log Hg and size in the blue shark in zone A (northeast of the Azores, white diamonds) compared with the other sampling zones (B, D and F, black squares).

Fig. 4.

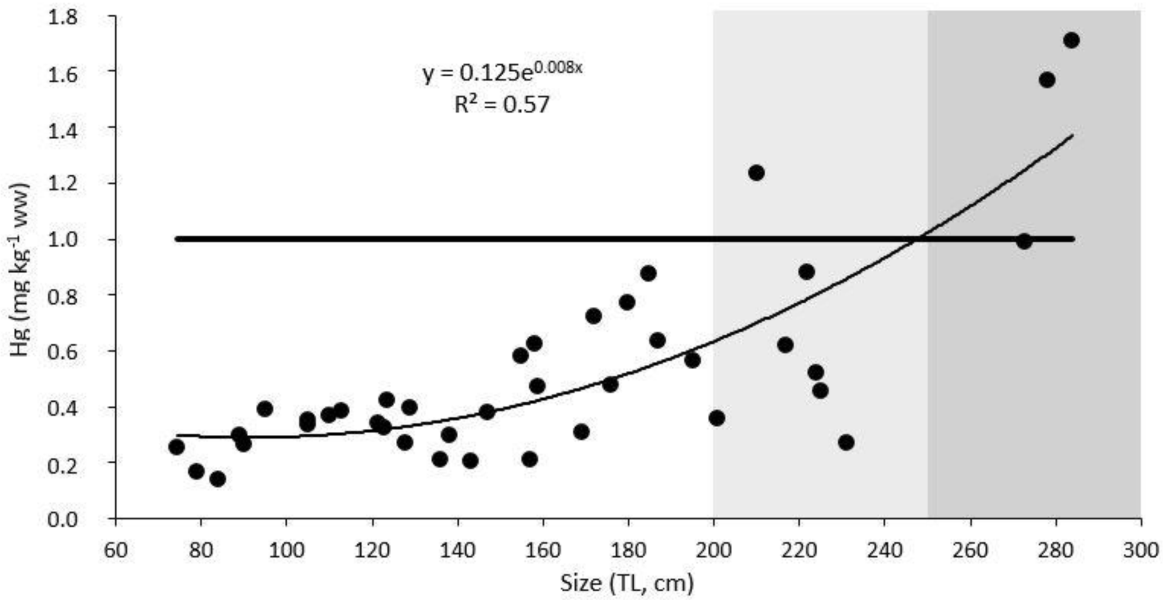

Fig. 4. Correlation between Hg level (mg kg^{-1} ww) and total length (TL, cm) in the blue shark. The horizontal bold line corresponds to the European regulatory threshold (1.0 mg kg^{-1} ww) for commercialization. The light grey area indicates the *size range of potential risk* (from which several sharks might be above the threshold) and the dark grey area starts at *the size at risk* (from which most sharks exceed the threshold).

Fig. 5.

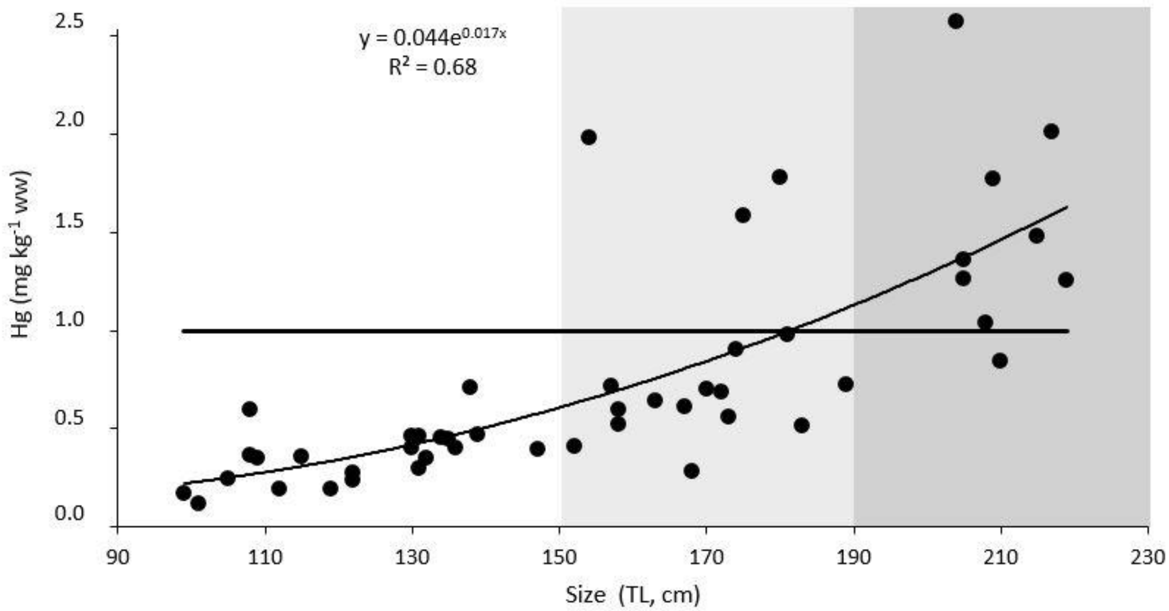

Fig. 5. Correlation between Hg level (mg kg^{-1} ww) and total length (TL, cm) in the shortfin mako. The horizontal bold line corresponds to the European regulatory threshold (1.0 mg kg^{-1} ww) for commercialization. The light grey area indicates the *size range of potential risk* (from which several sharks might be above the threshold) and the dark grey area starts at *the size at risk* (from which most sharks exceed the threshold).