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1 **Mercury in blue shark (*Prionace glauca*) and shortfin mako (*Isurus oxyrinchus*) from**
2 **north-eastern Atlantic: implication for fishery management**

3
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14
15 **Short abstract (150 words)**

16
17 Pelagic sharks (blue shark *Prionace glauca* and shortfin mako *Isurus oxyrinchus*) caught by
18 long-line fleets in the NE Atlantic were analysed for total mercury (Hg). Hg concentration in
19 muscle increased with size and weight in both species, but at a higher rate in shortfin mako.
20 Spatial variation was observed only in the blue shark with higher Hg values in the North of the
21 Azorean archipelago. These high-level predators are particularly susceptible to bioaccumulate
22 Hg. However, a significant positive relationship between Hg concentration and trophic level
23 ($\delta^{15}\text{N}$) of individuals was observed only in the shortfin mako. Most sharks landed were juveniles
24 which presented Hg concentration lower than the maximum limit allowed by the European
25 Union for marketing. However, concentrations above this threshold were most recorded in blue
26 sharks larger than 250 cm total length and in shortfin makos larger than 190 cm, raising the
27 question of the commercialization of large-sized individuals.

28
29
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31

32 Abstract

33

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

48

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

51

52 1. Introduction

53

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

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

63 6 years) and males at 180 cm TL (4-5 years) (Moreno, 2004; Compagno et al., 2005). The
64 shortfin mako presents a heavier body at similar size than the blue shark, a longer maximum size
65 (440 cm) and a longer life span (30 years max) (Natanson et al., 2006). Median size and age at
66 maturity would be about 280 cm TL and 7-10 years for females, which present a larger size than
67 males, and 200 cm TL, 5-6 years for males (Moreno, 2004; Barreto et al., 2016). However,
68 information on age and growth of both shark species is conflicting and still a matter of debate
69 due to difficult and random sampling of these predators (Skomal and Natanson, 2003; Barreto et
70 al., 2016). These two shark species are highly mobile predators able to migrate over thousands of
71 kilometers in the north Atlantic Ocean (Kohler et al., 2002). Spanish and Portuguese long-liners
72 catch mostly small individuals therefore juveniles represent the major part of shark landings at
73 Vigo fish market for both species (73% of blue sharks and 94% of shortfin makos) (Biton-
74 Porsmoguer, 2015).

75 Sharks position as high-level predators in the marine food web (Ferretti et al., 2010) makes
76 them especially susceptible to contain high concentration of contaminants and particularly
77 mercury (Hg) (Storelli et al., 2002), as Hg is known to bioamplify along food webs, increasing
78 with the trophic level of organisms (Harmelin-Vivien et al., 2009, 2012; Lavoie et al., 2013).
79 Trophic level of organisms is routinely estimated by the nitrogen isotopic ratio ($^{15}\text{N}/^{14}\text{N}$),
80 expressed relative to a standard as $\delta^{15}\text{N}$, which tends to increase with the size of individuals and
81 from prey to predator. $\delta^{15}\text{N}$ is then used to follow the transfer and accumulation of contaminants
82 like Hg or PCB in organisms and food webs (Cabana and Rasmussen, 1994; Booth and Zeller,
83 2005; Cossa et al., 2012). Mercury is a highly toxic trace element present in all compartments of
84 the biosphere. It enters marine food webs from natural and anthropogenic sources (Cossa et al.,
85 2009), and foraging pathway is recognized as being the main Hg contamination way (Mathews
86 and Fisher, 2009). Hg is susceptible to impact aquatic ecosystems including commercial species
87 (McKinley and Johnston, 2010), which could result to adverse health effects on humans like
88 toxic effects on the nervous, digestive, cardiovascular and immune systems, and alterations of
89 fetal neurodevelopment (Castoldi et al., 2003; Diez, 2008). As consumption of marine organisms
90 contributes to most Hg intake in humans, a maximum acceptable level in marine products have
91 been laid down by European Commission regulations and set at 1 mg kg^{-1} wet weight (ww) for
92 high-level pelagic predators (European Commission, 2006: Regulation N^o 1881/2006). Fisheries
93 Department from Galician region is supposed to apply the European regulation and must control

94 sanitary state for all landed sea products (Law 11/2008, December 3rd 2008; Xunta da Galicia,
95 2008). But are the sharks landed and commercialized in Galicia fulfil all these requirements?

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

100

101

102 **2. Material and methods**

103

104 *2.1. Sampling and stomach content analysis*

105

106 Sharks were caught by Spanish and Portuguese long-line vessels in the north-eastern Atlantic
107 in five zones (A to F) between the Iberian Peninsula and the Azores archipelago (15°-35°W and
108 30°-45°N), in 2012 and 2013 (Fig. 1). A total of 40 blue shark (*Prionace glauca*) and 48 shortfin
109 mako (*Isurus oxyrinchus*) landed at the fish market of Vigo (Spain) were sampled (Table 1).
110 Blue shark and shortfin mako respectively measured from 74 to 284 cm and from 99 to 219 cm
111 total length (TL). White muscle samples were extracted one centimeter beneath the skin from
112 each individual, put in plastic bags and stored frozen at -20°C. Once at the laboratory, samples
113 were cleaned with distilled water before freeze-drying, grinding and analyzing for total mercury
114 (Hg) and nitrogen stable isotopes ($\delta^{15}\text{N}$). Shark stomachs were extracted and stored at -20°C.
115 After identification and weighing (wet weight, ww) of the prey found in stomach contents, those
116 recently consumed (in good state of conservation) were freeze-dried and analyzed for Hg and
117 $\delta^{15}\text{N}$ in the same way as shark muscle samples. Total wet weights of partially digested prey were
118 reconstructed according to the size of their hard pieces (beaks for cephalopods and otoliths or
119 vertebrae for teleost fish) using pre-established relationships (Biton-Porsmoguer, 2015).
120 Reconstructed weight percentages (% ww) of the main prey types found in stomach contents
121 were then determined for both shark species.

122

123 *2.2. Mercury and stable isotope analyses*

124

125 Total Hg concentrations were determined with a semi-automated atomic absorption
126 spectrophotometer AMA 254 (Altec Ltd, Prague, Czech Republic) with a detection limit of 0.003
127 ng/mg, following the procedure described in Cossa et al. (2009). Hg quantification procedure
128 consisted in three automatic sequences: (1) ashing at 550°C of the freeze-dried sample for Hg
129 volatilisation, (2) evolved elemental Hg amalgamation on a gold trap, (3) atomic absorption
130 spectrophotometric measurement of the Hg collected after heating the gold trap at 800°C. The
131 accuracy of measurement was assessed every ten samples using certified reference materials
132 from the National Research Council of Canada (fish muscle tissues DORM-4). Hg concentration
133 level of samples was initially expressed as Hg dry weight (dw) concentration in fish muscle or
134 prey muscle samples. However, as Hg concentrations are expressed in wet weight (ww) in
135 international and European regulations, dry weight concentrations were converted into wet
136 weight concentrations, considering that dw concentration = 5 ww concentration (Cresson et al.,
137 2014). The relevancy of this factor has been corroborated with the samples of this study
138 (personal data).

139 Stable isotope analyses were performed with a continuous flow mass spectrometer (Delta V
140 Advantage, Thermo Scientific®, Bremen, Germany) coupled to an elemental analyzer (Flash EA
141 1112 Thermo Scientific®, Milan, Italy). N stable isotope ratios were expressed in the standard δ
142 notation: $\delta^{15}\text{N}\text{‰} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$, where R is the ratio $^{15}\text{N}/^{14}\text{N}$ in sample and
143 standard (atmospheric air for nitrogen). Accuracy of measurement was estimated by replicate
144 measurements of internal standard (acetanilide) and was lower than 0.1‰.

145

146 2.3. Data analysis

147

148 *Quantification of Hg concentrations in sharks' diet:* The diets of the blue shark and shortfin
149 mako in the sampling area have been previously studied (Biton-Porsmoguer et al., 2013, 2015).
150 The blue shark mainly fed on cephalopods, then teleost fish and rarely cetaceans, while the
151 shortfin mako mainly preyed on teleost fish, then cephalopods, and occasionally cetaceans and
152 chelonians. The importance in weight (% dw) of each prey type was determined for each species
153 and Hg concentration of their diet was quantified with the equation as in Harmelin-Vivien et al.
154 (2012):

155

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

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

$$160 \quad \delta^{15}\text{N}_{\text{diet}(i)} = \Sigma[\delta^{15}\text{N}_{\text{prey}(x)} \times W_{\text{prey}(x)(i)}]$$

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

165
166 *Biomagnification factor calculations:* A mean biomagnification factor (BMF) was determined
167 for the two sharks in the north-eastern Atlantic following Fisk et al. (2001), based on mean Hg
168 concentration and trophic level of sharks and their diet, using the following equation:

$$169 \quad \text{BMF}_{\text{shark}(i)} = [(C_{\text{shark}(i)}/C_{\text{diet}(i)})/(\delta^{15}\text{N}_{\text{shark}(i)}/\delta^{15}\text{N}_{\text{diet}(i)})]$$

170 Where $C_{\text{shark}(i)}$ is the Hg concentration in the shark species (i), $C_{\text{diet}(i)}$ is the Hg concentration in
171 the diet of the (i) shark species, $\delta^{15}\text{N}_{\text{shark}(i)}$ is the trophic level of the (i) shark species, and
172 $\delta^{15}\text{N}_{\text{diet}(i)}$ is the trophic level of the diet of the (i) shark species. This factor is based on the
173 assumption that mercury concentration in a predator depends on those of its prey, corrected for
174 trophic level difference between predator and prey. We replace the trophic level estimation used
175 by Fisk et al. (2001) by $\delta^{15}\text{N}$ values, which represents a more accurate estimation of organism
176 trophic position.

177
178 *Statistical analysis:* As the number, size, weight and sex of individuals differed between
179 sampling zones and species, differences were tested by ANCOVA to remove the effects of these
180 parameters and consider only the factor tested. When significant differences were observed
181 Tukey post-hoc tests were performed. Relationships between Hg content and sharks size, weight
182 and $\delta^{15}\text{N}$ were tested by Pearson's linear correlation on \log_{10} transformed Hg concentrations to
183 linearize the regression and stabilize the variances. Differences in slope and elevation were tested
184 by appropriate *t*-test. *T*-test was also used for interspecific comparisons of Hg and $\delta^{15}\text{N}$ values.

185
186

187 3. Results

188

189 3.1. Influence of biological parameters on Hg level

190

191 In both species, mercury concentration did not vary with sex for individuals of similar size or
192 weight (ANCOVA, all $p > 0.05$). Thus, sexes were combined for further analyses. Total Hg level
193 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
194 ww in the shortfin mako (Table 2). Mean Hg concentration and mean $\delta^{15}\text{N}$ value were
195 significantly higher in the shortfin mako than in the blue shark (t-tests, $t = -2.213$, $p = 0.029$ and t
196 $= -2.848$, $p = 0.006$ respectively) (Table 2). Hg concentration increased significantly with size
197 and weight in both species, as significant linear relationships were observed between log Hg and
198 TL, (cm) and between log Hg and total weight (W kg ww) (log Hg = 0.009 W - 0.555, $R^2 = 0.59$,
199 $p < 0.001$ in blue shark; log Hg = 0.012 W - 0.630, $R^2 = 0.66$, $p < 0.001$ in shortfin mako).
200 Whatever the parameter considered (size or weight), the slope of the regression was significantly
201 higher (t-test, $p < 0.05$) for the shortfin mako than the blue shark, suggesting different
202 bioaccumulation capacities between the two species. However, the relationship between Hg
203 concentration and the trophic level of individual, expressed as $\delta^{15}\text{N}$ values, was significant only
204 for the shortfin mako (log Hg = 0.169 $\delta^{15}\text{N} - 2.181$, $R^2 = 0.10$, $p = 0.036$), but not for the blue
205 shark (log Hg = -0.069 $\delta^{15}\text{N} + 0.422$, $R^2 = 0.02$, $p = 0.197$).

206

207 3.2. Geographical and temporal variations

208 In blue sharks of similar size, significantly higher mean Hg concentrations (x1.6) were
209 recorded in individuals collected in zone A, than in zone B, D and F, in which blue shark had
210 similar lower concentrations (ANCOVA $F = 3.46$, $p = 0.026$) and weight (ANCOVA $F = 3.37$, p
211 $= 0.029$). Pearson's linear correlations between log Hg and size presented similar slopes in all
212 zones, but a higher intercept value in zone A (Fig. 3), suggesting the influence of environmental
213 differences between zone A in the North of the Azores archipelago and the other zones, rather
214 than a biological difference between individuals. In contrast, no difference of Hg level among
215 zones was observed for the shortfin mako, taken into account differences in size or weight of
216 individuals in the different zones (ANCOVA, $F = 2.46$, $p = 0.086$ for size; $F = 1.49$, $p = 0.242$
217 for weight). In both species and all zones combined, Hg concentration in muscle did not

218 significantly vary with year (2012 vs 2013) nor season (spring vs autumn) (ANCOVA, all $p >$
219 0.05).

220

221 3.3. Biomagnification factor and influence of diet

222

223 The biomagnification factor BMF takes only into account the last trophic level of the food web
224 analyzed (the predator and its prey). Hg level and $\delta^{15}\text{N}$ of the main prey ingested by these two
225 sharks displayed highly variable inter- and intraspecific values (Table 3). The lowest mean Hg
226 concentrations were recorded in pelagic teleost prey fish, such as *Scomber* sp. ($0.02 \text{ mg kg}^{-1} \text{ ww}$)
227 and *Scomberesox saurus* ($0.08 \text{ mg kg}^{-1} \text{ ww}$), and the highest ones in some cephalopods (*Illex* sp.,
228 $2.24 \text{ mg kg}^{-1} \text{ ww}$) and the cetacean *Delphinus delphis* ($1.77 \text{ mg kg}^{-1} \text{ ww}$). The lowest $\delta^{15}\text{N}$ value
229 was observed in the teleost fish *Euthynnus alletteratus* (9.7‰) and the highest one in the
230 cephalopod *Ancistroteuthis lichtensteinii* (12.9‰). No significant correlation was found between
231 log Hg and $\delta^{15}\text{N}$ in prey, or $\delta^{15}\text{N}$ in prey and sharks combined ($R^2 = 0.06$, $p > 0.05$ for both
232 regressions). The blue shark and shortfin mako ingested then prey with various Hg burden, some
233 presenting higher Hg concentration and trophic level than them. The blue shark mainly fed on
234 cephalopods (76% by weight), teleost fish (18%) and cetaceans (0.03%), while the shortfin mako
235 preyed mainly on teleost fish (66% by weight), cephalopods (27%), cetaceans (0.05%) and sea
236 turtles (0.03%). When growing, the blue shark consumed less cephalopods and more teleosts and
237 cetaceans, while the shortfin mako ingest less teleosts and more cetaceans and chelonians. Mean
238 Hg concentration and $\delta^{15}\text{N}$ in diet was higher for the blue shark than for shortfin mako, globally
239 and in most size classes (Table 4), while the reverse was observed in shark muscle with generally
240 higher Hg level in shortfin mako muscle than in blue shark muscle. Mean BMF value was low
241 (<1) in the blue shark, but higher than 1 in the shortfin mako (Table 4), suggesting Hg
242 bioamplification from diet to predator in the shortfin mako only. When the different size classes
243 were considered, it was observed that BMF tended to increase with the size of individuals in both
244 species, but not in a linear way. BMF remained always <1 in the blue shark suggesting no Hg
245 bioamplification in this species. In the shortfin mako, no bioamplification was recorded in the
246 smaller individuals ($<130 \text{ cm TL}$) with $\text{BMF} <1$, while Hg bioamplification occurred for larger-
247 sized individuals (particularly $>200 \text{ cm TL}$) with BMF largely >1 . Such results indicated no

248 straightforward relationship between Hg level of prey and predator, but rather suggested
249 differences in metabolism and/or prey consumption rate between the two shark species.

250

251 3.4. *Mercury in sharks and food safety*

252

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

263

264 4. Discussion

265

266 4.1. *Bioaccumulation of Hg in blue shark and shortfin mako*

267

268 In both shark species Hg concentration in muscle was positively correlated with size and thus
269 age (Fig. 2), independently of sex, suggesting similar feeding habits in males and females, as
270 observed by Biton-Porsmoguer et al. (2016) with stomach content analysis. Increase of Hg level
271 in organism as they were growing and getting older is a well-known bioaccumulation process in
272 marine organisms, particularly in teleost fish (Cossa et al., 2012; Cresson et al., 2014) and high-
273 level predators like sharks (Storelli et al., 2001, 2002; Branco et al., 2004, 2007; Torres et al.,
274 2017) and cetaceans (André et al., 1991). This age-related increase in Hg content is related to an
275 efficient accumulation of mercury due to the decrease of both detoxification capability and
276 growth rate (Amlund et al., 2007; Dang and Wang, 2012). But many parameters can influence
277 the bioaccumulation of Hg in organism along with age, such as growth rate, reproduction,
278 metabolic activity, Hg concentration in prey and feeding rate (Trudel and Rasmussen, 2006;

279 Cossa et al., 2012). Diet is recognized as the main pathway of Hg intake in high-level predators
280 (Boening, 2000). Both shark species ingested prey of various Hg content and $\delta^{15}\text{N}$ values, some
281 being more contaminated and positioned at a higher trophic level than sharks (Table 3). Hg
282 concentration and $\delta^{15}\text{N}$ in diet did not increase with shark size (Table 4). Thus, the hypothesis of
283 an increase in Hg uptake when sharks were growing linked to an increase in Hg content of diet
284 was not supported by our study. Larger sharks did not always seem to feed at a higher more
285 contaminated trophic level than smaller ones, as observed by McMeans et al. (2010) in Arctic
286 sharks. Feeding on larger prey did not imply to feed on more contaminated or higher trophic
287 level organisms. No relationship between Hg concentration and $\delta^{15}\text{N}$ was found in the blue
288 shark, as also observed by Torres et al. (2017) south of the Azores and Escobar-Sánchez et al.
289 (2010) at Baja California, or by Rumbold et al. (2014) for coastal sharks in Florida. Conversely,
290 positive relationship between Hg and $\delta^{15}\text{N}$ was observed in shortfin mako. This result was also
291 observed in a few shark species analyzed in Australia (Pethybridge et al., 2011) or in the Celtic
292 sea (Domi et al. 2005). Hg is efficiently accumulated in shark tissues with a very slow
293 elimination rate as in teleost fish (Amlund et al., 2007). In addition, the fractionation and turn-
294 over of $\delta^{15}\text{N}$ between diet and shark tissue seems to be also slower in sharks than teleost fish
295 (Hussey et al. 2012), suggesting particular metabolic activity related to nitrogen cycling in
296 elasmobranchs. Torres et al. (2017) recorded also similar Hg concentrations and $\delta^{15}\text{N}$ values in
297 male and female blue sharks. The absence of influence of sex, season and year on mercury level
298 in both species can be explained by the high mobility of these sharks regardless of sex (Queiroz
299 et al., 2016) and the large spectrum of prey consumed at different seasons during the two years
300 (Biton-Porsmoguer et al., 2013, 2015).

301 A higher increase in Hg content with size was observed in the shortfin mako compared with
302 blue shark (Fig. 2), suggesting different bioaccumulation processes between the two species
303 (age, diet, activity and/or metabolism). While the blue shark and the shortfin mako are long-lived
304 species (with a higher life span for shortfin mako) (Skomal and Natanson, 2003; Natanson et al.,
305 2006), the age of specimens analyzed in this study ranged likely from 1 to 10 years for both
306 species with a majority of juveniles (87.5%), especially in the shortfin mako (Biton-Porsmoguer,
307 2015). Shortfin makos were heavier than blue sharks at the same size, invalidating the possibility
308 of a dilution of Hg by growth in the blue shark to explain its lower Hg content. Higher Hg
309 content of diet in shortfin mako was also not supported by our results (Table 4). Thus, the higher

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

321

322 *4.2. Regional differences*

323

324 Mercury levels recorded in the blue shark in this study (0.14-1.71 mg kg⁻¹ ww) were in
325 agreement with values previously reported in different studies performed in the north Atlantic
326 Ocean (0.16-1.84 mg kg⁻¹ ww) and Tasmania (SW Pacific Ocean) (0.27-1.20 mg kg⁻¹ ww), but
327 not on the south coast of Brazil (0.46-2.40 mg kg⁻¹ ww) (De Carvalho et al., 2014) where higher
328 Hg concentrations were recorded (Table 5). This may be related to the high mobility of these
329 sharks, their ability to cover large areas for feeding and their dietary opportunism. However,
330 Branco et al. (2007) record a higher accumulation rate of total mercury with size in the blue
331 shark from the equatorial Atlantic (0.68-2.50 mg kg⁻¹ ww) compared with specimens from the
332 Azores archipelago (0.22-1.30 mg kg⁻¹ ww). They relate this pattern to higher Hg concentration
333 in prey, as well as differences in quantity and type of food eaten in the Equator. Such a
334 hypothesis was not relevant in our study, as the rate (regression slope) of Hg increase in blue
335 shark with size was similar in all zones (Fig. 3). Higher Hg concentrations in the north of Azores
336 could not be attributed to gender, as no difference with sex was observed. In mid-Atlantic (south
337 of the Azores), Torres et al. (2017) record lower Hg concentrations in the blue shark than we did
338 for individuals of similar size range in the same area (0.33 ± 0.02 and 0.41 ± 0.02 respectively).
339 The higher intercept value of the linear regression for specimens from the north of the Azores
340 suggested rather the influence of environmental conditions with probably higher Hg

341 concentrations in sea water and prey in this zone, but no difference in metabolism, feeding rate
342 or prey types between populations. The Azores archipelago is a volcanic region with naturally
343 high mercury level in sea water and sediment (Guest et al., 1999) that may explain the
344 significantly higher levels of Hg found in the blue sharks of zone A. Moreover, the Azores
345 archipelago constitutes probably a nursery area for the blue shark and juveniles can stay in this
346 area at least for two years (Vandeperre et al., 2014), accumulating thus a higher Hg burden
347 during this time. However, in the absence of Hg concentration analysis in seawater and in a
348 higher number of prey in the different zones, we cannot test this hypothesis.

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

354

355 *4.3. Biomagnification of Hg in sharks*

356

357 Mercury, under its organic monomethylmercury form, is one of the few trace metals that
358 biomagnifies along food webs (Gray, 2002). Biomagnification is assumed when BMF factor
359 (concentration in predator/concentration in diet corrected from their respective trophic level) is
360 higher than 1 (Fisk et al., 2001). Biomagnification of Hg was observed only in the shortfin mako,
361 not in the blue shark (Table 4). These two sharks consumed prey with a great variability in both
362 Hg concentration and $\delta^{15}\text{N}$ value (Table 3). For the blue shark, Hg content in diet was higher
363 than in shark muscle for all size classes, leading up to a BMF always <1, while slightly
364 increasing with size. For the shortfin mako, higher Hg content in shark muscle compared with
365 diet and BMF values >1 were observed from individuals larger than 130 cm, and more
366 significantly larger than 200 cm. However, BMF calculation should be considered cautiously.
367 First, the reduced number of prey analysed in this study is not sufficient to characterize the
368 global diet of the two species and a broader study should be conducted to conclude. Second, the
369 same mean Hg and $\delta^{15}\text{N}$ values of prey groups were used for all size-classes of both sharks.
370 However, as observed by Biton-Porsmoguer et al. (2015), they could prey on different species.
371 Finally, the time lag between ingestion and assimilation of prey was not considered. Stomach

372 content offered a snap shot of shark's diet when caught, while muscle Hg concentration reflected
373 food assimilation over several months. Nonetheless, similar results are reported by Maz-Courrau
374 et al. (2012) at Baja California with Hg biomagnification in the shortfin mako but not in the blue
375 shark. The absence of any Hg biomagnification in blue shark is also observed in the Pacific
376 Ocean of México by Escobar-Sánchez et al. (2011). Difference in BMF factor between the two
377 species, with Hg biomagnification occurring only in the shortfin mako is probably related with
378 their metabolic differences (endothermy in shortfin mako vs ectothermy in blue shark)
379 (Shadwick and Goldbogen, 2012) as discussed above, with a positive relationship between Hg
380 content and $\delta^{15}\text{N}$ in the shortfin mako and not in blue shark.

381

382 4.4. Food safety and fishery implication

383

384 The mean values of mercury level in the muscle of blue shark and shortfin mako analyzed in
385 this study were lower than the upper limit allowed by the European Union in high-level pelagic
386 predators for human consumption ($1 \text{ mg kg}^{-1} \text{ ww}$) (Table 2), as also observed by Torres et al.
387 (2017) for these species. However, Hg concentration exceeded the legal EU standard in larger
388 individuals (Fig. 4 and 5). Above 250 cm TL in *P. glauca* and 190 cm TL in *I. oxyrinchus* most,
389 if not all, individuals exhibited Hg content well above the EU sanitary risk limit, representing a
390 *size at risk* for the consumption of these species. Measuring mercury concentration above
391 European and international regulatory thresholds in shark muscle is regularly reported in the
392 literature, whatever the ecology, geographic distribution or feeding habits of species (Storelli et
393 al., 2002; Branco et al., 2004, 2007; Pethybridge et al., 2010; Escobar-Sánchez et al., 2011; De
394 Carvalho et al., 2014; Cresson et al., 2014; Mc Kinney et al., 2016, and references in Table 5).
395 All authors raise the health issue of shark, and more generally high-level predator, consumption.
396 However, Hg concentration value alone does not seem to be sufficient to evaluate the toxicity of
397 marine organisms for human consumption, but rather the molar ratio Se:Hg, as selenium is
398 known to play a protective role against the toxic effects of Hg and enhance detoxifying
399 mechanisms (Branco et al., 2007; Khan and Wang, 2009). But studies on sharks record low
400 Se:Hg values (Kaneko and Ralston, 2007; Escobar-Sánchez et al., 2011) or no relationship
401 between Se and Hg concentrations (Torres et al., 2017), which leaves unsold the problem of Hg
402 toxicity in larger sharks.

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

417

418 **5. Conclusions**

419

420 Mercury concentrations in muscle of the blue shark *P. glauca* and the shortfin mako *I.*
421 *oxyrinchus* in north-eastern Atlantic were in the range of values reported in other regions for
422 these widely-distributed sharks. An increase in Hg burden with length and weight was observed
423 in both species, but at a higher rate in the shortfin mako. Bioaccumulation of Hg with individual
424 trophic level ($\delta^{15}\text{N}$) and biomagnification from prey to predator was only observed in the shortfin
425 mako, and likely related to its endothermic metabolism and higher feeding activity. The absence
426 of significant difference between sexes, seasons and years in both species could be explained by
427 the high mobility of these sharks and their feeding adaptation to different environmental
428 conditions. Most blue shark and shortfin mako landed and sold at the fish market of Vigo (Spain)
429 exhibited Hg concentration in muscle lower than the maximum limit allowed by the European
430 Union for human consumption ($1 \text{ mg kg}^{-1} \text{ ww}$), as they were juveniles. However, Hg content
431 above this legal threshold was recorded in the majority of adult blue shark larger than 250 cm TL
432 and shortfin mako larger than 190 cm TL. Then, we recommend avoiding the capture and
433 commercialization of individuals exceeding these respective lengths for the two species. In

434 addition, a size range of potential risk was defined (from 200 to 250 cm for the blue shark and
435 from 150 to 190 cm for the shortfin mako) in which some individuals might present Hg
436 concentration above the legal limit. The landing of blue shark and shortfin mako in Vigo during
437 the next decade should assume the implementation of management measures for the sustainable
438 fishing exploitation and the conservation of these species.

439

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441

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448

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625

626 **Tables**

627

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

630

631

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

632

633

634

635

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

638

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

639

640

641 Table 3. Mean (SD) Hg concentration (mg kg⁻¹ ww) and trophic level (δ¹⁵N) in the various prey
 642 of blue shark and shortfin mako. N = number of samples analyzed; unid. = unidentified
 643

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

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

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Species	N	Hg-shark (mg kg^{-1} ww)	$\delta^{15}\text{N}$ -shark (‰)	Hg-diet (mg kg^{-1} ww)	$\delta^{15}\text{N}$ -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

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

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665 Table 5. Range of total Hg levels in the blue shark (*Prionace glauca*) and the shortfin mako (*Isurus*
666 *oxyrinchus*) in the Atlantic and other oceans. * = mean values; TL = total length of individuals (cm); Hg =
667 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)
Hawai (Pacific Ocean)	-	-	105-240	0.40-3.10	(12)
Adriatic Sea (Mediterranean)	-	0.38*	-	-	(13)

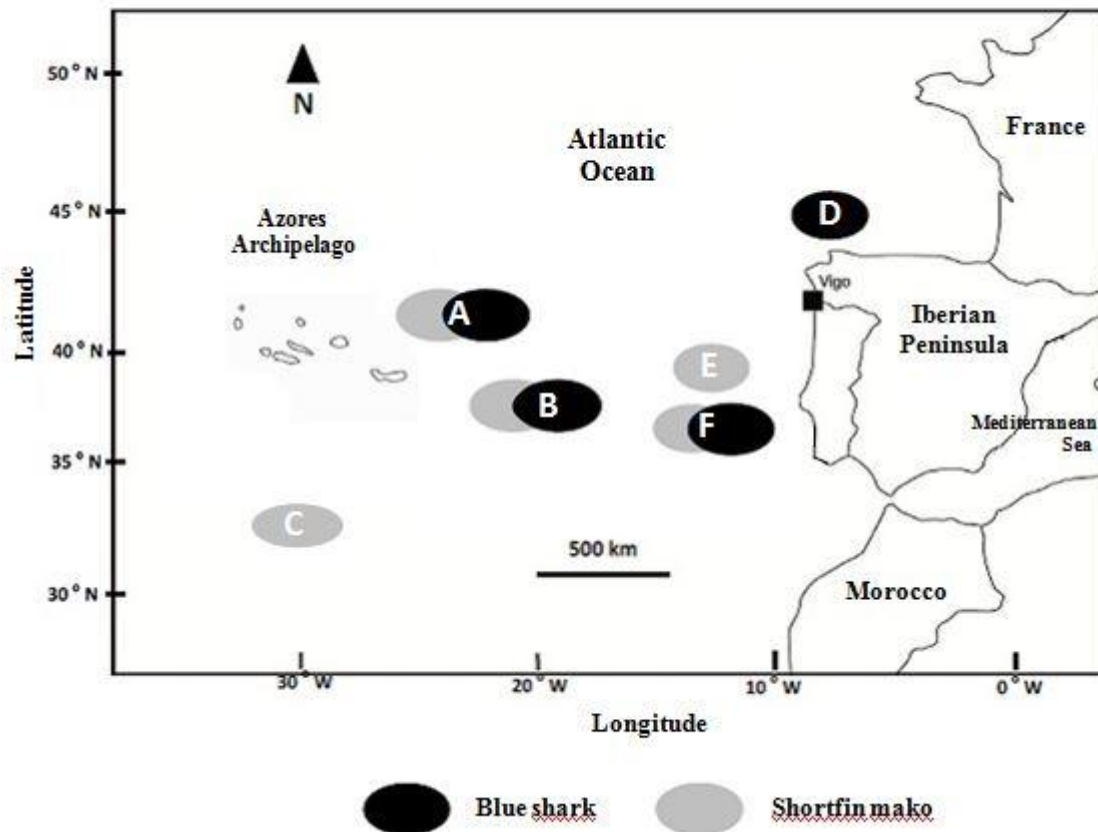
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669 (1) Present study, (2) Branco et al. (2004), (3) Branco et al. (2007), (4) IEO (2003), (5) Teffer (2014), (6)
670 Torres et al. (2016), (7) Dias et al. (2008), (8) De Carvalho et al. (2014), (9) McKinney et al. (2016), (10)
671 Davenport (1995),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

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681 Fig. 1.

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686 Fig. 1. Map of the 6 sampling areas (A to F) between the Azores Archipelago and the Iberian

687 Peninsula (North-eastern Atlantic Ocean) and two sampled species (the blue shark in black areas

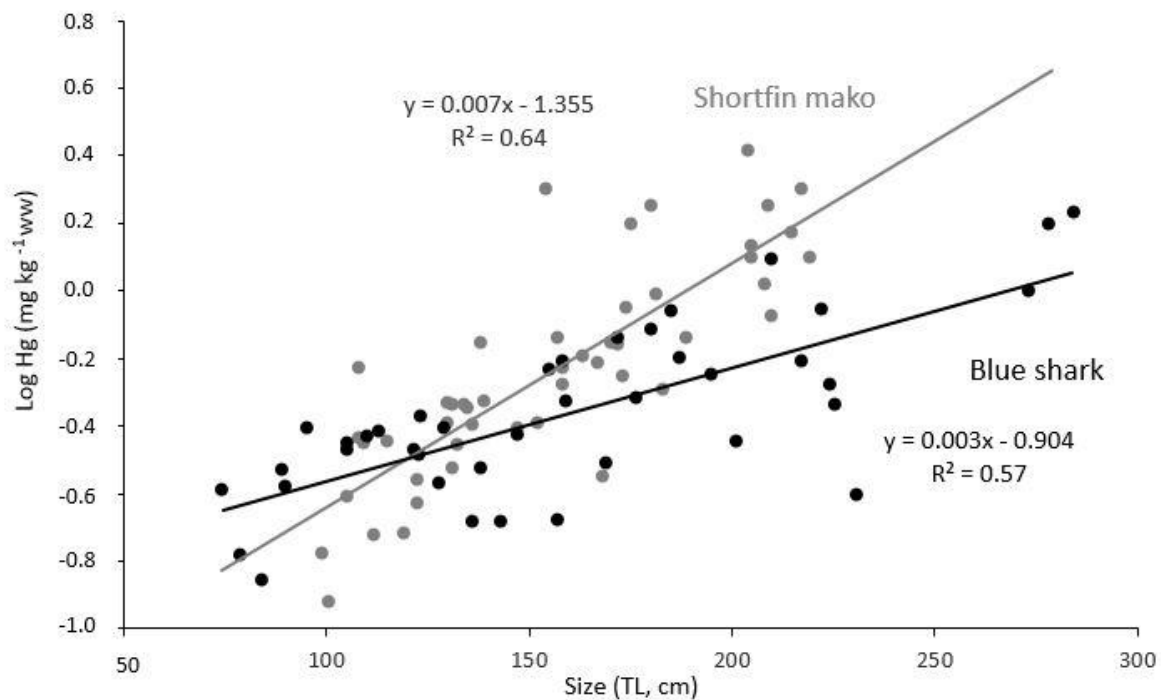
688 and the shortfin mako in grey areas)

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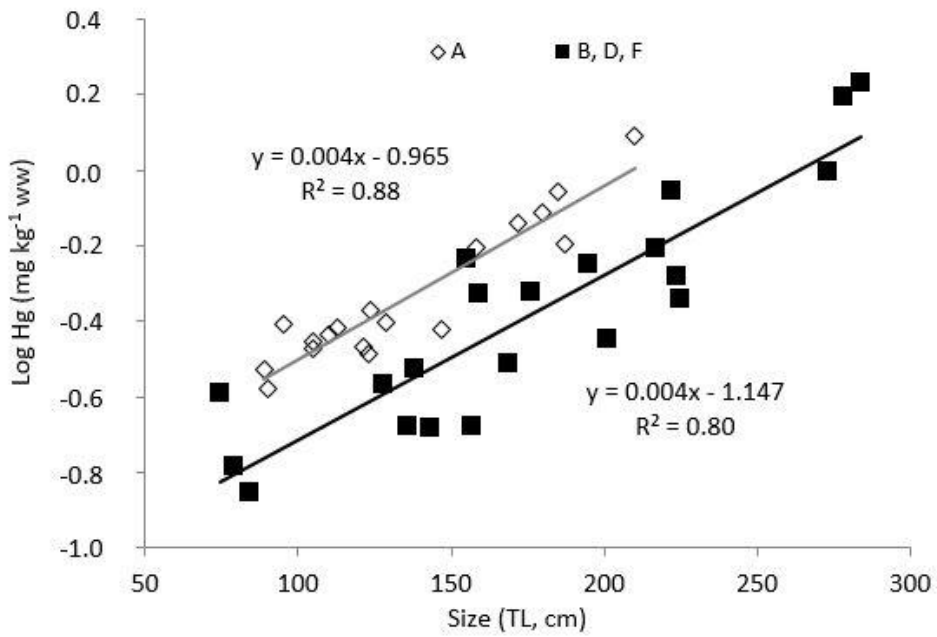
692 Fig. 2.
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695 Fig. 2. Correlation between log₁₀ Hg and size in blue shark (N=40, black line and circles) and
696 shortfin mako (N=48, grey line and circles)

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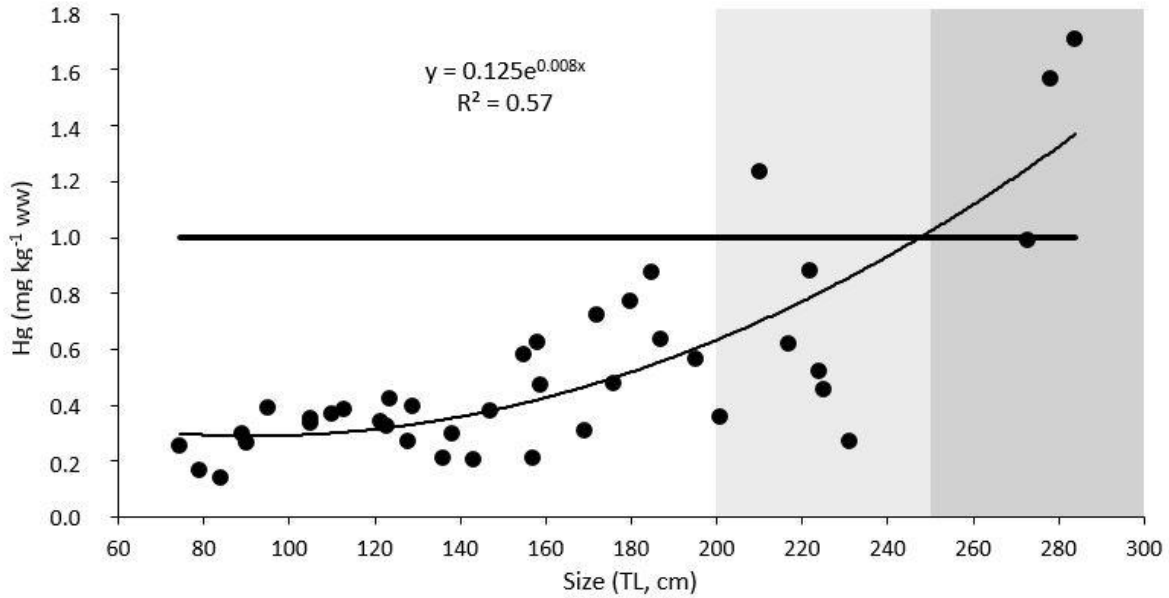
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710 Fig.3. Spatial difference of correlation between log Hg and size in the blue shark in zone A
711 (northeast of the Azores, white diamonds) compared with the other sampling zones (B, D and F,
712 black squares).

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Fig. 4.

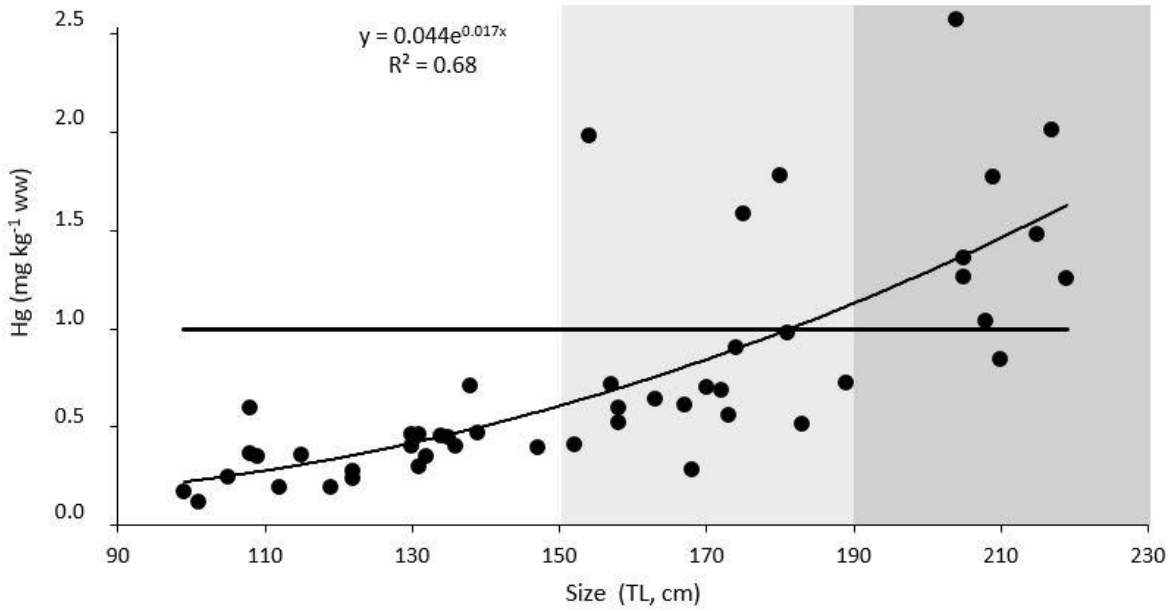


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Fig. 4. Correlation between Hg level (mg kg⁻¹ ww) and total length (TL, cm) in the blue shark. The horizontal bold line corresponds to the European regulatory threshold (1.0 mg kg⁻¹ ww) for commercialization. The light grey area indicates the *size 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).

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Fig. 5.



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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).