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## Mercury isotopes as tracers of ecology and metabolism in two sympatric shark species

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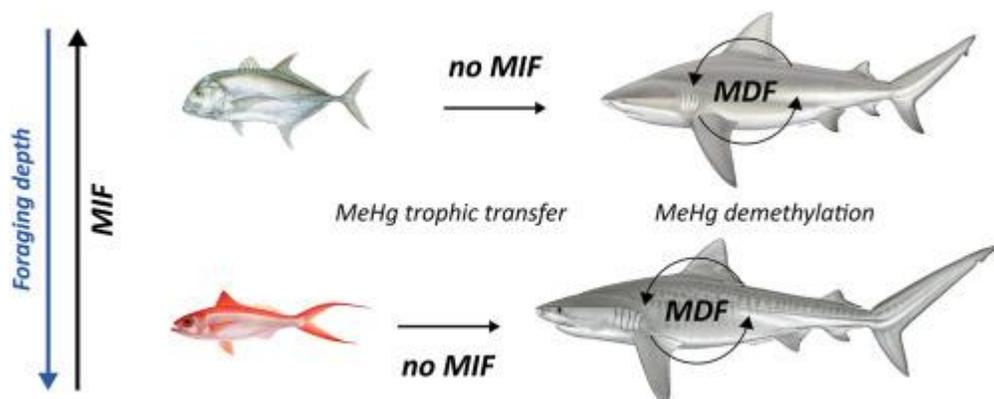
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### Abstract :

In coastal ecosystems, top predators are exposed to a wide variety of nutrient and contaminant sources due to the diversity of trophic webs within coastal areas. Mercury contamination could represent an additional threat to shark populations that are declining worldwide. Here we measured total mercury, carbon and nitrogen isotopes as well as mercury isotopes in two co-occurring shark species (the bull shark *Carcharhinus leucas* and the tiger shark *Galeocerdo cuvier*) and their prey from a coastal ecosystem of the western Indian Ocean (La Réunion Island), to (i) determine their main trophic Hg source and (ii) better characterize their diet composition and foraging habitat. Hg isotope signatures ( $\Delta^{199}\text{Hg}$  and  $\delta^{202}\text{Hg}$ ) of shark prey suggested that bull sharks were exposed to methylmercury (MeHg) produced in the water column while tiger sharks were exposed to mesopelagic MeHg with additional microbial transformation in slope sediments.  $\Delta^{199}\text{Hg}$  values efficiently traced the ecology of the two predators, demonstrating that bull sharks targeted coastal prey in shallow waters while tiger sharks were mainly foraging on mesopelagic species in the slope deeper waters. Unexpectedly, we found a positive shift in  $\delta^{202}\text{Hg}$  ( $>1\text{‰}$ ) between sharks and their prey, leading to high  $\delta^{202}\text{Hg}$  values in the two shark species (e.g.  $1.91 \pm 0.52\text{‰}$  in the bull shark). This large shift in  $\delta^{202}\text{Hg}$  indicates that sharks may display strong MeHg demethylation abilities, possibly reflecting evolutionary pathways for mitigating their MeHg contamination.

## Graphical abstract



## Highlights

- ▶ Mercury isotopes were analyzed in bull and tiger sharks from the Indian Ocean.
- ▶ Hg MIF signature revealed that bull sharks target coastal prey in shallow water.
- ▶  $\Delta^{199}\text{Hg}$  values showed that tiger sharks forage on deeper habitat on the island slope.
- ▶  $\delta^{202}\text{Hg}$  shift between prey and predator may indicate MeHg demethylation in sharks.

## 22 1. Introduction

23 Mercury (Hg) contamination constitutes a major global environmental concern,  
24 resulting in deleterious effects on marine organisms due to exposure to the toxic  
25 methylmercury (MeHg) form (Cáceres-Saez et al., 2018; Krey et al., 2015; Scheuhammer et  
26 al., 2015). Located at the interface between the atmosphere, lands, rivers and oceans,  
27 coastal areas are exposed to a wide variety of Hg sources and are at the same time used  
28 extensively and increasingly for a large number of activities, including resources exploitation  
29 or tourism. Within these complex ecosystems, long-lived organisms at the top of food webs  
30 usually display the highest Hg levels, due to bioaccumulation and biomagnification  
31 processes, which correspond to the increase of MeHg and other contaminant concentration  
32 over time and with the trophic position, respectively. Populations of marine predators are  
33 declining worldwide (Baum et al., 2003; Ferretti et al., 2008; Myers and Worm, 2003),  
34 including coastal sharks (Hammerschlag et al., 2019; Roff et al., 2018). The harmful effects of  
35 MeHg could thus represent an additional threat to the most depleted shark populations.

36 The bull shark *Carcharhinus leucas* and the tiger shark *Galeocerdo cuvier* are among  
37 the largest marine top-predators, playing a key role in tropical marine ecosystems (Roff et  
38 al., 2016). Due to their high trophic position and long life span, they are known to  
39 accumulate high MeHg levels (McKinney et al., 2016). The two species can be temporarily  
40 sympatric (i.e. co-occurring) in coastal ecosystems, but they also exhibit different habitat use  
41 involving incursions in freshwater and strong affinity for coral reefs for the bull shark  
42 (Espinoza et al., 2016; Matich and Heithaus, 2015; Werry et al., 2012) and large offshore  
43 movements for tiger sharks (James S. E. Lea et al., 2015; Meyer et al., 2018), during which  
44 they can dive in deep waters (Afonso and Hazin, 2015). Despite similar trophic positions, the

45 two species demonstrate segregation in their feeding habitats. While bull sharks can show  
46 high individual specialization and a strong dependence on coastal resources, tiger sharks are  
47 generalist predators able to rely on both coastal and oceanic prey (Dicken et al., 2017;  
48 Trystram et al., 2016).

49 Top predators using coastal ecosystems are known to feed on different food webs  
50 (Bird et al., 2018), inducing exposure to different sources and concentrations of  
51 contaminants (Le Croizier et al., 2016, 2019b). To characterize dietary resources, nitrogen  
52 and carbon stable isotopes ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) are traditionally used in ecotoxicological studies  
53 (Le Bourg et al., 2019; Le Croizier et al., 2020), reflecting respectively the trophic position  
54 (Pethybridge et al., 2018) and foraging habitat (Bird et al., 2018) of consumers. However,  
55 these tools show limitations, such as the variability of N and C isotopic fractionation during  
56 trophic transfers (Kim et al., 2012; Malpica-Cruz et al., 2012). More recently, new techniques  
57 such as Hg stable isotope analyses have been applied to characterize the sources and  
58 transfer routes of this contaminant (Gehrke et al., 2011; Sackett et al., 2017; Senn et al.,  
59 2010).

60 In seawater, MeHg is subject to mass-independent fractionation ("MIF", generally  
61 represented by  $\Delta^{199}\text{Hg}$ ) under the influence of solar radiation, which induces a preferential  
62 photochemical demethylation of the light and odd-mass isotopes. In the photic zone (i.e. the  
63 part of the water column exposed to light), the remaining MeHg pool available to marine  
64 organisms is therefore enriched in the heavy and odd-mass Hg isotopes. This process induces  
65 a  $\Delta^{199}\text{Hg}$  gradient from the surface (photic or epipelagic zone, between 0 and 200 m deep) to  
66 depth (twilight or mesopelagic zone, between 200 and 1000 m deep), with higher  $\Delta^{199}\text{Hg}$   
67 values near the surface. This isotopic gradient allows to trace the vertical foraging habitat of

68 marine consumers (Blum et al., 2013; Madigan et al., 2018; Sackett et al., 2017). Moreover,  
69  $\Delta^{199}\text{Hg}$  constitutes a trophic tracer of major interest since  $\Delta^{199}\text{Hg}$  values are conserved  
70 between prey and predators due to the absence of MIF during trophic interactions or  
71 metabolic processes (Kwon et al., 2016, 2012; Masbou et al., 2018).

72 Hg isotopes are also subject to mass-dependent fractionation (“MDF”, generally  
73 represented by  $\delta^{202}\text{Hg}$ ) that takes place during physico-chemical processes such as  
74 photoreduction (Bergquist and Blum, 2007) and volatilization (Zheng et al., 2007), but also  
75 during biological processes such as methylation (Janssen et al., 2016) and demethylation  
76 (Perrot et al., 2016).  $\delta^{202}\text{Hg}$  can be used to assess the Hg metabolism, such as the  
77 transformation of MeHg into less toxic inorganic Hg (iHg) by demethylation in the liver of  
78 marine organisms (Bolea-Fernandez et al., 2019). Indeed, the preferential demethylation of  
79 light Hg isotopes induces an increase in  $\delta^{202}\text{Hg}$  in the remaining MeHg pool which is  
80 ultimately accumulated in other tissues such as muscle (Perrot et al., 2016). Thereby,  
81 significant enrichment of  $\delta^{202}\text{Hg}$  is generally found in mammal muscle and human hair  
82 relative to trophic Hg sources, reflecting in vivo demethylation of MeHg (Laffont et al., 2011;  
83 Li et al., 2014; Perrot et al., 2012).

84 In the western Indian Ocean, only a few studies have documented Hg concentrations  
85 in coastal species (Le Bourg et al., 2019; Sardenne et al., 2017), with most studies focusing  
86 on offshore organisms (Bodin et al., 2017; Kiszka et al., 2015). In addition to trophic position  
87 and body length, foraging habitat was found to influence Hg levels in various shark species  
88 sampled across different locations in the southwestern Indian Ocean, including La Réunion  
89 Island (Le Bourg et al., 2019). Indeed, higher Hg concentrations were observed in offshore  
90 and deep-sea species compared to coastal species, suggesting different sources of MeHg

91 exposure between sharks feeding in nearshore shallow waters and those feeding in offshore  
92 deep waters (Kiszka et al., 2015; Le Bourg et al., 2019). On the other hand, high Hg  
93 concentrations were found in large coastal shark species from the east coast of South-Africa  
94 without apparent influence of foraging depth (McKinney et al., 2016). The role of feeding  
95 depth in MeHg exposure therefore remains to be clarified for predators using nearshore  
96 ecosystems in the western Indian Ocean. Moreover, while hepatic demethylation of MeHg is  
97 well known in marine mammals (Bolea-Fernandez et al., 2019; Perrot et al., 2016), bony fish  
98 do not appear to have such metabolic capacities. Although it has recently been suggested  
99 that sharks may have protective mechanisms that mitigate Hg toxicity (Merly et al., 2019), to  
100 date there is no evidence of MeHg demethylation in sharks.

101 In this context, the present study focused on two sympatric predator species, the bull  
102 and the tiger shark, sampled in a coastal ecosystem of the Indian Ocean (La Réunion Island).  
103 The two major objectives were to:

- 104 1) Use Hg MIF signatures ( $\Delta^{199}\text{Hg}$  values) as diet and foraging depth tracers to  
105 determine whether different MeHg sources contribute to MeHg burdens in the two  
106 shark species.
- 107 2) Use Hg MDF signatures ( $\delta^{202}\text{Hg}$  values) as tracers of Hg metabolism to evaluate the  
108 MeHg demethylation abilities in sharks.

109

## 110 **2. Material and methods**

### 111 **2.1 Sampling**

112 The study was carried out in the coastal ecosystem of La Réunion Island, an oceanic  
113 island located in the southwestern Indian Ocean. The island is of volcanic origin and its  
114 topography is characterized by abrupt slopes inducing close proximity between coastal and  
115 offshore ecosystems. This remote island is far from continental masses and therefore far  
116 from the main sources of industrial pollution.

117 Muscle samples from 20 bull sharks *Carcharhinus leucas* (10 males, 10 females) and 20 tiger  
118 sharks *Galeocerdo cuvier* (8 males, 12 females) were collected from individuals caught by  
119 local fishermen along the island slope on the west coast of La Réunion Island in 2015 (Figure  
120 1). To minimize the impact of ontogenetic changes in diet and habitat on Hg isotope values,  
121 most of the sharks were sub-adults and adults (i.e. > 259 cm for tiger sharks (Werry et al.,  
122 2014) and > 160 cm for bull sharks (Werry et al., 2011) (SI Global dataset), and were thus  
123 larger than the size at which dietary shifts occur (i.e. 200-230 cm for tiger sharks and 140 cm  
124 for bull sharks) (Habegger et al., 2012; Lowe et al., 1996).

125 At La Réunion island, teleost fish species dominate the preys consumed by both bull  
126 and tiger sharks, based on stomach contents (i.e. respectively 93% and 78% in terms of mass  
127 percentage) (Trystram et al., 2016). However, the large diversity of prey (i.e. 56 and 148  
128 species for bull and tiger sharks, respectively) (Trystram et al., 2016) makes it very difficult to  
129 cover the full prey spectrum of sharks. In this context, we collected muscle samples from fish  
130 with known ecology and representative of the different habitats that sharks could use for  
131 foraging and by which Hg exposure could occur (SI Table S3). In a similar coastal ecosystem  
132 (Hawaii, USA), terrestrial freshwater inputs to coastal sediments were previously identified  
133 as the primary source of tissue Hg in the giant trevally *Caranx ignobilis* (Sackett et al., 2017).  
134 In the present study, this fish species was then used as a proxy of Hg sources from the

135 demersal nearshore habitat. The yellow-edged lyretail *Variola louti*, a reef-associated  
136 grouper was sampled here to represent the shallow coral ecosystem (Emslie et al., 2017)  
137 while the great barracuda *Sphyraena barracuda* and the wahoo *Acanthocybium solandri*  
138 were collected to represent the epipelagic nearshore habitat (Trystram, 2016). The  
139 deepwater longtail red snapper *Etelis coruscans* was identified as primarily exposed to MeHg  
140 through deepwater sediments (Sackett et al., 2017) and was thus used as a proxy of MeHg  
141 sources from slope waters. Regarding pelagic offshore Hg sources, tuna species (i.e. the  
142 yellowfin tuna *Thunnus albacares* and the skipjack tuna *Katsuwonus pelamis*) and the lantern  
143 fish *Ceratoscopelus warmingii* were used as proxies of respectively epipelagic and  
144 mesopelagic Hg sources, according to their known ecology and previously published Hg  
145 isotope values (Blum et al., 2013; Madigan et al., 2018). *Ceratoscopelus warmingii* was  
146 collected during the “La Pérouse” and “MAD-RIDGE-1” scientific cruises in 2016 aboard the  
147 RV Antea off La Réunion Island and off southern Madagascar, respectively. All the other fish  
148 species were sampled between January 2012 and December 2014 off the west coast of La  
149 Réunion Island by local fishermen (Figure 1).

150 All samples were kept in a cool box following collection and thereafter frozen at  $-20\text{ }^{\circ}\text{C}$  in  
151 the laboratory until further analysis. Muscle samples were freeze-dried and ground into a  
152 fine and homogeneous powder using an automated grinder.

## 153 **2.2 Muscle $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values**

154 Urea and lipid extraction were not applied on shark samples. Although shark muscle  
155 generally contains low lipid content ( $<1\%$ ) (Meyer et al., 2017), lipids are depleted in  $^{13}\text{C}$  and  
156 shark osmoregulatory strategy induce a significant urea retention which is depleted in  $^{15}\text{N}$ .  
157 This may cause bias in the interpretation of the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , and therefore requires a

158 mathematical normalization for the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values following urea and lipid extraction  
159 in several species of sharks (Y. Li et al., 2016). Here, we applied the normalization equations  
160 established for the bull and the tiger shark from La Réunion Island, for each species and each  
161 sex (Martin and Jaquemet, 2019).

162 Approximately 0.5 mg of muscle were weighed and packed into tin capsules. Isotopic  
163 composition (with a precision of 0.1‰ for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) and carbon percentage and  
164 nitrogen percentage content were measured at the Stable Isotope Laboratory, GNS Science,  
165 Lower Hutt, New Zealand, using an Isoprime isotope ratio mass spectrometer interfaced to  
166 an EuroEA elemental analyzer in continuous-flow mode (EA-IRMS). Results are expressed in  
167 standard  $\delta$  notation based on international standards (Vienna Pee Dee Belemnite for  $\delta^{13}\text{C}$   
168 and atmospheric nitrogen for  $\delta^{15}\text{N}$ ) following the equation  $\delta^{13}\text{C}$  or  
169  $\delta^{15}\text{N} = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 10^3$  (in ‰), where R is  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$ .

### 170 **2.3 Hg concentration and speciation**

171 Total Hg (THg) determination was carried out on an aliquot (around 20 mg) of dried  
172 fish or shark muscle by combustion, gold trapping and atomic absorption spectrophotometry  
173 detection using a DMA80 analyzer (Milestone, USA). Mercury concentrations in muscle  
174 samples are expressed on a dry weight basis ( $\mu\text{g}\cdot\text{g}^{-1}$  dw). Only one analysis was performed  
175 per sample, but the accuracy and reproducibility of the method were established using two  
176 freeze-dried certified biological materials: a tuna fish flesh homogenate reference material  
177 (IAEA 436, IRMM) and a lobster hepatopancreas reference material (TORT 3, NRCC). The  
178 certified values for IAEA 436 ( $4.19 \pm 0.36 \mu\text{g}\cdot\text{g}^{-1}$  dw) were reproduced (measured value:  
179  $4.20 \pm 0.09 \mu\text{g}\cdot\text{g}^{-1}$  dw, n=10) within the confidence limits. The certified values for TORT 3

180 (0.292 ± 0.022 µg·g<sup>-1</sup> dw) were also reproduced (measured value: 0.286 ± 0.024 µg·g<sup>-1</sup> dw,  
181 n=10) within the confidence limits. The detection limit was 0.005 µg·g<sup>-1</sup> dw.

182 THg is known to be almost exclusively in the MeHg form in shark and fish muscle  
183 (Bosch et al., 2016; de Carvalho et al., 2014; Pethybridge et al., 2010; Ruiz-de-Cenzano et al.,  
184 2014), including species sampled in this study (e.g. giant trevally and deepwater snapper  
185 (Sackett et al., 2015), bull shark (Matulik et al., 2017)). However, a subset of muscle samples  
186 from 10 bull sharks were analyzed for MeHg, specifically to verify that MeHg accounted for  
187 the majority of THg in our shark muscle samples and to evaluate the link between δ<sup>202</sup>Hg  
188 values and Hg speciation. We focused on the bull shark, which showed the highest δ<sup>202</sup>Hg  
189 variability, and selected 5 individuals with the lowest δ<sup>202</sup>Hg values and 5 sharks with the  
190 highest δ<sup>202</sup>Hg values (SI Figure S3). MeHg concentrations in shark samples were obtained by  
191 the selective and quantitative extraction of MeHg followed by cold-vapor atomic  
192 fluorescence spectroscopy (CV-AFS) determinations according to the procedure described by  
193 (Masbou et al., 2013). MeHg fraction was expressed as the percentage of MeHg to THg ratio.  
194 A tuna fish flesh homogenate reference material (BCR 464, IRMM) was tested to ensure  
195 measurement accuracy and reproducibility, and a MeHg recovery of 95 ± 1% (n=2) was  
196 obtained relative to certified concentration.

#### 197 **2.4 Hg isotopes**

198 Aliquots of approximately 20 mg of dry muscle were left over night at room  
199 temperature in 3 mL of concentrated bi-distilled nitric acid (HNO<sub>3</sub>). Samples were then  
200 digested on a hotplate for 6h at 85°C in pyrolyzed glass vessels closed by Teflon caps. One  
201 mL of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) was added and digestion was continued for another 6h at  
202 85°C. One hundred µL of BrCl was then added to ensure a full oxidation of MeHg to inorganic

203 Hg. The digest mixtures were finally diluted in an inverse aqua regia (3 HNO<sub>3</sub>: 1 HCl, 20 vol.%  
 204 MilliQ water) to reach a nominal Hg concentration of 1 ng·g<sup>-1</sup>. Three types of certified  
 205 reference materials (TORT 3, BCR 464, IAEA 436) and blanks were prepared in the same way  
 206 as tissue samples (SI Table S1).

207 Mercury isotope compositions were measured at the Observatoire Midi-Pyrénées  
 208 using multicollector inductively coupled plasma mass spectrometry (MC-ICP-MS, Thermo  
 209 Finnigan Neptune Plus) with continuous-flow cold vapor (CV) generation using Sn(II)  
 210 reduction (CETAC HGX-200), according to a previously published method (Enrico et al., 2016;  
 211 Goix et al., 2019; Masbou et al., 2015). Hg isotope composition is expressed in  $\delta$  notation  
 212 and reported in parts per thousand (‰) deviation from the NIST SRM 3133 standard, which  
 213 was determined by sample-standard bracketing according to the following equation:  $\delta^{xxx}\text{Hg}$

$$214 (\text{‰}) = [ ( (^{xxx}\text{Hg}/^{198}\text{Hg})_{\text{sample}} / (^{xxx}\text{Hg}/^{198}\text{Hg})_{\text{NIST 3133}} ) - 1 ] \times 1000$$

215 where xxx represents the mass of each mercury isotope. <sup>204</sup>Hg was not measured due to  
 216 limitations in MC-ICP-MS cup configuration.  $\delta^{202}\text{Hg}$  is used as a measure of MDF. Measures  
 217 of MIF are calculated as the difference between a measured  $\delta$ -value, and the predicted  $\delta$ -  
 218 value that is calculated by multiplying the measured  $\delta^{202}\text{Hg}$  value by the kinetic MDF  
 219 fractionation factor for each isotope (Bergquist and Blum, 2007). MIF is expressed by the  $\Delta$   
 220 notation:

$$221 \Delta^{199}\text{Hg} (\text{‰}) = \delta^{199}\text{Hg} - (\delta^{202}\text{Hg} \times 0.252)$$

$$222 \Delta^{200}\text{Hg} (\text{‰}) = \delta^{200}\text{Hg} - (\delta^{202}\text{Hg} \times 0.502)$$

$$223 \Delta^{201}\text{Hg} (\text{‰}) = \delta^{201}\text{Hg} - (\delta^{202}\text{Hg} \times 0.752)$$

224 Total Hg in the diluted digest mixtures was monitored by MC-ICP-MS using <sup>202</sup>Hg signals:  
 225 mean recoveries of 95 ± 6% (n = 72) for samples and 93 ± 12% (n = 10) for certified reference

226 materials were found, ensuring efficient digestion of samples. Hg levels in blanks were below  
227 the detection limit. Reproducibility of Hg isotope measurements was assessed by analyzing  
228 UM-Almadén (n = 13), ETH-Fluka (n = 12) and the biological tissue procedural standards NRC  
229 TORT-3, ERM-BCR-464, and IAEA-436 (n = 10) (SI Table S1). Only one analysis was performed  
230 per sample, but measured isotope signatures as well as analytical reproducibility of  
231 standards were found to be in agreement with previously published values (Blum et al.,  
232 2013; Jiskra et al., 2017; M. Li et al., 2016; Masbou et al., 2013).

## 233 **2.5 Statistics**

234 Data was first checked for normality (Shapiro–Wilks test) and homogeneity of  
235 variances (Bartlett test). When these conditions were met, one-way ANOVAs were  
236 performed to test for differences between groups, followed by Tukey's HSD tests when more  
237 than two groups were compared. Otherwise, non-parametric analogues were used, i.e.  
238 Kruskal–Wallis tests (KW), followed by Conover–Iman multiple comparison tests with  
239 Bonferroni's adjustment in the presence of several groups.

240 Depending on data distribution (Shapiro–Wilks test), Pearson or Spearman correlation tests  
241 were used to investigate the correlation between shark THg concentration and other  
242 variables (length, mass,  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ).

243 Using  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, standard ellipse areas encompassing 95% of the data ( $\text{SEA}_B$ ,  
244 Bayesian SEA) were used to quantify the niche width and overlap between the two shark  
245 species, using the “SIBER” package (Jackson et al., 2011). The  $\text{SEA}_B$  constitutes a proxy of the  
246 feeding niche occupied by one species while the overlap between  $\text{SEA}_B$  quantifies the trophic

247 competition between species. The  $SEA_B$  overlap is expressed as a proportion of the non-  
248 overlapping area of the two ellipses.

249 Linear regressions were used to assess the relationships between i) Hg metabolism (using  
250  $\delta^{202}\text{Hg}$  as a proxy for MeHg demethylation) and shark age (using length as a proxy for age)  
251 (Bolea-Fernandez et al., 2019), ii) Hg MIF ( $\Delta^{199}\text{Hg}$ ) and Hg MIF ( $\Delta^{201}\text{Hg}$ ) as an indicator of  
252 MeHg photodemethylation *versus* iHg photoreduction in seawater (Bergquist and Blum,  
253 2007), iii) Hg MIF ( $\Delta^{199}\text{Hg}$ ) and Hg MDF ( $\delta^{202}\text{Hg}$ ) as an indicator of Hg photodegradation  
254 *versus* microbial transformation (Bergquist and Blum, 2007).

255 Mean values are expressed with one standard deviation (1SD) unless otherwise stated (2SD).

256 All statistical analyses were performed using the open source software R (version 3.6.1, R  
257 Core Team, 2019).

258

### 259 **3. Results**

#### 260 **3.1 Comparison of the two shark species**

261 Tiger sharks had higher length (ANOVA;  $p < 0.001$ ) but similar body mass (KW;  $p >$   
262  $0.05$ ) than bull shark (SI Table S2). The bull shark showed significantly higher THg  
263 concentration ( $4148 \pm 3069 \text{ ng}\cdot\text{g}^{-1}$ ) (KW;  $p < 0.01$ ), as well as higher muscle  $\delta^{15}\text{N}$  ( $14.22 \pm$   
264  $0.47 \text{ ‰}$ ) and  $\delta^{13}\text{C}$  values ( $-16.00 \pm 0.58 \text{ ‰}$ ) (ANOVA;  $p < 0.001$ ) compared to the tiger shark  
265 ( $3186 \pm 1252 \text{ ng}\cdot\text{g}^{-1}$ ,  $13.27 \pm 0.54 \text{ ‰}$ ,  $-17.01 \pm 0.49 \text{ ‰}$  for THg,  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values,  
266 respectively) (SI Table S2). THg was positively correlated with total length and body mass for  
267 both the bull (Pearson;  $p < 0.001$ ) and the tiger shark (Spearman;  $p < 0.05$ ). THg was not  
268 correlated with  $\delta^{15}\text{N}$  or  $\delta^{13}\text{C}$  values for either species. The two shark species showed similar

269 carbon and nitrogen isotopic niche area: 5.23 ‰<sup>2</sup> for the bull shark and 4.19 ‰<sup>2</sup> for the tiger  
270 shark (SEA<sub>B</sub> with 95% credible interval), and a small area overlap of 18%. (SI Figure S1).  
271 Concerning Hg isotopes, muscle  $\Delta^{199}\text{Hg}$  and  $\Delta^{201}\text{Hg}$  values, as well as  $\delta^{202}\text{Hg}$  values were  
272 significantly higher in bull sharks than in tiger sharks ( $\Delta^{199}\text{Hg}$  and  $\Delta^{201}\text{Hg}$ : ANOVA,  $p < 0.001$ ;  
273  $\delta^{202}\text{Hg}$ : KW,  $p < 0.001$ ) (SI Table S2). No difference was found between species for  $\Delta^{200}\text{Hg}$   
274 (0.08 ‰ and 0.06 ‰ for bull and tiger sharks respectively; ANOVA,  $p > 0.05$ ). The two sharks  
275 showed similar variability in  $\Delta^{199}\text{Hg}$  values (Bartlett test,  $p > 0.05$ ) (Figure 2A), whereas bull  
276 sharks displayed higher variability for  $\delta^{202}\text{Hg}$  values (from 1.04 to 2.82 ‰) compared to tiger  
277 sharks (from 0.74 to 1.7 ‰) (Bartlett test,  $p < 0.001$ ) (Figure 2B).  $\Delta^{199}\text{Hg}$  and  $\delta^{202}\text{Hg}$  values  
278 were not correlated with  $\delta^{15}\text{N}$  or  $\delta^{13}\text{C}$  values for either species ( $p > 0.05$ ).  $\Delta^{199}\text{Hg}$  values were  
279 not correlated with body length in the two shark species ( $p > 0.05$ ). A positive correlation  
280 was found between length and  $\delta^{202}\text{Hg}$  values for the two species ( $p < 0.001$ ;  $R^2=0.70$  for the  
281 bull shark, and  $R^2=0.31$  for the tiger shark) (Figures 3A and 3B), and between THg and  $\delta^{202}\text{Hg}$   
282 only for the bull shark ( $p < 0.001$ , SI Figure S2). MeHg accounted for  $95 \pm 8\%$  of THg in the  
283 tiger shark and the MeHg fraction was not correlated with length nor  $\delta^{202}\text{Hg}$  values (SI Figure  
284 S3).

### 285 3.2 Comparison between sharks and prey

286  $\Delta^{199}\text{Hg}$  in prey species exhibited a wide range of values, from 1.94‰ in the  
287 deepwater snapper to 2.72‰ in the lyretail grouper (SI Table S3 and Figure 2A). The bull  
288 shark displayed similar  $\Delta^{199}\text{Hg}$  as the yellowfin tuna and the giant trevally, while the tiger  
289 shark shared similar  $\Delta^{199}\text{Hg}$  with the deepwater snapper (ANOVA;  $p > 0.05$ ) (Figure 2A). The  
290 two shark species showed different  $\Delta^{199}\text{Hg}$  values from all the other prey species (*i.e.* lyretail  
291 grouper, wahoo, great barracuda, skipjack tuna, lantern fish) (ANOVA or KW;  $p < 0.05$ ).

292 Muscle  $\delta^{202}\text{Hg}$  in prey species ranged from 0.20‰ in the yellowfin tuna to 1.05‰ in the  
293 great barracuda (SI Table S3). The bull shark showed significantly higher  $\delta^{202}\text{Hg}$  than all the  
294 prey species (ANOVA or KW;  $p < 0.05$ ) (Figure 2B).  $\delta^{202}\text{Hg}$  in the tiger shark was also higher  
295 than in most of the prey species (ANOVA;  $p < 0.001$ ) except for the lyretail grouper and the  
296 great barracuda (ANOVA;  $p < 0.05$ ).

### 297 **3.3 Hg sources**

298 The linear regression between  $\Delta^{199}\text{Hg}$  and  $\Delta^{201}\text{Hg}$  for the overall fish and shark  
299 samples displayed a slope of  $1.23 \pm 0.05$  ( $R^2 = 0.87$ ,  $p < 0.001$ ) (SI Figure S4) while the linear  
300 regression between  $\Delta^{199}\text{Hg}$  and  $\delta^{202}\text{Hg}$  for teleost fish only displayed a slope of  $0.64 \pm 0.18$   
301 ( $R^2 = 0.28$ ,  $p < 0.01$ ) (SI Figure S5). The mesopelagic offshore lantern fish displayed similar  
302  $\Delta^{199}\text{Hg}$  and  $\delta^{202}\text{Hg}$  values than the giant trevally, as well as similar  $\Delta^{199}\text{Hg}$  but higher  $\delta^{202}\text{Hg}$   
303 than the deepwater snapper (SI Table S3). Compared to the previous study of Sackett *et al.*,  
304 2017 in Hawaii (Sackett et al., 2017), significant higher  $\delta^{202}\text{Hg}$  was found in the deepwater  
305 snapper in the present study (ANOVA,  $p < 0.05$ ) (SI Table S4). Despite no significant  
306 difference in  $\delta^{202}\text{Hg}$  and  $\Delta^{199}\text{Hg}$  for the giant trevally between our study and Sackett *et al.*,  
307 2017 (KW,  $p > 0.05$ ), our values showed lower variance for both  $\delta^{202}\text{Hg}$  and  $\Delta^{199}\text{Hg}$  (Bartlett  
308 test,  $p < 0.05$ ).

309

## 310 **4. Discussion**

### 311 **4.1 Hg isotopes as tracers of shark ecology**

312 **- Foraging depth**

313 According to the previously documented decrease in  $\Delta^{199}\text{Hg}$  values with depth (Blum  
314 et al., 2013), the higher  $\Delta^{199}\text{Hg}$  found in bull sharks is likely reflecting the use of shallower  
315 waters as main feeding habitat compared to tiger sharks, which would forage in deeper  
316 waters (Figure 2A). In previous studies in coastal environments, estuarine fish were  
317 characterized either by higher (M. Li et al., 2016) or lower MIF signature (Senn et al., 2010)  
318 than open ocean fish, depending on light penetration as a function of water turbidity. Except  
319 during episodes of heavy rain, the water clarity in the coastal ecosystem of La Réunion Island  
320 implies that  $\Delta^{199}\text{Hg}$  values may possibly vary more over a vertical gradient (i.e. depending on  
321 depth) than a horizontal gradient (i.e. depending on the distance to the shore). This  
322 hypothesis is supported by the fact that epipelagic fish (i.e. living in the upper part of the  
323 water column) from the inshore (e.g. wahoo and great barracuda) and offshore habitat (e.g.  
324 skipjack and yellowfin tunas) did not differ in  $\Delta^{199}\text{Hg}$  values (SI Table S3). Here,  $\Delta^{199}\text{Hg}$  values  
325 fully corroborate what was previously observed by telemetry studies at this site, showing a  
326 higher presence of bull sharks in the coastal environment of La Réunion Island compared to  
327 tiger sharks (Blaison et al., 2015). Similarly, bull sharks showed a preferential use of shallow  
328 waters in the Indian Ocean when not migrating (usually less than 50 m with deepest dive at  
329 164 m) (J. S. E. Lea et al., 2015), while tiger sharks have been recorded in oceanic waters  
330 down to more than 1000 m (Afonso and Hazin, 2015).

### 331 - Diet composition

332 Numerous studies have confirmed that  $\Delta^{199}\text{Hg}$  values are conserved between prey  
333 and predators due to the absence of photochemical Hg MIF during trophic interactions or  
334 metabolic processes (Kwon et al., 2016, 2012; Masbou et al., 2018), which makes this tool a  
335 trophic tracer of major interest. At La Réunion Island, teleost fish species represent the large

336 majority of the prey consumed by both bull and tiger sharks, based on stomach contents (i.e.  
337 respectively 93% and 78% in terms of mass percentage) (Trystram et al., 2016). Carangid  
338 species such as the giant trevally constitute the most consumed family by bull sharks with  
339 more than 40% of the ingested mass, while tiger sharks are more generalist predators and  
340 target a large number of teleost families. Stable isotope tracers were in accordance with  
341 stomach contents, as the bull shark had similar  $\Delta^{199}\text{Hg}$  values (Figure 2A) but higher  $\delta^{15}\text{N}$   
342 values (as a proxy for trophic position) than the giant trevally ( $\delta^{15}\text{N} = 14.22\text{‰}$  and  $12.53\text{‰}$   
343 for bull sharks and giant trevally respectively; ANOVA,  $p < 0.001$ ), supporting the idea that  
344 carangid species are common prey for bull sharks. Alternatively, the largest giant trevallies  
345 could also share the feeding habitat of bull sharks leading to competition for resources and  
346 similar  $\Delta^{199}\text{Hg}$  values. Associations between the two species have indeed been observed by  
347 video and acoustic (Loiseau, unpublished data). On the other hand, taking into account that  
348 epipelagic fish from the inshore and offshore habitats did not differ in MIF values (SI Table  
349 S3), the  $\Delta^{199}\text{Hg}$  overlap between the bull shark and yellowfin tuna may only reflect the use of  
350 the same water depth across different food webs (i.e. respectively inshore and offshore)  
351 rather than trophic interactions, since tuna species were not identified among bull shark  
352 prey at La Réunion Island (Trystram et al., 2016). This underlines the importance of  
353 considering the information given by stomach contents and trophic positions to interpret  
354  $\Delta^{199}\text{Hg}$  values, which are preserved throughout food webs. Here, the tiger shark and the  
355 deepwater snapper shared the same  $\Delta^{199}\text{Hg}$  (Figure 2A) but different trophic positions (i.e.  
356 higher  $\delta^{15}\text{N}$  for the tiger shark; ANOVA,  $p < 0.001$ ). As this bottom-associated snapper is  
357 found in waters around 300 m depth (Trystram et al., 2015), it suggests that tiger sharks are  
358 primarily targeting prey on the island slope. The two different isotopic approaches used in  
359 this study (i.e.  $\Delta^{199}\text{Hg}$  and  $\delta^{15}\text{N}/\delta^{13}\text{C}$ ) both lead to the conclusion that the two shark species

360 occupy different trophic niches (different  $\Delta^{199}\text{Hg}$  and small  $\text{SEA}_b$  overlap) (Figure 2A and S2)  
361 but also that their trophic niches are similar in size (similar variance for  $\Delta^{199}\text{Hg}$  (Bartlett Test,  
362  $p < 0.05$ ) and similar  $\text{SEA}_b$ ). In a previous study based on  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, a trophic  
363 independence of the epipelagic fish community (including both the bull and the tiger shark)  
364 and the mesopelagic fish community was observed (Trystram et al., 2015). Here, Hg isotopes  
365 confirm segregation between bull sharks and deep species such as the deepwater snapper,  
366 but in contrast, provide evidence that tiger sharks rely on mesopelagic resources.

367 Overall, these results highlight the interest of Hg isotopes to address fundamental ecological  
368 questions, such as habitat use and trophic energy transfers (Tsui et al., 2020).

## 369 **4.2 Hg isotopes as tracers of shark metabolism**

### 370 **- Comparison between sharks and bony fish**

371 While  $\Delta^{199}\text{Hg}$  values in sharks were comparable to those of other fish species in this  
372 area (Figure 2A),  $\delta^{202}\text{Hg}$  values were generally higher in sharks compared to their potential  
373 teleost prey (Figure 2B). More precisely, both shark species stand out from their prey by  
374 more than 1 ‰ in  $\delta^{202}\text{Hg}$  while having similar  $\Delta^{199}\text{Hg}$  values. To our knowledge, such high  
375  $\delta^{202}\text{Hg}$  values (i.e. 1.9 ‰ in the bull shark and 1.2 ‰ in the tiger shark) have never been  
376 reported in fish. However, similar high values have been documented in mammals, including  
377 humans, that are able to metabolize MeHg to inorganic Hg by demethylation in the liver  
378 (Laffont et al., 2011; Masbou et al., 2018, 2015; Perrot et al., 2016). The preferential  
379 demethylation of light Hg isotopes induces an increase in  $\delta^{202}\text{Hg}$  in the remaining MeHg pool  
380 that is ultimately accumulated in other tissues such as muscle (Perrot et al., 2016). This  
381 increase in MeHg  $\delta^{202}\text{Hg}$  following demethylation varies from about 0.3 ‰ in terrestrial

382 mammals (Ma et al., 2018; Masbou et al., 2018), to around 1 ‰ in aquatic mammals (Perrot  
383 et al., 2016, 2012) and around 2 ‰ in humans (Laffont et al., 2011, 2009). Previous studies  
384 on mercury speciation have concluded that MeHg demethylation can occur in fish liver and  
385 intestine (Gonzalez et al., 2005; Wang et al., 2017). However, experimental studies using Hg  
386 isotopes in fish muscle do not allow to draw clear conclusions, with either no change in MDF  
387 signature between Hg trophic source and consumer (Feng et al., 2015; Kwon et al., 2012), or  
388 a positive (+ 0.35 ‰) (Kwon et al., 2013) or a negative shift in  $\delta^{202}\text{Hg}$  values (- 0.19 ‰) (Kwon  
389 et al., 2016). In our study,  $\delta^{202}\text{Hg}$  values in sharks were well above fractionation values  
390 previously published in fish (e.g. + 1.4 ‰ between the bull shark and the giant trevally), with  
391 the bull shark having higher  $\delta^{202}\text{Hg}$  than all teleost species analyzed. This study represents  
392 the first isotopic investigation of Hg metabolism in sharks and supports the existence of  
393 MeHg demethylation in these predators.

394 High THg concentrations (around  $150 \mu\text{g}\cdot\text{L}^{-1}$ ) were found in the blood plasma of South  
395 African white sharks *Carcharodon carcharias*, at levels considered as toxic in other  
396 vertebrates (Merly et al., 2019). Despite this contamination, no alteration of the body  
397 condition nor negative effects on health parameters were observed suggesting that sharks  
398 may have protective mechanisms that mitigate Hg toxicity. Demethylation in sharks could  
399 therefore represent a mechanism limiting the accumulation of MeHg and its toxic effects.  
400 Moreover, MeHg concentrations are generally much higher in muscle compared to other  
401 organs such liver, kidney and brain in shark species (Bergés-Tiznado et al., 2015; Nam et al.,  
402 2011), suggesting that muscle could represent a storage tissue less sensitive to MeHg toxicity  
403 in sharks.

404 **- Hg metabolism over age**

405  $\delta^{202}\text{Hg}$  values increased with length, a proxy for age, in the two shark species studied  
406 here (Figures 3A and 3B). Most of the sharks were sub-adults and adults, and larger than the  
407 size at which the ontogenetic change in habitat and diet occurs (Habegger et al., 2012; Lowe  
408 et al., 1996). Furthermore, in the two shark species, none of the trophic markers analyzed  
409 ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ,  $\Delta^{199}\text{Hg}$ ) were correlated with size.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were also not correlated with  
410  $\delta^{202}\text{Hg}$ , which does not support the influence of ontogenetic diet changes on  $\delta^{202}\text{Hg}$  values.  
411 On the other hand, an enhancement of the demethylation rate with age was recently  
412 identified in the long-finned pilot whale *Globicephala melas*, leading to the decrease of the  
413 MeHg fraction in the muscle of older whales (from 99% to 65%) (Bolea-Fernandez et al.,  
414 2019). Since MeHg has higher  $\delta^{202}\text{Hg}$  values compared to inorganic Hg (iHg) (Perrot et al.,  
415 2016), the decrease in MeHg fraction alongside the increase in iHg fraction in pilot whales  
416 was accompanied by a drop in  $\delta^{202}\text{Hg}$  values (around 1 ‰ decrease) (Bolea-Fernandez et al.,  
417 2019). In our bull shark samples, MeHg accounted for 95% of total Hg, which is consistent  
418 with previous studies on this species (Matulik et al., 2017) or on other sharks (de Carvalho et  
419 al., 2014; Nam et al., 2011; Pethybridge et al., 2010). Moreover, no correlation was found  
420 between MeHg fraction and  $\delta^{202}\text{Hg}$  or shark length (SI Figure S3A and S3B). These results  
421 imply that the change in  $\delta^{202}\text{Hg}$  with age in shark muscle is unlikely to be the result of a  
422 variation in Hg speciation (i.e. MeHg versus iHg fraction). We thus hypothesize that the  
423 increase in  $\delta^{202}\text{Hg}$  values with length could reflect a higher extent of MeHg demethylation in  
424 the liver of older sharks, before preferential storage of the remaining MeHg over iHg in  
425 muscle. By contrast, the aging muscle in pilot whale may gradually favor the storage of iHg  
426 over MeHg (Bolea-Fernandez et al., 2019). Moreover, a positive correlation was also  
427 observed between the  $\delta^{202}\text{Hg}$  values and THg (as a proxy for MeHg) concentration only in  
428 the bull shark muscle (SI Figure S2). Such a positive correlation between  $\delta^{202}\text{Hg}$  and THg was

429 previously observed in teleost fish species and were attributed to the excretion of  
430 isotopically light Hg by fish during Hg bioaccumulation (Bergquist and Blum, 2007). In the  
431 present study, the shift in Hg MDF signatures between sharks and their prey (e.g. + 1.4 ‰ for  
432  $\delta^{202}\text{Hg}$  between the bull shark and the giant trevally) is well above the values attributed  
433 experimentally to excretion in teleost fish (e.g. + 0.35 ‰ between the amberjack *Seriola*  
434 *dumerili* compared to its food) (Kwon et al., 2013). However, preferential efflux of MeHg  
435 with light Hg isotopes over time, through urinary or biliary excretion (Le Croizier et al.,  
436 2019a, 2018), may also contribute to the increase in  $\delta^{202}\text{Hg}$  values observed in shark muscle.  
437 In addition, as previously proposed in pilot whales (Bolea-Fernández et al., 2019), it is  
438 possible that muscle MeHg can be recycled to the liver and undergo additional  
439 demethylation. Thus, older sharks may have experienced more MeHg exchanges between  
440 liver and muscle, leading to higher  $\delta^{202}\text{Hg}$  values in the remaining MeHg. This hypothesis  
441 would explain the increase in  $\delta^{202}\text{Hg}$  with shark length and THg concentration which both  
442 increase with age, respectively due to growth and bioaccumulation.

443         According to length, the age of individual sharks is supposed to vary from around 5  
444 years to more than 25 years for bull sharks (Natanson et al., 2014) and from around 2 years  
445 to more than 15 years for tiger sharks (Meyer et al., 2014). As bull sharks are on average  
446 older and display a broader year range between individuals, this could explain their higher  
447  $\delta^{202}\text{Hg}$  values and higher intraspecific variability compared to tiger sharks (Figure 2B), caused  
448 by an increase in demethylation and / or more liver-muscle exchanges over time.

#### 449 **4.3 Hg exposure sources to sharks**

##### 450         - Hg cycle in the shark environment

451 In open oceans, Hg inputs to the surface waters mainly occur through rainfall wet  
452 deposition of inorganic Hg (iHg) and atmospheric dissolution of gaseous Hg (Hg(0)) (Zhang et  
453 al., 2014). Although the processes responsible for the mass-independent fractionation of  
454 even Hg isotopes remain uncertain,  $\Delta^{200}\text{Hg}$  is thought to result from high-altitude Hg photo-  
455 oxidation in the tropopause (Chen et al., 2012). iHg wet deposition is characterized by a  
456  $\Delta^{200}\text{Hg}$  between 0 and 0.3 ‰ while  $\Delta^{200}\text{Hg}$  of atmospheric Hg(0) ranges from -0.11 to -0.01  
457 ‰ (Enrico et al., 2016; Gratz et al., 2010). In the present study,  $\Delta^{200}\text{Hg}$  values were similar  
458 between the two shark species (i.e.  $0.08 \pm 0.04$  ‰ in the bull shark and  $0.06 \pm 0.04$  ‰ in the  
459 tiger shark) (SI Table S2), implying that the iHg precursor to MeHg in sharks originates from a  
460 common source, which may correspond to a combination of rainfall iHg and gaseous Hg(0)  
461 (with positive and negative  $\Delta^{200}\text{Hg}$  values, respectively). Although to our knowledge there is  
462 still no data on the isotopic signature of Hg(0) in this region, preliminary  $\Delta^{200}\text{Hg}$  in rainwater  
463 collected at La Réunion Island is 0.12 ‰ (Araujo et al., unpublished data), which suggests  
464 that MeHg in sharks is mostly derived from the methylation of iHg from wet deposition to  
465 the marine ecosystem, as previously observed in other aquatic ecosystems (Lepak et al.,  
466 2018).

467 Under the action of solar radiation, dissolved MeHg will be transformed into iHg by  
468 photodemethylation, while dissolved iHg will be transformed into Hg(0) by photoreduction.  
469 Photodemethylation of MeHg is characterized by a  $\Delta^{199}\text{Hg}/\Delta^{201}\text{Hg}$  ratio of 1.36 while  
470 photodegradation of inorganic Hg leads to a ratio of 1.0 (Bergquist and Blum, 2007). In the  
471 present study, overall fish and shark values displayed a mean  $\Delta^{199}\text{Hg}/\Delta^{201}\text{Hg}$  ratio of 1.23  
472 indicating the dominance of MeHg demethylation over iHg photoreduction. This ratio is

473 consistent with those previously reported in coastal and pelagic ecosystems of oceanic  
474 islands such as Hawaii (Blum et al., 2013; Sackett et al., 2017).

475 Experimental studies have shown that Hg photochemical degradation leads to an  
476 approximate  $\Delta^{199}\text{Hg}/\delta^{202}\text{Hg}$  slope of 2.4 whereas microbial transformation (no MIF) is  
477 characterized by a slope of 0 (Bergquist and Blum, 2007). In our study, the Hg isotopic  
478 signatures of the teleost prey displayed a  $\Delta^{199}\text{Hg}/\delta^{202}\text{Hg}$  slope of 0.64 (SI Figure S5),  
479 indicating the dominance of microbial transformation (i.e. methylation and/or  
480 demethylation) over photochemical degradation, which is similar to previous studies on  
481 Hawaiian marine bottomfish (Sackett et al., 2017) and coastal marine fish from the Gulf of  
482 Mexico (Senn et al., 2010).

#### 483 **- Hg isotope signatures in shark prey**

484 The main trophic MeHg vector for the bull shark at La Réunion Island is thought to be  
485 constituted by nearshore fish such as the giant trevally (Trystram et al., 2015) which shared  
486 similar  $\Delta^{199}\text{Hg}$  and  $\delta^{202}\text{Hg}$  with offshore epipelagic species such as the skipjack tuna (SI Table  
487 S3, Figure 4). This result would suggest that MeHg to which bull sharks are exposed via  
488 trophic transfer originates from the offshore environment (Figure 4). This observation  
489 contrasts with the findings of a previous study in Hawaii where terrestrial freshwater inputs  
490 to coastal sediments were the primary MeHg source for the giant trevally (Sackett et al.,  
491 2017), resulting in higher inter-individual variability for both  $\Delta^{199}\text{Hg}$  and  $\delta^{202}\text{Hg}$  compared to  
492 our values (Bartlett test,  $p < 0.05$ ; SI Table S4). This strengthens the hypothesis of a limited  
493 input of MeHg by freshwater runoff of estuarine waters into the coastal ecosystem of La  
494 Réunion Island. Rather, MeHg around La Reunion originates from methylation of iHg that is  
495 supplied by rainwater to the marine environment, as suggested by  $\Delta^{200}\text{Hg}$  values.

496 According to  $\Delta^{199}\text{Hg}$  values, mesopelagic species such as the deepwater snapper  
497 constitute the main vector of Hg trophic contamination for tiger sharks (Figure 2A). The  
498 deepwater snapper only shared similar  $\Delta^{199}\text{Hg}$  with the lantern fish (SI Table S3, Figure 4).  
499 This is in full agreement with stomach content observations, which revealed that  
500 mesopelagic teleosts and especially lantern fish are the major prey of the deepwater  
501 snapper, accounting for around 60% of the total prey abundance (Trystram, 2016). In Hawaii,  
502 Hg isotopic signatures of bottom fish including deepwater snappers revealed the  
503 incorporation of mesopelagic offshore MeHg sources into the slope sediments, where MeHg  
504 was subject to additional negative MDF (due to microbial methylation and/or  
505 demethylation) before transfer to the marine consumers (Sackett et al., 2017). Here, a lower  
506  $\delta^{202}\text{Hg}$  was similarly found in the deepwater snapper compared to the lantern fish (SI Table  
507 S3, Figure 4). We thus suggest that the decrease in  $\delta^{202}\text{Hg}$  between the mesopelagic source  
508 and the deepwater snapper is related to its partial consumption of benthic prey (around 20%  
509 of the total prey abundance) (Trystram, 2016) and consequent exposure to a MeHg pool  
510 having undergone microbial MDF (i.e. methylation and/or demethylation) in sediments  
511 (Figure 4). The consumption of benthic prey could also have exposed the deepwater snapper  
512 to another source of MeHg produced in the sediments and characterized by a lower  $\delta^{202}\text{Hg}$   
513 values than mesopelagic MeHg.

514 As previously observed for marine fish species in the central Pacific (Blum et al., 2013;  
515 Sackett et al., 2017), both bull and tiger sharks were exposed to two different MeHg pools  
516 derived from the same main source of oceanic origin, produced from deposited atmospheric  
517 iHg. The bull shark was exposed to epipelagic offshore MeHg, which was dietary transferred  
518 to the epipelagic nearshore habitat (Figure 4). On the other hand, the tiger shark was

519 exposed to a mixed pool of (i) mesopelagic offshore MeHg and (ii) benthic MeHg subject to  
520 bacterial transformation in sediments.

521

## 522 **5. Conclusion**

523 Hg MIF signatures allowed to characterize the vertical feeding habitat of sharks, revealing  
524 that bull sharks forage in shallow water while tiger sharks use deeper habitat on the island  
525 slope. Our study highlights the potential of  $\Delta^{199}\text{Hg}$  values for tracing the ecology of marine  
526 species and brings important new perspectives on the habitat use of coastal predators in a  
527 context of increasing human-shark interactions. Using Hg MDF signatures, our results also  
528 revealed for the first time the capacity of sharks to breakdown MeHg via demethylation. This  
529 may limit MeHg concentrations in these vulnerable species, among the most contaminated  
530 in the animal kingdom.

531

## 532 **Acknowledgments:**

533 Gaël Le Croizier was supported by a postdoctoral grant from the French National Research  
534 Institute for Sustainable Development (IRD). This work was financially supported by the  
535 French National Research Agency project ANR-17-CE34-0010 MERTOX. Shark samples were  
536 collected as part of the Charc (Feder Fund convention 2011 Presage N°33021) and Ecoreco-  
537 Run (DEAL-Réunion BOP113) projects, fish samples were collected during DIPPLO (FEP,  
538 Regional council and TCO funds), ANCRE-DMX2 (FEP fund N°40055/DMSOI/2013), La  
539 Pérouse cruise (DOI: 10.17600/16004500) and MAD-RIDGE-2 cruise (DOI:  
540 10.17600/16004900). C. Trystram contributed to the data collection and laboratory

541 processes at GNS Sciences under the supervision of K. Rogers. We thank Laure Laffont and  
542 Jérôme Chmeleff for expert management of the OMP mercury and mass spectrometry  
543 facilities. We finally thank Marc Dando for allowing us to use his shark illustrations.

544

Journal Pre-proof

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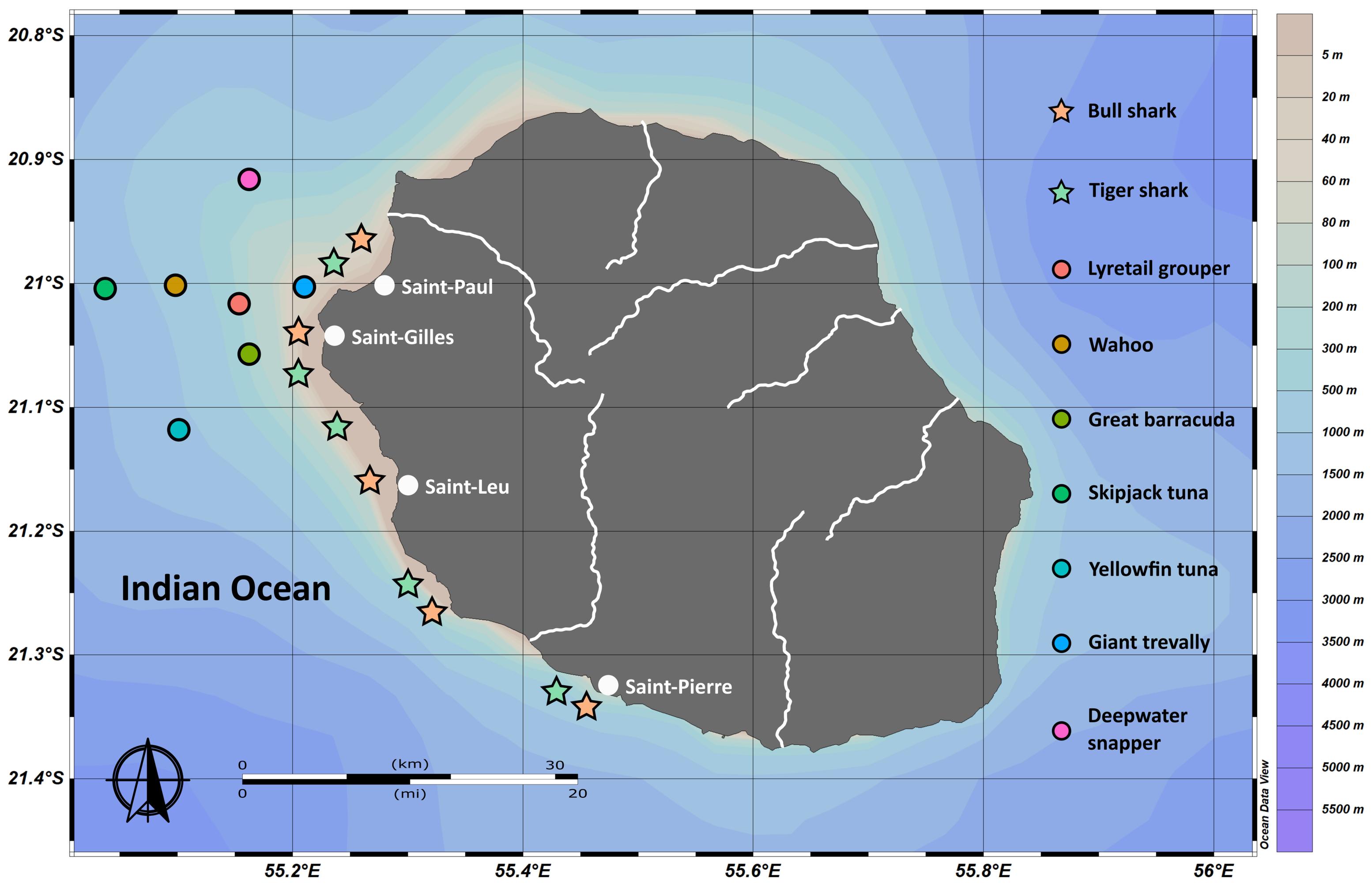
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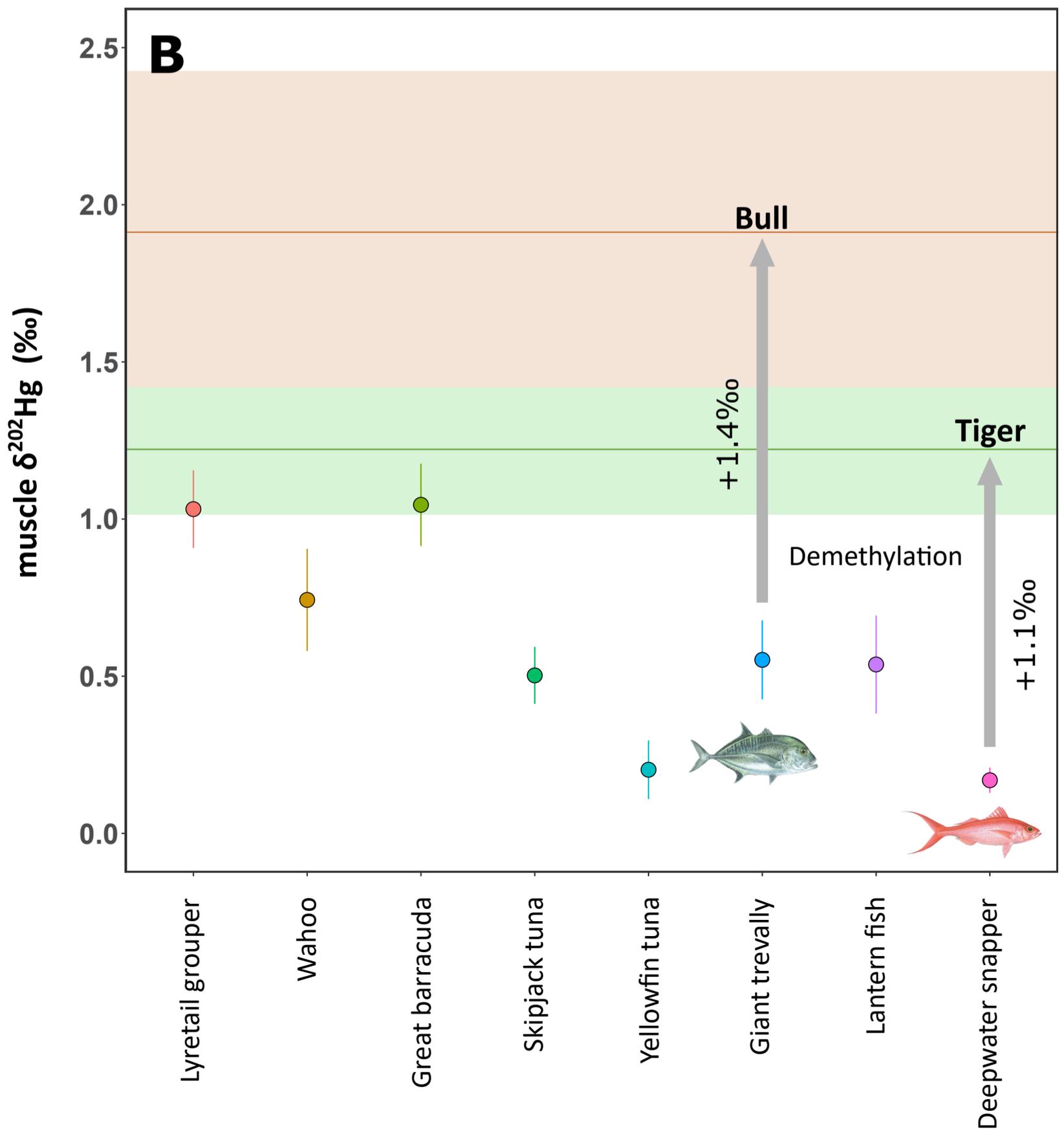
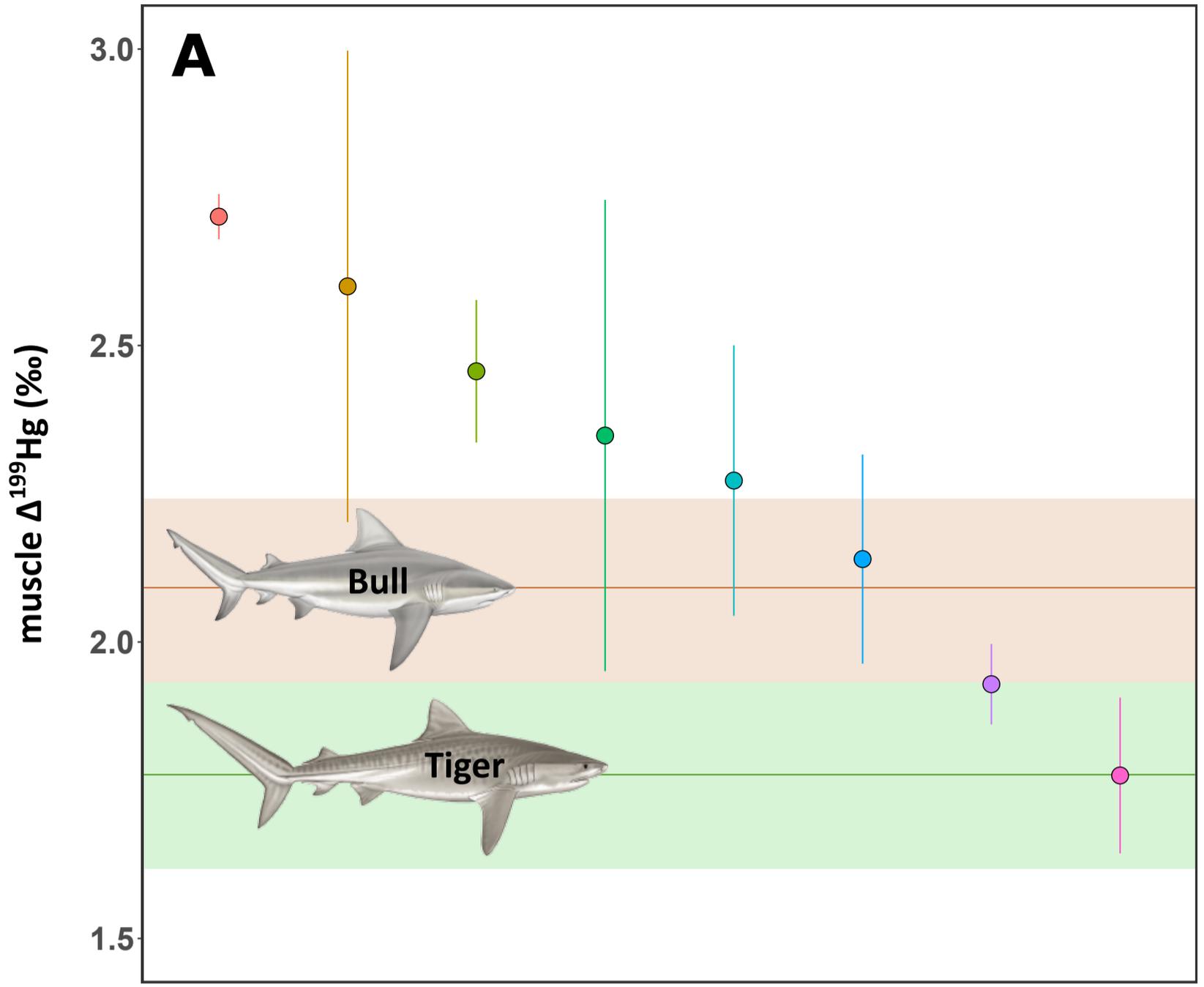
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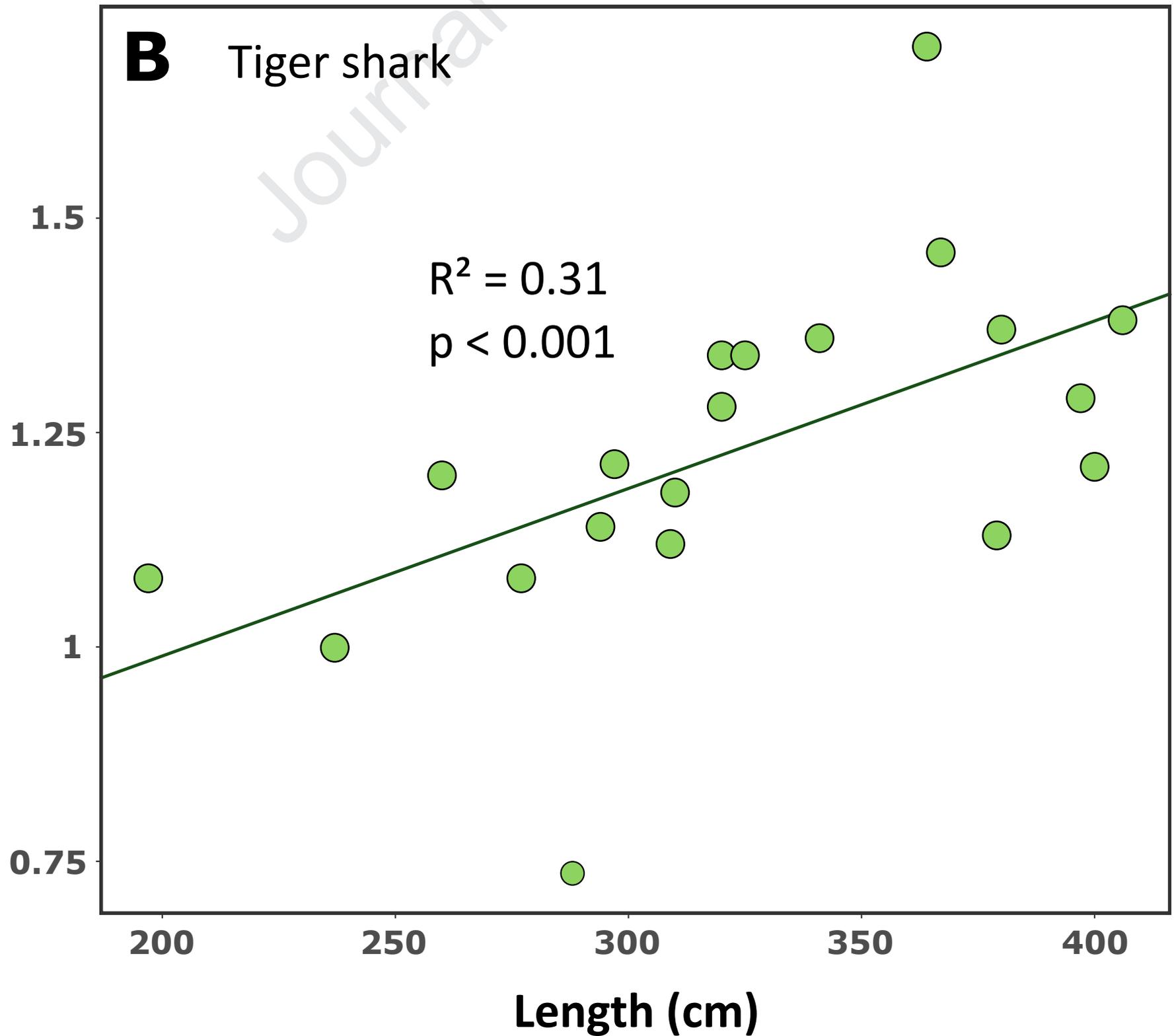
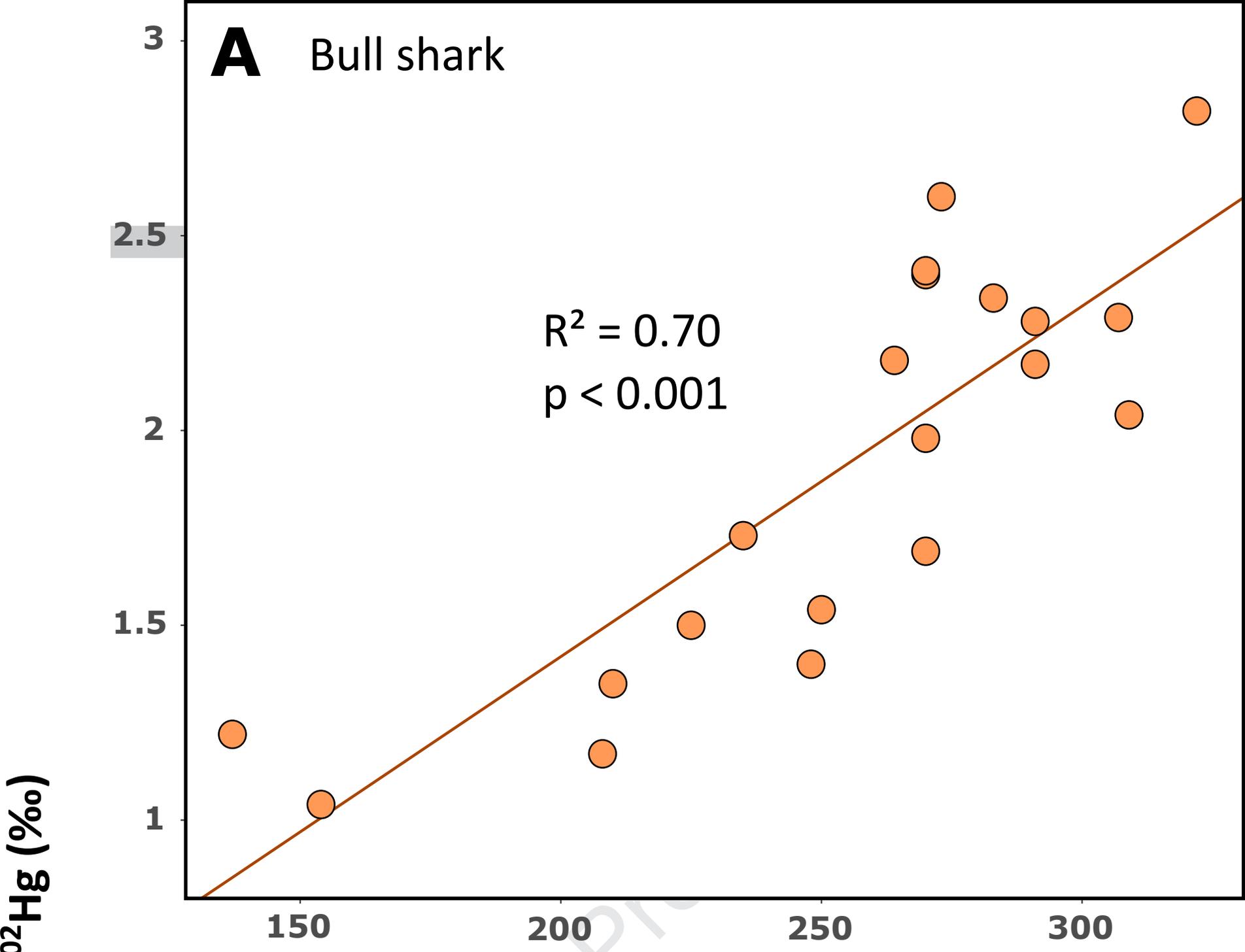
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## Figure captions

**Figure 1:** Map of sampling locations of the shark and prey species along the west coast of La Réunion Island. Major rivers are figured in white lines. Lantern fish are not shown but were caught in the area (off La Réunion Island and off southern Madagascar).

**Figure 2: A:**  $\Delta^{199}\text{Hg}$  (‰) in the bull shark (orange bar, mean  $\pm$  SD), the tiger shark (green bar, mean  $\pm$  SD) and prey species (dots, mean  $\pm$  SD) from La Réunion Island. The prey fish are ordered according to their  $\Delta^{199}\text{Hg}$  values. The bull shark had higher (ANOVA,  $p < 0.001$ )  $\Delta^{199}\text{Hg}$  than the tiger shark. The bull shark shared similar  $\Delta^{199}\text{Hg}$  with the yellowfin tuna and the giant trevally while the tiger shark shared similar  $\Delta^{199}\text{Hg}$  with the deepwater snapper.

**B:**  $\delta^{202}\text{Hg}$  (‰) in the bull shark (orange bar, mean  $\pm$  SD), the tiger shark (green bar, mean  $\pm$  SD) and prey species (dots, mean  $\pm$  SD). Bull shark had higher (KW,  $p < 0.001$ ) and more variable (Bartlett test,  $p < 0.001$ )  $\delta^{202}\text{Hg}$  than tiger shark. Bull shark had higher  $\delta^{202}\text{Hg}$  than its prey (e.g. giant trevally; KW,  $p < 0.001$ ). Similarly, the tiger shark displayed higher  $\delta^{202}\text{Hg}$  than its prey (e.g. deepwater snapper; ANOVA,  $p < 0.001$ ).

**Figure 3:** Muscle  $\delta^{202}\text{Hg}$  values versus total length for the bull shark (**A**) and the tiger shark (**B**). Data fits a linear regression in A and B.

**Figure 4:** Simplified representation of the Hg cycle in the environment of bull and tiger sharks. Atmospheric inorganic Hg (iHg) is deposited to water before being methylated to methyl-Hg (MeHg) along the water column, which induces mass-dependent fractionation (MDF). MeHg can then be demethylated by solar radiation in the photic zone of the water column, inducing mass-independent fractionation (MIF). Hg can reach the deep layers through particle sinking, either in the MeHg form, or in the iHg form. iHg can also be methylated in the twilight zone. Epipelagic MeHg is directly incorporated into the bull shark food web, while the tiger shark food web is exposed to a mixed pool of (i) mesopelagic offshore MeHg and (ii) benthic MeHg subject to bacterial transformation in slope sediments. MeHg is finally demethylated in sharks, leading to high  $\delta^{202}\text{Hg}$  values. Reduction of iHg into gaseous  $\text{Hg}^0$  and subsequent volatilization is not represented.

**Highlights:**

Mercury isotopes were analyzed in bull and tiger sharks from the Indian Ocean

Hg MIF signature revealed that bull sharks target coastal prey in shallow water

$\Delta^{199}\text{Hg}$  values showed that tiger sharks forage on deeper habitat on the island slope

$\delta^{202}\text{Hg}$  shift between prey and predator may indicate MeHg demethylation in sharks

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**Declaration of interests**

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

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

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