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# **Effect of body length, trophic position and habitat use on mercury concentrations of sharks from contrasted ecosystems in the southwestern Indian Ocean**

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## **Abstract**

The non-essential metal mercury (Hg) can have deleterious effects on health of organisms, and tends to bioaccumulate with age in long-lived organisms and to biomagnify along food chains. Because sharks are fished for human consumption and their Hg levels are frequently above the maximum Hg concentration recommended for fish consumption, understanding the drivers of Hg concentration is of considerable interest. Total Hg concentrations were analysed in muscle tissues of 14 shark and 2 batoid species (n = 339 individuals) sampled across multiple habitats (coastal, oceanic and bathyal) in the southwestern Indian Ocean. Stable isotope ratios of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) were analysed to assess whether relative trophic position and foraging habitats affected Hg concentrations. Hg concentrations increased with  $\delta^{15}\text{N}$  and body length, highlighting the mechanisms of bioaccumulation and biomagnification in relation with the trophic position and size of the individuals. Habitats where elasmobranchs were collected also affected their Hg concentrations. Bathyal sharks had high Hg concentrations that were almost similar to those of oceanic species, despite their lower relative trophic position. Higher bioavailability of Hg due to its enhanced methylation in deeper waters was considered as the most likely explanation for this result. These results highlight that multiple factors contribute to mercury accumulation in elasmobranchs.

**Keywords:** Hg, elasmobranchs, stable isotopes, trophic ecology, foraging habitat, body size.

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## 1. Introduction

Elasmobranchs are present in many habitats and at various depths. They exhibit a range of foraging strategies and tactics, including planktivorous filter-feeding and predatory species (e.g. Bird et al. 2018; Kiszka and Heithaus 2014). They also have long lifespans (Cortés 1999) and some species grow to large body sizes. Sharks are heavily fished for human consumption (Clarke et al. 2006). However, mercury (Hg) concentrations reported in sharks are frequently above the maximum values recommended for fish consumption ( $1 \mu\text{g}\cdot\text{g}^{-1}$  wet weight; Escobar-Sánchez et al. 2011; Hueter et al. 1995; Kousteni et al. 2006; McKinney et al. 2016). Hg is toxic to organisms at low concentrations with known sublethal effects including reduced growth, impacts on reproduction (e.g. reduced fertility and spawning success), abnormal behaviour (e.g. increased lethargy) and tissue damages (Depew et al. 2012; Díez 2008; Sandheinrich and Wiener 2011). Hg is released in the environment by both natural and anthropogenic sources (Fitzgerald et al. 2007; Selin 2009). It is known to be present in aquatic organisms mainly in the form of highly toxic methylmercury ( $\text{CH}_3\text{Hg}$ ) form (Bloom 1992; Jones et al. 2013; Pethybridge et al. 2010; Storelli et al. 2002; 2003). Due to its high assimilation rate and affinity for proteins,  $\text{CH}_3\text{Hg}$  bioaccumulates in organisms through time and biomagnifies up food webs (Atwell et al. 1998; Mason et al. 1995; Power et al. 2002).

Various biological and ecological factors influence Hg concentrations in aquatic animals including elasmobranchs. Body length (a proxy for animal size) and age may be considered as the main factors determining Hg concentrations. Hg concentrations increase with size in various taxa such as cephalopods (e.g. Chouvelon et al. 2011), predatory teleosts (Cresson et al. 2014; Kojadinovic et al. 2006; Sackett et al. 2013) and elasmobranchs (Cresson et al. 2014; Hueter et al. 1995; McKinney et al. 2016; Pethybridge et al. 2010; 2012; Rumbold et al. 2014). Differences in life-history traits may also influence Hg dynamics in elasmobranchs (e.g. Le Bourg et al. 2014; Lyons and Lowe 2013; Pethybridge et al. 2010). Relationships between trophic position metrics and Hg concentrations at

the levels of species (Cresson et al. 2014; Pethybridge et al. 2012), taxonomic groups (McKinney et al. 2016; Pethybridge et al. 2012) and communities (Atwell et al. 1998; Campbell et al. 2008; Lavoie et al. 2010; Power et al. 2002) show that feeding ecology of organisms dramatically influences Hg concentrations. Spatial variations of Hg concentrations in organisms (Hisamichi et al. 2010; Jones et al. 2013; Kojadinovic et al. 2006; Sackett et al. 2013) or correlations between depth of occurrence appears to be particularly important in driving Hg concentrations (Blum et al. 2013; Chouvelon et al. 2012; Choy et al. 2009; Monteiro et al. 1996), but the relative importance of environmental drivers on Hg concentrations in marine predators is still relatively poorly understood. Furthermore, Hg concentrations are poorly documented for a number of ocean regions such as in the western Indian Ocean (but see Chouvelon et al. 2017; McKinney et al. 2016; Kojadinovic et al. 2006).

The aim of this study was to investigate the effects of body size, trophic position, and broad habitat characteristics on Hg concentrations in sharks and other elasmobranchs of the south-western Indian Ocean (coastal, open ocean and bathyal). We used stable isotope ratios of carbon ( $^{13}\text{C}:^{12}\text{C}$ , denoted  $\delta^{13}\text{C}$ ) and nitrogen ( $^{15}\text{N}:^{14}\text{N}$ , denoted  $\delta^{15}\text{N}$ ) to respectively assess the effect of foraging habitat and trophic positions on observed Hg concentrations (Hobson 1999; Michener and Kaufman 2007). Increasing Hg concentrations with body length because of bioaccumulation and with  $\delta^{15}\text{N}$  because of bioamplification are expected. Furthermore, high Hg concentrations are expected in sharks from the deep sea because of the enhanced methylation processes in the deep-sea waters (Blum et al. 2013).

## **2. Material and methods**

### *2.1. Sampling and samples preparation*

White muscle samples were collected from the dorsal region of 14 shark and 2 batoid species in the south-western Indian Ocean from three sampling locations: southwestern Madagascar, La Réunion

Island and the oceanic waters of the southwestern Indian Ocean, from southern Madagascar to the Mascarene archipelago (between 43° 30' E and 70° 00' E and 16°30' S and 28° 30'S). Elasmobranchs sampled in Madagascar were caught between April 2009 and May 2010 by traditional fishers (non-motorised boats) with gillnets and longlines and brought back to five landing sites where local data collectors took elasmobranch muscle samples for stable isotope and Hg analysis. Details on sampling in Madagascar are provided in Kiszka et al. (2014). Elasmobranchs of the oceanic waters of the region were sampled during scientific longline fishing cruises or by observers on commercial longline fishing vessels from January 2009 to November 2010. Details on sampling locations in the open ocean are available in Kiszka et al. (2015). Bathyal sharks and bull sharks (*Carcharhinus leucas*) of La Réunion were caught in November 2011 and between June and July 2013, respectively, using longlines and drumlines. For all individuals, species, sex and basic morphometric measurements, including total and fork lengths were recorded and muscle samples were collected and frozen at – 20°C until further processing. A total of 339 samples were collected. Each species was assigned to a preferred habitat (coastal, open ocean and bathyal) according to ecological characteristics indicated in the literature and the location where it was captured (Table A.1). Most scalloped hammerheads (*Sphyrna lewini*) were juveniles and were caught in the coastal waters of Madagascar (Humber et al. 2017). Therefore, although adult scalloped hammerhead sharks tend to be oceanic, those in this study were considered coastal (Compagno et al. 1984b; Hussey et al. 2011; Kiszka et al. 2014). In the laboratory, samples were freeze-dried and then grounded into a homogenous powder using a porcelain mortar and a pestle previously decontaminated in an acid bath.

## 2.2. Stable isotope analyses

Subsamples of homogenised elasmobranch muscles were repeatedly (2 to 3 treatments) shaken for one hour in 4 ml of cyclohexane in order to remove lipids, which can affect  $\delta^{13}\text{C}$  values, and trimethyl-amine oxide (TMAO), which can affect  $\delta^{15}\text{N}$  values (Hussey et al. 2012). They were then

centrifuged for 5 min at 4000 g, and the supernatant containing lipids was discarded. Lipid-free subsamples (0.35-0.45 mg) were dried and then then weighted in tin cups and analysed with a continuous flow isotope-ratio mass spectrometer (Delta V Advantage, Thermo Scientific) coupled to an elemental analyser (Flash EA 1112, Thermo Scientific). Reference gas was calibrated against International Reference Materials (IAEA-N1, IAEA-N2 and IAEA-N3 for nitrogen; NBS-21, USGS-24 and IAEA-C6 for carbon). Results are expressed in ‰ in the  $\delta$  notation relative to PeeDee Belemnite and atmospheric N<sub>2</sub> for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , according to the equation:  $\delta X = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 10^3$ , where X is <sup>13</sup>C or <sup>15</sup>N and R is the isotope ratio <sup>13</sup>C:<sup>12</sup>C or <sup>15</sup>N:<sup>14</sup>N. Percent C and N elemental composition of tissues were obtained using the elemental analyser and the calculated C:N ratios were always below 3.5, indicating good lipid removal efficiency.

Trophic position (TP) was calculated for elasmobranchs (except the spinetail devil ray *Mobula japanica*) according to Hussey et al. (2014a; 2014b) and the baseline values used in Kiszka et al. (2015):

$$TP = \frac{\log(\delta^{15}\text{N}_{\text{lim}} - \delta^{15}\text{N}_{\text{base}}) - \log(\delta^{15}\text{N}_{\text{lim}} - \delta^{15}\text{N}_{\text{TP}})}{k} + TP_{\text{base}}$$

$\delta^{15}\text{N}_{\text{base}}$  and  $TP_{\text{base}}$  are the known  $\delta^{15}\text{N}$  value and TP of a consumer (9.7 and 3, respectively). Here, the values are based on four individuals of the species *Mobula mobular* (planktivorous) whose mean  $\pm$ SD  $\delta^{15}\text{N}$  values in white muscle were  $9.7 \pm 0.1$  ‰.  $\delta^{15}\text{N}_{\text{TP}}$  is the  $\delta^{15}\text{N}$  value of the consumer of interest.  $\delta^{15}\text{N}_{\text{lim}}$  is the  $\delta^{15}\text{N}$  value at which <sup>15</sup>N incorporation and <sup>15</sup>N elimination are equal (21.9). k is the averaged rate at which the ratio between <sup>15</sup>N incorporation and <sup>15</sup>N elimination changes relative to dietary  $\delta^{15}\text{N}$  averaged across the food web (0.14).



### 2.3. Hg analyses

Total Hg measurements were calculated using a solid sample atomic absorption spectrometer AMA-254 (Advanced Mercury Analyser-254; Altec®), except for the bull shark samples from La Réunion Island. At least two aliquots of 5–15 mg of homogenised dry muscle subsamples for each individual were analysed. The analytical quality (*i.e.* accuracy and reproducibility) of the Hg measurements by the AMA-254 was assessed by the analyses of blanks and certified reference material (CRM) TORT-2 (Lobster Hepatopancreas from the National Research Council of Canada; [www.nrc-cnrc.gc.ca](http://www.nrc-cnrc.gc.ca); certified Hg concentration:  $0.27 \pm 0.06 \mu\text{g}\cdot\text{g}^{-1}$  dw) at the beginning and at the end of the analytical cycle, and by running controls for every 10 samples (Bustamante et al. 2006). Measured values were  $0.26 \pm 0.02 \mu\text{g}\cdot\text{g}^{-1}$  dry weight (dw),  $n = 20$ . Mass of the CRM was adjusted to represent an amount of Hg similar to that in muscle samples. Blanks were analysed at the beginning of each set of samples and the limit of detection was  $0.005 \mu\text{g}\cdot\text{g}^{-1}$  dw. For bull sharks, Hg concentrations were measured from proton-induced X-ray emission (PIXE) analyses at GNS Sciences, New Zealand. Hg concentrations in tissues reported are expressed in  $\mu\text{g}\cdot\text{g}^{-1}$  dw but can be converted to wet weight by using a factor of 5 corresponding to an average 80% moisture (Cresson et al. 2014) to compare our results with previously published ones.

### 2.4. Statistical analysis

Hg concentrations were  $\log(x+1)$ -transformed (indicated as log-transformed in the following) to obtain a normal distribution of the data. As the maximum length differs between shark species and habitat, fork length values were standardised between 0 (juveniles) and 1 (adults) by dividing them by a theoretical maximum fork length for each species. The theoretical maximum fork length  $FL_{\max}$  was estimated by using the following fork length-total length relationship:

$$FL_{\max} = a \times TL_{\max} + b$$

with  $TL_{max}$  being the maximum total length reported for each species as the total length value and  $a$  and  $b$  being species specific constants (Binohlan et al. 2011; Kohler et al. 1996; table A.1). In several species, the standardised length of the largest individuals may be higher than 1 if using the highest total length reported. Consequently, the standardisation was done by dividing fork length values by the maximum fork length value we measured for these species. Furthermore, as both species of spurdog (*Squalus* spp.) were not precisely identified, the standardisation of fork length for these sharks was done by dividing fork length values by a same maximum fork length value we got for both species combined (Table A.1).

Relationships between the continuous variables (log-transformed Hg concentrations, standardised fork length,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values) were preliminary investigated with a principal component analysis (PCA). Because the sampling was concentrated on restricted areas for coastal and bathyal elasmobranchs, latitude and longitude were not included in the PCA. However, because open ocean elasmobranchs have a broad distribution, the relationship between log-transformed Hg concentrations and longitude of sampling was investigated for this group. A general linear model (GLM) followed by type III analysis of covariance (ANCOVA, F) was performed to assess the influence of standardised fork length,  $\delta^{15}\text{N}$  values and the habitat (either coastal/inshore, open ocean and bathyal) on log-transformed Hg concentrations. Second order interactions and non-significant first order interactions were progressively removed from the model and a post-hoc Scheffe analysis was performed on the habitat factor. Species with low sample size ( $n < 5$ ) were not included in the model. For each habitat, the influence of standardised fork length,  $\delta^{15}\text{N}$  values and species on log-transformed Hg concentrations were then assessed with GLM followed by type III ANCOVAs. Sex was not included in the models because no significant effects were observed in a first preliminary ANCOVA from which were removed five unsexed individuals and the pelagic thresher shark *Alopias pelagicus* for which only males were sampled. Pearson correlation coefficients between log-transformed Hg concentrations and standardised fork length were computed for each species, excepting those with  $n < 10$  individuals. The same analysis was done to study the relationship of

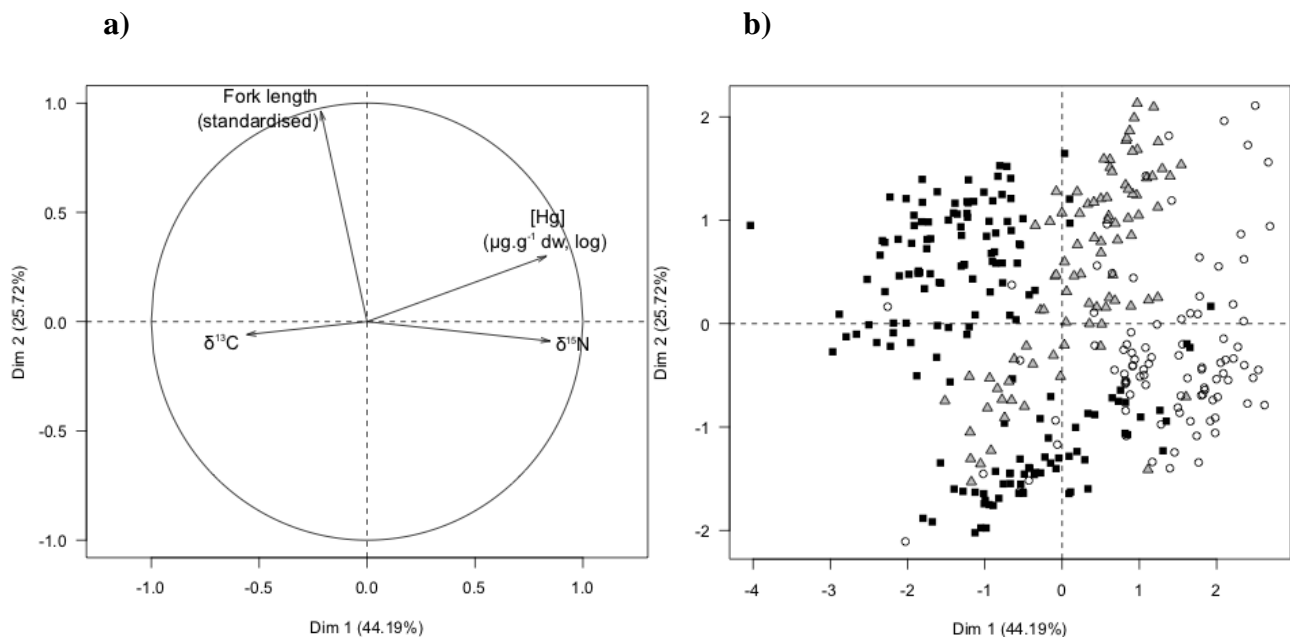
log-transformed Hg concentrations with  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values. All analyses were performed using R 3.3.3 (R development Core Team).

### 3. Results

#### 3.1. Factors influencing Hg concentration differences between habitats

The PCA performed on Hg concentration, standardised size of the individuals,  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values explained almost 70% of the variance of the data set with the two first axes. Results of the PCA showed that Hg concentrations were positively and strongly correlated to  $\delta^{15}\text{N}$  (proxy of trophic level) and negatively correlated to  $\delta^{13}\text{C}$  values (Fig. 1a). This suggested a potential effect of habitat on Hg concentrations, with habitats segregating along the first axis (Fig. 1b and A.1). Oceanic individuals had higher Hg concentrations and higher relative  $\delta^{15}\text{N}$  values. Coastal and bathyal elasmobranchs had lower Hg concentrations and lower  $\delta^{15}\text{N}$  values (Table 1).

No relationship between Hg concentrations and longitude was observed for oceanic sharks ( $r = -0.051$ ,  $P = 0.625$ ; Fig. A.2).



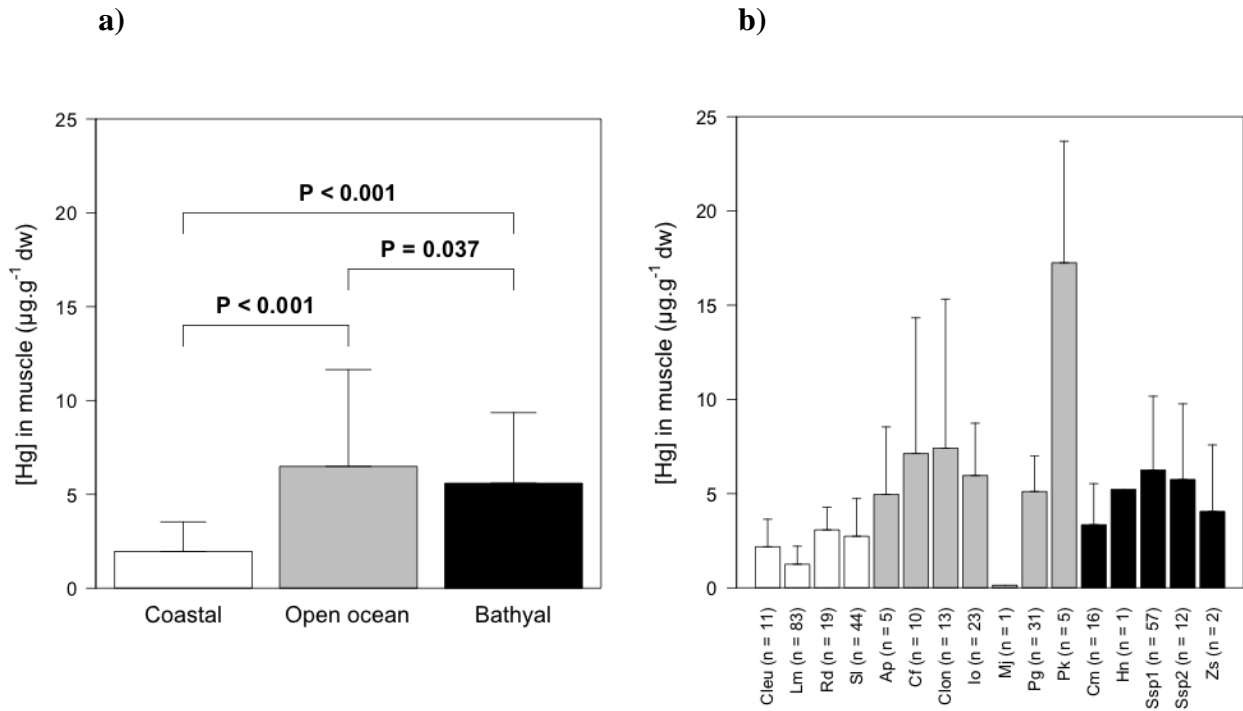
**Fig. 1.** Results of PCA: a) correlations between log-transformed Hg concentrations ( $\mu\text{g.g}^{-1}$  dw), standardised fork length,  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values (‰). b) plot of the individuals resulting from the PCA with symbols indicating the habitat: black squares: coastal, white points: open ocean, grey

triangles: bathyal. For a more detailed plot, see Fig. A.1.

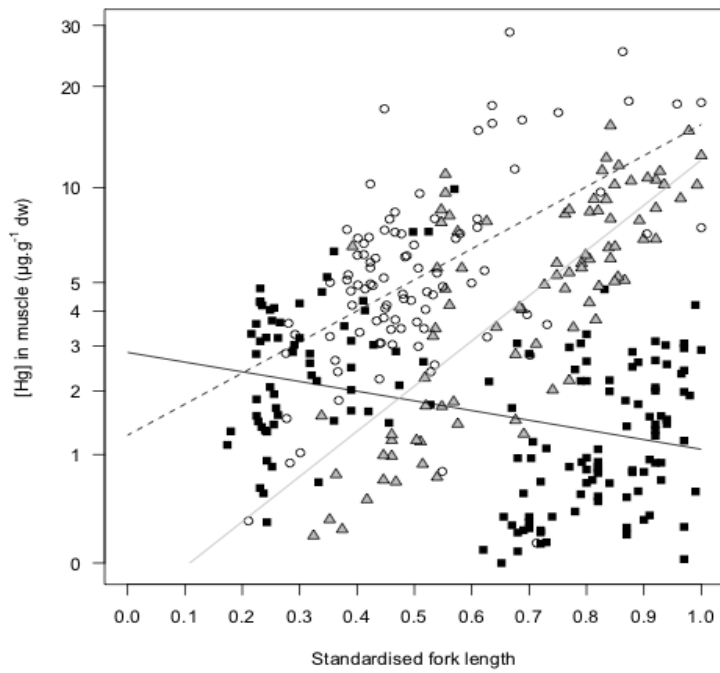
**Table 1.** Length ranges, mean  $\pm$  SD stable isotope values, Hg concentrations and trophic positions of elasmobranchs sampled in south-western Indian Ocean.

	Abbreviation	n	Fork length range (cm)	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)	Hg ( $\mu\text{g}\cdot\text{g}^{-1}$ dw)	TP
<b>Coastal</b>		<b>157</b>	<b>58.0-325.0</b>	<b>12.0 <math>\pm</math> 0.9</b>	<b>-15.5 <math>\pm</math> 1.0</b>	<b>1.95 <math>\pm</math> 1.58</b>	<b>3.68 <math>\pm</math> 0.31</b>
<i>Carcharhinus leucas</i>	Cleu	11	214.0-325.0	12.6 $\pm$ 0.6	-15.5 $\pm$ 0.7	2.18 $\pm$ 1.46	3.86 $\pm$ 0.21
<i>Loxodon macrorhinus</i>	Lm	83	62.0-100.0	11.7 $\pm$ 0.6	-15.4 $\pm$ 1.0	1.25 $\pm$ 0.96	3.55 $\pm$ 0.18
<i>Rhynchobatus djiddensis</i>	Rd	19	65.0-190.0	11.9 $\pm$ 0.4	-14.7 $\pm$ 0.4	3.07 $\pm$ 1.20	3.63 $\pm$ 0.13
<i>Sphyrna lewini</i>	Sl	44	58.0-190.0	12.7 $\pm$ 1.2	-15.9 $\pm$ 1.1	2.74 $\pm$ 2.01	3.90 $\pm$ 0.43
<b>Open ocean</b>		<b>94</b>	<b>73.0-304.0</b>	<b>13.8 <math>\pm</math> 1.2</b>	<b>-16.8 <math>\pm</math> 1.0</b>	<b>6.49 <math>\pm</math> 5.15</b>	<b>4.30 <math>\pm</math> 0.44</b>
<i>Alopias pelagicus</i>	Ap	5	147.0-268.0	13.9 $\pm$ 0.7	-16.7 $\pm$ 0.4	4.97 $\pm$ 3.57	4.32 $\pm$ 0.26
<i>Carcharhinus falciformis</i>	Cf	10	73.0-260.0	12.7 $\pm$ 1.2	-15.0 $\pm$ 1.4	7.13 $\pm$ 7.20	3.91 $\pm$ 0.42
<i>Carcharhinus longimanus</i>	Clon	13	99.0-229.0	13.4 $\pm$ 1.1	-16.5 $\pm$ 0.5	7.41 $\pm$ 7.91	4.14 $\pm$ 0.38
<i>Isurus oxyrinchus</i>	Io	29	122.0-304.0	14.6 $\pm$ 0.7	-16.7 $\pm$ 0.6	5.96 $\pm$ 2.78	4.62 $\pm$ 0.29
<i>Mobula</i> sp.	Mj	1	221	9,7	-17,4	0,14	NA
<i>Prionace glauca</i>	Pg	31	160.0-269.0	13.6 $\pm$ 1.1	-17.5 $\pm$ 0.5	5.11 $\pm$ 1.88	4.21 $\pm$ 0.42
<i>Pseudocarcharias kamoharai</i>	Pk	5	82.0-95.0	14.3 $\pm$ 0.6	-16.6 $\pm$ 0.1	17.25 $\pm$ 6.45	4.49 $\pm$ 0.24
<b>Bathyal</b>		<b>88</b>	<b>26.0-120.0</b>	<b>12.3 <math>\pm</math> 0.6</b>	<b>-16.9 <math>\pm</math> 0.2</b>	<b>5.59 <math>\pm</math> 3.76</b>	<b>3.74 <math>\pm</math> 0.20</b>
<i>Centrophorus moluccensis</i>	Cm	16	29.0-77.5	12.0 $\pm$ 0.5	-16.7 $\pm$ 0.4	3.35 $\pm$ 2.18	3.65 $\pm$ 0.15
<i>Hexanchus nakamurai</i>	Hn	1	120	12,8	-16,1	5,22	3,92
<i>Squalus</i> sp. 1	Ssp 1	57	26.0-69.5	12.3 $\pm$ 0.6	-16.9 $\pm$ 0.1	6.25 $\pm$ 3.92	3.74 $\pm$ 0.17
<i>Squalus</i> sp. 2	Ssp 2	12	31.0-64.0	12.2 $\pm$ 0.6	-16.9 $\pm$ 0.2	5.75 $\pm$ 4.02	3.71 $\pm$ 0.20
<i>Zameus squamulosus</i>	Zs	2	25.7-29.8	14.3 $\pm$ 0.4	-16.8 $\pm$ 0.2	4.06 $\pm$ 3.53	4.46 $\pm$ 0.18

The GLM and subsequent ANCOVA results (Table 2) supported PCA results, with Hg concentrations increasing with  $\delta^{15}\text{N}$  values and varying with habitat. Open ocean elasmobranchs had the highest Hg concentrations, closely followed by bathyal species. In contrast, coastal elasmobranchs had the lowest Hg concentrations recorded (Fig. 2a; Table 1). Influence of standardised fork length, as well as its interaction with the habitat, on Hg concentrations were also observed. The interaction between habitat and fork length showed that an increase of Hg concentrations with standardised size occurred in open ocean and bathyal sharks but that Hg concentrations decreased with standardised size in coastal species (Fig. 3 and A.3). This decrease occurred because most of the elasmobranchs with the highest standardised length were sliteye sharks (*Loxodon macrorhinus*) which also have the lowest Hg concentrations in the coastal habitat (see section 3.2).



**Fig. 2.** Hg concentrations ( $\mu\text{g.g}^{-1}$  dw, mean + SD) in muscle of elasmobranchs by a) preferred habitat and b) by species (Cleu: *Carcharhinus leucas*, Lm: *Loxodon macrorhinus*, Rd: *Rhynchobatus djiddensis*, Sl: *Sphyrna lewini*, Ap: *Alopias pelagicus*, Cf: *Carcharhinus falciformis*, Clon: *Carcharhinus longimanus*, Io: *Isurus oxyrinchus*, Mj: *Mobula japonica*, Pg: *Prionace glauca*, Pk: *Pseudocarcharias kamohrai*, Cm: *Centrophorus moluccensis*, Hn: *Hexanchus nakamurai*, Ssp1: *Squalus* sp. 1, Ssp2: *Squalus* sp. 2, Zs: *Zameus squamulosus*). White: coastal, grey: open ocean, black: bathyal. P values are the results of the post-hoc Scheffe analysis on the habitat factor.



**Fig. 3.** Relationship between the standardised fork length and Hg concentrations ( $\log(x+1)$ -scale,  $\mu\text{g}\cdot\text{g}^{-1}\text{ dw}$ ) in muscle of elasmobranchs with symbols and lines indicating the habitat: black squares and black line: coastal; white points and dashed line: open ocean; grey triangles and grey line: bathyal. For a more detailed plot, see Fig. A.3.

**Table 2.** Effects of standardised fork length,  $\delta^{15}\text{N}$  values, habitat and species of elasmobranch on log-transformed Hg concentrations. Bold results are significant at the  $P = 0.05$  level.

	df	F	P
<b>All</b>			
Standardised fork length	<b>1,328</b>	<b>106.269</b>	<b>&lt; 0.001</b>
$\delta^{15}\text{N}$ (‰)	<b>1,328</b>	<b>71.382</b>	<b>&lt; 0.001</b>
Habitat	<b>2,328</b>	<b>28.243</b>	<b>&lt; 0.001</b>
Standardised fork length : Habitat	<b>2,328</b>	<b>69.645</b>	<b>&lt; 0.001</b>
<b>Coastal</b>			
Standardised fork length	<b>1,145</b>	<b>9.468</b>	<b>0.003</b>
$\delta^{15}\text{N}$ (‰)	1,145	1.406	0.238
Species	<b>3,145</b>	<b>8.057</b>	<b>&lt; 0.001</b>
Standardised fork length : Species	<b>3,145</b>	<b>9.670</b>	<b>&lt; 0.001</b>
$\delta^{15}\text{N}$ (‰) : Species	<b>3,145</b>	<b>5.499</b>	<b>0.001</b>
<b>Open ocean</b>			
Standardised fork length	<b>1,80</b>	<b>67.469</b>	<b>&lt; 0.001</b>
$\delta^{15}\text{N}$ (‰)	1,80	3.216	0.077
Species	<b>5,80</b>	<b>3.034</b>	<b>0.015</b>
$\delta^{15}\text{N}$ (‰) : Species	<b>5,80</b>	<b>3.352</b>	<b>0.008</b>
<b>Bathyal</b>			
Standardised fork length	<b>1,80</b>	<b>34.203</b>	<b>&lt; 0.001</b>
$\delta^{15}\text{N}$ (‰)	<b>1,80</b>	<b>9.920</b>	<b>0.002</b>
Species	2,80	1.703	0.189

### 3.2. Factors influencing Hg concentrations within habitats

Within each habitat, Hg concentrations were influenced by standardised size thanks to the significant correlations between the standardised size and Hg concentrations within all species (tables 2 and 3). Variation in Hg concentrations amongst species occurred in the coastal habitat, with the sliteye shark (*L. macrorhinus*) having lower Hg concentrations ( $1.25 \pm 0.96 \mu\text{g.g}^{-1} \text{ dw}$ ) than the other species, and in the open ocean habitat, with the crocodile shark (*Pseudocarcharias kamoharai*) having much higher Hg concentrations ( $17.25 \pm 6.45 \mu\text{g.g}^{-1} \text{ dw}$ ) than the other species, but not in the bathyal habitat (Fig. 2b; Tables 2 and 3). The interaction between species and standardised size also influenced Hg concentrations in the coastal habitat thanks to the negative correlation between the standardised size and Hg concentrations observed in the giant guitarfish

(*Rhynchobatus djiddensis*). Hg concentrations increased with  $\delta^{15}\text{N}$  values in bathyal sharks only but the interaction between the species and  $\delta^{15}\text{N}$  values influenced Hg concentrations in coastal and open ocean elasmobranchs (Table 2). The significant and positive correlation between  $\delta^{15}\text{N}$  values and Hg concentrations within all bathyal species and the lack of correlation for several coastal and open ocean species (*Carcharhinus leucas*, *Carcharhinus longimanus*, *Isurus oxyrinchus* and *R. djiddensis*; Table 3) may explain this pattern.

Lastly, a significant positive correlation between Hg concentrations and  $\delta^{13}\text{C}$  values appeared only in the pelagic silky sharks (*Carcharhinus falciformis*) and blue sharks (*Prionace glauca*) and in the bathyal *Centrophorus moluccensis* and *Squalus* sp. 1 (Table 3), maybe as the result of ontogenetic change in foraging habitat in these species.

In a few cases, the correlations in Table 3 were not highly significant (or even not significant, if a Bonferroni correction was applied), but this does not modify these conclusions.

**Table 3.** Results of Pearson correlation tests between log-transformed Hg concentrations in elasmobranch muscles and standardised fork length,  $\delta^{15}\text{N}$  values and  $\delta^{13}\text{C}$  values. Bold results are significant at the  $P = 0.05$  level. Italic results are no more significant if applying Bonferroni correction, when the 0.05 P-level is divided by the number of tests (33 tests and  $P = 0.0015$ , Rice 1989).

	Standardised fork length			$\delta^{15}\text{N}$			$\delta^{13}\text{C}$		
	df	r	P	df	r	P	df	r	P
<b>Coastal</b>									
<i>Carcharhinus leucas</i> (n = 11)	<b>9</b>	<b>0.612</b>	<b>0.045</b>	9	0.010	0.976	9	-0.562	0.072
<i>Loxodon macrorhinus</i> (n = 83)	<b>81</b>	<b>0.379</b>	<b>&lt; 0.001</b>	<b>81</b>	<b>0.517</b>	<b>&lt; 0.001</b>	81	0.059	0.598
<i>Rhynchobatus djiddensis</i> (n = 19)	<b>17</b>	<b>-0.758</b>	<b>&lt; 0.001</b>	17	0.313	0.192	17	-0.096	0.696
<i>Sphyrna lewini</i> (n = 44)	<b>42</b>	<b>0.625</b>	<b>&lt; 0.001</b>	<b>42</b>	<b>0.495</b>	<b>&lt; 0.001</b>	42	-0.011	0.941
<b>Open ocean</b>									
<i>Carcharhinus falciformis</i> (n = 10)	<b>8</b>	<b>0.662</b>	<b>0.037</b>	<b>8</b>	<b>0.669</b>	<b>0.034</b>	<b>8</b>	<b>0.692</b>	<b>0.027</b>
<i>Carcharhinus longimanus</i> (n = 13)	<b>11</b>	<b>0.756</b>	<b>0.003</b>	11	0.453	0.120	11	-0.128	0.677
<i>Isurus oxyrinchus</i> (n = 29)	<b>27</b>	<b>0.630</b>	<b>&lt; 0.001</b>	27	0.064	0.742	27	0.072	0.709
<i>Prionace glauca</i> (n = 31)	<b>29</b>	<b>0.675</b>	<b>&lt; 0.001</b>	<b>29</b>	<b>0.638</b>	<b>&lt; 0.001</b>	<b>29</b>	<b>0.697</b>	<b>&lt; 0.001</b>
<b>Bathyal</b>									
<i>Centrophorus moluccensis</i> (n = 16)	<b>14</b>	<b>0.941</b>	<b>&lt; 0.001</b>	<b>14</b>	<b>0.642</b>	<b>0.007</b>	<b>14</b>	<b>0.762</b>	<b>&lt; 0.001</b>
<i>Squalus</i> sp. 1 (n = 57)	<b>55</b>	<b>0.723</b>	<b>&lt; 0.001</b>	<b>55</b>	<b>0.667</b>	<b>&lt; 0.001</b>	<b>55</b>	<b>0.641</b>	<b>&lt; 0.001</b>
<i>Squalus</i> sp. 2 (n = 12)	<b>10</b>	<b>0.808</b>	<b>0.001</b>	<b>10</b>	<b>0.881</b>	<b>&lt; 0.001</b>	10	0.157	0.626



#### 4. Discussion

In this study, the influence of various factors on Hg concentrations in elasmobranchs from Indian Ocean was assessed. Foraging habitat, body length and trophic level appeared to contribute significantly to the bioaccumulation of Hg in elasmobranchs.

With the exception of the crocodile shark (*P. kamoharai*), mean Hg concentrations reported in elasmobranch muscles in this study were lower than those reported for sharks sampled off eastern South Africa (McKinney et al. 2016). By contrast, lower Hg concentrations were reported in western South Africa than in the waters between La Réunion and Madagascar in albacore tuna *Thunnus alalunga* (Chouvelon et al. 2017). Thus, local factors may explain variation in Hg concentrations between regions. For coastal sharks, Hg concentrations reported in bull sharks (*C. leucas*) were two to four times lower than those reported near South Africa (McKinney et al. 2016) and off Florida (Adams and McMichael 1999; Hueter et al. 1995; Rumbold et al. 2014). For open ocean sharks, Hg concentrations reported in mako sharks (*I. oxyrinchus*) were three times lower than those reported near South Africa (McKinney et al. 2016) but higher than those reported in Mexican Pacific waters (Escobar-Sánchez et al. 2011; Maz-Courrau et al. 2012) and in the Atlantic Ocean (Biton Porsmoguer et al. 2018). Similarly, higher Hg concentrations were reported in our study than those in Mexican Pacific waters (Escobar-Sánchez et al. 2011; Maz-Courrau et al. 2012) and in the Atlantic Ocean (Biton Porsmoguer et al. 2018) for blue sharks (*P. glauca*). For bathyal sharks, Hg concentrations reported in spurdogs (*Squalus* spp.) were similar to those reported in Australia (Pethybridge et al. 2010), but lower than those reported in Brazilian waters (de Pinho et al. 2002).

In our study, sharks from bathyal and open ocean habitats appear to have almost similar Hg concentrations while coastal elasmobranchs had the lowest Hg concentrations. Influence of habitat

on Hg concentrations was reported in sharks (Chouvelon et al., 2012), teleosts (Monteiro et al. 1996; Chouvelon et al., 2018; Choy et al. 2009), cephalopods (Bustamante et al. 2006; Chouvelon et al. 2011), crustaceans (Chouvelon et al., 2012) and seabirds (Monteiro and Furness 1996), with higher Hg concentrations reported in organisms with deeper occurrence or foraging.

Differences in Hg concentrations between habitats may be explained by the species composition, size and trophic ecology in each habitat. Indeed, the low Hg levels of coastal elasmobranchs can be explained by the fact that most sampled coastal individuals are small sliteye sharks (*L. macrorhinus*), which are small mesopredatory sharks (Cortés 1999; Jabado et al. 2015), followed by mostly juvenile scalloped hammerhead (*S. lewini*), which are generalist feeders targeting smaller prey than adults (Bush, 2003; Florez-Martínez et al. 2017). Such a diet composition is supported by lower  $\delta^{15}\text{N}$  values in most coastal elasmobranchs. Conversely, the open ocean species we sampled included larger apex predators, which had bioaccumulated Hg to high levels and whose high  $\delta^{15}\text{N}$  values may reflect a diet composed of large and high trophic level prey (Biton Porsmoguer et al. 2015; 2017; Camhi et al. 2008; Kiszka et al. 2015). However, Hg concentrations in bathyal sharks were almost similar to those of oceanic species despite the sampled bathyal shark species being smaller generalist feeders (Braccini et al. 2005; Ebert et al. 1992). Furthermore, the Hg concentrations in bathyal sharks are higher than in coastal species despite their similar trophic ecology and their smaller size. This is consistent with the observation of increasing Hg concentrations with depth in other taxa and in other locations (Chouvelon et al. 2012; Choy et al. 2009; Monteiro et al. 1996) and this indicates that other factors than the trophic ecology of the sampled species explain the differences of Hg concentrations in sharks between habitats. All bathyal species were sampled near La Réunion, a remote island with active volcanism likely to enrich surrounding waters in Hg (Chiffolleau et al. 2011). The geographical origin of these animals might therefore explain this pattern and this is supported by Hg analyses in coastal waters of La Réunion that reveals unexplained high Hg concentrations (Chiffolleau et al. 2011). Yet, coastal bull sharks (*C.*

*leucas*), which were sampled in La Réunion too, did not exhibit high Hg concentrations compared to both oceanic and bathyal species. Furthermore, the absence of relationship between Hg concentration and longitude in elasmobranchs from the open ocean also indicates that geographical origin is not a primary driver of mercury accumulation in elasmobranchs of the Indian Ocean. Consumption of prey in a restricted latitude range and high mobility may explain the absence of link between Hg concentration and geographical origin for oceanic elasmobranchs (Bird et al. 2018). Hg concentrations in bathyal sharks could also be explained by the Hg distribution in the water column. Increasing concentrations of Hg with depth were observed in the water column of various regions (Hammerschmidt and Bowman 2012; Horvat et al. 2003; Kim and Fitzgerald 1988), including in the southwestern Indian Ocean (Sunderland et al. 2011). In particular, a maximum level of highly bioavailable CH<sub>3</sub>Hg may be present in the mesopelagic environment (*ca* 200-1000 m; Hammerschmidt and Bowman 2012; Horvat et al. 2003). This process is due to the higher net CH<sub>3</sub>Hg production below the mixed layer by microorganisms' activity than at the surface where a large portion of methylmercury is photodegraded (Blum et al. 2013). Consequently, considering that increasing levels of CH<sub>3</sub>Hg with depth were observed in southwestern Indian Ocean (Sunderland et al. 2011), that bathyal sharks rely on organic matter produced at the surface which is degraded and methylated while sinking (Bird et al. 2018; Blum et al. 2013) and that most of the Hg present in muscles of sharks is CH<sub>3</sub>Hg (Pethybridge et al. 2010; Storelli et al. 2003), similar Hg levels in open ocean and bathyal elasmobranchs despite differences of trophic positions are likely the result of higher bioavailability of CH<sub>3</sub>Hg in deep-sea waters as highlighted by higher proportions of CH<sub>3</sub>Hg relative to total Hg in deep-sea sharks (Pethybridge et al. 2010). Such a situation could also explain why bull sharks in coastal waters of La Réunion Island showed low Hg concentrations despite surface waters having high Hg concentrations (Chiffolleau et al. 2011), as the photodegradation of CH<sub>3</sub>Hg would make Hg less bioavailable.

Although sample size is limited, *P. kamoharai* appeared to have very high Hg concentrations and

$\delta^{15}\text{N}$  values despite the small body size of this species. The maximum concentration of  $\text{CH}_3\text{Hg}$  in the mesopelagic environment may explain the high Hg levels observed in *P. kamoharai*, which have a mesopelagic distribution (Compagno 1984a; Last and Stevens 1994), and high Hg concentrations previously reported in organisms foraging in the mesopelagic environment (Choy et al. 2009; Kojadinovic et al. 2007; Monteiro et al. 1996). Another hypothesis to explain this finding would be the existence of a longer food chain at smaller body sizes in the mesopelagic environment, as indicated by the high  $\delta^{15}\text{N}$  values and estimated trophic position for this species and the higher rates of carnivory for mesopelagic zooplankton (Hannides et al. 2013). A third hypothesis would be that the high  $\delta^{15}\text{N}$  values in *P. kamoharai* would be the result of higher  $\delta^{15}\text{N}$  values in lower trophic positions and baseline sources of the mesopelagic food web where this species forages, as highlighted by increasing  $\delta^{15}\text{N}$  values in suspended particles and zooplankton along depth (Hannides et al. 2013).

Hg concentrations consistently increased with fork length at the scales of the whole sampling area, of the habitats and of the species. This pattern has previously been documented in several species of sharks in different marine environments (Cresson et al. 2014; Hueter et al. 1995; Pethybridge et al. 2010; 2012; Rumbold et al. 2014). Body length may be used as a proxy of age in elasmobranchs (e.g. Cotton et al. 2011; Natanson et al. 2006; Pajuelo et al. 2011; Piercy et al. 2007; Skomal and Natanson 2003). Consequently, we may consider that increasing Hg concentrations with length results from ageing: because Hg is more efficiently assimilated than eliminated, it accumulates in the body with age. However, age alone may not explain this phenomenon, because the trophic level of organisms tends to increase with body length (Chouvelon et al. 2014; Hussey et al. 2011). Our results actually indicate that Hg concentrations and  $\delta^{15}\text{N}$  values, and thus trophic level, are generally linked at the scale of the whole sampling area and within species. However, the link between Hg concentrations and  $\delta^{15}\text{N}$  values appeared only in the bathyal habitat because there are still some species for which no relationship between Hg concentrations and  $\delta^{15}\text{N}$  values occurred in the two other habitats. Nevertheless, large-scale variations of trophic level may explain regional

differences of Hg concentrations. Indeed, higher Hg concentrations in South Africans *C. leucas* and *S. lewini* may be linked to higher trophic levels for these species in this region than in our study area (McKinney et al. 2016). In contrast, and in accordance with previous studies (e.g. Domi et al. 2005; Pethybridge et al. 2012; Rumbold et al. 2014), positive correlation between Hg concentrations and  $\delta^{13}\text{C}$  values are rarely found. This lack of relationship may suggest that all the sampled individuals in each species forage inside a single preferred habitat with no ontogenetic changes of feeding habitat or individual foraging specialisation or segregation (e.g. size or sexual segregation). By comparison, a relationship between Hg concentrations and  $\delta^{13}\text{C}$  values were observed in the wandering albatross *Diodema exulans*. This relationship results from the foraging segregation between males preying mainly in Antarctic waters and females preying in subtropical areas (Carravieri et al. 2014).

In conclusion, Hg concentrations in elasmobranchs living in three different types of habitat of the southwestern Indian Ocean appear to be linked to three factors: 1) trophic position, as shown by the relationship between Hg concentration and  $\delta^{15}\text{N}$  values at the whole sample level, and by top predator sharks from the open ocean usually having higher Hg levels than the mesopredatory coastal elasmobranchs, 2) habitat, as shown by mesopredatory bathyal species having almost similar Hg concentrations as the top predator species of the open ocean despite their lower trophic position, 3) body size, as Hg concentrations increased with body length.

Many shark species are currently threatened by human activities (Clarke et al. 2006; Dulvy et al. 2014) and the Hg accumulation in their bodies could have additional detrimental effects on the dynamics of their populations (Depew et al. 2012; Sandheinrich and Wiener 2011). The results of the present study suggest that bathyal and oceanic sharks should be considered more carefully for human consumption. It is now well-established that the toxicity of Hg in teleost fish decreased when selenium (Se) concentrations are elevated (Kaneko and Ralston 2007; Ralston et al. 2008;

Ralston and Raymond, 2010). However, the role of Se in commercial sharks could be minor, because its concentrations are usually not linked with Hg concentration, nor exceed it, particularly in oceanic blue and mako sharks (Domi et al. 2005; Escobar-Sánchez et al. 2011; Kaneko and Ralston 2007; Torres et al. 2017).

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