Plasticity of trophic interactions among sharks from the oceanic south-western Indian Ocean revealed by stable isotope and mercury analyses

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Sharks are a major component of the top predator guild in oceanic ecosystems, but the trophic relationships of many populations remain poorly understood. We examined chemical tracers of diet and habitat (δ15N and δ13C, respectively) and total mercury (Hg) concentrations in muscle tissue of seven pelagic sharks: blue shark (Prionace glauca), short-fin mako shark (Isurus oxyrinchus), oceanic whitetip shark (Carcharinus longimanus), scalloped hammerhead shark (Sphyra lewini), pelagic thresher shark (Alopias pelagicus), crocodile shark (Pseudocarcharias kamoharai) and silky shark (Carcharhinus falciformis), from the data poor south-western tropical Indian Ocean. Minimal interspecific variation in mean δ13C values and a large degree of isotopic niche overlap – driven by high intraspecific variation in δ15N values – was observed among pelagic sharks. Similarly, δ13C values of sharks overlapped considerably for all species with the exception of P. glauca, which had more 13C-depleted values indicating possibly longer residence times in purely pelagic waters. Geographic variation in δ13C, δ15N and Hg were observed for P. glauca and I. oxyrinchus. Mean Hg levels were similar among species with the exception of P. kamoharai which had significantly higher Hg concentrations likely related to mesopelagic feeding. Hg concentrations increased with body size in I. oxyrinchus, P. glauca and C. longimanus. Values of δ15N and δ13C varied with size only in P. glauca, suggesting ontogenetic shifts in diets or habitats. Together, isotopic data indicate that – with few exceptions – variance within species in trophic interactions or foraging habitats is greater than differentiation among pelagic sharks in the south-western Indian Ocean. Therefore, it is possible that this group exhibits some level of trophic redundancy, but further studies of diets and fine-scale habitat use are needed to fully test this hypothesis.

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1. Introduction

Sharks are a major component of the top predator guild in open-ocean ecosystems along with tunas, billfish, and cetaceans. These “pelagic sharks” occur from cold temperate to tropical waters, from the surface to 1000 m depth or more (Compagno, 2008) and include some of the most wide-ranging marine predator species (Pitkitch et al., 2008). Their extensive movements and ocean-basin scale migrations are most likely related to oligotrophy and the patchy nature of food resources in open-ocean ecosystems as well as directed movements for social and reproductive purposes. Many pelagic sharks use coastal/continental shelf waters in addition to open oceanic waters throughout ontogeny although certain biological functions, for example, gestation may be restricted to the open ocean (Compagno, 1984; Bonfil, 2008; Nakano and Stevens, 2008). Even though pelagic sharks commonly occur in an environment far from human populations, they commonly overlap and interact with offshore fisheries. Currently, three-quarters of pelagic elasmobranchs are classified as Threatened or near Threatened (IUCN Red List Status), and 11 species are globally threatened with a high risk of extinction (Dulvy et al., 2008). Despite clear evidence for shark population declines, including in oceanic ecosystems (Baum et al., 2003; Myers and Worm, 2003; Ferretti et al., 2010), relatively little is known on the feeding ecology of many species and the ecological importance of this guild is poorly understood (Ferretti et al., 2010; Heithaus et al., 2010; Kitchell et al., 2002).

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Sharks can play important roles in marine ecosystems through diverse mechanisms, but their relative importance may vary significantly among ecosystems, species and contexts (Heithaus et al., 2008, 2010). While the decline of large predatory sharks in some coastal ecosystems may initiate trophic cascades and affect overall community structure (see Heithaus et al., 2008; Ferretti et al., 2010 for reviews), it is still unclear whether the removal of pelagic sharks affects community structure in open-ocean ecosystems (Stillwell and Kohler, 1982; Ward and Myers, 2005). Evidence from ecosystem models from the central Pacific Ocean suggest pelagic shark declines may be mitigated by compensatory increases in populations of large teleosts (Kitchell et al., 2002). However, these models generally categorize pelagic sharks into a single homogenous trophic group when in fact there may be interspecific variation in habitat use and trophic interactions resulting in greater trophic complexity and less trophic redundancy than assumed for pelagic ecosystems. More ecological data on the relative trophic position and trophic interactions of oceanic sharks is required to test such model assumptions. If species are functionally redundant (i.e. they occupy similar trophic niches, hence consume the same prey base and perform the same functional role; Walker, 1992), the loss of one or several may not result in large scale community rearrangements because of compensation by other species (e.g. Kitchell et al., 2002), but rather density compensation effects among the remaining species (Walker, 1992). Recent studies suggest that sympatric shark species show considerable variation in trophic interactions (Hussey et al., 2011; Kinney et al., 2011; Speed et al., 2011). However, patterns of trophic redundancy within a given community are likely to be biologically and environmentally context-dependent (e.g. ocean productivity, intra-guild species richness), and are expected to vary accordingly.

In the tropical Indian Ocean (between 10°N and 10°S), the most commonly caught pelagic sharks are blue (*Prionace glauca*), silky (*Carcharhinus falciformis*), pelagic thresher (*Alopias pelagicus*) and short-fin mako sharks (*Isurus oxyrinchus*). Between 10°S and 25°S, sharks are less abundant and the community is dominated by *P. glauca* and *I. oxyrinchus* (Huang and Liu, 2010). Despite evidence for high bycatch levels of pelagic sharks in the Indian Ocean (Romanov, 2002; Huang and Liu, 2010), there is a paucity of reliable data to facilitate assessing historical changes in shark catch rate trends (Smale, 2008). Similarly, knowledge on the trophic ecology of pelagic sharks is limited in the Indian Ocean (and globally), particularly at the community level (Rabehagasoa et al., 2012).

The use of naturally occurring carbon and nitrogen stable isotopes provide chemical tracers to examine the ecology of organisms in a given ecosystem. Carbon and nitrogen stable isotopic ratios (δ¹³C and δ¹⁵N) can depict the food webs in which consumers are foraging and their relative trophic position, respectively (Hobson, 1999). Stable isotope analyses may provide complementary or, in some cases, an alternative method to traditional diet (e.g. based on analyses of stomach contents) and costly habitat and movement methodologies (e.g. telemetry). They have already been successfully used to understand elasmobranch ecology, including community trophic interactions and ontogenetic variation in trophic position and foraging habitats (Hussey et al., 2011; Kinney et al., 2011; Vaudo and Heithaus, 2011).

Mercury (Hg) is a non-essential metal that is released from natural (e.g. volcanism) and anthropogenic sources (e.g. discharge by hydroelectric industries, mining), reaching the open ocean through runoff, riverine input and atmospheric deposition (Fitzgerald et al., 2007). Mercury provides an indicator of foraging habitats and trophic position of large marine predators because body burden concentrations are highly correlated to size/age, environmental

![Fig. 1. Distribution of sampled oceanic sharks in the south-western Indian Ocean (n=92).](image-url)
parameters and geographic location (Rivers et al., 1972; Power et al., 2002; Colaço et al., 2006; Cai et al., 2007). Total Hg levels in pelagic fishes increase with median depth of occurrence in the water column and mesopelagic habitats are probably major entry points of mercury into marine food webs as a result of increased methylation at these depths (Monteiro et al., 1996; Choy et al., 2009; Chouvelon et al., 2012).

The aim of this work was to investigate the trophic interactions and potential overlap in foraging habitats of pelagic sharks from the south-western Indian Ocean using three complementary chemical tracers: δ15N and δ13C and Hg. Because tropical oceanic ecosystems are characterized by low productivity, we hypothesized that trophic plasticity would drive isotopic niche overlap among pelagic shark species, similarly to tropical seabirds and epipelagic tunas from the same region (Catry et al., 2008; Cherel et al., 2008; Koajdinovic et al., 2008). As a consequence of diet overlap, we hypothesized that Hg levels would be comparable among species reflecting a similar rate of Hg bioaccumulation. In addition, we also hypothesized that that size and δ15N values would be positively correlated with Hg concentrations due to its bioamplification within marine food webs.

2. Materials and methods

2.1. Sampling

Shark muscle samples were collected in the south-western Indian Ocean, from southern Madagascar to the Mascarene Islands (Fig. 1). Sharks were sampled during scientific longline fishing cruises or by observers on commercial longline fishing vessels from January 2009 to November 2010. The ecological characteristics of the pelagic shark species sampled are summarized in Table 1. For each individual shark caught, white muscle tissue was sampled from the base of the dorsal fin and immediately stored frozen. Once at the laboratory, the samples were freeze-dried, ground into a homogenous powder using a porcelain mortar and pestle, and then analyzed for stable isotopes (Fig. 1). Sharks were sampled during scientific longline fishing cruises or by observers on commercial longline fishing vessels from January 2009 to November 2010. The ecological characteristics of the pelagic shark species sampled are summarized in Table 1. For each individual shark caught, white muscle tissue was sampled from the base of the dorsal fin and immediately stored frozen. Once at the laboratory, the samples were freeze-dried, ground into a homogenous powder using a porcelain mortar and pestle, and then analyzed for stable isotopes (δ15N and δ13C) and Hg. Because tropical oceanic ecosystems are characterized by low productivity, we hypothesized that trophic plasticity would drive isotopic niche overlap among pelagic shark species, similarly to tropical seabirds and epipelagic tunas from the same region (Catry et al., 2008; Cherel et al., 2008; Koajdinovic et al., 2008). As a consequence of diet overlap, we hypothesized that Hg levels would be comparable among species reflecting a similar rate of Hg bioaccumulation. In addition, we also hypothesized that that size and δ15N values would be positively correlated with Hg concentrations due to its bioamplification within marine food webs.

2.2. Stable isotope analyses

Because lipids are highly depleted in 13C relative to other tissue components (De Niro and Epstein, 1977) and urea and trimethylamine oxide (TMAO) present in shark muscle tissue can alter δ15N values, samples were lipid and urea/TMAO-extracted by two successive extractions prior to stable isotope analysis (1 h shaking in 4 ml cyclohexane at room temperature and subsequent centrifugation). This standardized data among individuals and across species within the food web (Hussey et al., 2012a, 2012b). After drying, lipid-free sub-samples (0.35–0.45 mg ± 0.01 mg) were weighed in tin cups for stable isotope analysis. Stable isotope measurements were performed with a continuous-flow isotope-ratio mass spectrometer (Delta V Advantage, Thermo Scientific, Germany) coupled to an elemental analyzer (Flash EA1112 Thermo Scientific, Italy). 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 the δ notation relative to PeeDee Belemnite and atmospheric N2 for δ13C and δ15N, respectively, according to the following equation:

\[
\delta X = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 10^3
\]

where X is 13C or 15N and R is the isotope ratio 13C/12C or 15N/14N, respectively. Replicate measurements of a laboratory standard (acetanilide) indicated that analytical errors were < 0.1% for δ13C and δ15N. Percent C and N elemental composition of tissues were obtained using the elemental analyzer and the calculated C:N ratios were always below 3.5, indicating good lipid removal efficiency (Table 2).

2.3. Hg analyses

Total Hg measurements were performed using a solid sample atomic absorption spectrometer AMA-254 (Advanced Mercury Analyser-254 from Altec©). At least two aliquots of 5–15 mg of homogenized dry muscle tissue subsamples for each individual were analyzed. The analytical quality (i.e. accuracy and reproducibility) of the Hg measurements by the AMA-254 was assessed by the analyses of blanks and TORT-2 Certified Reference Material (Lobster Hepatopancreas Reference Material from the National Research Council of Canada) at the beginning and at the end of the analytical cycle, and by running it every 10 samples. Results of quality controls showed a satisfactory precision with a relative standard deviation of 6.0%. The detection limit was 0.005 μg g⁻¹ dry weight (dwt). All Hg concentrations in tissues reported below are expressed in μg g⁻¹ dwt.

2.4. Trophic position (TP15N) calculation

Trophic position (TP) was calculated using a scaled Δ15N framework approach based on a dietary δ15N value-dependent

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Table 1

<table>
<thead>
<tr>
<th>Species</th>
<th>Habitat</th>
<th>Depth range (m)</th>
<th>Diet</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alopias pelagicus</td>
<td>Epipelagic, oceanic</td>
<td>0–152</td>
<td>Small- medium sized fish and squids</td>
<td>Compagno (1984), Smith et al. (2008)</td>
</tr>
<tr>
<td>Carcharhinus falciformis</td>
<td>Epipelagic, oceanic</td>
<td>0–500</td>
<td>Large squids, pelagic fishes, pelagic crabs</td>
<td>Compagno (1984), Bondil (2008), Cabrera-Chavez et al. (2010)</td>
</tr>
<tr>
<td>Carcharhinus longimanus</td>
<td>Epipelagic, oceanic</td>
<td>0–152</td>
<td>Large epipelagic teleosts and squids</td>
<td>Compagno (1984), Bondil et al. (2008)</td>
</tr>
<tr>
<td>Prionace glauca</td>
<td>Epi- to mesopelagic, oceanic</td>
<td>0–600</td>
<td>Small pelagic fishes, squids and elasmobranchs</td>
<td>Compagno (1984), Nakano and Stevens (2008)</td>
</tr>
<tr>
<td>Isurus oxyrinchus</td>
<td>Epi- to mesopelagic, oceanic</td>
<td>0–600</td>
<td>Pelagic fishes, squids and elasmobranches</td>
<td>Stevens (2008)</td>
</tr>
<tr>
<td>Pseudocarcharias kamohara</td>
<td>Mesopelagic</td>
<td>0–600</td>
<td>Deep-water fishes, squids and shrimps</td>
<td>Compagno (1984), Stevens (2010)</td>
</tr>
<tr>
<td>Sphyrna lewini</td>
<td>Coastal and semi- oceanic</td>
<td>0–450</td>
<td>Pelagic to demersal fishes, squids and elasmobranches</td>
<td>Compagno (1984), Kimley (1993), Hussey et al. (2011)</td>
</tr>
</tbody>
</table>

* Minimal depth range.
* Major prey types.
Table 2
Characteristics of pelagic sharks sampled in the south-western Indian Ocean. Values are mean (± SD). TP_{15N}—trophic position based on Hussey et al. (2014a, 2014b).

<table>
<thead>
<tr>
<th>Species</th>
<th>n</th>
<th>Fork Length (cm)</th>
<th>Sex ratio (m:f)</th>
<th>Site (°S)</th>
<th>δ15N (% C) Mean ± SD</th>
<th>δ15C (% C) Mean ± SD</th>
<th>C:N Mean ± SD</th>
<th>Hg (μg g⁻¹ dw) Mean ± SD</th>
<th>TP_{15N} Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alopis pelagicus</td>
<td>5</td>
<td>200</td>
<td>1:0</td>
<td>30–40</td>
<td>−13.91 ± 0.69</td>
<td>−16.71 ± 0.43</td>
<td>2.9 ± 0.2</td>
<td>4.97 ± 3.60</td>
<td>4.4 ± 0.3 (3.9–4.7)</td>
</tr>
<tr>
<td>Carcharhinus falciformis</td>
<td>3</td>
<td>114</td>
<td>96–148</td>
<td>30–40</td>
<td>−12.35 ± 0.36</td>
<td>−16.53 ± 0.35</td>
<td>2.9 ± 0.1</td>
<td>2.43 ± 2.15</td>
<td>3.8 ± 0.1 (3.7–3.9)</td>
</tr>
<tr>
<td>Carcharhinus longimanus</td>
<td>13</td>
<td>161</td>
<td>99–229</td>
<td>30–40</td>
<td>−13.39 ± 0.03</td>
<td>−16.46 ± 0.44</td>
<td>2.9 ± 0.1</td>
<td>7.14 ± 7.44</td>
<td>4.2 ± 0.4 (3.5–4.7)</td>
</tr>
<tr>
<td>Prionace glauca</td>
<td>31</td>
<td>202</td>
<td>160–269</td>
<td>30–40</td>
<td>−13.57 ± 1.10</td>
<td>−17.47 ± 0.49</td>
<td>2.8 ± 0.2</td>
<td>5.30 ± 2.17</td>
<td>4.2 ± 0.4 (3.6–5.0)</td>
</tr>
<tr>
<td>Isurus oxyrinchus</td>
<td>29</td>
<td>185</td>
<td>122–204</td>
<td>30–40</td>
<td>14.62 ± 0.08</td>
<td>−16.74 ± 0.58</td>
<td>2.9 ± 0.1</td>
<td>5.96 ± 2.78</td>
<td>4.7 ± 0.3 (3.9–5.3)</td>
</tr>
<tr>
<td>Pseudocarcharias kamoharai</td>
<td>5</td>
<td>87</td>
<td>82–95</td>
<td>30–40</td>
<td>14.35 ± 0.57</td>
<td>−16.58 ± 0.57</td>
<td>2.9 ± 0.1</td>
<td>17.25 ± 6.45</td>
<td>4.5 ± 0.2 (4.3–4.8)</td>
</tr>
<tr>
<td>Sphyra lewini</td>
<td>6</td>
<td>152</td>
<td>100–190</td>
<td>30–40</td>
<td>−13.88 ± 1.01</td>
<td>−16.12 ± 0.09</td>
<td>3.0 ± 0.1</td>
<td>5.86 ± 2.73</td>
<td>4.4 ± 0.4 (3.9–5.0)</td>
</tr>
</tbody>
</table>

Δ15N model (Hussey et al., 2014a, 2014b). In brief, with knowledge of the δ15N value of a known baseline consumer (δ15NB), the δ15N value of the consumer of interest (δ15N_{i}), the dietary δ15N value at which 15N incorporation and δ15N elimination are equal (δ15Nlim), and the rate at which the ratio between 15N incorporation and δ15N elimination changes relative to dietary δ15N averaged across the food-web (k), TP is calculated as follows:

$$TP = \frac{\log (δ_{15N_{lim}} - δ_{15NB}) - \log (δ_{15N_{lim}} - δ_{15N_{i}})}{k} + TP_{base}$$

Mobula japonica, a zooplanktivore (Last and Stevens, 2009), was used as the baseline consumer (TP=3). Stable isotope values in white muscle samples from four individuals caught in offshore waters off southern Madagascar, during the sampling period, were (mean δ15N ± SD) 9.7 ± 0.1, similar to those reported for Mobula spp. off the east coast of South Africa (9.9 ± 0.5; Hussey et al., 2011). A value of k=0.14 and δ15N_{lim}=21.9 were used following the meta-analysis of experimental isotopic studies conducted by Hussey et al. (2014a, 2014b).

2.5. Data analysis

We used Shapiro–Wilk tests to assess the assumptions of parametric tests. Hg data were log-transformed to reduce skewness and fit parametric requirements. ANOVA were used to test the influence of size (fork length), latitude, longitude, sex and species on δ13C and δ15N values and on log-transformed Hg concentrations. Post-hoc Tukey HSD (Honesty Significance Difference) tests were used to investigate paired differences for significant factors. To investigate isotopic niche space and isotopic niche overlap among species we employed a Bayesian approach based on multivariate ellipse metrics (SIBER – Stable Isotopes Bayesian Ellipses within the R-package siar; Jackson et al., 2011). Standard ellipses are bivariate data calculated from the variance and covariance of the data matrix (x, y parameters) and represent the core isotopic niche for a species. This analysis generates standard ellipse areas corrected for small sample sizes (SEAc) which can be compared among species to determine differences in core isotopic niche space and core niche overlap (Jackson et al., 2011). The calculation of standard ellipses (SEAc) was undertaken for δ13C vs. δ15N data, but also for combinations of δ13C vs. Hg and δ15N vs. Hg data to provide complementary tracer information. The degree of niche overlap among species for each combination of the above data parameters, i.e. the overlap in species’ standard ellipse (SEAc) areas was then calculated using the OVERLAP command. Pearson’s correlations were used to examine δ13C and δ15N values vs. log Hg concentrations, as well as the relationship between δ13C and δ15N values. Statistical analyses were performed using R 2.12.0 (R Development Core Team, 2010).

3. Results

3.1. Inter-species variations

Values of δ15N and δ13C and Hg concentrations varied significantly among species (one-way ANOVA, F_{6, 85} = 5.94; p < 0.001; F_{6, 85} = 11.23, p < 0.001; F_{6, 85} = 5.41, p < 0.001, respectively; Figs. 2 and 3, Table 2). There were, however, relatively few significant pairwise comparisons (Table 3). For δ15N, I. oxyrinchus had significantly higher values than C. falciformis (p < 0.01), C. longimanus (p < 0.01) and P. glauca (p < 0.001). For δ13C, the only significant pairwise comparison was between P. glauca and all species except C. falciformis. For log Hg concentrations, pairwise comparisons between P. kamoharai and the other species were all significant (Table 3). Also, log Hg concentrations were significantly higher in L. oxyrinchus than in C. falciformis (p < 0.01, Table 3).

Standard δ15N–δ13C ellipses (SEAc) identified considerable niche overlap among species, with few exceptions (Fig. 4, Table 4). For P. glauca, no ellipse overlap occurred with C. longimanus, P. kamoharai and S. lewini and there was very limited overlap with A. pelagicus (Fig. 4). S. lewini showed a high degree of niche overlap with C. longimanus and P. kamoharai. Isotopic niche space represented by ellipse size was the largest for S. lewini (27.3), similar for L. oxyrinchus, P. glauca, C. longimanus and A. pelagicus (10.2, 1.5 and 1.2, respectively) and smallest for P. kamoharai (0.3, Fig. 4; Table 4). When considering the δ13C vs. Hg and δ15N vs. Hg standard ellipses (SEAc; Figs. 5 and 6), P. kamoharai showed significant niche space separation from all other species with the exception of C. longimanus (Tables 6 and 7). Most species showed considerable niche overlap in terms of Hg vs. δ13C and Hg vs. δ15N space (Figs. 5 and 6; Tables 5 and 6). For both δ13C vs. Hg and δ15N vs. Hg, C. longimanus had the largest ellipse area (12.3 and 27.4, respectively), while P. glauca had the smallest (2.3 and 5.3, respectively; Figs. 5 and 6; Tables 5 and 6).

Estimated TPs of all pelagic shark species based on δ15N values (TP_{15N}) spanned 1.8 trophic positions ranging from TP 3.5 for a 99 cm FL C. longimanus to TP 5.3 for a 173 cm FL I. oxyrinchus (Table 2). Overall I. oxyrinchus fed at the highest mean TP (4.7 SD ± 0.3) and C. falciformis at the lowest mean TP (3.8 ± 0.1), with all other species feeding at a similar level (mean TP ~4.3). High intraspecific variation in TP_{15N} was observed in most species estimated to be feeding over one trophic level.

3.2. Geographic, size and sex-related variation

Values of δ13C, δ15N and Hg, increased with increasing longitude for I. oxyrinchus (F_{1, 90} = 6.41, p < 0.05). For P. glauca, there were significant increasing log Hg concentrations when latitude increased (F_{1, 90} = 13.93, p < 0.001) and δ15N values decreased with increasing latitude (F_{1, 90} = 10.31, p < 0.01). A significant positive effect of longitude on δ15N values was also detected in P. glauca (F_{1, 90} = 8.12, p < 0.01). When considering all species combined, there
was a significant positive effect of size on log Hg concentrations (one-way ANOVA, $F_{1,90} = 7.03, p < 0.01$) and on $\delta^{13}C$ values ($F_{1,90} = 6.48, p < 0.05$), but not for $\delta^{15}N$ ($F_{1,90} = 3.70, p > 0.05$).

At the species level, the effect of size on log Hg concentrations was significant for *I. oxyrinchus* ($F_{1,27} = 16.1, p < 0.001$, Fig. 7a), *P. glauca* ($F_{2,9} = 23.7, p < 0.001$, Fig. 7b) and *C. longimanus* ($F_{1,11} = 15.9, p < 0.01$, Fig. 7c). There was a positive relationship between size and both $\delta^{15}N$ and $\delta^{13}C$ in *P. glauca* ($F_{1,11} = 11.3, p < 0.01$ and $F_{1,11} = 25.1, p < 0.001$, respectively, Fig. 7b), but not for any other species examined. There was no detectable variation in $\delta^{15}N$ and $\delta^{13}C$ values and Hg concentrations between sexes (one-way ANOVA for log Hg, $\delta^{13}C$ and $\delta^{15}N$; all $p > 0.05$).

### 3.3. Relationships between $\delta^{15}N$, $\delta^{13}C$ and Hg concentrations

For all data combined, there was a significant correlation between log Hg concentrations and $\delta^{15}N$ values (Pearson: $r = 0.38$, df = 90, $p < 0.001$) but not for $\delta^{13}C$ ($r = 0.12$, df = 90, $p > 0.05$, Table 7). At the species level, positive correlations between log Hg and $\delta^{15}N$, and log Hg and $\delta^{13}C$ were only significant for *P. glauca* ($r = 0.64$, df = 29, $p < 0.001$ and $r = 0.71$, $p < 0.001$, respectively). There was a significant positive correlation between $\delta^{13}C$ and $\delta^{15}N$ for *C. falciformis* ($r = -0.99, p < 0.05$), *P. glauca* ($r = 0.71$, $p < 0.001$) and *I. oxyrinchus* ($r = 0.67$, df = 27, $p < 0.001$; Table 7).

### 4. Discussion

Understanding interspecific variation in trophic interactions and foraging base of pelagic sharks based on size, sex and geographic location is important for elucidating the role of this predator guild in pelagic ecosystems. Traditionally considered to be a trophically redundant group, our data suggest the potential for high levels of intraspecific variation in trophic interactions or foraging locations that obscure differences among species within the guild as a whole. These data identify species trophic plasticity as would be predicted in the oligotrophic pelagic realm of the western Indian Ocean. In addition, this study extends our understanding of ontogenetic shifts (studied for *P. glauca* in the Mozambique Channel and off eastern Madagascar; Rabehagasoa et al., 2012), and provides the first Hg concentrations for this data poor region. However, considering the limited sample size for a number of species, results should be interpreted cautiously for these taxa.

#### 4.1. Patterns of isotopic niche partitioning

Intraspecific variation in estimated trophic positions was considerable, with most species feeding over more than a single trophic level. However, as hypothesized and assumed by models of pelagic ecosystems, interspecific variation in average trophic positions was limited among species in our study area. The notable exception to this pattern was *I. oxyrinchus*, which exhibited significantly higher $\delta^{15}N$ values, likely because of their propensity to forage on large predatory fish, including small elasmobranchs and marine mammals (Cliff et al., 1990). Our mean species TP results provided similar estimates to those based on stomach contents by Cortes (1999), but overall the TP range was slightly higher, likely a result of using the more accurate scaled $\Lambda^{15}N$ framework (Hussey et al., 2014a, 2014b). Nonetheless, our results suggest that pelagic sharks from the study region generally occupy similar trophic positions, and that intraspecific variation is much greater than interspecific variation. Surprisingly, calculated TP$^{15}N$ for *P. kamoharai* was relatively high (mean value = 4.5) given the small body size of this species ( < 100 cm). Stomach contents of *P. kamoharai* suggest a diet of small mesopelagic teleosts, cephalopods and shrimps (Compagno, 1984). The high $\delta^{15}N$ values and estimated trophic position for this species may be a result of a higher nitrogen baseline value in the deep-sea food web where this species forages. This would bias the comparison of $\delta^{15}N$ values and estimated TP among species, given the other species feed predominantly in the epipelagic zone. Alternatively, this could suggest the existence of a longer food chain at smaller body sizes in these deep-water environments. Further analyses are required in the future to test these hypotheses.

Most species had similar $\delta^{13}C$ values suggesting a high degree of overlap in habitat use. The principal exception was *P. glauca* which was more depleted in $^{13}C$ compared to the other species. There is a limited diversity of carbon sources in open-ocean systems, particularly in oligotrophic environments, and detrital carbon sources are likely to be the most available. $\delta^{13}C$ values commonly decrease from productive continental shelf waters to pelagic systems ( Cherel et al., 2008; Kiszka et al., 2011; Chouvelon et al., 2012). The $\delta^{13}C$ values of most shark species were intermediate to these environments, indicating the use of both pelagic and coastal waters (Hussey et al., 2011). Lower $\delta^{13}C$ values of *P. glauca* indicate this species spends longer periods of time in pelagic vs. coastal waters and/or forages in more...
13C-depleted deeper waters. This is supported by satellite tracking data of *P. glauca* in the North Atlantic (Campana et al., 2011).

Overall, our results confirm high core isotopic niche overlap among pelagic sharks in the western Indian Ocean. A similar pattern was previously reported for other pelagic sharks in the temperate waters of the North Atlantic, specifically *P. glauca* and *I. oxyrinchus* (Estrada et al., 2003) and other oceanic top predator guilds, including tunas and tropical seabirds in the western Indian Ocean (Catry et al., 2008; Cherel et al., 2008; Kojadinovic et al., 2008). This degree of overlap in core isotopic niches of pelagic sharks and other pelagic predator guilds is likely driven largely by the homogeneity of carbon sources in oligotrophic open-ocean ecosystems, characterized by low productivity and little seasonality (Cherel et al., 2008).

### Table 3

Mean differences and p-values based on post hoc tests Tukey's tests for paired comparisons of Hg concentrations (top), δ15N (middle), and δ13C (bottom). Significant differences are in bold.

<table>
<thead>
<tr>
<th>Species</th>
<th>C. pelagicus</th>
<th>C. falciformis</th>
<th>C. longimanus</th>
<th>I. oxyrinchus</th>
<th>P. glauca</th>
<th>P. kamoharai</th>
<th>S. lewini</th>
<th>A. pelagicus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log Hg</td>
<td>–0.701, 0.612</td>
<td>0.246, 0.981</td>
<td>0.368, 0.824</td>
<td>1.069, &lt;0.05</td>
<td>0.122, 0.995</td>
<td>–0.122, 0.995</td>
<td>0.096, 0.999</td>
<td>–1.101, &lt;0.05</td>
</tr>
<tr>
<td>δ15N</td>
<td>–1.561, 0.243</td>
<td>–0.523, 0.931</td>
<td>0.710, 0.684</td>
<td>2.271, &lt;0.01</td>
<td>1.233, &lt;0.01</td>
<td>–1.050, &lt;0.001</td>
<td>0.304, 0.989</td>
<td>–0.470, 0.980</td>
</tr>
<tr>
<td>δ13C</td>
<td>0.175, 0.999</td>
<td>0.244, 0.973</td>
<td>0.068, 0.999</td>
<td>0.730, 0.001</td>
<td>0.278, 0.676</td>
<td>–0.278, 0.676</td>
<td>0.304, 0.989</td>
<td>–0.470, 0.980</td>
</tr>
</tbody>
</table>

### Table 4

Percentage of δ13C vs. δ15N overlap of corrected standard ellipses (SEAc) among the six species investigated; note two overlap values per species comparison are given, e.g. *I. oxyrinchus* vs. *C. longimanus* and vice versa.

<table>
<thead>
<tr>
<th>Species</th>
<th>C. longimanus</th>
<th>I. oxyrinchus</th>
<th>P. glauca</th>
<th>P. kamoharai</th>
<th>S. lewini</th>
<th>A. pelagicus</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. longimanus</em></td>
<td>23.9</td>
<td>1.8</td>
<td>62.5</td>
<td>38.6</td>
<td>65.7</td>
<td></td>
</tr>
<tr>
<td><em>I. oxyrinchus</em></td>
<td>15.4</td>
<td>*</td>
<td>5.7</td>
<td>81.4</td>
<td>16.9</td>
<td>32.0</td>
</tr>
<tr>
<td><em>P. glauca</em></td>
<td>2.9</td>
<td>1.4</td>
<td>*</td>
<td>0</td>
<td>1.5</td>
<td>8.7</td>
</tr>
<tr>
<td><em>P. kamoharai</em></td>
<td>11.3</td>
<td>23.5</td>
<td>0</td>
<td>*</td>
<td>8.5</td>
<td>17.2</td>
</tr>
<tr>
<td><em>S. lewini</em></td>
<td>71.1</td>
<td>48.4</td>
<td>1.7</td>
<td>87.0</td>
<td>*</td>
<td>64.7</td>
</tr>
<tr>
<td><em>A. pelagicus</em></td>
<td>54.1</td>
<td>41</td>
<td>4.5</td>
<td>78.2</td>
<td>28.9</td>
<td>*</td>
</tr>
</tbody>
</table>

### 4.2. Hg as a complementary dietary tracer

Hg levels which provide insights into the trophic position of organisms (e.g. McMeans et al., 2010), were relatively similar among species. A positive relationship between Hg concentrations and δ15N has been previously documented for many marine taxa, including pelagic sharks from other regions in the Atlantic and Pacific Oceans (e.g. Branco et al., 2004; Maz-Courrau et al., 2012). Our results for *P. glauca, I. oxyrinchus* and *C. longimanus* (species with satisfactory sample size) are consistent with this pattern. Overall, Hg concentrations in muscle tissues were very high in pelagic sharks sampled in the south-western Indian Ocean (mean=6.34 µg g⁻¹ dw), compared to other pelagic fish from the same region, including *Xiphias gladius* (mean=3.97 µg g⁻¹ dw), *Thunnus albacares* (1.15 µg g⁻¹ dw), *Thunnus obesus* (0.67 µg g⁻¹ dw) and *Coryphaena hippurus* (0.21 µg g⁻¹ dw) (Kojadinovic et al., 2006). The total Hg levels in blue shark muscle samples of a similar size were also much higher in the south-western Indian Ocean compared to those reported off Baja California (central Pacific Ocean) (1.96 ± 1.48 µg g⁻¹ dw vs. 5.3 ± 2.17 in our study, Maz-Courrau et al., 2012). These concentrations highlight not only the high trophic position of these species but the potential risks of shark meat consumption for human health that need to be further investigated.

The high Hg concentrations observed in *P. kamoharai* (mean=17.25 µg g⁻¹ dw) were unexpected, since this species appears to feed at a similar trophic position to most other pelagic species, based on δ15N despite being much smaller in
methylion of inorganic Hg in deep oceanic waters (Blum et al., 2013), resulting in its enhanced bioaccumulation in mesopelagic organisms (Monteiro et al., 1996; Chouvelon et al., 2012). Therefore, top marine predators feeding on mesopelagic prey, such as seabirds and large predatory fishes, exhibit significantly higher concentrations than epipelagic predators (Thompson et al., 1998; Kojadinovic et al., 2006; Choy et al., 2012). Therefore, top marine predators feeding on mesopelagic prey, such as seabirds and large predatory fishes, exhibit significantly higher concentrations than epipelagic predators (Thompson et al., 1998; Kojadinovic et al., 2006; Choy et al., 2009). In this respect, Hg can be considered as an indicator of the feeding depth (Carravieri et al., 2013). Higher Hg concentrations in P. kamoharai, are most likely due to its occupying a significantly deeper feeding habitat than the other, more surface-oriented, pelagic species sampled.

### 4.3. Spatial and ontogenetic variation

Spatial variation in δ13C and δ15N values and Hg concentrations of individual sharks sampled were not consistent, especially for P. glauca and I. oxyrinchus. Drawing conclusions on these inconsistent patterns is difficult because there are currently no complementary independent datasets from this region to provide insights into isoscapes or geographical patterns of Hg concentrations. Information on size and sex-based differences in trophic interactions of marine top predators, including sharks, is critical to better understand ecosystem function and to assess the ecological role of these organisms. Ontogenetic shifts in diet are common in elasmobranchs, for large predatory species such as tiger (Galeocerdo cuvier, Lowe et al., 1996) and great white sharks (Carcharodon carcharias, Estrada et al., 2006) to zooplanktivorous whale sharks (Rhincodon typus, Borrell et al., 2011).

Ontogenetic variation in the foraging ecology and habitat use of most pelagic sharks investigated in this study are poorly known, except for P. glauca, including in the western Indian Ocean. Previous studies suggest a significant increase in δ13C and δ15N values with body length in P. glauca in the surrounding oceanic waters of Madagascar, but not in C. falciformis (Rabehagasoa et al., 2012). Our data suggest an identical pattern for P. glauca (not tested for C. falciformis due to small sample size), which is also consistent with a significant dietary shift.

### Table 5

<table>
<thead>
<tr>
<th>Species</th>
<th>C. longimanus</th>
<th>I. oxyrinchus</th>
<th>P. glauca</th>
<th>P. kamoharai</th>
<th>S. lewini</th>
<th>A. pelagicus</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. longimanus</td>
<td>*</td>
<td>68.3</td>
<td>8.1</td>
<td>34.7</td>
<td>59.8</td>
<td>75.9</td>
</tr>
<tr>
<td>I. oxyrinchus</td>
<td>29.3</td>
<td>*</td>
<td>25.8</td>
<td>2</td>
<td>39.3</td>
<td>69.6</td>
</tr>
<tr>
<td>P. glauca</td>
<td>1.8</td>
<td>13.1</td>
<td>*</td>
<td>0</td>
<td>1.4</td>
<td>1.5</td>
</tr>
<tr>
<td>P. kamoharai</td>
<td>10.0</td>
<td>1.4</td>
<td>0</td>
<td>*</td>
<td>1.1</td>
<td>2.7</td>
</tr>
<tr>
<td>S. lewini</td>
<td>29.0</td>
<td>44.4</td>
<td>3.1</td>
<td>1.8</td>
<td>*</td>
<td>20.5</td>
</tr>
<tr>
<td>A. pelagicus</td>
<td>26.0</td>
<td>55.7</td>
<td>2.4</td>
<td>3.2</td>
<td>14.5</td>
<td>*</td>
</tr>
</tbody>
</table>

### Table 6

<table>
<thead>
<tr>
<th>Species</th>
<th>C. longimanus</th>
<th>I. oxyrinchus</th>
<th>P. glauca</th>
<th>P. kamoharai</th>
<th>S. lewini</th>
<th>A. pelagicus</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. longimanus</td>
<td>*</td>
<td>36.4</td>
<td>63.6</td>
<td>6.3</td>
<td>82.4</td>
<td>91.1</td>
</tr>
<tr>
<td>I. oxyrinchus</td>
<td>8.1</td>
<td>*</td>
<td>33.2</td>
<td>6.6</td>
<td>38.2</td>
<td>28</td>
</tr>
<tr>
<td>P. glauca</td>
<td>21.7</td>
<td>50.6</td>
<td>*</td>
<td>1.2</td>
<td>56.7</td>
<td>44.9</td>
</tr>
<tr>
<td>P. kamoharai</td>
<td>3.3</td>
<td>1.6</td>
<td>1.9</td>
<td>*</td>
<td>7.6</td>
<td>6.4</td>
</tr>
<tr>
<td>S. lewini</td>
<td>32.4</td>
<td>67.2</td>
<td>65.4</td>
<td>5.7</td>
<td>*</td>
<td>77.9</td>
</tr>
<tr>
<td>A. pelagicus</td>
<td>34.1</td>
<td>46.9</td>
<td>49.3</td>
<td>4.5</td>
<td>74.2</td>
<td>*</td>
</tr>
</tbody>
</table>

**Fig. 5.** Standard ellipses for corrected sample sizes (SEAc, in %, δ13C) and Hg concentrations (in μg g⁻¹ dw) of A. pelagicus, C. longimanus, P. glauca, S. lewini, I. oxyrinchus and P. kamoharai.

**Fig. 6.** Standard ellipses for corrected sample sizes (SEAc, in %, δ15N) and Hg concentrations (in μg g⁻¹ dw) of A. pelagicus, C. longimanus, P. glauca, S. lewini, I. oxyrinchus and P. kamoharai.
with increasing size based on stomach content analyses in Baja California (Markaida and Sosa-Nishizaki, 2010).

5. Conclusion

In this study, we found a high degree of isotopic niche overlap among pelagic sharks in the oligotrophic waters of the southwestern Indian Ocean that is consistent with previous studies on other epipelagic marine top predators. Nevertheless, our data also found large intra-species variation in Δ15N values identifying dietary and foraging habitat plasticity that masks variation among species. This large intra-species variation in Δ15N values would suggest pelagic species are either opportunistic feeders or have evolved variable and complex feeding behaviors, potentially related to variable regional prey bases and the migratory nature of these animals. Nevertheless a high degree of dietary plasticity would be expected in oligotrophic waters and intra-species variation should be investigated further in other pelagic guilds. Although, at the species level, significant core isotopic and Hg niche overlap among species is consistent with the hypothesis of trophic redundancy, these results must be viewed with caution due to limited differentiation in carbon pools in this ecosystem coupled with high intra-species variation.

Acknowledgments

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Table 7

Correlations between Δ13C, Δ15N values and log Hg concentrations of pelagic sharks based on Pearson’s correlations. Significant values are bold.

<table>
<thead>
<tr>
<th>Species</th>
<th>Log Hg/Δ13N</th>
<th></th>
<th>Log Hg/Δ15N</th>
<th></th>
<th>Δ15N/Δ13C</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t, p-Value</td>
<td>r</td>
<td>t, p-Value</td>
<td>r</td>
<td>t, p-Value</td>
<td>r</td>
</tr>
<tr>
<td>A. pelagicus</td>
<td>-0.79, 0.49</td>
<td>-0.41</td>
<td>2.67, 0.08</td>
<td>0.84</td>
<td>-0.35, 0.75</td>
<td>-0.20</td>
</tr>
<tr>
<td>C. falciformis</td>
<td>1.61, 0.35</td>
<td>0.85</td>
<td>-1.77, 0.33</td>
<td>-0.87</td>
<td>25.06, 0.05</td>
<td>0.99</td>
</tr>
<tr>
<td>C. longimanus</td>
<td>1.86, 0.09</td>
<td>0.49</td>
<td>-0.47, 0.65</td>
<td>-0.14</td>
<td>1.71, 0.11</td>
<td>0.46</td>
</tr>
<tr>
<td>I. oxyrinchus</td>
<td>0.32, 0.75</td>
<td>0.06</td>
<td>0.28, 0.78</td>
<td>0.05</td>
<td>4.67, 0.001</td>
<td>0.67</td>
</tr>
<tr>
<td>P. glauca</td>
<td>4.49, &lt; 0.001</td>
<td>0.64</td>
<td>5.37, &lt; 0.001</td>
<td>0.71</td>
<td>5.43, &lt; 0.001</td>
<td>0.71</td>
</tr>
<tr>
<td>P. kamoharai</td>
<td>0.97, 0.40</td>
<td>0.49</td>
<td>-1.13, 0.34</td>
<td>-0.55</td>
<td>-1.50, 0.23</td>
<td>-0.65</td>
</tr>
<tr>
<td>S. lewini</td>
<td>0.15, 0.89</td>
<td>0.07</td>
<td>-1.39, 0.24</td>
<td>-0.57</td>
<td>0.19, 0.86</td>
<td>0.10</td>
</tr>
<tr>
<td>All species</td>
<td>3.84, &lt; 0.001</td>
<td>0.38</td>
<td>1.11, 0.27</td>
<td>0.12</td>
<td>4.27, &lt; 0.001</td>
<td>0.41</td>
</tr>
</tbody>
</table>

Fig. 7. Influence of size (FL: fork length) on log Hg concentrations (top), Δ15N (middle) and Δ13C values (bottom) in (a) I. oxyrinchus, (b) P. glauca and (c) C. longimanus.


