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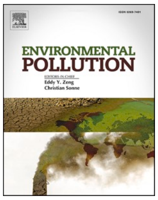

Effect of body size, feeding ecology and maternal transfer on mercury accumulation of vulnerable silky shark *Carcharhinus falciformis* in the eastern tropical pacific☆

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ABSTRACT

The silky shark *Carcharhinus falciformis* is a large pelagic species distributed in the global oceans and was recently listed as “Vulnerable” by the IUCN because of its decline in population due to overfishing. As an apex predator, the silky shark can accumulate elevated quantities of mercury (Hg), posing a potential risk to its remaining population. In this study, total Hg (THg) concentrations were determined in silky shark muscle, liver, dermis, red blood cells (RBC) and plasma sampled from the eastern tropical Pacific, and $\delta^{15}N$ values were measured to explore the influence of feeding ecology on Hg accumulation. The highest THg concentrations were in muscle (7.81 ± 6.70 μg g^{-1} dry weight (dw) or 2.14 ± 1.83 μg g^{-1} wet weight (ww)) and liver (7.88 ± 10.22 μg g^{-1} dw or 4.66 ± 6.04 μg g^{-1} ww) rather than dermis, RBC and plasma. The THg concentrations in all tissue types were significantly correlated with fork length and showed faster accumulation rates after maturity. Maternal THg transfer was observed in silky sharks with embryos having 33.16% and 1.98% in muscle and liver compared with their respective mothers. The potentially harmful THg concentrations in silky shark tissues and embryos may lead to health problems of sharks and consumers. THg concentrations were negatively correlated with $\delta^{15}N$ values for all tissues, indicating likely baseline variations in $\delta^{15}N$ values that reflect changes in the foraging habitats or regions of silky sharks with size or age. Lastly, strong correlations were observed among THg concentrations of all tissue types, indicating that nonlethal sampling of muscle and dermis tissue can be used effectively to quantify THg concentration of other internal tissues.

1. Introduction

Mercury (Hg) is a toxic, nonessential element that is naturally occurring, but anthropogenic activities have increased its occurrence in the environment, notably in marine food webs (Blanchfield et al., 2022). Once Hg enters the marine environment it is known to biomagnify, with higher trophic organisms at a greater risk of accumulating potentially unhealthy concentrations (Gworek et al., 2016). Hg has long been known to have deleterious effects on fish by altering behavior (Ceccatelli et al., 2010), reducing growth rates (Sandheinrich and Drevnick, 2016), impairing reproduction (Grieshaber et al., 2021), causing oxidative stress (Barrera-Garcia et al., 2012) and damaging nervous system (Pan et al., 2022) and immune system (Chuang et al., 2022). The most common way humans are exposed to Hg is through seafood consumption with the recommended levels of methylmercury (MeHg) human consumption, a neurotoxic form of Hg, being 1 μg g^{-1} wet weight (or ∼ 4 μg g^{-1} dry weight, dw) by the US Environmental Protection Agency (EPA, 2022) and Food and Drug Administration (FDA, 2020).

Large sharks are long-lived species, occupying high trophic positions and accumulating substantial concentrations of Hg, in which over 95% of total mercury (THg) consists of the highly toxic form of MeHg (Tiktak et al., 2020). Many shark species are reported to have THg

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concentrations in their flesh (muscle tissue) that exceed the recommended safety levels including pelagic thresher shark *Alopias pelagicus* (4.97 μg g^{-1} dw, Kiszka et al., 2015), oceanic whitetip shark *Carcharhinus longimanus* (16.80 μg g^{-1} dw, Gelsleichter et al., 2020) and smooth hammerhead shark *Sphyrna zygaena* (12.15 μg g^{-1} dw, Storelli et al., 2003). Typically, large pelagic sharks have more elevated THg concentrations than coastal sharks of similar size and trophic position (Tiktak et al., 2020), posing potential higher health risks to them and humans that consume them (Garcia Barcia et al., 2020). For most shark species, however, there lacks an understanding of the main environmental and biological drivers of mercury accumulation, warranting further studies.

The silky shark, *Carcharhinus falciformis*, as an apex predator in oceanic waters, has been reported to accumulate moderate to elevated concentrations of THg across its distribution with means ranging from 0.6 μg g^{-1} dw in the Southern Mexican Pacific (Rodriguez-Gutierrez et al., 2020) to 7.1 μg g^{-1} dw in the Galapagos (Maurice et al., 2021) and Southwestern Indian Oceans (Le Bourg et al., 2019). Silky shark is one of the most heavily fished species in the world and is captured in huge numbers by a wide range of artisanal and industry fisheries, but particularly as a target or bycatch species of high-seas tuna long-liners. They are the second most traded species in the Hong Kong retail dried fin market and globally the third largest landed oceanic shark species globally (Cardeñosa et al., 2018; FAO, 2020). Most parts of the silky shark are consumed by humans (e.g., fin, meat, and liver oil) and liver oil was commonly used as nutritional supplements for children and elders (Palmieri et al., 2014; EPA, 2022). In 2017, silky shark was listed as "Vulnerable" by the International Union for Conservation of Nature highlighting a need to better understand all aspects of its ecology and nutrition, including the presence of potentially lethal chemicals such as mercury and that nonlethal methods for acquiring information are urgently needed.

The main objectives of this study, focused on silky sharks, were to: (1) determine concentrations of THg in multiple tissues (muscle, liver, dermis, RBC and plasma) and in embryos in which to explore maternal transfer, and (2) examine relationships between THg accumulation, growth (body size) and feeding ecology (as derived from stable nitrogen isotope, δ^{15}N values). The THg concentrations of the five tissue types were compared with the results of previous studies on silky shark and other shark species to examine the inter/intraspecific variations. Correlations between THg concentrations of five tissue types were tested to assess the efficacy of the nonlethal sampling methods in the future. Given that silky sharks are top predators they represent environmental monitoring for bioaccumulation and biomagnification of THg in oceanic food webs from the eastern tropical Pacific (ETP), which is an economically important fishing area for numerous countries.

2. Materials and methods

2.1. Sample collection

All samples were collected as bycatch of Chinese tuna longline fishing vessels working in the ETP from September 2019 to January 2020 (−6° N ~ 8° S, 98 ~ 113° W) (Fig. 1). The fork length (FL) was measured to the nearest cm, and to assess maturity state, we used a combination of FL and macroscopic examination of female reproductive tract or male clasper length and calcification. Muscle and dermis tissues were collected from the dorsal region. Liver samples were collected from the tip of any lobe. Approximately 5 g of these three tissues were sampled for laboratory analysis. Blood samples were collected using sterilized syringes from the caudal vein, transferred to 5 ml sterile blood collection tubes lined with lithium heparin anticoagulant, and were spun and separated immediately into RBC and plasma components in a portable centrifuge at 3000 rpm for 3 min. RBC and plasma layers were pipetted into separate 5 ml blood collection tubes. Through internal dissection, 15 whole embryos were obtained from four pregnant females. All tissue samples were stored frozen at −20 °C onboard and immediately archived in an ultralow temperature freezer (−80 °C) upon

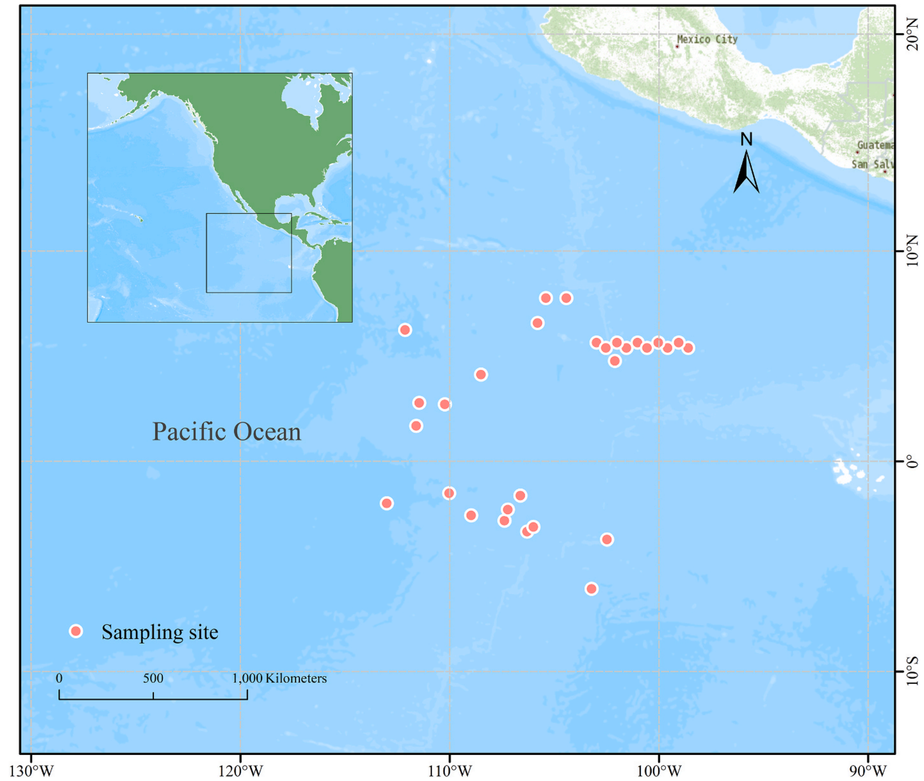

Fig. 1. Sampling locations for silky shark (*Carcharhinus falciformis)* in the eastern tropical Pacific collected as bycatch in the Chinese pelagic longline fishery targeting tuna . .

return to the laboratory.

2.2. Stable isotope analysis

Deionized water was used to rinse muscle and liver tissues repeatedly to remove urea, while the process of removing urea and lipids will lead to a loss of free amino acids in RBC or plasma, resulting in large isotopic effects (Li et al., 2016a; Weideli et al., 2019). All samples were freeze-dried at −50 °C for ≥24 h and then ground into a fine powder using a Mixer Mill MM 400 (Retsch). Before and after drying, all samples were weighed to calculate the water content of samples to assist with converting concentrations from wet to dry weight.

Dried samples were weighed (~1.5 mg) into tin capsules and analyzed using an IsoPrime 100 isotope ratio mass spectrometer (IsoPrime Corporation; Cheadle, UK) and vario ISOTOPE cube elemental analyzer (Elementary Analysensysteme GmbH; Hanau, Germany) at Shanghai Ocean University. The δ^{15}N values of the samples were calculated according to the following equation: $\delta^{15}N(‰) = [((^{15}N/^{14}N)_{sample}/(^{15}N/^{14}N)_{standard})\text{-}1] \times 1000$; where ‰ is parts per thousand, and where $^{15}N/^{14}N$ represent the atomic ratios of ^{15}N of the sample and the standard, respectively (Li et al., 2016a). Reference standards USGS 26 (53.7 ± 0.4‰ V-AIR) were used to quantify ^{15}N stable isotope values, and a laboratory reference (fish protein, 5.96‰ for nitrogen) was used for calibration every twenty samples. The analytical errors of δ^{15}N values were ±0.20‰.

2.3. THg analysis

THg concentrations in silky shark muscle, liver, dermis, RBC, and plasma were determined via thermal decomposition (combustion), amalgamation, and atomic absorption spectrometry using a calibrated DMA-80 Direct Mercury Analyzer (Milestone, Italy). Dried and crushed samples previously prepared for stable isotope analysis were used to measure THg concentrations. Approximately 0.02 g of crushed sample was loaded into the DMA-80, dried and burned at a temperature of 650 °C in an oxygen atmosphere (Maurice et al., 2021). The measurements in tissues were conducted as follows: drying time 100 s, decomposition time 150 s, and waiting time 10 s. Quality control procedures included analysis of laboratory method blanks, duplicate tissue samples, and certified reference materials (DORM-4) (O'Bryhim et al., 2017). The precision of duplicate samples averaged ±6.56%, and percentage recovery for the certified reference materials ranged from 95% to 108%.

2.4. Data analysis

Data were separated by tissue types and analyzed using descriptive statistics to determine mean THg concentrations for comparison with previous studies on silky sharks and other sharks near the study area. Differences of THg concentrations and δ^{15}N values between tissue types were examined by paired *t*-test. Patterns of Hg accumulation were examined by Pearson's correlation coefficient (r) to determine if there was a significant correlation between muscle THg concentrations and FL (used as a proxy for age) and δ^{15}N values (used as a proxy of trophic position). Correlations between FL and THg concentrations in other tissue types were also analyzed to determine whether Hg concentrations in these tissues appeared to reflect long-term Hg accumulation patterns. After normalized transformation of THg concentrations and FL, THg concentrations in five tissues were linear regressed with the FL to evaluate their THg accumulation rates. The values of THg concentrations in liver, RBC, and plasma as indicators of internal THg burden were evaluated by using linear regressions to determine if there were significant correlations between these values and those measured in muscle and dermis using natural log-transformed data.

3. Results

A total of 32 silky sharks were examined in the present study, half of which were male. The average FL was 140.2 ± 29.1 (ranging from 65 to 182) cm. Not all individuals were able to provide samples of all tissue types. Therefore, sample sizes varied for different sample matrices and for correlation analyses.

The THg concentrations in the muscle, liver, RBC, plasma, and dermis of silky sharks sampled from the ETP are shown in Fig. 2 with mean THg concentrations (±SD) presented in Table 1. The highest means and variability in THg concentrations were detected in the muscle and liver while the plasma and dermis statistically had the lowest mean THg concentrations (Fig. 2, $P < 0.05$). THg concentrations ranged from 0.43 to 6.56 μg g^{-1} ww in muscle, 0.14–20.72 μg g^{-1} ww in liver, 0.01–1.08 μg g^{-1} ww in RBC, 0.002–0.07 μg g^{-1} ww in plasma, and 0.01–0.45 μg g^{-1} ww in dermis. Water content and thus wet to dry weight conversion factors varied greatly between the different tissue types ranging from 1.69 for liver to 10.5 for plasma, with mean THg concentrations in dry and wet weight reported in Table 2. The THg concentrations in muscle and liver exceeded the US EPA and FDA recommended levels of human consumption (1.0 mg kg^{-1} ww, EPA, 2022; FDA, 2020). There was no statistical difference between THg concentrations in males and females in any tissue type ($P > 0.05$).

THg concentrations of maternal and embryonic tissues were shown in Table 2. The mean THg concentrations in embryonic muscle and liver were 5.72 ± 1.81 and 0.54 ± 0.17 μg g^{-1} dw, respectively, and were lower than their mothers (17.15 and 28.20 μg g^{-1} dw). Embryos contained 33.16 ± 4.17% (ranging from 21.73 to 41.76%) of the THg concentrations observed in their respective mothers in muscle and 1.98 ± 0.69% (ranging from 0.93 to 3.41%) in liver. The THg concentrations in the muscle and liver of embryos within each litter were similar (indicated by low standard deviations) and there was no statistical difference between sexes across litters ($P > 0.05$). THg concentrations were higher in embryonic (5.72 ± 1.81 μg g^{-1} dw) than in juvenile individuals (2.84 ± 1.23 μg g^{-1} dw, FL < 137 cm, $P < 0.05$).

There were exponential increases of THg concentrations in all tissue types with shark FL, with highest concentrations in mature individuals greater than 137 cm FL (Fig. 3). Positive Pearson correlation coefficient (r) values varied from 0.65 for liver to 0.97 for RBC. There were no

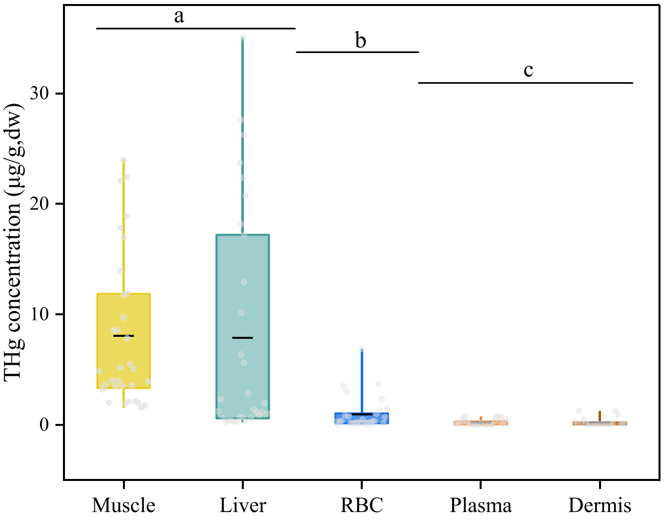

Fig. 2. Total Hg (THg) concentrations in muscle, liver, red blood cells (RBC), plasma, and dermis (μg g^{-1}, dry weight) in silky shark *Carcharhinus falciformis* sampled from the eastern tropical Pacific. Different letters represent statistical differences (paired *t*-test, $P < 0.05$) between tissue types with no differences detected in THg concentrations between muscle and liver or between plasma and dermis. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Total Hg (THg) concentrations and $\delta^{15}N$ values (mean ± standard deviation) of five tissue types in the silky shark *Carcharhinus falciformis* from the eastern tropical Pacific. The sample size (N) and the range of THg concentrations in parentheses are also presented. Different letters represent statistical differences (paired *t*-test, $P < 0.05$) between tissue types.

Tissue	N	THg concentration ($\mu g\ g^{-1}$)		$\delta^{15}N$ (‰)
		Dry weight	Wet weight	
Muscle	32	7.81 ± 6.70^a (1.56–23.98)	2.14 ± 1.83^a (0.43–6.56)	12.74 ± 1.69^a (9.87–15.1)
Liver	31	7.88 ± 10.22^a (0.23–35.06)	4.66 ± 6.04^a (0.14–20.72)	11.09 ± 1.61^b (8.00–14.38)
RBC	31	0.74 ± 1.02^b (0.03–6.85)	0.22 ± 0.23^b (0.01–1.08)	11.74 ± 1.42^c (8.76–14.37)
Plasma	31	0.21 ± 0.23^c (0.02–0.77)	0.02 ± 0.02^b (0.002–0.07)	10.44 ± 1.66^d (6.91–14.64)
Dermis	23	0.23 ± 0.36^c (0.02–1.27)	0.08 ± 0.12^b (0.01–0.45)	12.37 ± 0.90^e (10.66–13.57)

Table 2
Total Hg (THg) concentrations ($\mu g\ g^{-1}$ dw) of the four female silky sharks and their associated embryos.

No.	Pregnant female			Embryo			
	Fork length (cm)	THg concentration		Fork length (cm)	Sex	THg concentration	
		Muscle	Liver			Muscle	Liver
1	175	10.15	19.41	49.4	M	3.02	0.26
				46.6	F	3.18	0.28
				46.4	F	3.09	0.36
				47.4	F	4.74	0.46
				49.7	F	2.95	0.36
2	170	18.52	35.06	49.4	M	4.08	0.40
				46.6	F	4.81	0.38
				46.4	F	6.88	0.41
				47.4	F	4.03	0.44
				49.7	F	4.68	0.32
3	167	22.09	34.61	16.0	M	8.69	0.78
				21.0	M	7.96	0.61
4	175	17.84	23.72	43.8	M	7.22	0.81
				42.8	F	6.92	0.78
				45.0	F	4.61	0.57

differences in the THg - FL relationship between males and females. The slope of the relationship between normalized THg concentrations and FL suggested that accumulation rates were higher in the liver than the other 4 tissues (Fig. 4).

The THg concentrations in all the tissue types were significantly correlated with each other (Fig. 5). Higher concentrations in one tissue corresponded to comparably higher concentrations in another, with very similar correlations (r) values observed between all tissue relationships ($r = 0.87$ to 0.94).

Stable nitrogen ($\delta^{15}N$) values, commonly used as a proxy for trophic position, varied between tissue types with the lowest mean values detected in plasma and highest values in muscle (Table 1). THg concentrations were significantly negatively correlated with $\delta^{15}N$ values in all tissue types (Fig. 6). THg concentrations of the tissues of silky sharks decreased with increasing $\delta^{15}N$ values. Similarly, we found a significant negative relationship between FL and $\delta^{15}N$ values in all tissue types ($P < 0.05$).

4. Discussion

This is the first attempt to systematically consider Hg bioaccumulation of multiple tissues in silky sharks, an endangered oceanic shark species, and consider the influence of key ecological and growth factors. The elevated THg concentrations in silky shark found in this study, particularly in the muscle and liver, are higher than the recommended safety levels for human consumption and thus pose a potential health risk to both silky sharks and humans that consume them.

The THg concentrations in muscle and liver observed in this study greatly exceeded the average THg concentrations reported for elasmobranchs (1.43 $\mu g\ g^{-1}$ in muscle and 0.52 $\mu g\ g^{-1}$ in liver, ww) reviewed by Tiktak et al. (2020). This was consistent with the results of Maurice et al. (2021) that indicated that silky shark contained one of the highest THg concentrations among six pelagic shark species. Our reported THg concentrations for the ETP were also some of the highest concentrations reported for silky sharks worldwide, particularly in respect to the liver for which there are fewer comparative studies available (Table 3). For muscle, comparable THg concentrations have been reported in silky sharks of a similar size range from the Galapagos marine reserve and South western Pacific whereas much lower concentrations are reported in the Southern Mexican Pacific and south-western Indian Ocean. These results indicate that THg concentrations in silky shark do vary in space and time which, similar to other marine top predators, is likely due to a spatial variation in number of complex extrinsic factors, such as natural or anthropogenic sources of Hg inputs, depth of the oxygen minimum zone, and food web dynamics including prey availability and food chain length. A recent study of the spatial distribution of THg in skipjack tuna within the Pacific Ocean, showed that the highest THg concentrations were in the north western and eastern Pacific which was attributed to increased deposition of atmospheric Hg (Médieu et al., 2022).

The present study showed a strongly positive and exponential relationship of THg concentrations with size suggesting that THg accumulation rates proceed more quickly after maturation or at ~140 cm FL (Fig. 3). This kind of relationship has been observed in silky shark populations in southern Mexican Pacific (Rodriguez-Gutierrez et al., 2020) and in other shark species around the world (Pethybridge et al., 2010; Grant et al., 2018; Le Croizier et al., 2020). These patterns of THg concentrations can be associated with known ontogenetic shifts in diet or feeding habitat along with increased rates of dietary uptake to sustain large metabolic requirements such as migration or reproduction. Juvenile silly sharks in the ETP are reported to feed mainly on jumbo squid *Dosidicus gigas* (0.041 ± 0.009 THg $\mu g\ g^{-1}$ ww, Xie et al., 2021), whereas the adults consumed chub mackerel *Scomber japonicus* with much higher THg concentrations (0.11 ± 0.02 THg $\mu g\ g^{-1}$ ww, Bae et al., 2011).

As the trophic position of a consumer is thought to be an important parameter to explain Hg accumulation in marine food webs (Lavoie et al., 2013), it was somewhat surprising that this study found significant negative correlations between THg concentrations and $\delta^{15}N$ values in all five tissue types of silky shark. This results likely indicates baseline effects of different forging depth or habitats on the predators' $\delta^{15}N$ values instead of their trophic behaviors only (Kiszka et al., 2015; Le Bourg et al., 2019), potentially reflecting an increased use of epipelagic and/or oceanic resources with size or age (Walker et al., 1999; Kelly, 2000; Chen et al., 2009; Karimi et al., 2013). Certainty juvenile silky sharks are thought to lead a demersal or semi-pelagic lifestyle and have a greater reliance on mesopelagic suspended particulate-based food webs with higher baseline ecosystem $\delta^{15}N$ values rather than surface productivity (Bonfil, 1997; Choy et al., 2015). As silky sharks mature, they migrate to pelagic waters such as the central tropical/subtropical pacific which could be associated with areas of nitrogen fixation that is known to decrease baseline, and thus consumer, $\delta^{15}N$ values (Popp et al., 2007; Bonfil, 2008). A similar pattern was reported in dermal tissue of silky and blue sharks in the northeast central Pacific, where larger-sized sharks exhibited depleted ^{15}N which was linked to changes in foraging habitat (Li et al., 2016b). While spatial and temporal changes in diet and habitat use are likely explanatory factors, $\delta^{15}N$ values of sharks can also be influenced by other factors such as maternal transfer, or periods of starvation or strenuous metabolic expenditure (e.g. during migration or reproduction) (Trueman and Glew, 2019; McMeans et al., 2009; Vaudo et al., 2010). To better understand the trophic ecology and Hg accumulation of sharks, future studies could look to new techniques and

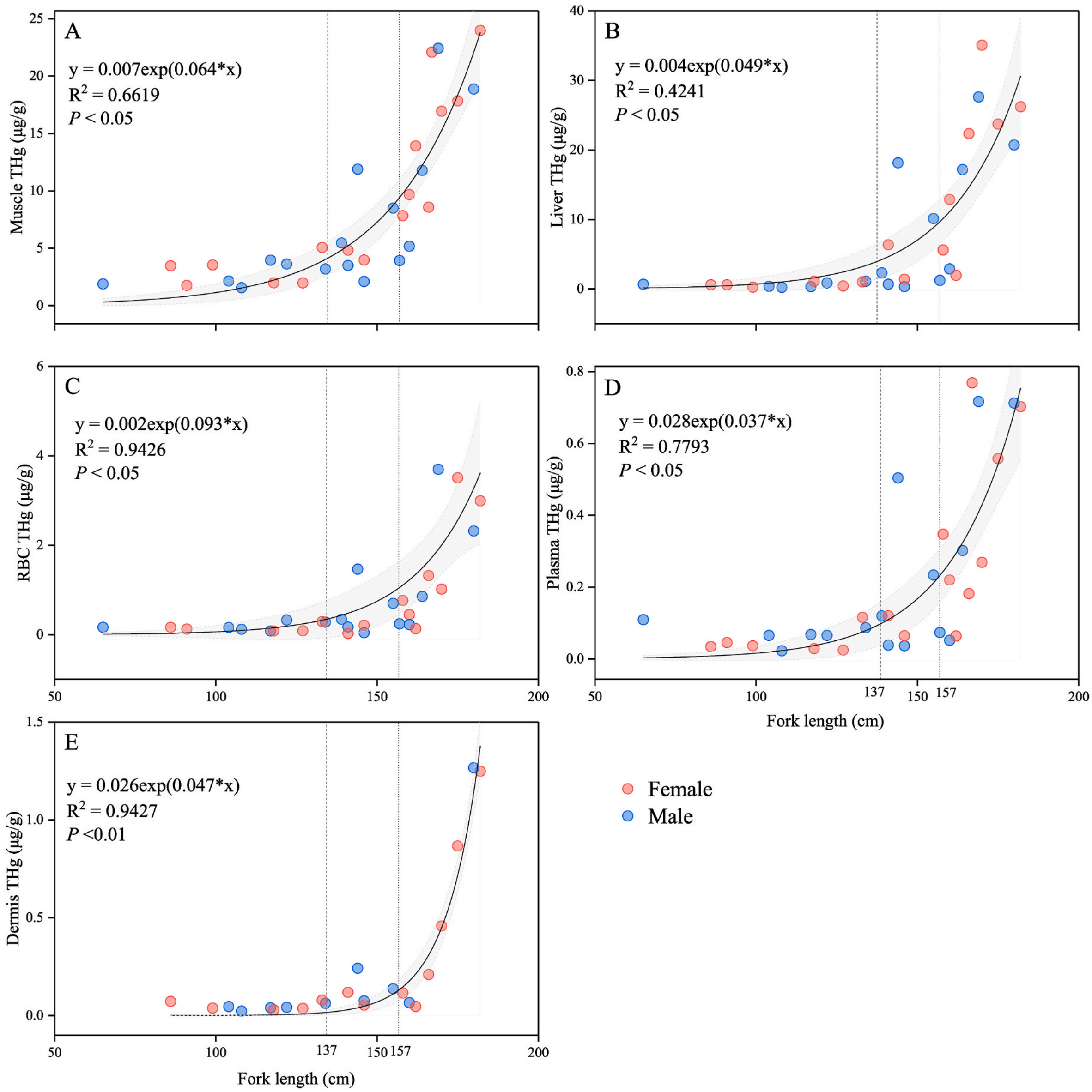

Fig. 3. Exponential relationships between fork length (cm) and total Hg (THg) concentrations in muscle (A), liver (B), red blood cells (RBC) (C), plasma (D) and dermis (E) in silky sharks *Carcharhinus falciformis* from the eastern tropical Pacific. The correlations were not significantly different between males and females ($P >$ 0.05). The mature lengths of males and females were 137 and 157 cm, respectively, as indicated by vertical grey lines. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

multidisciplinary approaches such as the Hg stable isotope analysis and satellite tags (Hutchinson et al., 2019; Maurice et al., 2021).

Maternal transfer of THg to embryos is thought to be an important exposure pathway that can directly influence offspring development while also providing a potential means for offloading by mothers (Chételat et al., 2020). Our results showed that there was a high maternal transfer of THg to embryos in silky sharks, particularly in the muscle where concentrations were higher in embryos than in juvenile individuals. This suggests that silky sharks have a very high starting point of THg concentration for neonates. As placental viviparous species, silky sharks transfer nutrition by ova and umbilical cord (Endo et al., 2015), which likely provides for the continuous and high transfer of Hg to embryos. As there were no gender differences in THg concentrations of embryo, juvenile or adult individuals, our results suggest that the maternal transfer process doesn't significantly reduce Hg loads of pregnant silky sharks. We observed only 3–5% of THg burden was transferred from mother to embryos (e.g., total 650 mg in mother vs. total 23 mg in all 5 embryos, according to the estimated body weight and THg concentration in muscle of No.1 pregnant shark and its embryos). These transfer percentages are within the range of those reported in other shark species (Lyons and Lowe, 2013; van Hees and Ebert, 2017; Lopes et al., 2019). For example, the THg concentrations in embryonic muscle and liver were 27.10% and 0.62% in silvertip shark (*Carcharhinus albimarginatus*) and 18.44% and 1.39% in tiger shark (*Galeocerdo cuvier*) compared with maternal tissues. Although differences in trophic ecology and physiology may influence maternal transfer of Hg comparable results across species suggests potentially similar Hg offloading processes in Carcharhinidae sharks (Frías-Espericueta et al., 2015; Endo et al., 2015, 2016).

Despite the high THg concentrations in shark meat, it is still widely used in many traditional dishes by coastline populations around the world with more than 700 million tons of sharks caught in 2018 (Tiktak et al., 2020; FAO, 2020). The high THg concentrations observed in both muscle and liver of silky shark exceeded the safe consumption limits (1.0 μg g^{-1} ww) set by governmental and human health organizations, when up to 95% of THg in muscle is present as MeHg (Pethybridge et al.,

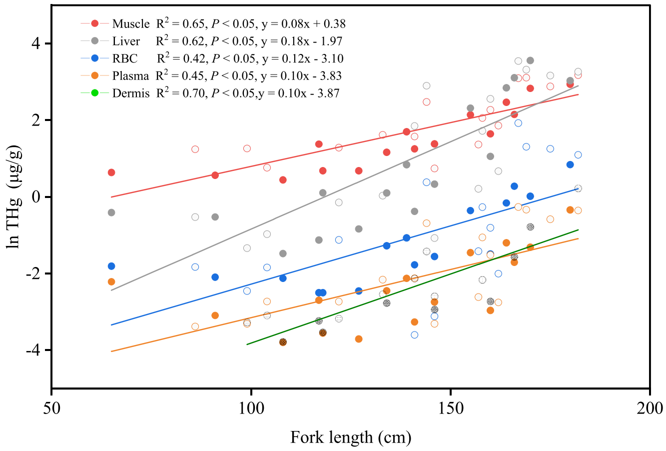

Fig. 4. Accumulation of THg (after normalized transformation) with body size in the five tissues of silky sharks *Carcharhinus falciformis* given by the linear regression and coefficient of determination (R^2). The males are denoted by solid circles, whereas females are represented by open circles.

2010; FDA, 2020; EPA, 2022). When the recommended intake exceeds limits, high Hg concentrations in humans can induce a series of physiological responses including alterations in membrane permeability and immunology systems, cardiovascular and hematological systems, renal and digestive systems, immune and nervous systems, endocrine and reproductive systems, and fetal health (Rice et al., 2014). In addition, Nalluri et al. (2014) reported that even just one standard 226.8 g bowl of shark fin soup contains Hg at levels comparable to the US EPA reference dose (RfD).

As a potent neurotoxicant, elevated THg concentrations may also affect the nervous system of silky shark at the organismal, tissue, biochemical, and physiological levels, which may implicate an individual's ability to catch and ingest prey (Wood et al., 2012). MeHg readily passes the blood-brain barrier and results in brain contamination (Rodrigues et al., 2022) so maternal transfer of Hg to embryos may potentially jeopardize early development and survivorship (van Hees and Ebert, 2017). High Hg concentrations could also result in other problems, such as oxidative stress, extensive damage to osmoregulation, histopathology and cellular function, and changes in the immune system (Wood et al., 2012). There is increasing evidences that selenium can effectively inhibit the toxicity of MeHg (Zhang et al., 2014) and that it is abundant in silky shark liver (Terrazas-Lopez et al., 2019), which may reduce the potential health risks of the high THg concentrations.

Differences in THg concentrations between tissues, as evident in this study, are reflective of the physiological functions of different tissue types and the way that MeHg is processed and stored internally. The majority of THg, of which approximately 90% is MeHg in sharks, is first absorbed through intestinal epithelial cells by diffusion, or by non-specific active uptake mechanisms of MeHg complexes. RBC and plasma then redistribute MeHg to be further processed or stored throughout the whole body (Giblin and Massaro, 1975), which likely explains the strong correlations observed among all tissue types assessed in this study. Importantly this result highlights that nonlethal sampling methods like muscle or dermis biopsies or fin clips (Sanderson et al., 2009) can provide a reliable means to predict THg concentrations of internal organs. This finding has stark implications for species of conservation status such as the silky shark and allows for better monitoring of THg contamination in live sharks collected from large-scale areas like the open ocean.

5. Conclusion

This study reported and compared THg concentrations in five types of tissues and embryos of silky shark caught from ETP with several important implications in respect to future monitoring studies and expanding our understanding of Hg cycling in oceanic ecosystems and contamination in top-order and conservation dependent marine predators. Various factors were linked to the elevated THg concentrations including increasing body size and differences in vertical habitat use.

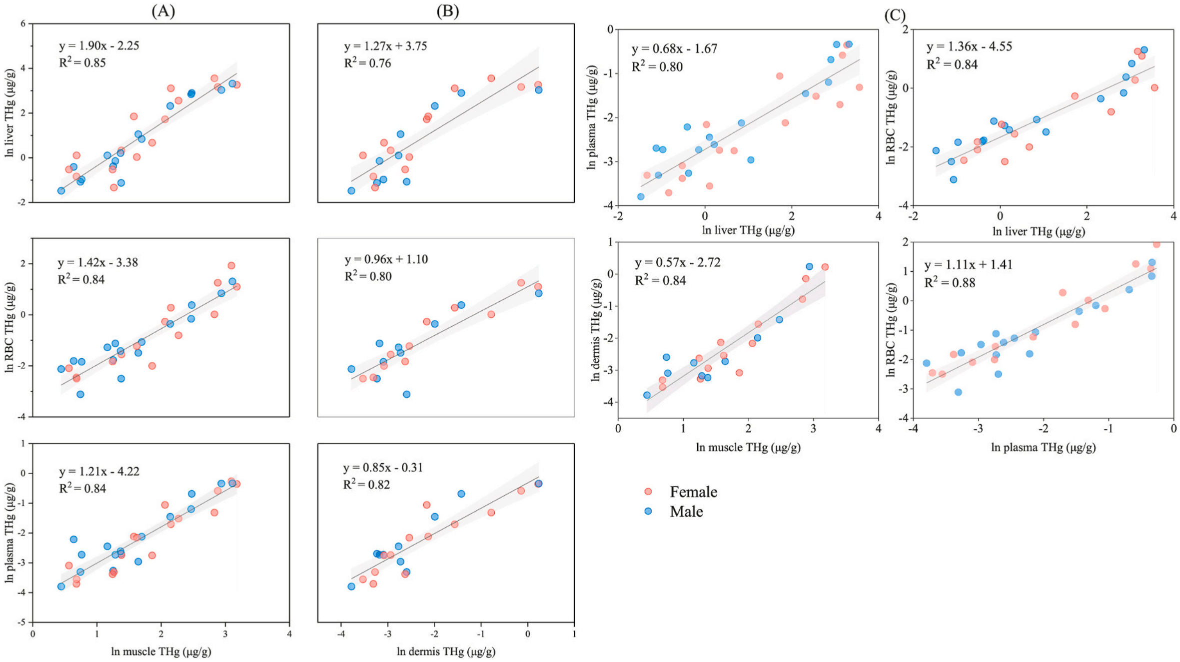

Fig. 5. Relationships between total Hg (THg) concentrations (μg g^{-1}) between different among muscle and liver, red blood cells (RBC) and plasma (A), dermis and liver, RBC and plasma (B), and other relationships among muscle, dermis, liver, RBC and plasma (C) in silky sharks *Carcharhinus falciformis* from the eastern tropical Pacific. All data were natural log transformed. The results of linear regression analysis, including the coefficient of determination (R^2), are shown for all datasets and all the p-values were <0.05. The red and blue points represent female sharks and male sharks, respectively. Shadow area: 95% confidence intervals. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

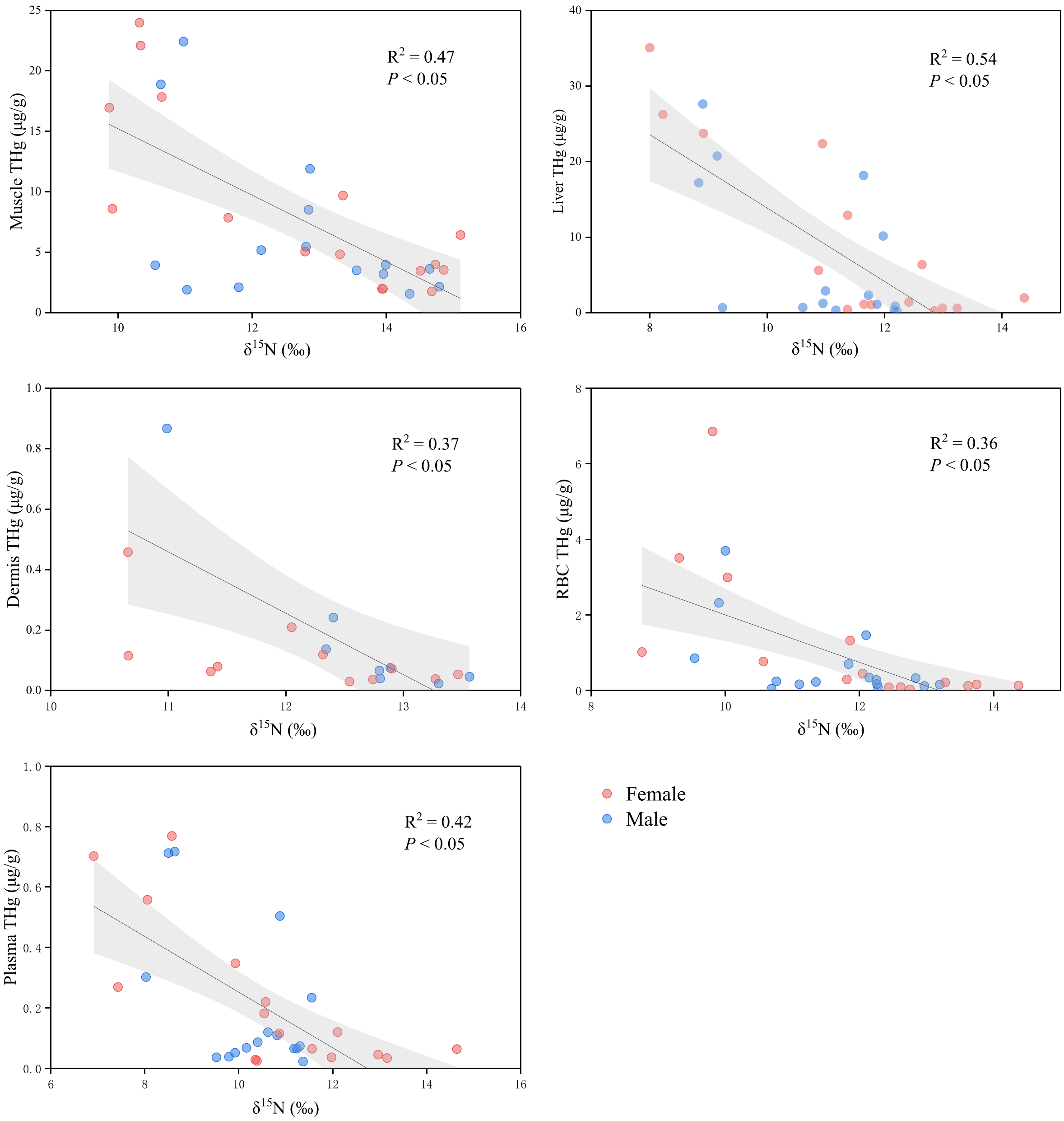

Fig. 6. Correlations of δ^{15}N values and total Hg (THg) concentrations (μg g^{-1}, dry weight) in muscle, liver, red blood cells (RBC) and plasma in the silky shark *Carcharhinus falciformis* from the eastern tropical Pacific. The coefficient of determination (R^2) of the linear relationships are shown in the figure. The red and blue points represent female sharks and male sharks, respectively. Shadow area: 95% confidence intervals. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

The slower growth rates, higher metabolic requirements for migration and/or reproduction, and ontogenetic diet shifts may be responsible for the increased rates of THg bioaccumulation after maturation. The negative correlation between δ^{15}N values and Hg concentrations suggested the influence of a shifting isotopic baseline which may be related to changes in foraging habitat and related characteristics of the environment. Maternal transfer of Hg was evident in silky sharks with relatively high concentrations present that could be a potential risk to their development or survival. As there were no detection of any gender difference in THg concentrations of comparable sized individuals we don't think that there is significant offloading of THg by pregnant females or that there are large gender differences in the feeding ecology or movement patterns. Relatively high THg concentrations in silky sharks may increase the potential health risks to them and humans that consumer them. Lastly, the highly significant correlations observed among tissue THg concentrations mean that it is possible to use nonlethal methods, such as fin clips or collection of muscle punch biopsies, in future monitoring studies of silky shark.

Credit author statement

Zezheng Li and Yunkai Li: Conceptualization, Methodology, Software, Investigation, Formal Analysis, Writing - Original Draft; Feng Wu and Xiaojie Dai: Methodology, Validation, formal analysis, Investigation; Heidi R. Pethybridge and Yi Gong: Writing - Review & Editing; Yunkai Li: Funding Acquisition, Resources, Supervision, Writing - Review & Editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

Table 3
THg concentrations in muscle and liver of silky shark (*Carcharhinus falciformis*) from previous studies.

Area	Sample number	Fork length (cm)	THg concentrations (μg g^{-1} dw)		Reference
			Muscle	Liver	
Eastern Pacific	32	65 to 182	7.81 ± 6.70	7.88 ± 10.22	This study
Baja California	15	126 to 188	3.40 ± 1.42		Maz-Courrau et al. (2012)
Southwestern Indian Ocean	3	96 to 148	2.43 ± 2.15		Kiszka et al. (2015)
Western Atlantic	13	69 to 180	3.09 ± 2.38	2.10 ± 3.64	O'Bryhim et al. (2017)
South Baja California		76 to 203	3.04 ± 0.31	3.95 ± 1.33	Terrazas-Lopez et al. (2019)
Southwestern Indian Ocean	10	73 to 260	7.13 ± 7.20		Le Bourg et al. (2019)
Southern Mexican Pacific	136	43 to 144	0.60*		Rodriguez-Gutierrez et al. (2020)
Galapagos	13	62 to 182	7.05		Maurice et al. (2021)

Note: "*" indicates that we estimated THg concentration in dry weight since study results were reported in wet weight, using a water content of 75% based on this study.

the work reported in this paper.

Data availability

Data will be made available on request.

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