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Mercury bioaccumulation in thresher sharks from the eastern tropical Pacific: Influences of body size, maturation stage, and feeding habitat

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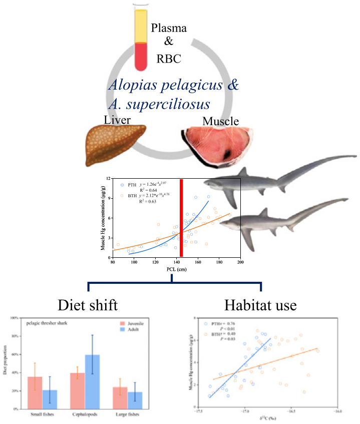
HIGHLIGHTS

- Hg in liver and muscle was assessed in two thresher sharks from the Pacific Ocean.
- Internal tissues of both thresher sharks exhibit a similar Hg distribution pattern.
- Growth, habitat use and diet shifts explained Hg accumulation patterns.
- Almost 100 % Hg in muscle tissue occurred as MeHg.

GRAPHICAL ABSTRACT



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ABSTRACT

Sharks, as top order predators, provide a guidance on how contaminants such as mercury bioaccumulate in marine environments. This study assessed the bioaccumulation of mercury (total mercury, THg) in the muscle, liver, red blood cells (RBC), and plasma of pelagic and bigeye thresher sharks (*Alopias pelagicus* and *A. superciliosus*) from eastern tropical Pacific. Additionally, the concentration of methylmercury (MeHg) in muscle was also determined to assess risks for human consumption. For both species, muscle THg concentrations (4.05 ± 2.15 and 4.12 ± 1.84 μg g^{-1} dry weight for pelagic and bigeye thresher shark) were higher than that in other tissues. THg concentrations for all tissues were significantly correlated with precaudal length, with higher accumulation rates after maturity in pelagic than bigeye thresher sharks, suggesting an associated dietary shift at maturation. Correlations among tissues in both species suggested similar transportation and distribution patterns in internal tissues. The δ^{13}C values in muscle, RBC and plasma suggested that habitat shifts influenced Hg accumulation, whereas trophic position, estimated by δ^{15}N values, had limited effects on patterns of Hg bioaccumulation. Diet shifts towards prey more cephalopods that content higher Hg than small fishes (large fishes: 1.77 μg g^{-1}; cephalopods: 0.66 μg g^{-1} and small fishes 0.48 μg g^{-1}, dry weight) increased Hg accumulation rates in adult pelagic thresher sharks. Concentrations of MeHg in the muscle of both thresher shark (3.42 ± 1.68 μg g^{-1} in *A. pelagicus* and 3.78 ± 2.13 μg g^{-1} in *A. superciliosus*) exceeded the recommended levels for human consumption. This research provides insight into the factors influencing mercury bioaccumulation in thresher sharks, which are essential for the management and conservation of these species.

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1. Introduction

Anthropogenic contributions of Hg have increased surface ocean Hg concentrations since the 19th-century Industrial Revolution (Doney, 2010; Driscoll et al., 2013). Once Hg enters the marine environment, it is biomagnified, with high trophic level organisms at a great risk of accumulating potentially elevated concentrations (Matulik et al., 2017). Marine predators, particularly long-lived shark species, are known to contain high levels of Hg (Tiktak et al., 2020). The high Hg content of sharks, especially the high percentage of methylmercury (MeHg), can pose significant risks to the health of the sharks and the human who consume them since certain pelagic sharks are among the most highly valued species in the commercial seafood market owing to the high quality of their meat, fin and liver oil (Cardeñosa et al., 2018).

Typically, large sharks have elevated Hg concentrations, posing potential higher health risks (Garcia Barcia et al., 2020). Concentrations of Hg can typically increase with fish age when the rate of dietary uptake is faster than that of elimination (Chételat et al., 2020; Amezcua et al., 2022). As sharks grow, they are able to feed on larger prey, and the concentrations of Hg in the predators typically increases proportionally with their length or mass (Lyons et al., 2013). Many shark species are reported to have total Hg (THg) concentrations in their muscle that exceed the recommended safety levels including silky shark *Carcharhinus falciformis* (7.81 μg g^{-1} dry weight, dw; Li et al., 2022a), oceanic whitetip shark *C. 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), in which main form is the most toxic organomercury compound MeHg. It is important to study Hg accumulation in oceanic sharks, as their populations have significantly declined due to high incidental bycatch in commercial fisheries. However, there are still knowledge gaps concerning the environmental and biological effects on Hg accumulation, which warrants further studies.

Thresher sharks were one of the most important commercial bycatch shark species in tuna fishery, and the pelagic thresher (*Alopias pelagicus*) (PTH) and bigeye thresher shark (*A. superciliosus*) (BTH) are commonly found in oceanic tropical and temperate seas (Smith et al., 2008). Both species feed on small fish and squid by stunning the prey with their long, scythe-like tails (Compagno, 2001). They both vertically migrate, though they occupy different depths at daytime, where they may encounter prey items with different THg concentrations (Coelho et al., 2015; Arostegui et al., 2020). Both species are ovoviviparous and have 1–2 embryos per litter and follow highly k-selected life-history traits, combining with the high interaction rates with fisheries, resulting in their endangered status (Smith et al., 2008). Accordingly, PTH and BTH are listed by the International Union for Conservation of Nature as vulnerable and endangered, respectively, and listed by Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora since 2017. Gaining insight into their Hg contaminant, especially the differences in accumulation rates and patterns between species, could provide valuable information about their feeding ecology, aiding in the protection of these endangered species.

Given the potentially increased Hg bioaccumulation and biomagnification in thresher sharks, we hypothesize that THg concentrations of the PTH and BTH in the eastern tropical Pacific are influenced by their growth patterns, feeding habitats and trophic position. By examining Hg concentrations, body size and carbon and nitrogen stable isotopes, this study intended to assess the presence of species-specific patterns of Hg accumulation between the two species. The effects of food habits were quantified for each species to determine the variations in Hg ingestion. Correlations between THg concentrations of four tissue types were used to assess the THg internal handling and the efficacy of the nonlethal sampling methods for the future. Finally, we examined the MeHg concentrations in shark muscle tissue to assess potential human health risks associated with its production and consumption.

2. Materials and methods

2.1. Study area and sampling collection

The eastern tropical Pacific is a tuna longline fishing ground, where tunas, sailfish and swordfish are the main catch species, and pelagic sharks are common bycatch. Between September 2019 to January 2020, a total of 30 PTH and 31 BTH were caught in the bycatch of Chinese tuna longline fishing vessels operating in the eastern tropical Pacific Ocean (−6°N ~ 8°S, 98–113°W, Fig. 1). The precaudal length (PCL) was measured to the nearest cm. To assess maturity states, we used the macroscopic examination of the female reproductive tract or male clasper calcification (Walker, 2005) (Table S1). Muscle tissues were collected from the dorsal region. Liver samples were collected from the tip of any lobe of the bulk liver. Blood samples were collected via sterilized needles and syringes from the caudal vein, transferred to sterile blood collection tubes lined with lithium heparin anticoagulant, and were spun and separated immediately into red blood cell (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. Stomach contents were identified to the lowest possible taxonomic level, and their muscle tissue was sampled for stable isotope and mercury analysis. All samples were stored frozen at −20 °C onboard and immediately archived in an ultralow temperature freezer (−80 °C) upon return to the laboratory.

2.2. Stable isotope analysis

Deionized water was used to rinse muscle and liver tissues repeatedly to remove urea (Li et al., 2016), while the process of removing urea will lead to a loss of free amino acids in RBC or plasma, resulting in large isotopic effects (Weideli et al., 2019). All samples were freeze-dried at −55 °C for 48 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 estimate the wet weight (ww) of Hg concentrations.

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 $\delta^{13}C$ and $\delta^{15}N$ values of samples were calculated according to the following equation: $\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$, where X is ^{13}C or ^{15}N, R_{sample} and $R_{standard}$ represent the ratios of $\delta^{13}C$ or $\delta^{15}N$ of the sample and the standard, respectively (Li et al., 2016). The standard references used were Pee Dee Belemnite (PDB) for carbon and atmospheric N_2 for nitrogen. A laboratory reference (Elemental Microanalysis Protein Standard OAS, −26.98 ‰ for carbon, and 5.94 ‰ for nitrogen) was used to calibration every twenty samples. Both analytical errors of $\delta^{13}C$ and $\delta^{15}N$ values were lower than ±0.20 ‰.

2.3. THg analysis

THg concentrations of all samples were determined via thermal decomposition (combustion), amalgamation, and atomic absorption spectrometry using a calibrated Direct Mercury Analyzer (DMA-80, Milestone, Italy). Dried and crushed samples previously prepared for stable isotope analysis were used to measure THg concentrations. Approximately 0.02 g of the crushed sample was loaded into the DMA-80, dried and burned at a temperature of 650 °C in an oxygen atmosphere. 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) were analyzed (Li et al., 2022a). The precision of duplicate samples averaged ±6.56 %, and percentage recovery for the certified reference materials ranged from 95 % to 108 %.

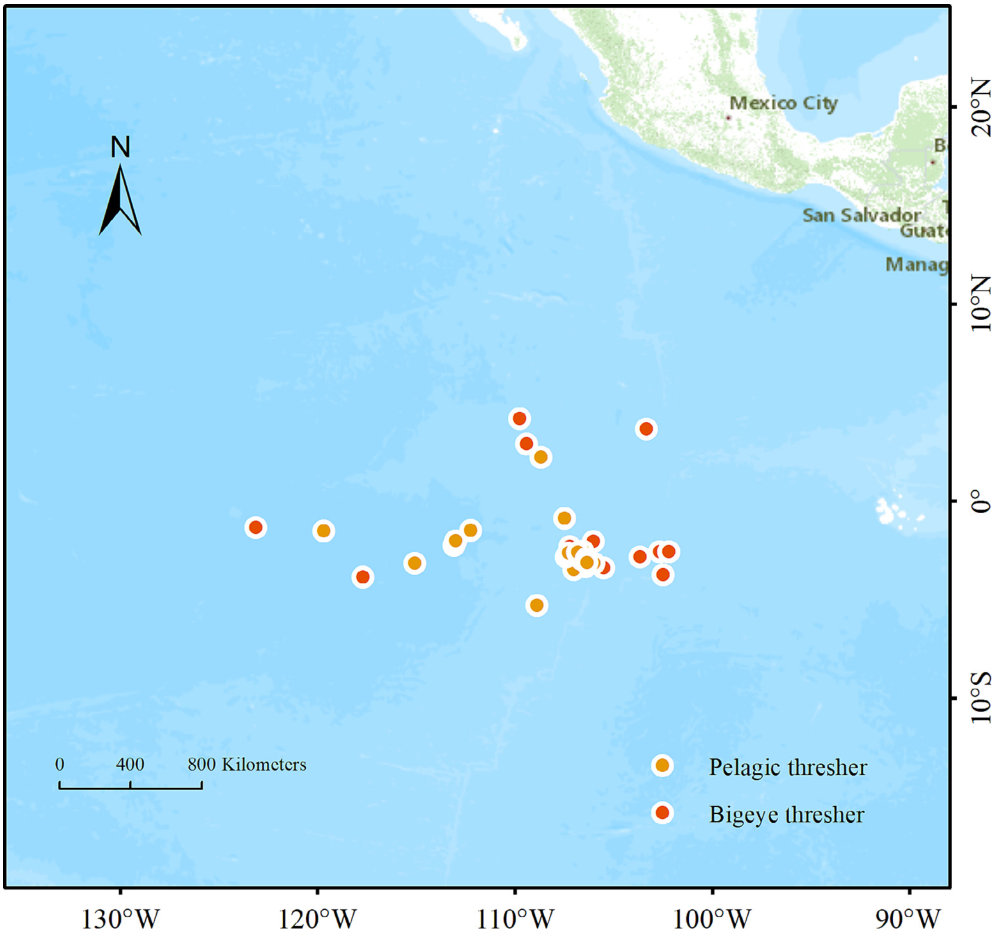

Fig. 1. Sampling locations for pelagic thresher (*Alopias pelagicus*) and bigeye thresher shark (*A. superciliosus*) in the eastern tropical Pacific from bycatch in the Chinese pelagic longline fishery.

2.4. Methylmercury analysis

MeHg extraction was performed and modified according to established method (Maggi et al., 2009). Approximately 0.5 g dried and ground muscle sample was weighed and transferred in 50 mL polypropylene tube with screw caps and hydrolyzed with 10 mL of HBr (47–49 %, AR). After 5 min of shaking using a mechanical shaker, 20 mL toluene (99.7 %, AR) was added following shake for 20 min. Sample was centrifuged (3000 rpm for 10 min) and the supernatant was separated to another 50 mL tube. The process was repeated once, and the combined organic extracts were subjected twice to back extraction with 6 mL of 1 % (v/w) L-cysteine aqueous solution (dissolved 1 % L-cysteinium chloride, 12.5 % anhydrous sodium sulfate and 0.775 % sodium acetate) to strip methylmercury from toluene. Then, the extract was immediately analyzed with DMA-80. The DORM-4 ($n = 3$) were analyzed and the percentage recovery for the certified reference materials ranged from 93 % to 98 %.

2.5. Data analysis

Data were separated by tissue types and analyzed using descriptive statistics to determine mean Hg concentrations in comparison with previous studies on thresher sharks and other shark species near the study area. Differences of Hg concentrations and isotope values among four tissues were examined by ANOVA with paired-*t*-test. Regressions of log-transformed Hg concentrations were used to explore internal Hg burden (linear correlations between muscle and other tissues) and indicators of Hg bioaccumulation (exponential correlations between tissue concentrations and PCL and isotope values). Between species differences in Hg concentration and isotope values of the four tissues were examined by the Kruskal-Wallis test. Effects of sex and maturity among species and tissues were also examined. *P*-values of <0.05 were considered to be statistically significant.

The Bayesian mixing model MixSIAR was used to determine the diet proportion of both thresher shark species following Carlisle et al. (2021). Model input data included $\delta^{13}C$ and $\delta^{15}N$ values of muscle tissues, and trophic discrimination factors (TDFs). Maturation was considered by separating sharks into two groups (Group 1: Juvenile, immature samples, and Group 2: Adult, mature samples) to find out the effects of feeding habits on THg accumulation rates. The preys of both PTH and BTH are similar, such as small- to medium-sized schooling fishes and pelagic invertebrates (Smith et al., 2008). The samples from 19 stomachs were deemed as prey for both PTH and BTH, and they were divided into three groups by their taxa and body size ("Small fishes", "Cephalopods", and "Large fishes", Table 1). Firstly, we separated them as the squids and fishes. Then the fish samples of which whole body size <30 cm were grouped into small fishes, and other samples were large fishes. For all models, the TDFs were applied from Kim et al. (2011) (1.7 ± 0.5 for $\delta^{13}C$ and 3.7 ± 0.4 for $\delta^{15}N$). The model was run with 300,000 Markov chain Monte Carlo (MCMC) simulations (200,000 burn-in) and showed good convergence based on Gelman-Rubin (all variables <1.01) and Geweke (no scores outside ± 1.96 in any chain) diagnostics. Uninformative priors were used. The differences between the diet proportions were performed with ANOVA.

Considering a provisional tolerable weekly intake (PTWI) of 1.6 μg MeHg kg^{-1} Human Body Weight (HBW) proposed by the World Health Organization (WHO, 2018), we calculated the maximum weekly intake of

Table 1

δ^{13}C, δ^{15}N and THg concentrations for each prey item found in the stomachs of both pelagic thresher (*Alopias pelagicus*, PTH) and bigeye thresher shark (*A. superciliosus*, BTH).

Species	Prey items	N	δ^{13}C values (‰)	δ^{15}N values (‰)	THg (μg g^{-1})	Sampled from
Taractichthys steindachneri	Small fishes	1	−19.15	7.42	0.27	BTH
Magnisudis atlantica	Small fishes	5	−17.97 ± 0.75	7.78 ± 0.97	0.51	PTH
Rosenblattichthys volucris	Small fishes	1	−18.74	5.88	0.78	PTH
Psenes cyanophrys	Small fishes	1	−17.91	8.36	0.33	BTH
Vinciguerria lucetia	Small fishes	11	−18.75 ± 0.90	6.58 ± 0.79	0.37	PTH/BTH
Brama japonica	Small fishes	1	−17.53	9.00	0.43	PTH
Scopelosaurus sp.	Small fishes	3	−18.07 ± 0.79	8.53 ± 0.72	0.69	PTH/BTH
Asperoteuthis acanthoderma	Cephalopods	1	−19.13	7.13	1.19	BTH
Taningia danae	Cephalopods	3	−18.23 ± 0.44	8.53 ± 0.63	1.90	BTH
Dosidicus gigas	Cephalopods	3	−18.00 ± 0.65	8.93 ± 1.99	0.39	PTH/BTH
Thysanoteuthis rhombus	Cephalopods	1	−17.11	7.38	0.40	PTH
Sthenoteuthis oualaniensis	Cephalopods	4	−18.21 ± 0.42	7.69 ± 0.11	0.52	PTH/BTH
Argonauta argo	Cephalopods	1	−17.50	8.47	0.38	PTH
Liguriella podophthalma	Cephalopods	1	−17.34	7.98	0.92	PTH
Ancistrocheirus lesueurii	Cephalopods	1	−17.19	8.98	0.40	BTH
Onykia loennbergii	Cephalopods	2	−18.04 ± 1.16	8.37 ± 1.26	0.33	BTH
Planctoteuthis sp.	Cephalopods	4	−18.48 ± 0.75	7.45 ± 0.89	0.40	PTH
Walvisteuthis sp.	Cephalopods	2	−17.19 ± 0.34	9.87 ± 0.28	0.45	PTH/BTH
Coryphaena hippurus	Large fishes	1	−16.78	9.39 ± 0.56	1.03	PTH
Lepidocybium flavobrunneum	Large fishes	2	−17.07 ± 0.15	9.76 ± 0.32	2.16	BTH
Xiphias gladius	Large fishes	1	−17.08	11.92 ± 1.63	3.04	PTH
Alepisaurus ferox	Large fishes	2	−18.15 ± 0.28	10.12 ± 2.18	0.88	PTH/BTH

shark meat (g/weekly) according to the following equation (Maurice et al., 2021):

$$\text{Intake} = (\text{PTWI} * \text{HBM})/\text{MeHg}$$

where: MeHg represents the average MeHg concentration (μg g^{-1} ww) in each shark species measured in this study. Human body mass (HBM) was equal to 70 kg, 60 kg and 15 kg for adult men, women, and children, respectively. All statistical analyses were performed with R statistical software (R Development Core Team 2013) and OriginPro 2022.

3. Results

The sampled sharks were identified as 16 females (9 of them were juvenile) and 14 males (6 were juvenile) of the PTH, 12 females (6 of them were juvenile) and 19 males (7 were juvenile) of the BTH. There was no statistical difference in the mean PCL (body size) of the two species in all tissues (range from 95 to 170 cm for PTH and 81–181 cm for BTH).

THg concentrations ranged from 0.04 to 9.25 μg g^{-1} dry weight (dw) in PTH, and from 0.02 to 7.43 μg g^{-1} dw in BTH (Table 2). The MeHg concentrations in muscle ranged from 0.90 to 8.42 μg g^{-1} dw for PTH, 1.28 to 7.17 μg g^{-1} dw for BTH and the fraction of THg as MeHg averaged (±SD) 96.1 ± 6.1 % (range: 84.1 to 100 %) and 98.1 ± 8.0 % (range: 86.6 to 100 %) for PTH and BTH, respectively. The MeHg concentrations in muscle were found mostly (67 % samples for PTH and 55 % samples for BTH) to exceed the U.S. FDA recommended levels of human consumption (1.0 μg g^{-1} ww, or 4.0 μg g^{-1} dw, FDA, 2020). The tolerable weekly intake of meat by pelagic and bigeye thresher shark consumptions were 141 g and 150 g for adult men, 121 g and 129 g for women, 30 g and 32 g for children, respectively.

Both THg and MeHg concentrations in the muscle, liver and RBC were not revealed significant difference between the two species, while for plasma the THg concentrations were higher in PTH than BTH. For both species, THg concentrations in muscle were the highest, following the plasma, RBC and liver (paired-*t*-test, $P < 0.05$, Table 2). There was no difference in Hg concentrations between males and females in any tissue type for either species (paired-*t*-test, $P > 0.05$). The adults showed higher Hg concentration than juveniles in both species ($P < 0.05$).

THg concentrations in all the tissue types were significantly correlated with each other, with the R^2 values were over 0.72 in all correlations (Fig. S1). The slopes of THg correlations among tissues were similar in both thresher sharks, especially among the muscle, liver and RBC. Positive correlations were observed between PCL and Hg concentrations in all four types of tissues of both PTH and BTH (Fig. 2). The THg accumulation rates in PTH were faster than that in BTH when the PCL were exceeded 140 cm (Fig. 2). THg concentrations were significantly correlated with the δ^{13}C values of all tissues studied for both shark species except for the livers, while the correlations between THg concentrations and δ^{15}N values were only observed in RBC of PTH (Fig. 3).

For prey species, the highest THg concentrations were observed for large fishes' muscle (average of 1.77 μg g^{-1}, dw), followed by cephalopods (0.66 μg g^{-1}, dw) and small fishes (0.48 μg g^{-1}, dw). Cephalopods were the predominant prey item in both shark species, followed by small fishes. For PTH, the small fishes were the main prey item for the juveniles and cephalopods were the main prey item for the adults. For BTH, cephalopods were the primary food resources in both juveniles and adults. The diet proportions were significantly different between juvenile and adult PTH, while it showed no difference in BTH (Fig. 4, $P < 0.05$).

4. Discussion

For both PTH and BTH, muscle THg concentrations in this study were similar to the same species documented for the Galápagos Marine Reserve, southwestern Indian Ocean, Baja California Sur and Colombian Pacific coast (Kiszka et al., 2015; Le Bourg et al., 2019; Lara et al., 2020; Maurice et al., 2021). The percentage of Hg burden presented as MeHg exceed 90 % in several studies regarding the muscle tissue (Matulik et al., 2017; Pethybridge et al., 2010), supporting the results that the Hg determined in both thresher shark species was apparently MeHg as their estimated mean was indistinguishable from 100 %. For PTH livers, the THg concentrations were lower than the previous studies sampled from Mexico Pacific coast (0.37 ± 0.31 μg g^{-1} ww) (Lara et al., 2020). The THg concentrations of the two thresher shark species are notably lower than the global mean values reported for Lamniformes (2.58 μg g^{-1} in muscle and 0.085 μg g^{-1} in liver, ww) compiled by Tiktak et al. (2020), and all sharks (1.51 μg g^{-1} in muscle, and 1.57 μg g^{-1} in liver ww) compiled by Amezcua et al. (2022). Such differences among species may demonstrate that thresher sharks have the ability to reduce their Hg levels by foraging different dietary sources or/and physiological capacities to eliminate Hg or MeHg by breaking it down to inorganic mercury and excreting it from the body (Chételat et al., 2020). There is growing evidences that selenium can effectively inhibit the toxicity of MeHg by forming inert mercury-selenide complexes, which leads to MeHg demethylation in the liver and eliminates through feces. Maternal transfer in pregnant PTH was

Table 2

Hg concentrations (μg g^{-1}, dry weight), δ^{13}C and δ^{15}N values (‰, mean ± standard deviation and the ranges) of four tissue types in the pelagic thresher (*Alopias pelagicus*, PTH) and bigeye thresher shark (*A. superciliosus*, BTH) from the eastern tropical Pacific.

Items	THg concentration (μg g^{-1})				δ^{13}C (‰)				δ^{15}N (‰)			
Species	PTH		BTH		PTH		BTH		PTH		BTH	
Muscle	4.05 ± 2.15^{a}	(1.00, 9.25)	4.12 ± 1.84^{a}	(1.29, 7.43)	−16.99 ± 0.20^{a}	(−17.38, −16.72)	−16.74 ± 0.30^{b}	(−17.34, −16.21)	10.67 ± 0.84^{a}	(9.93, 13.60)	11.45 ± 1.15^{a}	(9.59, 13.02)
Liver	0.42 ± 0.79^{a}	(0.04, 3.88)	0.29 ± 0.34^{a}	(0.02, 1.16)	−17.74 ± 0.83^{a}	(−17.59, −15.79)	−17.90 ± 0.46^{a}	(−18.67, −16.72)	8.99 ± 0.75^{a}	(8.47, 11.93)	9.69 ± 0.92^{b}	(7.61, 10.52)
RBC	0.84 ± 0.75^{a}	(0.12, 2.72)	0.71 ± 0.54^{a}	(0.09, 1.83)	−17.10 ± 0.53^{a}	(−18.81, −16.42)	−16.99 ± 0.20^{b}	(−17.59, −16.68)	8.84 ± 0.95^{a}	(8.13, 12.75)	9.65 ± 1.52^{b}	(6.92, 12.22)
Plasma	1.69 ± 1.13^{a}	(0.21, 4.01)	0.93 ± 0.86^{b}	(0.08, 3.46)	−17.21 ± 0.56^{a}	(−18.76, −16.55)	−17.19 ± 0.53^{a}	(−18.53, −16.44)	7.88 ± 0.90^{a}	(6.78, 9.66)	9.75 ± 0.81^{b}	(6.78, 9.66)

Note: PTH: pelagic thresher shark; BTH: bigeye thresher shark. Different letters represent statistical differences ($P < 0.05$) between species within the same tissue.

discovered as an offloading process, with THg embryonic muscle containing 2.18 μg g^{-1} dw (Li et al., 2022b). This phenomenon is also observed in the common thresher shark (*Alopias vulpinus*), wherein the Hg concentration in embryos was 6.6 % of that for mothers (Lyons and Lowe, 2013). A similar result may occur in BTH.

Mean THg and MeHg concentrations were comparable between the two species, although there were different interspecific Hg accumulation patterns. Furthermore, similar inter-tissue relationships and the same order of mean Hg loads among tissues (muscle > plasma, RBC and liver) are likely related to the redistribution and the processing and storing of Hg in these tissues (Schultz and Newman, 1997). Results of the highly positive correlations among four tissues provide a possible means for nonlethal sampling methods using muscle biopsies to predict THg concentrations of internal tissues (Fig. S1). It is believed that MeHg is mainly absorbed by the gut before being distributed to other tissues throughout the body via RBCs and plasma (Oliveira Ribeiro et al., 1999). Ultimately, observations of high muscular Hg concentrations (1.11 μg g^{-1} ww) are probably due to the affinity of MeHg binding and its linkage to thiol groups of proteins, which are abundant in the muscles (Taylor et al., 2014). In the liver, Se is regulated by homeostasis when Hg accumulates continuously in the muscle and is transferred to the liver, forming Se—Hg complexes and being eliminated with feces via biliary excretion (Eisler, 2000; Chételat et al., 2020). This suggests that Se—Hg interactions likely influence the distribution of Hg in different internal tissues since Se and Hg concentrations are closely related and Hg can bind strongly with Se (Eisler, 2000). The background levels of Hg in tissues of sharks are important parameters to assess the health status and quality conditions of marine organisms (Lu et al., 2019; Amezcua et al., 2022). In the eastern Pacific Ocean, the relatively low background Hg concentrations in muscle and liver might have little effect on the Hg accumulation patterns for the two shark species (Amezcua et al., 2022). Further research on background concentrations of Hg is needed to differentiate between anthropogenically-influenced and naturally-occurring contamination of ecosystems.

For all tissues, growth-related increases were observed in both species with larger (and likely older) individuals accumulating higher Hg levels, as is widely reported for other shark species (Pethybridge et al., 2010; Taylor et al., 2014; Le Croizier et al., 2020). Previous research has demonstrated that Hg bioaccumulation in marine fish is attributed to a high Hg intake relative to low depuration rates (Wang, 2012). It is also well documented that faster somatic growth rates in marine fish results in reduced overall Hg burdens through growth dilution, which is due to disproportionate increasing fish size relative to the Hg dietary intake in BTH (Taylor et al., 2014; Wang, 2012). A notable exponential rise in THg concentrations was observed at ~140 cm PCL for PTH and ~160 cm for BTH, coinciding with reduced growth rates after maturation and a consequent shift in Hg accumulation rates (Chen et al., 1997; Liu et al., 1999; Fernandez-Carvalho et al., 2011). The PCL at which 50 % attained maturity of females and males is reported between 145 and 150 cm and 140–145 cm for PTH, 175–180 cm and 150–155 cm for BTH in the Pacific (Chen et al., 1997; Liu et al., 1999). The dietary shifts after maturity (more cephalopods) indicated by our isotopic mixing model results were likely a key driver for the rapid THg accumulation rate increase in PTH because of the higher proportion of the Hg-rich cephalopods. These dietary shifts were observed by the stomach content analysis of PTH (Polo-Silva et al., 2013), width of jaw gape, and the development of sensory organs with increasing body size improved the ability to catch larger prey (Barley et al., 2019). We also noticed that the THg concentrations for PTH plasma were higher than that for BTH, which may be influenced by recent dietary intake since the plasma reflects the consumption of relatively Hg-rich foods (Gelsleichter et al., 2020).

As Hg contamination levels in predators reflect environmental pollution, the differences of δ^{13}C values in muscle and RBC between PTH and BTH showed variation in long-term habitat use, while δ^{13}C values in liver and plasma were similar between the two species, indicated a similarity of short-term habitat use. Turnover rates of Hg and isotopes are comparable in muscle (half-life estimated time 1 to 3 year) and plasma (several days to

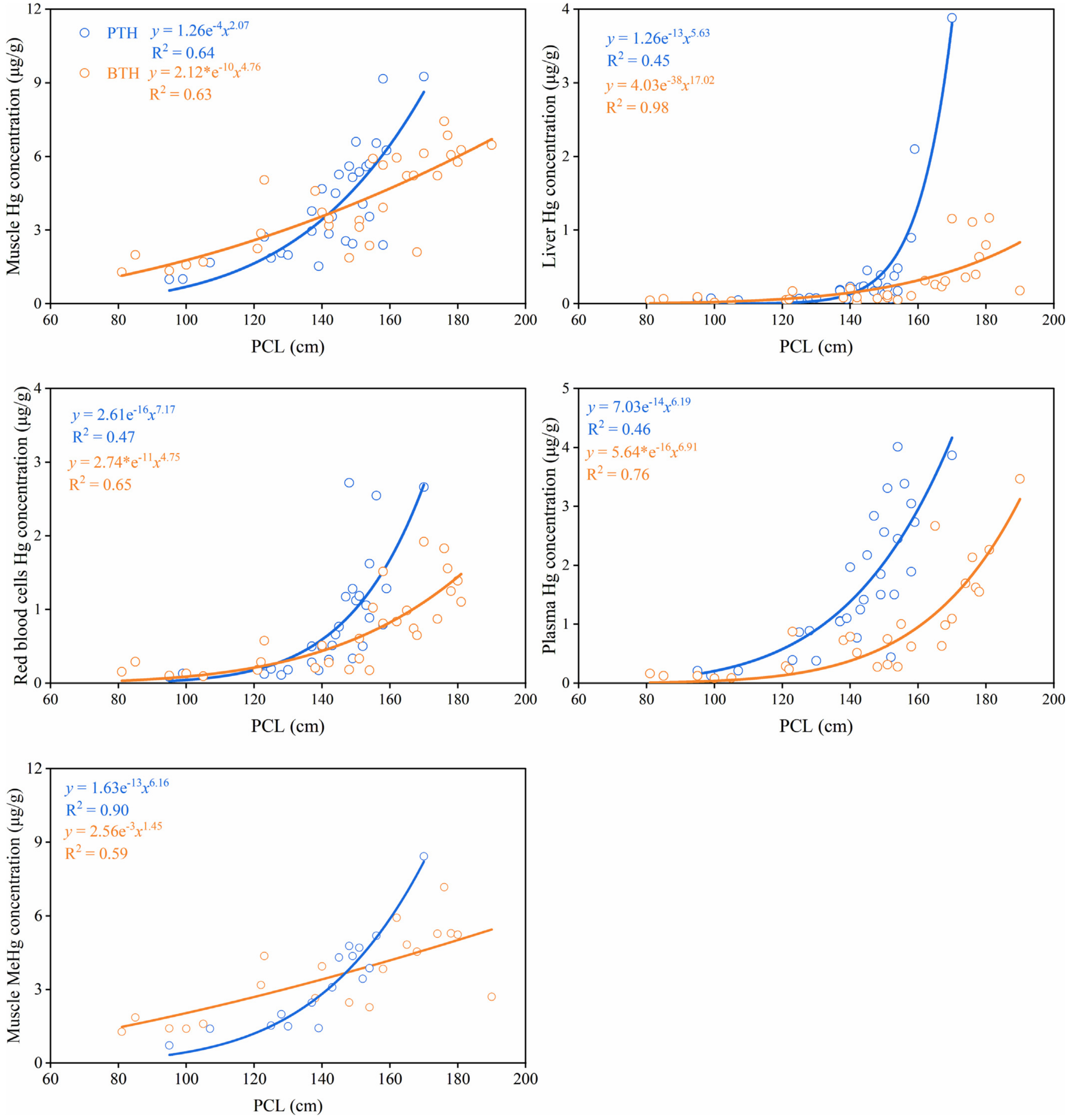

Fig. 2. Exponential relationships between precaudal length (PCL, cm) and Hg concentrations (THg or MeHg) in muscle, liver, red blood cells (RBC), and plasma in pelagic thresher (*Alopias pelagicus*, PTH) and bigeye thresher sharks (*A. superciliosus*, BTH) from the eastern tropical Pacific. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

weeks) (MacNeil et al., 2006; Van Walleghem et al., 2013). Interspecific differences in δ^{13}C values likely reflected the use of different feeding habitats, with the lower δ^{13}C values in offshore regions comparing to nearshore regions where the CO_2 concentration was reduced or high primary productivity was high, such as in coastal upwelling areas (Cullen et al., 2001; Graham et al., 2010; Páez-Rosas et al., 2021). THg concentrations showed no difference between the two species in muscle, which could be influenced by the high overlap of habitat utilization (Maurice et al., 2021; Li et al., 2016). The δ^{13}C values in muscle and plasma were significantly positively correlated with THg concentrations in both shark species, likely indicating that the sharks had experienced different regions with primary producer having distinct THg concentrations which are biomagnified up the food web and regulate THg in top predators (Newman et al., 2011).

Trophic position, estimated by δ^{15}N values, differed between the two species and among all tissues. The higher δ^{15}N values in most tissues of BTH compared to PTH may contribute to the elevated δ^{15}N values prey associated with in deeper waters (Voss et al., 2001). Both PTH and BTH undertake diel vertical movements, with foraging depths between 0 and 50 m after sunset (Musyl and Brill, 2011; Arostegui et al., 2020). However, BTH has been shown to spend more daytime at deeper depths (mean depth =

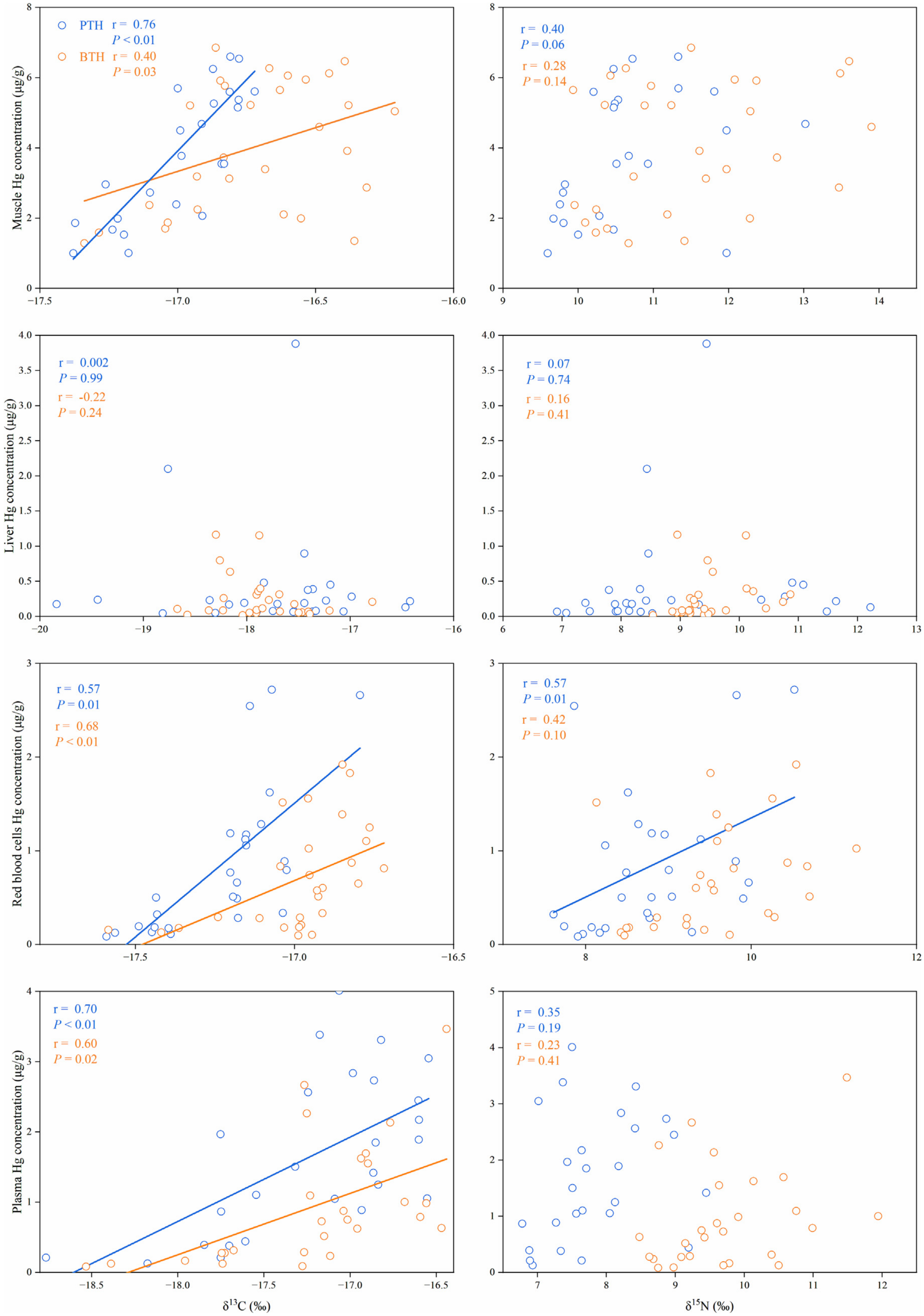

Fig. 3. Correlations of $\delta^{13}C$, $\delta^{15}N$ values and total Hg (THg) concentrations (μg g^{-1}, dry weight) in muscle, liver, red blood cells (RBC) and plasma in the pelagic thresher (*Alopias pelagicus*, PTH) and bigeye thresher sharks (*A. superciliosus*, BTH) from the eastern tropical Pacific. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

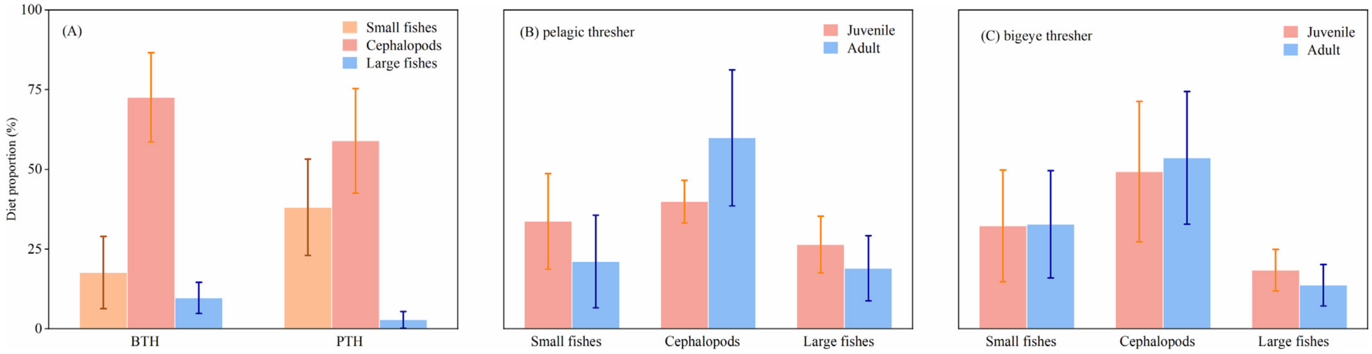

Fig. 4. Diet proportion inferred from isotope mixing models (MixSIAR) for (A) interspecies, juvenile and adult pelagic (B) and bigeye (C) thresher sharks from the eastern tropical Pacific.

353 m) than PTH sharks (mean depth = 250 m) (Coelho et al., 2015; Sepulveda et al., 2019; Arostegui et al., 2020). It is surprising that despite higher δ^{15}N values and deeper water occupancy, BTH showed lower THg concentrations in its plasma. This could be due to its physiology, which limited the amount of hemoglobin thiol groups binding Hg in plasma (Taylor et al., 2014). With the exception of PTH RBCs, no correlations between THg concentrations and δ^{15}N values of any tissues were found for the two shark species. Though juvenile and adult BTH demonstrated dietary shifts, their tropic positions changed little with body size indicating that these pelagic sharks adopt a generalist strategy over their life history. A lack of correlation has been reported by multiple studies (Newman et al., 2011; Endo et al., 2015, 2016; Matulik et al., 2017; Gelsleichter et al., 2020), reinforcing the hypothesis that trophic positions have limited effects on Hg accumulation in thresher sharks.

Shark samples in this study showed mean MeHg concentrations ranging from 0.16 to 2.23 μg g^{-1} ww in muscle, most of which exceeded the FDA reference value of 1 μg g^{-1} ww (FDA, 2020). In numerous cultures, women, men, and children can be exposed to MeHg contamination by regular seafood ingestion (via the diet or contaminated breast milk) all over the world (Tiktak et al., 2020). The weekly MeHg intake of shark consumption acceptable for humans varies depending on body weight and shark species. It is highly recommended to avoid regular consumption of thresher sharks' meat, particularly for vulnerable populations at risks such as pregnant women and young children (WHO, 2018; Liu et al., 2018). The results of this study revealed that consuming one portion of 100 g of shark fillet per week could be detrimental for human health at the advised weight of 60–80 g for pregnant women (Japan Ministry of Health Labour and Welfare, 2003). The USA recommends avoiding the consumption of shark meat for pregnant women and children (Han and Watanabe, 2012). Considering the human health risk assessment results, the regular consumption of thresher shark meat represents a serious human health risk for the populations.

5. Conclusions

Mercury accumulation in thresher sharks from the eastern tropical Pacific Ocean were found to influenced by a myriad of factors including body size, maturation stage, habitat use, and physiological capacities. There were few differences between the two species although rates of bioaccumulation were higher in PTH after 140 cm PCL (approximate size of maturation). Gender and trophic position did not seem to affect Hg accumulation in thresher sharks. With increased demand for shark products and global shark declines, shark conservation is critical for the thresher sharks. Shark products with high mercury concentration should be monitored by nonlethal sampling methods to reduce the exposure risk for human.

CRediT authorship contribution statement

Zezheng Li: Conceptualization, Methodology, Software, Investigation, Formal analysis, Writing – original draft. **Heidi R. Pethybridge:** Writing – review & editing. **Feng Wu:** Methodology, Formal analysis, Investigation. **Yunkai Li:** Conceptualization, Methodology, Funding acquisition, Resources, Supervision, Writing – review & editing.

Data availability

Data will be made available on request.

Declaration of competing interest

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

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Appendix A. Supplementary data

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