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The consumption of shark meat in the Amazon region and its implications for human health and the marine ecosystem

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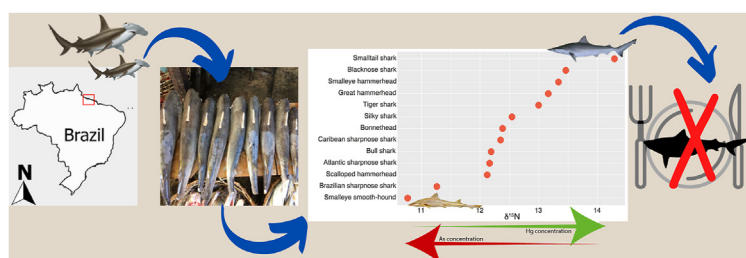
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HIGHLIGHTS

- Shark meat contained levels of arsenic and mercury unsafe for human consumption.
- Consumption of shark meat should be reduced to less than 416.39 g per day.
- Arsenic is biodiluted while mercury is biomagnified relative to $\delta^{15}\text{N}$ values.
- Consumption of shark meat is leading to the catch and sale of threatened species.

GRAPHICAL ABSTRACT



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ABSTRACT

Here, we evaluated the levels of As, Hg, Pb, and Cd in shark meat sold along the Amazon Coast of Brazil and used nitrogen stable isotope values to determine trophic position and to assess element biomagnification. From market samples, a total of 13 species were identified via molecular analysis, including those listed as endangered and vulnerable by the IUCN Red List. Arsenic was present in significantly higher concentrations than all other elements, followed by Hg, with the highest mean concentrations recorded in *M. higmani* (As: $19.46 \pm 8.79 \mu\text{g/g ww}$) and *C. acronotus* (Hg: $1.12 \pm 0.68 \mu\text{g/g ww}$). Lead and Cd were recorded at much lower levels in all species. The EWI of individual elements were above PTWI for all species when considering Hg, seven species for inorganic arsenic (iAs), and one species for Pb. The weekly consumption of 10 species should be reduced to less than 416.39 g, which is equivalent to the daily estimated fish consumption rate in the region. The mean ($\pm\text{SD}$) $\delta^{15}\text{N}$ values of species ranged from $10.7 \pm 0.51\text{‰}$ in *M. higmani* to $14.2 \pm 0.59\text{‰}$ in *C. porosus*, indicating feeding over >1 trophic level. Arsenic was negatively correlated with $\delta^{15}\text{N}$ values, while Hg was positively correlated indicating biodilution and biomagnification, respectively. Our results indicate that the sale and consumption of shark meat will expose consumers to potentially harmful levels of iAs and Hg, as well as contributing to the population decline of species including those that are currently categorized as threatened.

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Credit statement

Souza-Araujo, J and Giarrizzo, T, conceived of the presented idea. Souza-Araujo, J and Souza-Junior, O. G, made the field work. Guimarães-Costa, A performed the molecular analyses. Lima, M. O and Hussey, N, E verified the analytical methods. Giarrizzo, T, supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

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1. Introduction

Coupled with declines of shark populations associated with the fin trade (Fowler and Séret, 2010; Heithaus et al., 2010), the increasing use of shark meat as a food source is further impacting stocks worldwide (Taylor et al., 2014; Ong and Gan, 2016; McKinney et al., 2016). Brazil, where no specific licenses are required to catch sharks, is now among the top elasmobranch fishing nations, and could be considered to be the world's leading importer of shark meat (Barreto et al., 2015; Dent and Clarke, 2015; Dulvy et al., 2014). Since 2012, it is estimated that 8000 boats interacted in Brazilian fisheries targeting pelagic sharks, but this value is likely underestimated, since the number of illegal fishing vessels is unknown (Barreto et al., 2017).

Approximately 55 elasmobranch species targeted by Brazilian commercial offshore fisheries are listed under a threat category assigned by the IUCN: 19 species are listed as Vulnerable (VU), 8 as Endangered (EN), and 28 as Critically Endangered (CR) (ICMBio, 2016). A similar proportion (36%) of species are Data Deficient (DD). Globally, these species represent a quarter of the world's threatened sharks (Dulvy et al., 2014). Palmeira et al. (2013) for example, reported specimens of *Pristis perotteti*, a critically endangered sawfish, being sold in fish markets on the northern coast of Brazil, while Feitosa et al. (2018) used DNA sequences to identify that nine of seventeen species obtained from local fisheries were listed at risk on the IUCN Red list. In addition to directed shark fisheries, many species are also caught as bycatch, but this impact is largely unknown.

Aside from the ecological implications of shark fishing, removal and associated population declines, sharks are known to bioaccumulate high (and potentially harmful) concentrations of organohalogenated compounds and trace elements, through the process of biomagnification (Rumbold et al., 2014; Weijs et al., 2015). The ingestion of toxic metals [e.g., mercury (Hg), lead (Pb), and cadmium (Cd)] and metalloids [e.g., arsenic (As)] through the consumption of shark meat can have harmful effects on the human body, if frequently consumed in toxic quantities (Bosch et al., 2015; WHO, 2008; 2011a; 2011b). By measuring element concentrations in aquatic consumers and comparing these with national and international standards, risks to human health can be assessed (insert relevant ref). Concurrently, chemical tracers, such as the analysis of nitrogen stable isotopes ($\delta^{15}\text{N}$), provide a well-established technique to understand the flow of trace elements through food webs (Matulik et al., 2017). Through known fractionation at each trophic level, $\delta^{15}\text{N}$ values of consumers represent the assimilation of prey

resources across trophic levels (Fry, 2005) providing insight into diet and trophic relationships (Hussey et al., 2012, 2015) and allowing investigation of element biomagnification/biodilution dynamics (Endo et al., 2015; Huang, 2016).

While trace metals occur naturally in the environment, and may be introduced into marine ecosystems through a number of natural biogeochemical processes such as volcanic eruptions, sea-salt sprays, rock weathering, biogenic sources and wind-borne soil particles, the recent, ongoing increase in contamination levels is primarily attributed to urban and industrial effluents (Authman et al., 2015; Bosch et al., 2015). A range of anthropogenic pressures, including mining activities, deforestation, fires, and hydroelectric dams impact the Amazon basin and have raised concerns with regard to the release of metals into the region's rivers (Kasper et al., 2014; Patry et al., 2013; Scarpelli, 2005). This concern is based on the fact that the Amazon River discharges large volumes of water and sediments into the coastal region (Isaac and Ferrari, 2017), in which contaminants may become concentrated and then made available to high order consumers in the marine environment. For example, large amounts of total As (up to 95% in the non-toxic arsenobetane form in fish; Zhang et al., 2016), is transported from the Andes to the ocean via sediment and dissolved in water discharged from the Amazon river basin (Scarpelli, 2005). In addition, other nonessential elements such as Hg, Pb, Cd, Al, Ba, Tl, U (Souza-Araujo et al., 2016a, 2020), and even microplastics (Schmid et al., 2018), are recorded in other marine species from this region.

To address the knowledge gap over the levels of trace elements and element biomagnification in aquatic species in the Amazon coastal region, the present study aimed to (i) evaluate the concentration of As, Hg, Pb, and Cd in shark meat sold at the principal fish markets of the Amazon Coast, northern Brazil relative to international standards for human consumption and (ii) examine the relative trophic position of each species using nitrogen stable isotope ($\delta^{15}\text{N}$) data and determine the degree of biomagnification of trace elements using combined element concentrations and $\delta^{15}\text{N}$ values. Sharks are considered to play a significant role in structuring food webs (Ferretti et al., 2010; Heithaus et al., 2008), although fishing and the shark fin trade have impacted the conservation status of global populations (Dent and Clarke, 2015). In northern Brazil, the number of studies on elasmobranchs, in particular those focused on sharks, is limited, with most research restricted to the more developed regions in the south of the country (Barreto et al., 2015; Bornatowski et al., 2013). Along the northern coast of Brazil, an extremely productive region influenced by the Amazon estuary, these data provide the first measures of element concentrations and $\delta^{15}\text{N}$ -elements dynamics for 13 species of shark, including data on a large number of juveniles.

2. Material and methods

2.1. Study area

A total of 91 sharks were sampled at fish markets at five ports located along the Amazon Coast. This region is part of the Amazon Continental Shelf, which is known to be one of the world's most productive ecosystems, but is subject to overfishing, pollution, and rising ocean temperatures (Isaac and Ferrari, 2017). The region encompasses the largest continuous tracts of mangrove forest in the world, which cover an area of 8900 km² (Kjerfve and Lacerda, 1993). The Amazon rainforest biome covers more than 4.2×10^6 km² (Bernardes et al., 2012), and is located within the drainage basins of the Amazon, Orinoco, and other smaller rivers (Fig. 1).

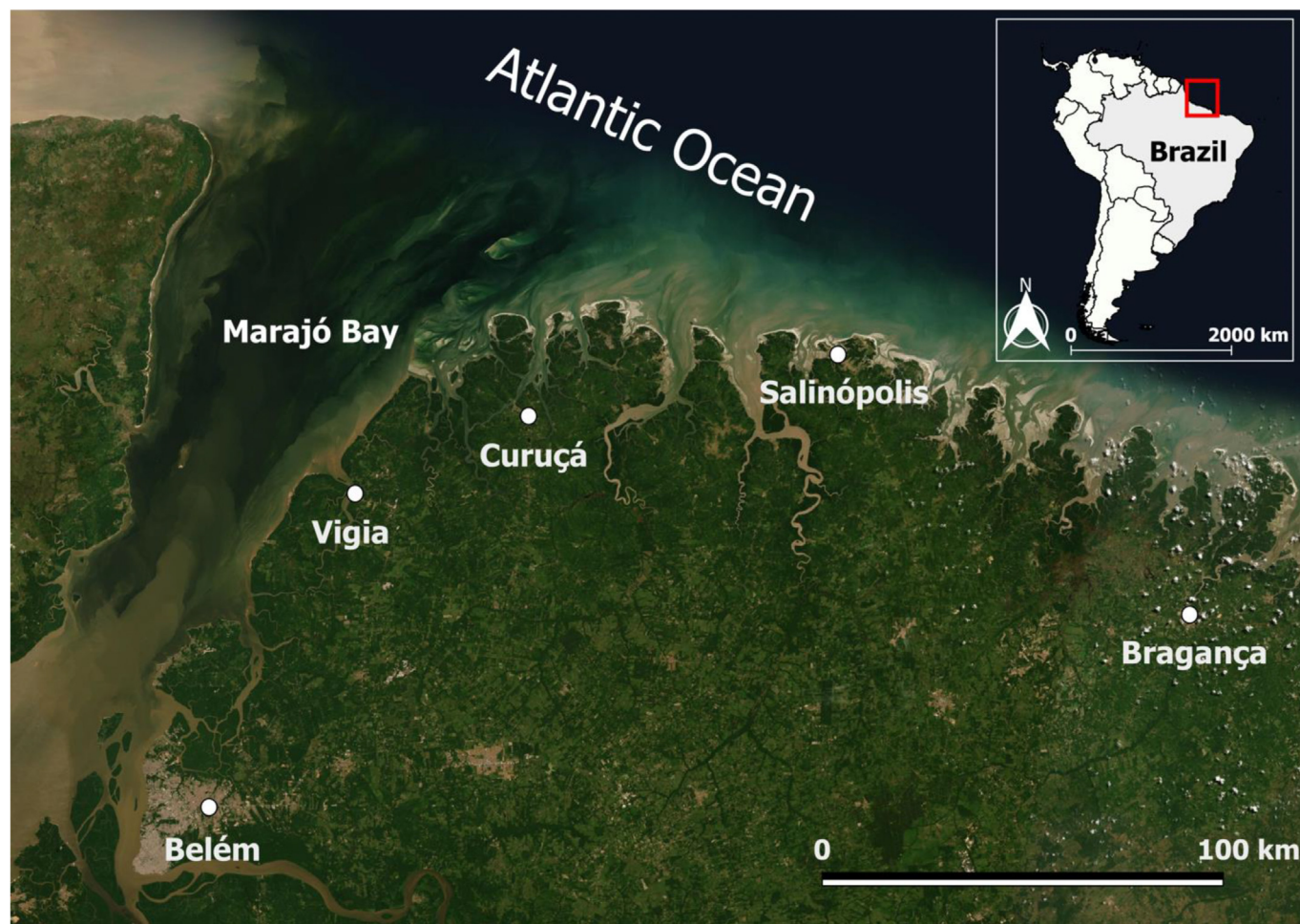


Fig. 1. The Pará coast, in the Amazon Coastal region, including the five most representative landing points for shark meat where samples were taken (white circles).

2.2. Sampling

Muscle tissue samples taken from the dorsal surface of sharks, known locally as “cação”, were obtained from individuals on display for sale in local markets in August of 2017. Samples ($n = 91$; mass = ~20 g) were placed in individual polyethylene bags on ice, transported back to the laboratory and kept frozen ($-20\text{ }^{\circ}\text{C}$) until elemental/isotope analysis. A tissue subsample was also reserved for species identification through genetic analysis. Total body length was not recorded because sharks were without head and fins, but most body trunks were less than 100 cm.

2.3. Species identification

To first identify species, total genomic DNA was extracted from muscle tissue using a Wizard Genomic DNA Purification Kit (Promega Corporation, Madison, WI – USA) following the manufacturer’s protocol. A fragment of the Cytochrome C Oxidase I gene (COI), standardized as DNA Barcoding, was amplified using the primers: COI 5’TCAACCAACCACAAAGACATTGGCAC3’ and COI 5’TAGACTTCTGGGTGGCCAAAGAATCA 3’ (Ward et al., 2005). The samples were amplified to a final volume of 25 μL , containing 4 μL of DNTP (1.25 mM), 2.5 μL of 10X buffer solution, 1 μL of MgCl_2 (25 mM), 0.25 μL of each primer (200 ng/ μL), 1–1.5 μL of genomic DNA (100 ng/ μL), 1 U of Taq DNA polymerase (5 U/ μL), and deionized water to complete the final reaction volume. The Polymerase Chain

Reactions (PCRs) were run in a thermocycler (Applied Biosystems) under the following thermal protocol: initial denaturation at $93\text{ }^{\circ}\text{C}$ for 3 min; 35 cycles of denaturation at $94\text{ }^{\circ}\text{C}$ for 30 s, annealing (at temperatures of $50\text{--}60\text{ }^{\circ}\text{C}$, depending on the species) for 45 s, and extension at $72\text{ }^{\circ}\text{C}$ for 45 s, with a final extension of 5 min at $72\text{ }^{\circ}\text{C}$. All positive reactions were sequenced in an ABI 3500 automatic sequencer (Applied Biosystems). Following DNA sequencing barcoding, each sample was identified to species level by cross referencing with those held in the following public databases: GenBank (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) and BoldSystems V4 (<http://www.boldsystems.org>).

2.4. Trace elements analysis

Concentrations of the trace elements As, Hg, Pb, and Cd in shark muscle tissue samples were determined by Induced Plasma Coupled Mass Spectrometry (ICP-MS). Muscle tissue was first homogenized with surgical scissors or a PTFE stick, and an aliquot of 0.1 g (wet weigh) of tissue was placed in a PTFE bottle with 1.5 ml of HNO_3 (65% PA). After 30 min, 0.5 ml of H_2O_2 was added and samples were heated in a microwave oven (MarsXpress, CEM Corporation) along a temperature ramp (1st step: 800 W, $180\text{ }^{\circ}\text{C}$, 10 min; 2nd step: 1200 W, $200\text{ }^{\circ}\text{C}$, 5 min; 3rd step: 1000 W, $100\text{ }^{\circ}\text{C}$, 10 min) and then cooled for 20 min in a cold bath. The digested solutions were then transferred to polyethylene bottles, which were topped up to 15 ml with HNO_3 (1%), and stored at $4\text{ }^{\circ}\text{C}$ until analysis by ICP-

MS. For quality control, a blank sample and a DORM-3 (0.05 g dw) Certified Reference Material (National Research Council, Canada) were analyzed every 30 samples, and triplicates run every 15 samples. The percentage recovery of DORM-3, DL and LOQ of the analytical method for each element are reported in Supplementary Material File 1.

2.5. Health risk assumption

An assessment of the human health risk posed from consuming shark meat with respect to trace element concentrations recorded was estimated using the following equations:

2.5.1. Estimated weekly intake (EWI)

$$EWI = \frac{te \times \text{weekly consumption of fish}}{\text{body weight}}$$

Where EWI ($\mu\text{g}/\text{kg}_{\text{bw}}/\text{week}$) is the estimated weekly intake; *te* is the mean trace element concentration recorded per species ($\mu\text{g}/\text{g}$ ww); weekly consumption of fish (g/week) is the average consumed [here a value of 2914.73 g was used according to Isaac et al. (2015)] and body weight is that for an average adult (a value of 70 kg was used). For As, USEPA (2000) suggests using the uptake of inorganic As (iAs) rather than total exposure to As for assessment of human health risks. For As, it was estimated that 10% of total As was iAs (USFDA, 1993). The obtained EWI values were compared with the Provisional Tolerable Weekly Intake (PTWI) values determined by the Joint Food and Agriculture Organization Expert Committee of Food Additives (JECFA, 2019). There are no recommended JECFA PTWI values for Pb, however the European Food Safety Authority (EFSA, 2010) states a value of 25 $\mu\text{g}/\text{kg}_{\text{bw}}/\text{week}$ as a regulatory PTWI guideline for the dietary intake of Pb.

2.5.2. Maximum amount of shark meat (MAS)

$$MAS = \frac{PTWI \times \text{body weight}}{te}$$

Where MAS is the Maximum Amount of Shark meat (g) that should be consumed per week to remain within the limits of the Provisional Tolerable Weekly Intake (PTWI).

2.6. Stable isotope analysis

To determine nitrogen stable isotope values ($\delta^{15}\text{N}$), samples were dried at 60 °C for 24 h, ground and homogenized to a fine powder using a porcelain mortar and pestle. Lipids were extracted by vortexing the homogenized powder in a cryovial with 1.9 ml of chloroform-methanol solution (1:2) for 1 min. Cryovials were then placed in a water bath at 30 °C for at least 24 h, after which, they were centrifuged for 4–6 min and solvent was filtered. New chloroform-methanol solution was then added, and the samples were shaken for 1 min and centrifuged once again for 4–6 min. The resulting filtrate was left under a fume hood for 24–48 h to evaporate the remaining solvent (Hussey et al., 2012). Following lipid extraction, urea was removed by shaking the resultant powdered tissue in a cryovial with 1.9 ml of deionized water for 1 min. Vials were then placed in a water bath at 30 °C for 24 h, after which, they were centrifuged for 4–6 min and water was extracted using a medical syringe. This process was repeated three times, and the samples once again dried. Approximately 710–890 μg of lipid and urea extracted muscle tissue for each sample was weighed and

compressed into 5 mm × 3.5 mm tin capsules. Nitrogen stable isotope values were then determined by combustion in a Continuous Flow Isotope Ratio Mass Spectrometer (IR-MS, Finnigan MAT Deltaplus, Thermo Finnigan, San Jose, CA, USA) equipped with an elemental analyzer (Costech, Valenica, CA, USA). The isotope values are expressed in delta notation (δ) and defined as parts per thousand (‰) in relation to a standard sample, as follows:

$$\delta X = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 1000$$

where R_{sample} and R_{standard} correspond to the stable isotope values ($^{15}\text{N}/^{14}\text{N}$) in the test and standard samples, respectively. Analytical precision was assessed by the standard deviation of the replicate analyses of four standards: NIST1577c, internal lab standard (tilapia muscle), USGS 40, and Urea ($n = 68$ in all cases), with $\delta^{15}\text{N} \leq 0.18\text{‰}$. Accuracy, based on the certified values of USGS 40 ($n = 68$) analyzed throughout runs and not used to normalize samples showed a difference of -0.05‰ for $\delta^{15}\text{N}$ from the certified value. Instrumentation accuracy was checked throughout the study period, based on NIST standards 8573, 8547, and 8574 ($n = 20$ for each). The mean $\delta^{15}\text{N}$ differences from the certified values were -0.17 , -0.10 and -0.14‰ , respectively.

2.7. Statistical analyses

To examine differences in the concentrations of trace elements among species, a univariate PERMANOVA based on Euclidean distances matrices with 9999 permutations and including the Monte Carlo correction for small sample size was conducted (Anderson, 2001). Only species with $n \geq 3$ individuals were used in the PERMANOVA. To assess biomagnification profiles of each trace element, the relationship between log transformed element concentrations (As, Hg, Pb and Cd) and $\delta^{15}\text{N}$ values was evaluated using Pearson's correlation coefficients. All analyses were conducted in Rstudio (Version 1.1.383) and PERMANOVA+ in the PRIMER-E software (Anderson et al., 2008).

3. Results

Of the 91 shark muscle samples collected from fish markets along the Amazon coast, DNA barcoding identified 13 species belonging to three families (Carcharhinidae, Sphyrnidae and Triakidae). Of these, two species are listed as endangered (EN), three are near threatened (NT), two are vulnerable (VU), four are least concern (LC), and two are data deficient, DD according to the IUCN RedList (Table 1).

The concentrations of trace elements in muscle tissue samples were highly variable across species (Pseudo-F = 128.9 $p < 0.001$) (Table 1), with significantly higher overall levels of As when compared with the other three elements (Hg: $t = 11.1$ $p < 0.001$; Pb: $t = 11.4$ $p < 0.001$; Cd: $t = 11.4$ $p < 0.001$). Recorded As concentrations were above the safe limits of 0.5 $\mu\text{g}/\text{g}$ for human consumption determined by WHO (2011a). The mean As (\pm SD) of the 91 samples combined was 12.1 ± 10.1 $\mu\text{g}/\text{g}$ (Table 1), with the highest concentration of As (42 $\mu\text{g}/\text{g}$) recorded in a sample of an individual *Mustelus higmani* (Fig. 2A). Among species, the highest mean (\pm SD) As concentration (19.46 ± 8.79 $\mu\text{g}/\text{g}$) was also recorded for *Mustelus higmani*, followed by *Carcharhinus leucas* (15.26 ± 15.80 $\mu\text{g}/\text{g}$), *Sphyrna tiburo* (10.61 ± 1.93 $\mu\text{g}/\text{g}$), *Sphyrna lewini* (9.77 ± 11.50 $\mu\text{g}/\text{g}$), and *Rhizoprionodon porosus* (9.58 ± 5.27 $\mu\text{g}/\text{g}$) (Fig. 2A).

Mercury concentrations were significantly higher than those recorded for Pb ($t = 5.7$ $p < 0.001$) and Cd ($t = 6.0$ $p < 0.001$) across all species, and exceeded the WHO recommendation of 0.5 $\mu\text{g}/\text{g}$

Table 1
Species identified and analyzed in the present study, IUCN category (EN = Endangered; VU = Vulnerable; NT = Near Threatened; LC = Least Concern; DD = Data Deficient), number of samples (N), mean ($\mu\text{g/g}$) and standard deviation (SD) of $\delta^{15}\text{N}$ values (‰), and the trace element concentrations recorded in 91 samples of shark meat obtained from markets along the Amazon Coastal region in 2017.

Species	Common name	IUCN	N	$\delta^{15}\text{N}$ (‰)		As ($\mu\text{g/g}$)		Hg ($\mu\text{g/g}$)		Pb ($\mu\text{g/g}$)		Cd ($\mu\text{g/g}$)	
				Mean \pm SD	Min - Max	Mean \pm SD	Min - Max	Mean \pm SD	Min - Max	Mean \pm SD	Min - Max	Mean \pm SD	Min - Max
All combined	Cação	-	91	-	-	12.1 \pm 10.1	0.43–42.98	0.34 \pm 0.52	<0.01–2.76	0.07 \pm 0.34	<0.001–3.11	0.03 \pm 0.17	<0.001–1.60
<i>Sphyrna lewini</i>	Scalloped hammerhead	EN	2	-	10.4–13.7	-	1.63–17.90	-	<0.01–0.1	-	<0.001–0.01	-	<0.001
<i>Sphyrna mokarran</i>	Great hammerhead	EN	3	13.16 \pm 0.54	12.5–13.5	5.13 \pm 4.09	1.81–9.70	0.32 \pm 0.16	0.18–0.50	0.04 \pm 0.03	0.01–0.07	0.01 \pm 0.007	<0.001–0.01
<i>Carcharhinus falciformis</i>	Silky shark	VU	2	-	11.7–13.2	-	0.78–4.66	-	0.12–1.59	-	0.01–0.02	-	<0.001–0.01
<i>Sphyrna tudes</i>	Smalleye hammerhead	VU	2	-	12.7–13.9	-	1.49–2.74	-	1.20–2.25	-	0.02–0.03	-	0.012
<i>Carcharhinus acronotus</i>	Blacknose shark	NT	5	13.45 \pm 2.06	9.8–14.7	3.01 \pm 4.32	0.60–10.70	1.12 \pm 0.68	0.08–1.67	0.64 \pm 1.37	0.01–3.10	0.011 \pm 0.006	<0.001–0.01
<i>Carcharhinus leucas</i>	Bull shark	NT	4	12.18 \pm 2.03	10.3–14.0	15.26 \pm 15.80	0.43–37.29	0.41 \pm 0.59	0.02–1.30	0.32 \pm 0.53	0.02–1.12	0.008 \pm 0.005	<0.001–0.01
<i>Galeocerdo cuvier</i>	Tiger shark	NT	1	12.99	-	1.74	-	0.45	-	0.02	-	0.012	-
<i>Mustelus higmani</i>	Smalleye smooth-hound shark	LC	40	10.76 \pm 0.51	9.8–11.4	19.46 \pm 8.79	6.19–42.98	0.13 \pm 0.11	<0.01–0.42	0.03 \pm 0.03	<0.001–0.1	0.05 \pm 0.2	<0.001–1.59
<i>Rhizoprionodon porosus</i>	Caribbean sharpnose shark	LC	5	12.34 \pm 1.35	10.0–13.4	9.58 \pm 5.27	2.19–15.39	0.37 \pm 0.66	0.01–1.55	0.01 \pm 0.01	<0.01–0.022	0.011 \pm 0.01	<0.001–0.02
<i>Rhizoprionodon terraenovae</i>	Atlantic sharpnose shark	LC	5	12.16 \pm 0.34	11.8–12.6	5.96 \pm 3.13	1.61–9.30	0.60 \pm 0.36	0.04–1.05	0.04 \pm 0.01	0.025–0.06	0.017 \pm 0.01	0.01–0.02
<i>Sphyrna tiburo</i>	Bonnethead	LC	10	12.38 \pm 0.49	11.8–13.5	10.61 \pm 1.93	7.01–12.95	0.09 \pm 0.05	0.02–0.22	<0.01 \pm 0.002	<0.001–0.006	0.002 \pm 0.005	<0.001–0.01
<i>Carcharhinus porosus</i>	Smalltail shark	DD	10	14.29 \pm 0.59	13.4–14.9	1.02 \pm 0.67	0.50–2.81	0.49 \pm 0.82	0.10–2.75	0.02 \pm 0.00	0.02–0.04	0.012 \pm 0.003	<0.001–0.02
<i>Rhizoprionodon lalandii</i>	Brazilian sharpnose shark	DD	2	-	11.2–11.4	-	1.51–4.94	-	0.09–0.70	-	0.01–0.03	-	<0.001–0.02

(WHO, 1990; 2008) in nine of 13 species (Table 1). Mean (\pm SD) Hg concentrations ranged from $0.07 \pm 0.10 \mu\text{g/g}$ in *S. phyrna lewini* to $1.72 \pm 0.74 \mu\text{g/g}$ in *Sphyrna tudes* with the highest Hg value ($2.75 \mu\text{g/g}$) recorded for an individual *Carcharhinus porosus* (Fig. 2B). The mean (\pm SD) Hg of the 91 combined samples was $0.34 \pm 0.52 \mu\text{g/g}$ (Table 1). Lead and Cd were recorded at much lower concentrations in all species. Mean Pb concentrations ranged from 0.0007 ± 0.002 in *Sphyrna tiburo* to $0.64 \pm 1.37 \mu\text{g/g}$ in *Carcharhinus acronotus*; the maximum value recorded of $3.10 \mu\text{g/g}$ was for an individual of the latter species (Fig. 2C) and the mean (\pm SD) of the 91 combined samples was $0.07 \pm 0.34 \mu\text{g/g}$ (Table 1). *Sphyrna tiburo* had the lowest mean Cd value (0.002 ± 0.005) while *Mustelus higmani* had the highest mean value ($0.05 \pm 0.25 \mu\text{g/g}$), with the maximum concentration recorded ($1.59 \mu\text{g/g}$) (Fig. 2D). The mean (\pm SD) Cd of the 91 combined samples was $0.03 \pm 0.17 \mu\text{g/g}$ (Table 1).

According to EWI (Table 2), the intake of iAs would exceed the PTWI ($15 \mu\text{g/kg}_{\text{bw/week}}$) for both combined samples ($\text{EWI} = 504.18 \mu\text{g/kg}_{\text{bw/week}}$) and for seven of the species examined, with values ranging from 7.28 to $81.06 \mu\text{g/kg}_{\text{bw/week}}$. For Hg, EWI exceeded the PTWI ($1.6 \mu\text{g/kg}_{\text{bw/week}}$) for combined samples ($14.22 \mu\text{g/kg}_{\text{bw/week}}$) and for all species; with values ranging between 3.30 and $71.94 \mu\text{g/kg}_{\text{bw/week}}$. The intake of Pb when considering combined samples did not exceed the EFSA (2010) guideline ($25 \mu\text{g/kg}_{\text{bw/week}}$) with only one species, *Carcharhinus acronotus*, having an EWI of $3.81 \mu\text{g/kg}_{\text{bw/week}}$. The EWI of Pb for the remaining 12 species ranging between 0.03 and $13.48 \mu\text{g/kg}_{\text{bw/week}}$. Neither combined samples or individual species exceed the Cd PTWI ($5.81 \mu\text{g/kg}_{\text{bw/week}}$). According to the estimated MAS values for the ingestion of a single trace element, the consumption of at least 10 species should be reduced to stay within the limits of the respective PTWI, with the exception of Cd (Table 2). However, based on the joint analysis of the ingestion of combinations of the four elements in each species – general MAS (the lowest MAS value in each specie), the weekly consumption of *Sphyrna tudes*, *Carcharhinus acronotus*, *Carcharhinus falciformis*, *Rhizoprionodon terraenovae*, *Carcharhinus porosus*, *Galeocerdo cuvier*, *Carcharhinus leucas*, *Rhizoprionodon lalandii*, *Rhizoprionodon porosus* and *Sphyrna mokarran* should be reduced to less than 416.39 g , which is equivalent to the daily estimated fish consumption rate in the region (Fig. 3).

Across species, mean (\pm SD) $\delta^{15}\text{N}$ values ranged from $10.7 \pm 0.51\text{‰}$ in *M. higmani* to $14.2 \pm 0.59\text{‰}$ in *C. porosus* (Table 1; Fig. 4) with significant differences observed among the eight species analyzed (Pseudo-F = 25.65; $p < 0.001$; see Supplementary Material). Pairwise tests revealed that *Carcharhinus acronotus* $\delta^{15}\text{N}$ values were only significantly different to *Mustelus higmani* while *M. higmani* $\delta^{15}\text{N}$ values were significantly different from all seven species tested (see Supplementary Material File 2). When considering $\delta^{15}\text{N}$ as an absolute measure of trophic position, large variation was observed among the smaller bodied shark complex, while *C. leucas* exhibited the largest intra-species variation (Fig. 4). Arsenic was negatively correlated ($r = -0.79$; $p < 0.001$) with $\delta^{15}\text{N}$ (Table 3) suggesting biodilution with increasing trophic position, whereas Hg was positively correlated ($r = 0.48$; $p < 0.001$) indicating biomagnification. On average, a 1‰ enrichment of $\delta^{15}\text{N}$ was associated with an As reduction of approximately $4.71 \mu\text{g/g}$ ($y = -4.7126x + 68.412$) (Fig. 5A), and a $0.15 \mu\text{g/g}$ increase in Hg ($y = 0.1503x - 1.4542$) (Fig. 5B). No systematic relationships were identified between Pb and Cd concentrations and nitrogen stable isotope values.

4. Discussion

4.1. Trace element concentrations in shark muscle tissue

Arsenic was recorded at the highest concentrations in all shark

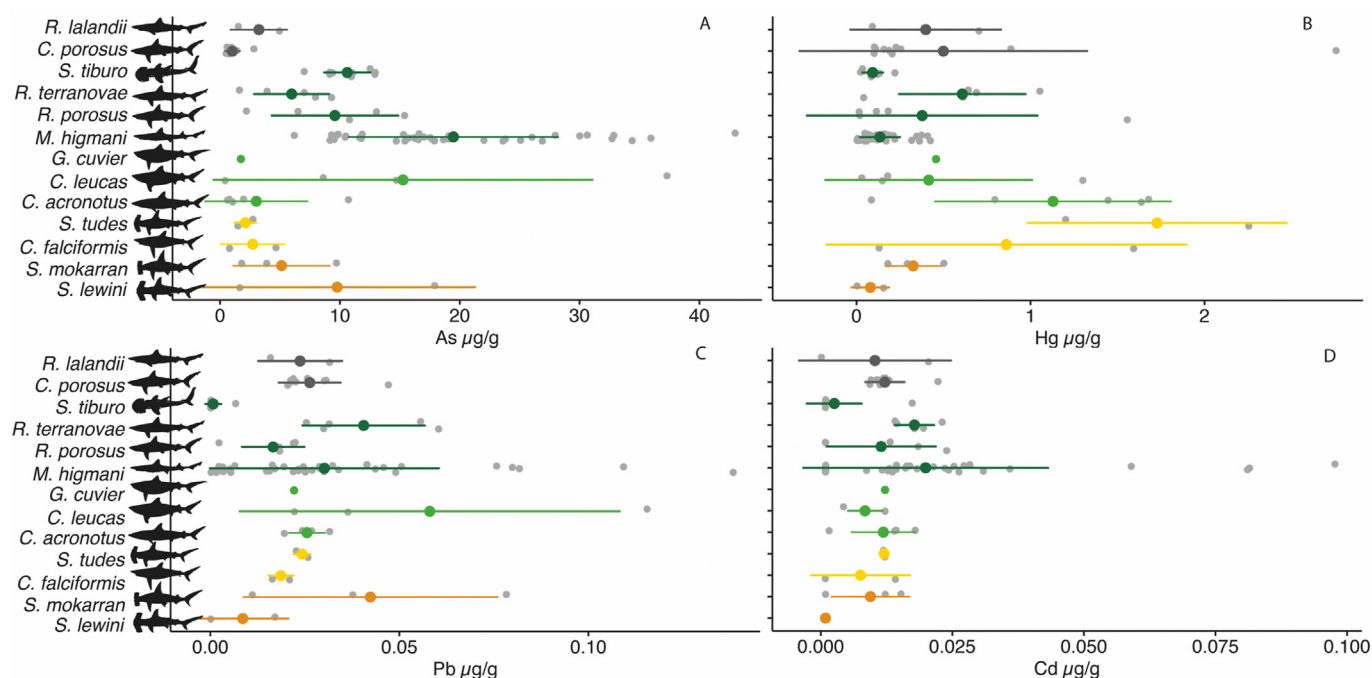


Fig. 2. Trace element concentrations recorded in 91 samples of shark meat obtained from fish markets along the Brazilian Amazon Coast in 2017: (A) arsenic [As]; (B) mercury [Hg], (C) lead [Pb] and; (D) cadmium [Cd]. The grey circles represent element concentrations in individual samples, the central circles are the mean for each species, and the horizontal lines represent the standard deviation.

Table 2

Estimated Weekly Intake [EWI ($\mu\text{g}/\text{kg}_{\text{bw}}/\text{week}$)] of trace elements in all samples combined, in the 13 shark species identified from markets along the Amazon Coastal region in 2017, and the maximum amount of shark meat [MAS(g)] that can be consumed per species to remain within the limits of the Provisional Tolerable Weekly Intake (PTWI).

Species	iAs		Hg		Pb		Cd	
	EWI ($\mu\text{g}/\text{kg}_{\text{bw}}/\text{week}$)	MAS (g)	EWI ($\mu\text{g}/\text{kg}_{\text{bw}}/\text{week}$)	MAS (g)	EWI ($\mu\text{g}/\text{kg}_{\text{bw}}/\text{week}$)	MAS (g)	EWI ($\mu\text{g}/\text{kg}_{\text{bw}}/\text{week}$)	MAS (g)
All combined	504.18 ^a	86.72 ^c	14.22 ^a	327.88 ^c	3.00	24255.95	1.31	12930.96
<i>Carcharhinus acronotus</i>	12.56	3481.11	47.00 ^a	99.23 ^c	26.71 ^a	2727.73 ^b	0.49	34351.85
<i>Carcharhinus falciformis</i>	11.35	3852.50	35.84 ^a	130.10 ^c	0.78	93582.89	0.31	54052.98
<i>Carcharhinus leucas</i>	63.55 ^a	688.01 ^b	17.27 ^a	269.96 ^c	13.48	5404.57	0.35	48439.17
<i>Carcharhinus porosus</i>	11.73	3728.56	20.76 ^a	224.59 ^c	1.09	66590.56	0.51	33505.75
<i>Galeocerdo cuvier</i>	7.28	6008.93	19.00 ^a	245.45 ^c	0.92	79185.52	0.51	33450.82
<i>Mustelus higmani</i>	81.06 ^a	539.38 ^b	5.60 ^a	832.64 ^b	1.26	58028.68	2.47	6878.19
<i>Rhizoprionodon lalandii</i>	13.45	3250.52	16.55 ^a	281.87 ^c	0.99	73684.21	0.43	39621.36
<i>Rhizoprionodon porosus</i>	39.93 ^a	1095.01 ^b	15.71 ^a	296.86 ^c	0.69	105294.83	0.48	35548.78
<i>Rhizoprionodon terraenovae</i>	24.85 ^a	1759.58 ^b	25.31 ^a	184.27 ^c	1.69	43167.24	0.74	22952.76
<i>Sphyrna lewini</i>	40.69 ^a	1074.42 ^b	3.30 ^a	1412.36 ^b	0.36	203488.37	0.04	453444.44
<i>Sphyrna mokarran</i>	21.37 ^a	2045.53 ^b	13.53 ^a	344.79 ^c	1.76	41306.06	0.39	43261.48
<i>Sphyrna tiburo</i>	44.20 ^a	989.21 ^b	3.86 ^a	1206.90 ^b	0.03	2272727.27	0.11	160039.22
<i>Sphyrna tudes</i>	8.82	4955.64	71.94 ^a	64.83 ^c	1.01	72164.95	0.50	33867.22
PTWI	15 ^c		1.6 ^c		25 ^e		5.81 ^d	

^a Higher than PTWI.

^b Lower than the average weekly consumption rate (2914.73 g).

^c Lower than the average daily consumption rate (416.34 g).

^d JECFA, 2019.

^e EFSA, 2010.

species caught along the Amazon coast, with values reported here similar to or higher than those reported for species from other global regions (South Africa: $28.31 \pm 18.79 \mu\text{g}/\text{g}$ – Bosch et al., 2015; Trinidad and Tobago: $0.13\text{--}0.15 \mu\text{g}/\text{g}$ – Mohammed and Mohammed, 2017). We note here that these values could be underestimated given our analytical recovery of As and Cd were 79% and 77% respectively (Supplementary Material File 1). Despite analyzing total As, our results identify that the discharge of arsenic via sediments transported from the Andes through the Amazon basin interferes with the accumulation of As in marine species that

occur on the Amazon coast. Annually, the coastal region at the mouth of the Amazon River receives approximately 5 tons of As via sediments discharged by the river (Scarpelli, 2005), due to the geological features present, but also as a result of seasonal effects and certain anthropogenic activities in the Andes region (Bundschuh et al., 2012; Tapia et al., 2019). Marine organisms inhabiting the Amazon coast may act as important ecological filters of As sources, metabolizing and mobilizing the element within the coastal food web (Huang, 2016).

Mustelus higmani and *C. leucas* had the highest recorded

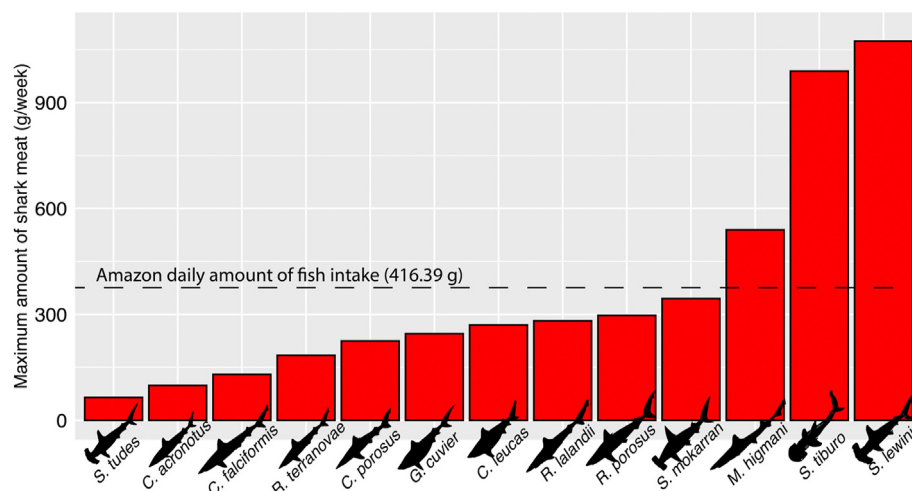


Fig. 3. Estimated maximum amount of shark meat (MAS) that can be consumed per species without exceeding the lowest Provisional Tolerable Weekly Intake (PTWI) among the elements As, Hg, Pb and Cd measured in samples collected in 2017. The dotted line represents the daily amount of fish typically consumed in the Amazon coastal region.

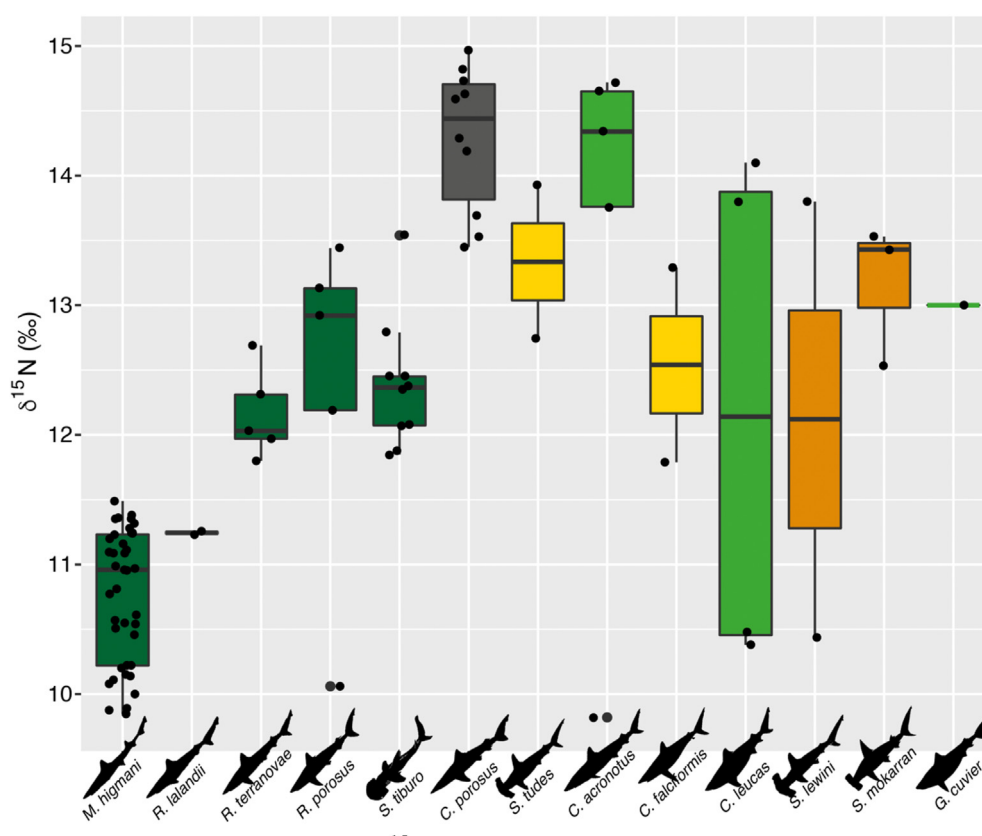


Fig. 4. Box plots of the $\delta^{15}\text{N}$ values recorded in 91 samples of shark meat comprising 13 individual species obtained from fish markets along the Amazon Coastal region in 2017. The central horizontal line is the mean $\delta^{15}\text{N}$ value for each shark species, while the boxes contain 50% of the data and the vertical lines correspond to the 95% confidence intervals. Colors represent conservation status of the species (see Table 1) and species are ordered by relative body size. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

concentrations of As, but appeared to feed at the lowest trophic level of all examined species, based on their $\delta^{15}\text{N}$ values. Unlike contaminants such as MeHg, which is known to biomagnify through the food web, the trophodynamics of As is poorly understood. In a review of published data, Huang (2016) concluded that As tends to be biodiluted in coastal systems, whereby, predators typically have lower concentrations than primary and secondary

consumers (Meador et al., 2004; Vizzini et al., 2013). Factors such as food habit or dietary preference may have influenced the accumulation of As in *M. higmani*; this species feeds primarily on lower trophic level crustaceans (Tagliafico et al., 2015). For *C. leucas*, the high As concentrations may relate to proximity to the source given this species commonly occurs close to the Amazon river mouth, and parturition and residency of young occurs in estuaries and rivers

Table 3

Pearson correlation coefficients for the relationship between trace element concentrations (As, Hg, Pb and Cd) and $\delta^{15}\text{N}$ values for the 91 samples of shark muscle obtained from fish markets along the Amazon Coastal region in 2017.

Element	$\delta^{15}\text{N}$	
	r	P
As	-0.79	<0.001
Hg	0.48	<0.001
Pb	-0.17	0.09
Cd	-0.09	0.37

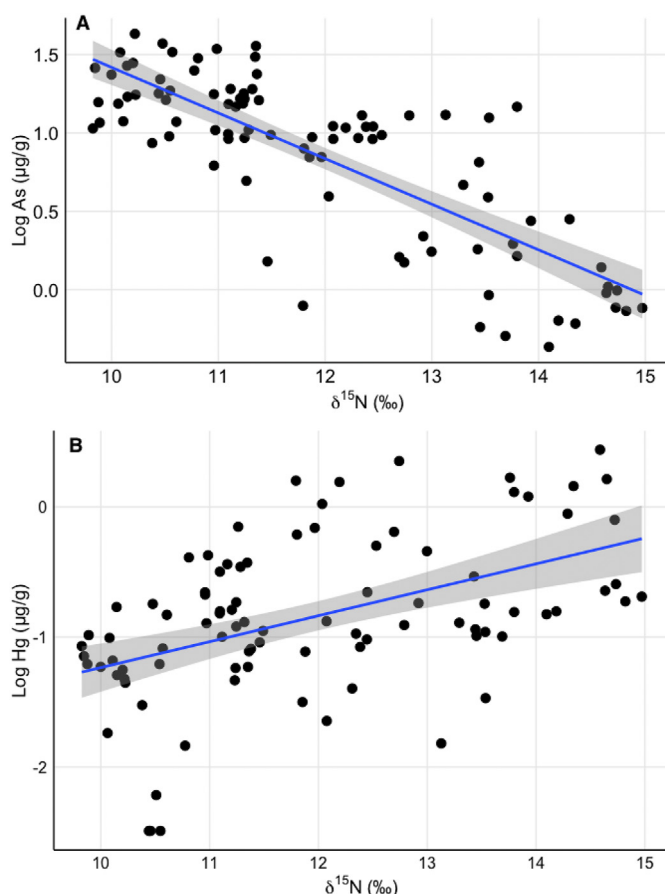


Fig. 5. Relationship between log transformed As (A) and Hg (B) concentrations [$\mu\text{g/g}$ (wet weight)] and $\delta^{15}\text{N}$ values (‰) recorded in 91 samples of shark meat obtained from fish markets along the Amazon Coastal region in 2017. Statistically significant correlations are presented (for the r and p values, see Table 3).

(Compagno et al., 2005; Pillans et al., 2006). As is largely found in the Amazon river (Scarpelli, 2005) supporting this point.

While Hg was the second most abundant element recorded across all species, the concentrations reported here are lower than those found in Kuwait ($4.37 \pm 3.31 \mu\text{g/g}$; Moore et al., 2015), Japan ($1.32 \mu\text{g/g}$; Endo et al., 2015) and Korea ($0.1\text{--}7 \mu\text{g/g}$; Kim et al., 2016). Nevertheless, Hg concentrations recorded were up to four times higher than the recommended limit established by the WHO for human consumption (WHO, 2008). Hg concentrations may reflect the life-stage examined and present in the region. Smaller individuals are gape limited and consequently feed on lower trophic level secondary consumers and small tertiary consumers (Lucifora et al., 2009). In contrast to As, there was a positive relationship between Hg and $\delta^{15}\text{N}$ values across species. This relationship identifies biomagnification whereby species feeding at a

higher trophic level had higher levels of Hg (Matulik et al., 2017). Similarly to our results, biomagnification of Hg has also been reported at the species level for *Carcharhinus leucas* and *Carcharhinus acronotus* from Florida Bay (Matulik et al., 2017), and *Carcharhinus leucas* and *Sphyrna lewini* from the southwestern Indian Ocean (Le Bourg et al., 2014).

The levels of Pb and Cd recorded were the lowest among elements analyzed in the present study, but fewer data are available on Cd and Pb concentrations in shark muscle tissue for comparison (Mohammed and Mohammed, 2017). Pb concentrations in the majority of species analyzed were lower than those reported for sharks sampled in Malaysia ($0.11 \pm 0.02\text{--}0.43 \pm 0.32$; Ong and Gan, 2016) and the Persian Gulf ($0.10 \pm 0.03\text{--}0.13 \pm 0.04$; Adel et al., 2016). Only *C. acronotus* and *C. leucas* had Pb concentrations that were higher than those reported for these locations. Similarly, Cd concentrations were lower than those reported in Japan ($0.03\text{--}7.59 \text{ ng/g}$; Endo et al., 2015) and South Africa ($0.04 \pm 0.02 \mu\text{g/g}$; Bosch et al., 2016).

4.2. Risk assessment of shark meat consumption

While most shark meat available for sale along the Amazon coast is derived from small bodied species, or juveniles of larger species, the estimated EWIs of iAs, Hg and Pb were up to 10-times higher than the Provisional Tolerable Weekly Intake (PTWI). This suggests regular consumption of shark meat poses a risk to human health (IRIS, 2019). Excluding occupational exposure, the primary route of trace element exposure in humans is through the consumption of contaminated drinking water and food, in particular fish and shellfish (ATSDR, 1999; 2007; Clarkson et al., 2007).

In the marine environment the major forms of As in seafood, namely arsenobetaine and arsenosugars, are considered nontoxic (Francesconi, 2010), with only 1–5% consisting of the iAs form, which is highly carcinogenic (Juncos et al., 2019; Peshut et al., 2008). We emphasize, however, that our EWI estimate is based on expected iAs (10% of the total As), following the recommendations of the USEPA (2000) to use the uptake of inorganic As rather than total As for the assessment of human health risk. Recent studies on speciation of As in marine fish have found that iAs can range from 0.6% to as much as 5% for elasmobranchs (Gao et al., 2018; Schmid et al., 2018), identifying the USEPA 10% approach is conservative. While consumption of all species would lead to high As exposure, the iAs value was higher than the PTWI when considering seven species, with the estimated iAs intake per 2914.73 g serving ranging between $21.37 \mu\text{g/kg}$ (*S. mokarran*) and $81.06 \mu\text{g/kg}$ (*M. higmani*) of body weight. Following the recommendations of the World Health Organization (WHO; 2011a) that the PTWI for As of $15 \mu\text{g/kg}_{\text{bw}}/\text{week}$ is no longer a relevant cut off for measuring health risk and that the intake of iAs reported here is higher than this value in more than half of the species analyzed, we suggest that there is an imminent risk of exposure to iAs from the consumption of shark meat along the Amazon coast.

Of the four elements analyzed, estimated EWI values for Hg were higher than PTWI for all species. Unlike total As, total Hg (THg) concentrations in fish muscle can be used to assess the risk of human exposure to methylmercury (MeHg), which is the organic form and most toxic to wildlife and humans at low concentration. Most THg in fish is MeHg (Souza-Araujo et al., 2016b; Watanabe et al., 2017; WHO, 1990; 2008), including in sharks, where more than 95% of THg in muscle tissue is MeHg (De Carvalho et al., 2014). The estimated Hg intake per 2914.73 g serving of shark meat derived from the Amazon coast would range between 3.30 (*S. lewini*) and $71.94 \mu\text{g/kg}$ (*S. tudes*) of body weight. According to WHO, the intake of MeHg up to $3.2 \mu\text{g/kg}_{\text{bw}}/\text{per week}$ may not represent a risk for developing neurotoxicity in healthy adults

(WHO, 2008). However, the intake of MeHg above the PTWI (1.6 $\mu\text{g/kg}_{\text{bw}}/\text{week}$) by women of childbearing age, pregnant females, young children and people with zinc (Zn), selenium (Se), glutathione and antioxidant nutritional deficiencies may present a risk and measures of intervention and risk management must be considered (Fuentes-Gandara et al., 2018; Ha et al., 2017; WHO, 2008). As a result, none of the species analyzed could be considered suitable for consumption by healthy adults or the identified risk groups, since the lowest EWI was above the maximum PTWI of no deleterious effect.

There are no formal recommended PTWI values for any metal that causes cancer by a mutagenic route; consequently it cannot be assumed that there is any threshold level below which they can safely be consumed (WHO, 2011b; Bat, 2017). As a result, our estimated EWI for Pb was compared with the regulatory PTWI guideline for the dietary intake of Pb (25 $\mu\text{g/kg bw}/\text{week}$; EFSA, 2010). Accordingly, our results showed that the weekly intake of Pb was lower than the PTWI for 12 of the 13 species analyzed, and it is approximately less than 16% of the regulatory guideline value. When compared to other European regulations, our values are still lower than the guideline intake of 0.57 $\mu\text{g/kg}_{\text{bw}}/\text{day}$ (Bat, 2017). Although consumption of *M. higmani* flesh might lead to some exposure to Cd, the intake per 2914.73 g serving across all shark species was far less than the PTWI.

Given the MAS for each species is calculated for each individual trace element, the weekly consumption of meat from most shark species (7 out of 13) would have to be drastically reduced for people to stay within the safe limit of iAs intake, and for all species when considering the safe limit of Hg. However, assessing risk exposure relative to species-specific consumption is problematic for human consumers in the region as an individual often buys shark meat with no knowledge of the species, i.e. if it is a high or low risk. Shark meat available is either from unidentified species or consists of mixed species catches (Bornatowski et al., 2015; Feitosa et al., 2018). When accounting for this through analyzing grouped data, the combined concentrations and EWI of the respective elements still demonstrate a possible health risk. Determining species specific health risks of examined elements was only possible here through molecular analysis to identify species. These latter points highlight that if the sale of shark meat is to continue, then regulations need to be established that vendors must label/identify the species for sale so species-specific risks can be observed.

A second important point aside from species-specific elements concentration profiles in sharks and associated risks identified here is the amount of fish consumed by the regional population. The per capita weekly consumption value for aquatic products in Brazil was 39.72 g/day in 2011 (IBGE, 2011), but in the Brazilian Amazon area the rate is estimated to be 416.39 g/day (Isaac et al., 2015) based on consumption of a diverse diet including teleost fish (reef and pelagic) and small bodied and juveniles of large sharks. Consequently, the risk assessment presented here based only on the concentrations of trace elements in sharks could underestimate the true or absolute quantity of elements ingested through overall diet and the health risks caused by chronic exposure.

Given the identified enhanced risk, it is recommended that people, especially pregnant women, breastfeeding mothers, young children, and those who regularly consume large amounts of fish avoid eating fish named “cação”. Additionally, the general public in Brazil should be made aware of the reported element levels in marine resources and provided with recommendations on the risks and benefits of fish consumption relative to established risk guidelines. In the US, for example, the Food and Drug Administration (USFDA, 2020) advises the general public over the risk of contaminant toxicity through classifying fish as “best choice, good

choice, or choice to avoid”. Moreover, a National Listing of Fish Advisories by state assists people to check how often it is safe to eat certain fish species. The Fisheries and Agriculture Organization (FAO/WHO, 2011) and European Food Safety Authority (EFSA, 2014) also recommend that consumers choose fish and seafood with known low pollutant levels, such as salmon, shrimp, cod, and sardines, and to avoid, for example, swordfish, marlin, shark, and rays.

4.3. Relative trophic ecology of sharks along the Amazon Coast

Overall, observed variation in $\delta^{15}\text{N}$ values among sampled sharks reflected their varying food habits and associated relative trophic position (Cortés, 1999). Among the eight species analyzed, *C. porosus*, *C. acronotus* and *S. mokarran* had the highest $\delta^{15}\text{N}$ values. Although *S. mokarran* is considered the largest species in the Sphyrnidae family, and an apex predator primarily consuming other sharks and rays (Raoult et al., 2019), its $\delta^{15}\text{N}$ values were significantly lower than *C. porosus*, a species that preys on small fish, crustaceans and cephalopods (Lessa and Almeida, 1997). The fact that the sharks sampled in this study were <100 cm TL indicates that *S. mokarran* were all juveniles. These data identify the diet of juveniles is different to adults and support an ontogenetic diet shift reported by Raoult et al. (2019).

Among small bodied coastal shark species, there were also marked differences in $\delta^{15}\text{N}$ values and hence relative trophic position. *M. higmani* (average length: 55 cm) had low $\delta^{15}\text{N}$ values (mean: 10.7‰) when compared to *R. terranova* (average length: 70 cm; mean $\delta^{15}\text{N}$ = 12.1‰) and *R. porosus* (average length: 75 cm; mean $\delta^{15}\text{N}$ = 12.3‰). These differences can be largely attributed to prey preference and habitat. *M. higmani* occur primarily in muddy, sandy and limestone environments feeding on decapod crustaceans and, occasionally, small fish, stomatopods and cephalopods (Tagliafico et al., 2015). In contrast, *Rhizoprionodon* spp. inhabit bays and estuaries and is classified as an opportunistic predator feeding on small bony fish, but also marine snails, squid and shrimp (Harrington et al., 2016). These combined data indicate these species likely have distinct ecological roles in the Amazonian marine ecosystem, but further work is required to contextualize $\delta^{15}\text{N}$ values for the broader food web.

Of all species examined, *C. leucas* had the most variable $\delta^{15}\text{N}$ values, ranging from 10.3 to 14‰. Stomach contents indicate that juveniles of this species are tertiary consumers, occupying a high trophic position in coastal, estuarine and riverine food webs (Estupiñán-Montañón et al., 2017). In an analysis of 81 juvenile sharks (70–162 cm in total length) in the Shark River estuary in the Everglades National Park, Florida, USA, Matich et al. (2010) reported a similar range of $\delta^{15}\text{N}$ values (11.0–13.2‰). This variation is likely a result of variation in prey types consumed related to distinct isotopic baselines between riverine, estuarine and marine ecosystems (Hussey et al., 2012). These data may therefore suggest that the Amazon Coast plays an important ecological role as a nursery area for this species in a region that is highly exploited by fisheries.

Diversity in trophic roles is important for food web structure and function (Hussey et al., 2015; Ferreira et al., 2017). In an analysis of the structure of the trophic web in southern Brazil, Bornatowski et al. (2014) found that species such as *G. cuvier* and *S. lewini* (included in the present study) have important ecological functions, and exert a major influence on lower trophic levels. Given the productivity of the Amazon Continental Shelf along the northern coast of Brazil and its importance for supporting regional fisheries, further research is necessary to evaluate the trophic role of the local elasmobranch assemblage and their influence on the trophic structure of the local marine-estuarine ecosystems (Kiszka et al., 2014; Myers et al., 2007). Systematic monitoring of biological

parameters such as sex ratios and body size, and the seasonality of the catches of commercially exploited shark species is also necessary for the long-term management of stocks and to provide further context for these nitrogen stable isotope data.

5. Conclusions

The results of the present study indicate that the sale and consumption of shark meat can expose consumers inhabiting the Amazon coastal region and eating shark meat on a regular basis to potentially harmful ingestion levels of iAs and Hg that are above those of recommended guidelines. Moreover, consumption of shark meat poses a regional threat to biodiversity conservation given it promotes fishers to catch individuals to meet market demand under the current scenario where no fisheries regulations are in place. This is ultimately leading to the catch and sale of threatened species including those that are endangered (e.g. *S. lewini*, *S. mokarran*, *S. tudes*, *C. falciformis*, *C. acronotus*, *C. leucas* and *G. cuvier*). For certain species, such as the highly endangered *Sphyrna tudes*, their habitat specialism through affinity to coastal regions will result in high catch rates that could lead to localized population declines and potential extirpation. High variability in $\delta^{15}\text{N}$ values among the sampled sharks, including multiple smaller bodied species and juveniles of larger species, suggests they occupy diverse ecological roles in the coastal environment. The scale and impact of shark removals in this region however, are unknown and more data will be required to assess if fisheries targeting these species are even sustainable. To our knowledge, these are the first data on trace element concentrations and risk assessments for the consumption of shark meat sold along the entire Brazilian North Coast. These combined results can be used by environmental and public health agencies to develop food safety guidelines, to build public awareness of consumption risks, to promote the conservation of threatened shark species in the region and form the basis for future studies to investigate the ecological importance of sharks in the Amazon coastal marine ecosystem.

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

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.chemosphere.2020.129132>.

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