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Nurse sharks, space rockets and cargo ships: Metals and oxidative stress in a benthic, resident and large-sized mesopredator, *Ginglymostoma cirratum*☆

Natascha Wosnick[a], Ana Paula Chaves[b], Renata Daldin Leite[a], Jorge Luiz Silva Nunes[c], Tatiana Dillenburg Saint'Pierre[d], Isabel Quental Willmer[e,f], Rachel Ann Hauser-Davis[e,*]

[a] *Programa de Pós-Graduação em Zoologia, Universidade Federal do Paraná, Paraná, Brazil*
[b] *Analytical and System Toxicology Laboratory, Faculdade de Ciências Farmacêuticas de Ribeirão Preto (USP), São Paulo, Brazil*
[c] *Laboratório de Organismos Aquáticos, Universidade Federal do Maranhão, Maranhão, Brazil*
[d] *Departamento de Química, Pontifícia Universidade Católica do Rio de Janeiro, Rio de Janeiro, Brazil*
[e] *Laboratório de Avaliação e Promoção da Saúde Ambiental, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz (Fiocruz), Rio de Janeiro, Brazil*
[f] *Laboratório de Biologia e Tecnologia Pesqueira, Centro de Ciências da Saúde, Instituto de Biologia, Departamento de Biologia Marinha, Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil*

A R T I C L E I N F O

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A B S T R A C T

It is widely recognized that apex predators, such as large sharks with highly migratory behavior, are particularly vulnerable to pollution, mainly due to biomagnification processes. However, in highly impacted areas, mesopredator sharks with resident behavior can be as vulnerable as apex sharks. In this context, this study evaluated cadmium (Cd), mercury (Hg), lead (Pb), and rubidium (Rb) concentrations, as well as the potentially protective effects of selenium (Se) and the behavior of two non-enzymatic biomarkers, metallothionein (MT) and reduced glutathione (GSH), employing the Atlantic nurse shark *Ginglymostoma cirratum* as a study model and compared the results with other resident benthic sharks, as well as highly mobile apex sharks. Muscle tissue samples from 28 nurse sharks opportunistically sampled from the Brazilian Amazon Coast were analyzed. Lower metal concentrations were observed for Pb, Rb and Se in the rainy season, while statistically significant correlations between metals were observed only between Hg and Cd and Pb and Se. Molar ratio calculations indicate potential protective Se effects against Pb, but not against Cd and Hg. No associations between MT and the determined metals were observed, indicating a lack of detoxification processes via the MT detoxification route. The same was noted for GSH, indicating no induction of this primary cellular antioxidant defense. Our results indicate that benthic/mesopredator sharks with resident behavior are, in fact, as impacted as highly mobile apex predators, with the traditional detoxification pathways seemingly inefficient for the investigated species. Moreover, considering the studied population and other literature data, pollution should be listed as a threat to the species in future risk assessments.

1. Introduction

Among the criteria used to assess species extinction risks both at the global and regional levels, population size reduction, labeled as criteria A by the International Union for Conservation of Nature red list committee, is the most commonly applied (IUCN, 2012). Among all threats to biodiversity, human exploitation has proven the main driving force for the extinctions observed in the last centuries (Pievani, 2014), as the removal of parental biomass from a population significantly compromises species recruitment (Ricker, 1954). Population reduction can also result from other direct or indirect anthropic pressures. More specifically, along with introduced taxa, hybridization, pathogens, competitors, or parasites, pollutants are also considered driving factors for population declines (IUCN, 2012) and conservation plans should consider the potential impacts of pollution on population health and resilience (Deem et al., 2001).

Allied to this, geographic distribution reduction, whether caused by habitat loss or overexploitation on a local scale, also significantly compromises biodiversity conservation. In the marine environment, pollution has been highlighted as a significant yet emerging contributing

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* Corresponding author.
E-mail address: rachel.hauser.davis@gmail.com (R.A. Hauser-Davis).

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factor to habitat degradation, as several compounds, both organic and non-organic, negatively affect environmental quality (McNeely, 1992). Several metals are of particular concern in this regard, as they are potentially toxic and tend to accumulate in living organisms.

A growing concern about the negative effects of pollution in sharks is noted, as these animals are apex predators and, in addition to bioaccumulation concerns, certain elements also display the ability to biomagnify through the food chain, leading to risks concerning this group (Escobar-Sánchez et al., 2011; Olmedo et al., 2013; Matulik et al., 2017). Studies indicate that non-essential metallic elements displaying potential toxicity accumulate mainly in shark liver and muscle tissue (Terrazas-López et al., 2016), although other organs, such as gonads, gills, kidneys, rectal gland and brain, can also undergo bioaccumulation processes (Frías-Espericueta et al., 2014; Gilbert et al., 2015; Wosnick et al., 2021).

Cadmium, mercury, and lead are noteworthy among contaminants with the potential to disrupt reproduction and, consequently, population recruitment in vertebrates (Kime, 1995; Massányi et al., 2020). These elements can also affect cardiac capacity, vascular dynamics (Evans and Weingarten, 1990; Wang et al., 1999), and regulatory processes associated to the homeostatic balance in sharks (Forrest et al., 1997; Kinne-Saffran and Kinne, 2001; Eyckmans et al., 2013; Wosnick et al., 2021). Furthermore, as some elements tend to biomagnify in higher trophic levels (Lozano-Bilbao et al., 2018; Tiktak et al., 2020), it is particularly interesting to assess their concentrations in the tissues of large-bodied sharks. Antother element, rubidium is used mainly as a space fuel and in the production of photoelectric cells. Variant forms, such as rubidium carbonate, are used in the glass industry. This element is also used in medicine, for the production of soporific and sedatives and in the treatment of epileptics. These sources comprise this element's main environmental inputs. In this context, although little attention has been given to rubidium, there is evidence of its potential to both bioaccumulate and biomagnify in marine organisms (Campbell et al., 2005). Furthermore, despite some evidence concerning a possible role as an essential element, Rb exposure may lead to deleterious effects, as this element has the potential to inhibit spermatogenesis and alter endocrine pathways in fish (Yamaguchi et al., 2007), reduce immune responses (Jones et al., 1990) and cause hepatic and nephrotoxicity (Usuda et al., 2014) in rodents.

Some mechanisms and molecules that aid in countering the toxicity of the aforementioned elements have been well studied (Ralston, 2008; Park and Mozaffarian, 2010). For example, selenium is an important micronutrient associated with antioxidant selenoproteins (Tapiero et al., 2003). This element competes with Hg for the same binding sites, transforming Hg into a hydrophilic complex and facilitating its excretion through an equimolar Hg–Se complex (Branco et al., 2012). Moreover, this complex has also been reported as binding to certain plasma proteins, such as Selenoprotein P, further assisting in metal detoxification, primarily in chronic exposure cases (Yoneda and Suzuki, 1997). Another efficient metal detoxification mechanism comprises interactions with non-enzymatic biomarkers, such as metallothionein (MT) and reduced glutathione (GSH). Metallothioneins, in particular, display high affinity for metal and metalloids (Chan et al., 2002). At high or toxic concentrations, several metals and metalloids may induce metalloprotein synthesis and complexation to several metals and metalloid elements, neutralizing and reducing their toxic effects (Kondoh et al., 2003; Ali et al., 2004). In addition to behaving as a detoxifying agent, MT are also responsible for the homeostatic balance of both essential and non-essential metals (Dabrio et al., 2002). Reduced glutathione (GSH), on the other hand, acts against oxidizing molecules and potentially harmful substances, including metals (Elia et al., 2003). Its main function is to sequester reactive oxygen species and excess elements, making them unavailable and, thus, protecting cells from possible adverse effects (Cao et al., 2012).

While highly mobile aquatic vertebrates tend to be exposed to a set of contamination gradients (Meador et al., 2010; Taylor et al., 2018), resident species may be more vulnerable to pollutant impacts, as they are chronically exposed when inhabiting degraded environments (Liu et al., 2013). Moreover, benthic vertebrates, even occupying lower trophic levels, may be more vulnerable to contaminant effects due to their substrate dependence (Hosseini et al., 2013). This is the case of the Atlantic nurse shark, *Ginglymostoma cirratum*, a benthic large-bodied opportunistic predator, feeding mostly on bottom invertebrates and small vertebrates (Compagno, 2001). This species is sedentary, displaying strong site fidelity and resident behavior, found mainly in coastal areas and insular shelves (Carrier and Pratt, 1998). Nurse sharks are robust and exhibit both enhanced physiological and behavioral responses to stressors (Jerome et al., 2018). In this context, this species is a good model for assessing phenotypic resilience to both environmental and anthropogenic stressors.

The Atlantic nurse shark is listed as Vulnerable in both the IUCN and Brazilian red lists (ICMBio, 2018; Carlson et al., 2021) and is protected by federal legislation throughout the entire Brazilian territory. The main threat to this species comprise coastal fisheries, as it is captured both targeted and as bycatch. There is strong evidence of population fragmentation, and the species has already become locally extinct in some states, such as São Paulo, and in some portions of Rio de Janeiro (Rosa et al., 2006). Some population remnants are protected in Brazilian marine reserves, among them the Atol das Rocas Biological Reserve (in the state of Rio Grande do Norte), Marine State Park of Parcel Manuel Luiz (in the state of Maranhão), Marine State Park of Risca do Meio (in the state of Ceará), National Marine Park of Fernando de Noronha (in the state of Pernambuco) and National Marine Park of Abrolhos (in the state of Bahia) (Rosa et al., 2006). However, most populations are still under constant threat, with reported declines of >80 % over the past three generations in the Southwest Atlantic (Carlson et al., 2021). To date, risk assessments for this species are based solely on population reduction due to overexploitation, and no information on additional contributing factors (*i.e.,* habitat loss, pathologies and pollution) are available. Nevertheless, as nurse sharks exhibit strong site fidelity, habitat degradation has the potential to negatively affect population dynamics, posing an extra challenge for the conservation of this species in Brazilian waters. In order to fill this knowledge gap and assess the potential impacts of pollution on this benthic, high site fidelity and resident mesopredator, the present study evaluated the concentrations of the toxic and non-essential metals Cd, Hg, Pb, and Rb, as well as the potentially protective effects of the semimetal Se and two non-enzymatic biomarkers, MT and GSH in the muscle tissue of nurse sharks from Brazilian waters.

2. Materials and methods

2.1. Shark sampling

The chosen nurse shark population is located in the state of Maranhão, inserted within the Brazilian Amazon Coast (BAC). The reason for choosing this population is multifold. First, the BAC is listed as a global hotspot for shark conservation (Dulvy et al., 2014), so it is imperative to adequately identify and monitor all potential threats to local populations. Second, part of this Amazonian population is protected by a state park, but its main portion is severely exposed to environmental and anthropogenic stressors outside the limits of the marine reserve. Third, according to local fishers, the nurse sharks sampled in the present study are residents to one of the most pollution-affected areas in the region and, thus, a promising focal group to assess the effects of long-term contamination exposure.

Muscle samples (~5 g) of 28 adult nurse sharks incidentally caught by artisanal fishers were obtained between May 2018 and September 2019. According to the fishers, all sharks were caught at the Canal do Navio, a dredging area on the continental shelf through which cargo ships have access to the state capital, the island of Maranhão (Fig. 1). Sharks were caught with longlines at depths ranging from 11 to 23 m. As

Fig. 1. Canal do Navio (dotted line), where the nurse sharks analyzed in the present study were caught, Maranhão (Brazilian Amazon Coast), Brazil.

sharks were landed fully processed, the sex, total length, and weight of sampled individuals could not be determined. Sampling was conducted with the approval of the Brazilian Ministry of Environment (IBAMA/ICMBio- SISBIO no. 60306-1).

2.2. Metal analyses

About 100 mg of each sample was weighed in 15 mL screw-capped polypropylene tubes, followed by the addition of 1.0 mL concentrated subboiled bidistilled nitric acid to each (HNO_3, 67 % v/v). After an initial acid-digestion overnight at room temperature, the samples were heated the next day at 100 °C for approximately 4 h in the capped (closed) polypropylene tubes for 4 h, avoiding loss of volatile elements, such as Hg and Se (USP, 2013). After cooling, the samples were diluted with ultra-pure water (resistivity> 18 MΩ cm) obtained from a Merck Millipore water purifying system (Darmstadt, Germany) to 10 mL. Elemental determinations were then performed using multi-elemental external calibration (Merck IV standard solution) by inductively coupled plasma mass spectrometry (ICP-MS), employing a NexIon 300X spectrometer (PerkinElmer, Norwalk, USA), with ^{103}Rh as the internal standard added online at 40 mg L^{-1}. Method accuracy was verified by the analysis of procedural blanks and two certified reference materials (CRM), ERM BB422 (European Commission, Joint Research Centre) and DORM-4 (NRC, Canada). All analyses were performed in triplicate and analytical curve correlation coefficients were always above 0.995. The recovery values for both CRM are displayed in Table 1. All results are expressed as mg kg^{-1} dry weight. The volatile elements determined in the present study (Hg and Se) presented only slightly higher CRM concentrations than the certified values, demonstrating that the sample preparation procedure is efficient and not prone to losses. In addition, the concentrations are also higher than the limits of quantification of the technique obtained through direct sample solution introduction. Therefore, the vapor generation technique, which is more time- and reagent-consuming, is not required (Bruno et al., 2021; Hauser-Davis et al., 2021). All certified reference material recovery values are considered adequate for this type of study, as per Eurachem standards (Eurachem, 1998; Ishak et al., 2015), further confirming the adequacy of the chosen technique.

The limits of detection (LOD) and limits of quantification (LOQ) for each investigated element were calculated according to the Brazilian National Institute of Metrology, Quality and Technology (Inmetro, 2016) using the following equations: LOQ = (3*SD*df)/slope of the line and LOQ = (10*SD*df)/slope of the line, where SD is the standard deviation of the ratio of the analytical signal to the internal standard signal of 10 blanks and df is the sample dilution factor applied. The respective determined LOD and LOQ for each investigated element in the present study were, in mg kg^{-1}: Cd (0.002 and 0.01), Hg (0.001 and 0.004), Pb (0.003 and 0.010), Rb (0.013 and 0.044) and Se (0.03 and 0.10).

2.3. Statistical treatment

An exploratory Principal Component Analysis was performed to first verify potential associations between the elements (*i.e.*, Cd, Hg, Pb, Rb, and Se) and MT concentrations between both sampling seasons (rainy and dry), based on other assessments of this kind for different biota reported in the literature (De Boeck et al., 2003; O'Brien et al., 1995; Amiard et al., 2008; Subotić et al., 2013; Aru et al., 2016; Okay et al., 2016; Wosnick et al., 2021). The number of PCA factors were chosen based on Kaiser's rule, where the Principal Components (PCs) with the highest explanatory power display eigen values higher than 1 (Méndez et al., 1993). Subsequently, to assess possible differences in metal and biomarker concentrations between seasons, a t-Student test (parametric data) or a Wilcoxon test (non-parametric data) was performed. No post-hoc corrections were applied, according to Perneger (1998) and Moran (2003). GSH was excluded from both analyses, as there were not enough samples to measure this marker in 16 sharks (three sharks

Table 1
Observed and certified values for the certified reference materials (in mg kg^{-1} dry weight) and their recoveries (%).

Element	ERM-BB422			DORM-4		
	Certified	Observed	Recovery (%)	Certified	Observed	Recovery (%)
Pb	–		–	0.404 ± 0.062	0.40 ± 0.13	99
Se	1.33 ± 0.13	1.54 ± 0.026	115	3.45 ± 0.40	3.63 ± 0.25	105
Cd	0.0075 ± 0.0018	0.0064 ± 0.0015	101	0.299 ± 0.018	0.360 ± 0.025	87
Hg	0.601 ± 0.030	0.671 ± 0.079	111	0.412 ± 0.0036	0.430 ± 0.035	104

captured during the rainy season and 13 captured during the dry season), preventing us from performed inter-seasonal comparisons. Lastly, to verify the potential correlation between the evaluated variables, Pearson correlation (parametric data) or Spearman (non-parametric data) correlation tests were performed. Statistical significance was set at $p < 0.05$. All analyses were performed employing the R software version 1.4.1103 (R Development Core Team, 2021), using the FactoMineR, factoextra and vegan packages.

3. Results

3.1. GSH, MT, and elemental concentrations

The GSH and MT concentrations determined in muscle samples of nurse sharks collected from Maranhão, on the Brazilian Amazon Coast, Brazil, are presented in Fig. 2. For GSH, concentrations ranged from 0.07 to 0.63 (0.25 ± 0.17 mg kg^{-1}; mean ± SD), while for MT, concentrations ranged from 0.028 to 0.084 (0.055 ± 0.01 mg kg^{-1}; mean ± SD). All results are presented as dry weight.

Considering the determined elements, the box plots presenting the means ± SD of the concentrations determined in the muscle of the same nurse shark samples are presented in Fig. 3. The concentration ranges and the means ± SD (in parenthesis) were, respectively: Cd from 0.013 to 2.32 mg kg^{-1} (0.196 ± 0.47 mg kg^{-1}); Hg from 6.65 to 30.1 mg kg^{-1} (14.2 ± 7.38 mg kg^{-1}); Pb from 0.009 to 0.94 mg kg^{-1} (0.164 ± 0.21 mg kg^{-1}); Rb from 2.04 to 14.3 mg kg^{-1} (5.44 ± 2.6 mg kg^{-1}); Se from 2.19 to 14.3 mg kg^{-1} (3.95 ± 2.4 mg kg^{-1}). Interestingly, the highest concentrations of Pb, Rb, and Se were detected in the same individual (0.94, 14.3, and 14.3 mg kg^{-1}, respectively). All results are presented as dry weight.

3.2. Metal and biomarker trends and seasonal influence

Concerning the PCA analysis (total explained variance = 65.5 %, Fig. 4), the elements displaying the highest contribution in PC 1 were Se (0.91), Pb (0.79) and Rb (0.64), while Cd (0.79) and Hg (0.41) contributed with the highest explanatory data variance power in PC2 and MT, in PC3 (0.93). For axis 1, the most representative variable was selenium, and for axis two, cadmium (Supp. Table 1). When a lack of statistical correlations between MT and metals is observed, a consensus exists in the literature indicating that MT plays no detoxification role in that specific situation, as reported in several marine fish assessments (Mieiro et al., 2011; Rotchell et al., 2001; Roméo et al., 1997)). This seems to be the case in the present study, as MT was locates in the opposite quadrant to all elements investigated herein in PC1, and in the same quadrant as Cd in PC2, although dispersed, with a higher contribution only in PC 3 not associated to any of the investigated elements. To test this hypothesis, correlations between MT and metals were also

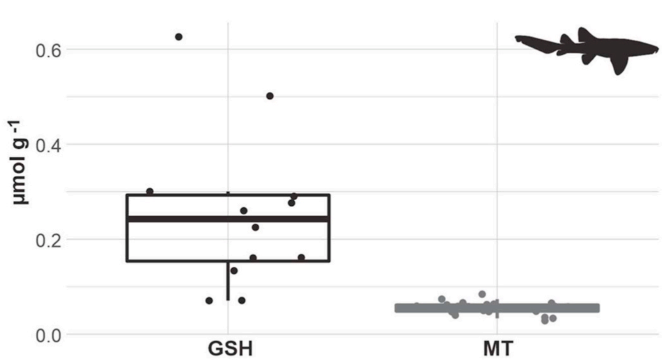

Fig. 2. Metallothionein (n = 28) and GSH (n = 12) concentrations in nurse shark muscle samples from the state of Maranhão (Brazilian Amazon Coast), Brazil.

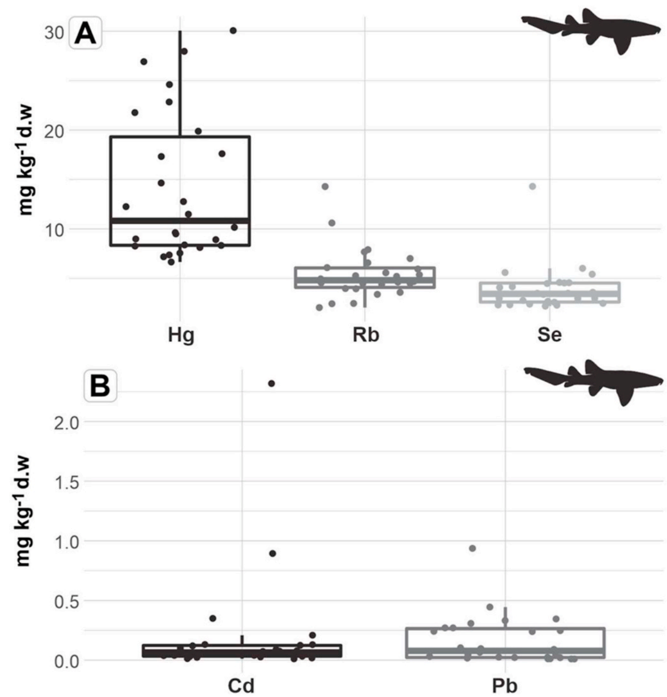

Fig. 3. Overall elemental concentrations determined in nurse shark muscle tissue samples in the present study (n = 28).

evaluated.

As for the potential influence of seasons upon elemental and biomarker concentrations, significant differences were identified between the rainy and dry periods for Pb (W: 12, p = 0.00005633), Rb (W: 43 p = 0.0356), and Se (W: 12, p = 0.00005633), While no statistical difference between seasons was detected for Cd (W = 78, p = 0.781), Hg (W = 78, p = 0.781), and MT (t = 1.7365, df = 24, p = 0.0953) (Fig. 5). For GSH (n = 12), the lowest detected concentration was 0.07 μmol g^{-1} and the highest, 0.63 μmol g^{-1}. As only one nurse shark sampled in the dry season was analyzed for GSH due to lack of samples, no seasonality pattern could be assessed for this biomarker. Regarding MT (n = 28), the lowest concentration was detected in the dry season (0.028 μmol g^{-1}) and the highest, in the rainy season (0.084 μmol g^{-1}).

For Cd (n = 28), both lowest and highest concentrations were detected in nurse sharks sampled in the rainy season (0.013 and 2.32 mg kg^{-1}, respectively). For Hg (n = 28), both the lowest and highest concentrations were detected in nurse sharks sampled in the dry season (6.65 and 30.1 mg kg^{-1}). Regarding Pb, Rb, and Se (n = 28), the lowest concentrations were detected in nurse sharks sampled in the rainy season (0.009, 2.04, and 2.19 mg kg^{-1}, respectively), and the highest were detected in the dry season (0.94, 14.3, and 14.3 mg kg^{-1}, respectively).

Concerning correlations between elements and biomarkers analyzed in the present study, positive correlations were detected between Cd and Hg ($R^2 = 0.57$, p = 0.002503), and between Se and Pb ($R^2 = 0.80$, p = 0.000002759) (Fig. 6; Supp. Table 2). Taking into account that Se is an essential element (albeit, toxic at high concentrations) and Pb, toxic, the molar ratio for this association was also calculated. These calculations are valuable tools to assess potential protective effects of essential elements against the effects of toxic metals and metalloids, where ratios higher than 1 for the former compared to the latter are considered protective (Ralston et al., 2007, 2008). Furthermore, considering the notably high Hg concentrations detected herein and the important relationship between Se and Hg reported in the literature for sharks (Nam et al., 2011; Bergés-Tiznado et al., 2015; Merly et al., 2019), the Se:Hg molar ratio was also calculated, even though no statistically significant correlation between these elements was noted. The calculations indicate a molar ratio of 64:1 for Se:Pb, and a 0.7:1 ratio for Se:Hg. The

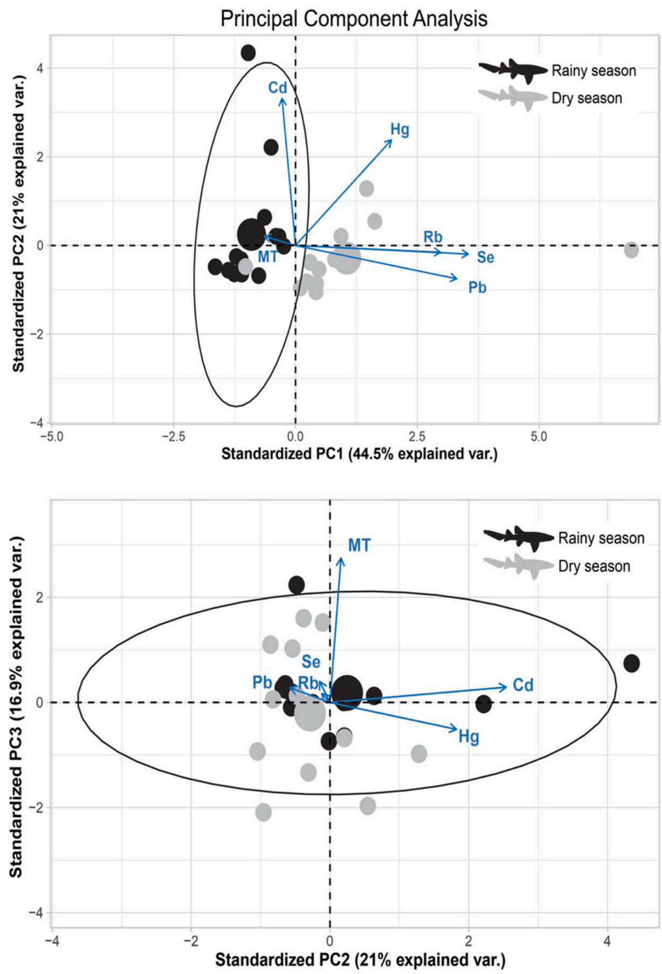

Fig. 4. Principal component analysis (PCA) for metals and MT determined in nurse shark muscle samples from the state of Maranhão (Brazilian Amazon Coast).

latter indicates an unfavorable balance and no protective effect against Hg, indicating these sharks may suffer toxic Hg effects (Ralston, 2008). The lack of significant MT correlations to any of the investigated metals corroborates the previously discussed PCA data, and is suggestive of no detoxification mechanisms in place by this metalloprotein, as mentioned previously.

4. Discussion

The present study reports the accumulation of non-essential and toxic elements and associations with an essential element in an Amazonian nurse shark population, as well as two non-enzymatic biomarkers. Moreover, our results seem to suggest accumulation of the investigated elements as, under optimum conditions muscle tissue tends to accumulate low metal concentrations due to lower metabolic activities compared to other organs, such as the liver, for example (Uluturhan and Kucuksezgin, 2007; Squadrone et al., 2013). However, as only muscle tissue was analyzed, future studies should be carried out to confirm bioaccumulation and biomagnification processes in this Amazonian shark population. All discussed data are in dry weight. This is the first time Se, GSH, and MT have been reported for nurse sharks, indicating a significant knowledge gap that should be better investigated in future assessments. Furthermore, few data on metal accumulation are available for this species. Compared to the scarce worldwide assessments available, Hg concentrations of 0.50 mg kg^{-1} in southeastern Brazil (Lacerda et al., 2016), 0.141 mg kg^{-1} in northeastern Brazil (Wosnick et al., 2021), 0.42 mg kg^{-1} in Florida (Hammerschlag et al., 2016), 0.069 mg kg^{-1} in Cuba (Montero-Alvarez et al., 2014), and 9.03 mg kg^{-1} in the Bahamas (Shipley et al., 2021) have been reported. To date, Cd and Pb concentrations for nurse sharks are available only for the Bahamian population (0.263 and 0.125 mg kg^{-1}, respectively) (Shipley et al., 2021). For Rb, our knowledge is even more incipient, with concentrations available only for two arctic species (Pacific sleeper shark, 0.79 mg kg^{-1}; Greenland shark, 0.66 mg kg^{-1}) (McMeans et al., 2007).

In the present study, Hg concentrations reached up to 30.1 mg kg^{-1} d.w. (14.2 ± 7.38 mg kg^{-1}d.w.; mean ± SD), 3-fold higher than the highest concentration detected for nurse sharks in previous assessments (Shipley et al., 2021). Such data is also interesting when compared with nurse sharks sampled from another region within the BAC (0.141 ± 0.09 mg kg^{-1}; mean ± SD - Wosnick et al., 2021), as Hg concentrations in the nurse sharks from the present study were 106-fold higher. These results indicate that the population evaluated herein suffers chronic exposure not experienced by other populations in the same region. Moreover, it is possible that a point-source or event has affected this population in particular but has not yet affected other populations. Cadmium concentrations (0.196 ± 0.467 mg kg^{-1} d.w.; mean ± SD) were similar to those detected in the Bahamian population (0.263 ± 0.301 mg kg^{-1}; mean ± SD - Shipley et al., 2021). Lead concentrations in the Amazonian nurse sharks (0.164 ± 0.206 mg kg^{-1} d.w.; mean ± SD) were also similar to those detected in the Bahamian individuals (0.108 ± 0.045 mg kg^{-1}; mean ± SD - Shipley et al., 2021). Taken together these results indicate that both populations are being affected in the same magnitude, at least regarding Cd and Pb. Contamination sources can be diverse, and further studies in this regard are needed, especially considering toxic effects already described for sharks.

Data on toxic metal concentrations are scarce for benthic sharks, with most reports focusing on Hg concentrations (Table 2). Mercury accumulation depends on several factors, including shark size, life stage, tissue type, and source of contamination (Walker et al., 2014). As Hg tends to accumulate and also biomagnify, higher concentrations are expected for large-sized predators (Escobar-Sánchez et al., 2011). However, Hg can also adsorb to sediments, becoming available at high concentrations for bottom feeding species (Nascimento and Chasin, 2001). Previous studies have investigated Hg concentrations in benthic sharks, among them *Triakis* spp. (0.22–0.32 mg kg^{-1} – van Hees and Ebert, 2017; Kim et al., 2019), and *Chiloscyllium* spp. (0.05–0.8 mg kg^{-1} - Ong and Gan, 2017; Adel et al., 2018). To date, apart from nurse sharks, the highest Hg concentration reported for a benthic species was 3.85 mg kg^{-1} in narrowmouth catsharks (*Schroederichthys bivius*) from Argentina (Marcovecchio et al., 1991). However, as all benthic sharks for which toxicological data is available are small-sized species, comparisons should be performed with caution. Data on Cd and Pb are scarcer (Table 2). For Cd, concentrations similar to those of the present study were detected for whitespotted bamboo shark (*Chiloscyllium plagiosum* - 0.174 mg kg^{-1}) in Malaysia (Ong and Gan, 2017) and for narrowmouth catsharks in Argentina (0.26 mg kg^{-1}; Marcovecchio et al., 1991), indicating that even small-sized benthic sharks can accumulate high Cd concentrations. Regarding Pb, high concentrations were also detected in whitespotted bamboo sharks from Malaysia (0.256 mg kg^{-1}; Ong and Gan, 2017). In fact, despite being much smaller (i.e., maximum reported size 93 cm in TL), Pb concentrations were significantly higher than those observed in both Amazonian and Bahamian nurse sharks, indicating that benthic habits may also favor Pb accumulation, even in smaller species. Regarding Se, data is available only for the banded houdshark (*Triakis scyllium* - 1.12 mg kg^{-1}; Kim et al., 2019), and for the port jackson shark (*Heterodontus portusjacksoni* - 0.75 mg kg^{-1}; Gibbs and Miskiewicz, 1995), and concentrations in nurse sharks from the present study were 3.5 and 5.2-fold higher, respectively, which might be a result of greater physiological demands or greater environmental availability.

Studies performed with apex predators, such as tiger sharks (*Galeocerdo cuvier*), reported lower Hg concentrations than those detected in

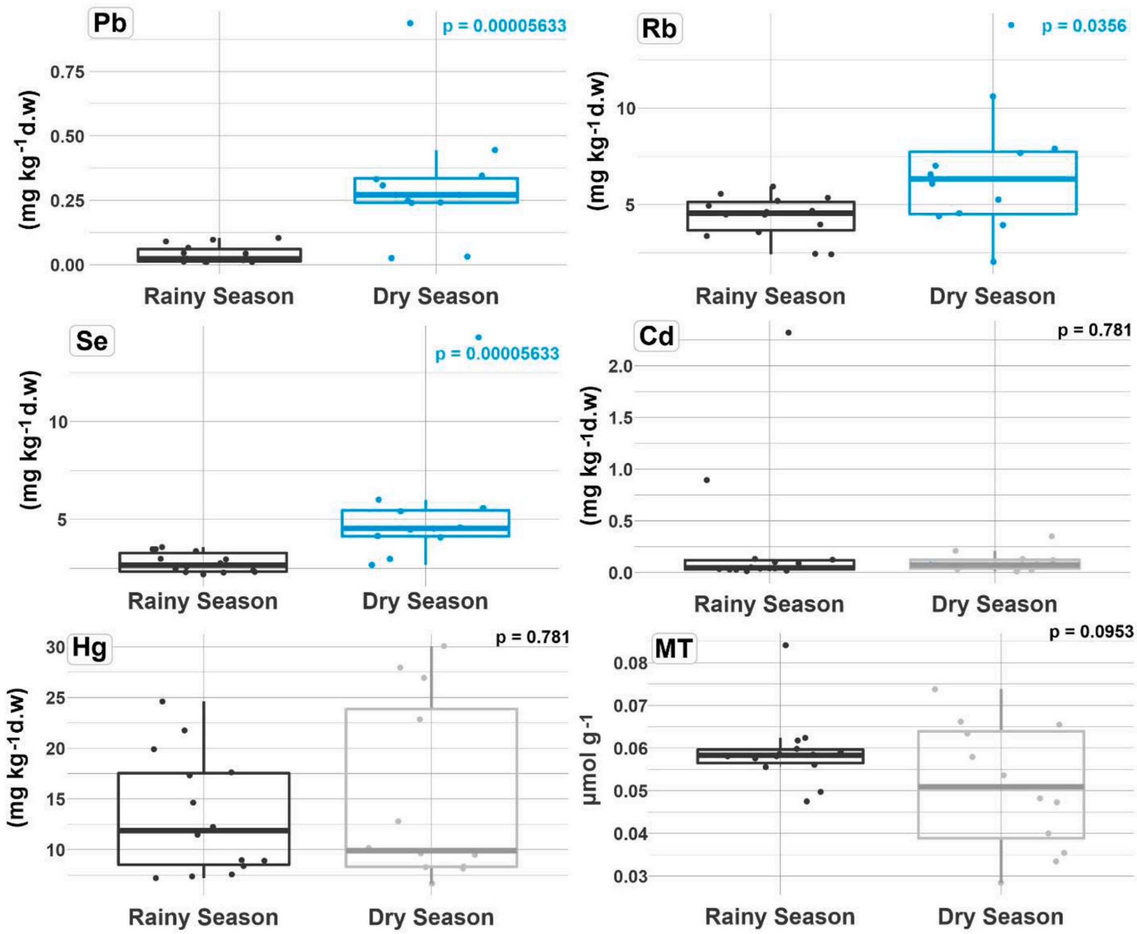

Fig. 5. Significant season-specific variations in lead, rubidium, and selenium. Box plots in blue are significantly higher in the dry season compared to the rainy one (Pb - $p = 0.00005633$; Rb – $p = 0.0356$; Se – $p = 0.00005633$). Only non-significant season-specific variations were noted for cadmium, mercury, and MT. Variations between seasons were not detected for GSH, as only one sample from the dry season was analyzed. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

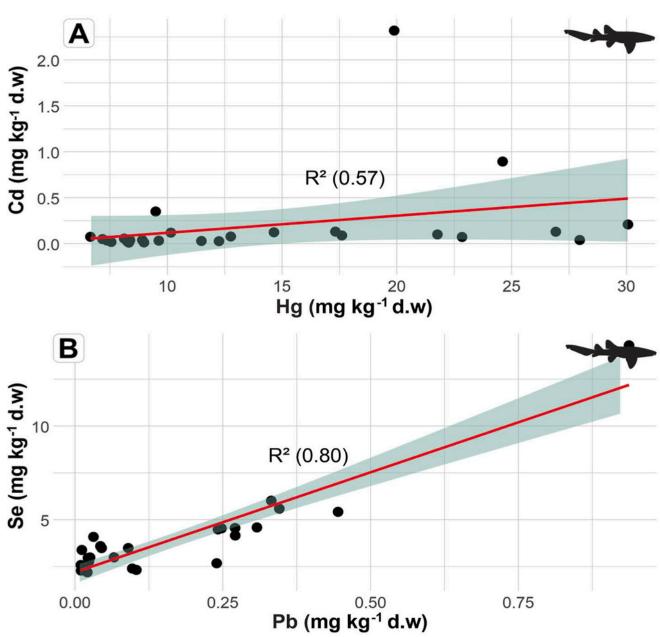

Fig. 6. Positive correlations between the measured elements determined in nurse shark muscle tissues from Maranhão (Brazilian Amazon Coast), Brazil. (A) Cd:Hg - $R^2 = 0.57$, $p = 0.002503$; (B) Se:Pb - $R^2 = 0.80$, $p = 0.000002759$.

the nurse sharks assessed in the present study, with means ranging from 0.78 to 4.44 mg kg^{-1} (Table 3). Mercury concentrations in nurse sharks were also higher than those reported for white sharks (*Carcharodon carcharias*) and bull sharks (*Carcharhinus leucas*) (means of 4.86 and 6.92 mg kg^{-1}, respectively).

Regarding Cd, the nurse sharks from the Canal do Navio exhibited mean concentrations (0.19 mg kg^{-1}) higher than those reported for white sharks (mean of 0.05 mg kg^{-1}), similar to those detected in tiger sharks (mean of 0.13 mg kg^{-1}; Table 3), but lower than those detected in bull sharks (mean of 2.8 mg kg^{-1}; Table 3). Moreover, Pb concentrations were higher when compared to tiger sharks (mean of 0.09 mg kg^{-1}), similar to white sharks (mean of 0.13 mg kg^{-1}), and lower than those detected in bull sharks (mean of 0.9 mg kg^{-1}; Table 3). Interestingly, Cd, Hg and Pb concentrations were remarkably higher for Amazonian nurse sharks when compared to tiger sharks (means of 0.78, 0.03, 0.02 mg kg^{-1}, respectively) also sampled within BAC (Pará state) (Table 3). Hg and Cd concentrations were also higher when compared to Amazonian bull sharks (means of 0.41 and 0.01 mg kg^{-1}, respectively), but lower for Pb (mean of 0.56 mg kg^{-1}) (Table 3). Taken together, these results reinforce the vulnerability of benthic sharks with resident behavior when living in highly polluted regions. In fact, even when comparing the nurse sharks with apex sharks (apart from Cd and Pb of bull sharks from Mexico, and Pb from Amazonian bull sharks), accumulation is similar or higher for all determined toxic elements. Such results also indicate that, when in a highly contaminated environment, direct assimilation has the potential to be more harmful than biomagnification through the food

Table 2
Hg, Cd, Pb, and Se concentrations in the muscle of benthic sharks worldwide.

Species	Hg	Cd	Pb	Se	Location	Source
Ginglymostoma cirratum	14.19	0.19	0.16	3.94	Maranhão, Brazil	The Present Study
Ginglymostoma cirratum	0.50	–	–	–	Rio de Janeiro, Brazil	Lacerda et al. (2016)
Ginglymostoma cirratum	0.42	–	–	–	Florida, USA	Hammerschlag et al. (2016)
Ginglymostoma cirratum	0.06	–	–	–	Cuba, Caribe	Montero-Alvarez et al., 2014
Ginglymostoma cirratum	9.03	0.26	0.10	–	Bahamas, Caribe	Shipley et al. (2021)
Triakis scyllium	0.22	0	0.03	1.12	Offshore waters, Korea	Kim et al. (2019)
Triakis semifasciata	0.31	–	–	–	California, USA	van Hees e Ebert (2017)
Squatina californica	0.43	–	–		Baja California Sur, Mexico	Escobar-Sánchez et al., 2011
Chiloscyllium plagiosum	0.81	0.17	0.25	–	Terengganu, Malaysia	Ong e Gan (2017)
Chiloscyllium arabicum	0.05	0.0	0.14	–	Khozestan, Iran	Adel et al. (2018)
Chiloscyllium plagiosum	–	0.01	<0.001	–	Hong Kong, China	Cornish et al. (2007)
Figaro boardmani	0.7	–	–	–	Sydney, Australia	Pethybridge et al. (2010)
Schroederichthys bivius	3.85	0.26	–	–	Bahia Blanca, Argentina	Marcovecchio et al. (1991)
Heterodontus portusjacksoni	–	0.008	0.01	0.75	Sydney, Australia	Gibbs and Miskiewicz (1995)

Results reported as wet weight were transformed to dry weight considering 75 % water content for muscle (Bosch et al., 2013).

Table 3
Hg, Cd, Pb, and Se concentrations in the muscle of three apex sharks, the tiger shark (*Galeocerdo cuvier*), the white shark (*Carcharodon carcharias*), and the bull shark (*Carcharhinus leucas*). ND = Not detected; (–) = Not accessed.

Species	Hg	Cd	Pb	Se	Location	Source
Ginglymostoma cirratum	14.19	0.19	0.16	3.94	Maranhão, Brazil	The Present Study
Galeocerdo Cuvier	4.44	0.13	0.09	–	Nassau, Bahamas	Shipley et al. (2021)
Galeocerdo Cuvier	0.78	0.03	0.02	–	Pará, Brazil	Souza-Araujo et al. (2020)
Galeocerdo Cuvier	1.22	ND	–	–	Ishigaki, Japan	Endo et al. (2015)
Galeocerdo Cuvier	1.50	ND	–	–	Ishigaki, Japan	Endo et al. (2008)
Carcharodon carcharias	4.86	0.05	0.13	1.12	California, USA	Mull et al. (2012)
Carcharhinus leucas	0.41	0.01	0.56	–	Pará, Brazil	Souza-Araujo et al. (2020)
Carcharhinus leucas	6.92	–	–	–	Florida, USA	Matulik et al. (2017)
Carcharhinus leucas	–	2.8	0.9	–	Sinaloa, Mexico	Ruelas-Inzunza and Paez-Osuna, 2007

Results reported as wet weight were transformed to dry weight considering 75 % water content for muscle (Bosch et al., 2013).

chain.

Concerning differences between seasons, in which significantly lower values were noted for Pb, Rb, and Se in the rainy season, this is probably due to increased rainfall rates, resulting in the dilution of waterborne contaminants (Szefer et al., 2004). During the dry season, higher metal levels are usually associated with a concentration effect, due to low rainfall volumes. In the present study, however, only one summer and one winter season were assessed, and it would be interesting to monitor further seasonal cycles, in order to allow for inferences concerning associations between rainfall indices and bioaccumulated metals.

Positive correlations between elements indicate similar accumulation behaviors, detoxification processes, and input sources (Pagenkopf, 1983; Playle, 1998; Ribeiro et al., 2009; Jerez et al., 2014). With regard to sharks, interelemental correlation assessments are still very scarce, and differential trends have been reported among species, probably due to variable metabolisms (Shipley et al., 2021). For example, some authors have reported no interelemental correlations in smooth-hound sharks (*Mustelus mustelus*) (Bosch et al., 2016), while others report significant correlations between toxic elements (*i.e.,* Hg and Pb in copper sharks, *Carcharhinus brachyurus*) (Kim et al., 2019) and between essential and toxic elements (Zn and Mn with Pb, As and Hg in the muscle of Caribbean reef sharks, *Carcharhinus perezi*) (Shipley et al., 2021).

The lack of MT relationships with any metal in the PCA seem to indicate a lack of detoxification processes via the MT detoxification route, as reported in other assessments, which may be due to metal concentrations lower than synthesis inducing thresholds in *G. cirratum*, although no baseline data and previous assessments in this regard are available. Furthermore, a relationship was noted for Cd and Hg, and for the three other analyzed metals (Pb, Rb and Se). Following the PCA, Spearman correlations were therefore performed in order to verify significant correlations among the assessed variables in nurse shark muscle tissue. The only significant interelemental correlations observed were between Se and Pb and Hg and Cd.

The positive correlation observed between Hg and Cd, both toxic elements, implies a similar geochemical input pathway to the study area, such as dust deposition, mining, industrial processing, and direct discharges into water or watersheds (Wright and Welbourn, 1994, Mager, 2011). This has also been observed for Caribbean reef sharks, and the authors postulated that this may be due to foraging dynamics, longevity, physiology, and a slower growth rate in older individuals (Shipley et al., 2021).

Selenium plays an important role against oxidative stress, both as an enzymatic cofactor and as a part of selenoproteins displaying antioxidant properties (Tanekhy, 2015), and some studies indicate that this element is able to decrease Pb-induced oxidative stress in several taxa, including fish. For example, one study assessed the potential ability of Se in alleviating oxidative stress in kidneys following lead nephrotoxicity induction daily exposure to lead for up to 10 weeks in tilapia *Oreochromis niloticus* (Hashish et al., 2015). Fish treated with Se administered as sodium selenite one week before Pb intoxication exhibited a significant amelioration of adverse lead toxicity effects, indicating antioxidant effects and the ability to improve kidney function after lead intoxication in this fish species. In another study, organic Se was administered to carp

Cyprinus carpio exposed to sublethal Pb concentrations for 14 days, and significant improvements in oxidative stress enzymatic and non-enzymatic biomarkers and oxidative stress endpoints were noted (Özkan-Yılmaz et al., 2014).

Significant correlations between Se and Pb indicate a protective effect of Se against Pb toxicity. Pb toxicity has been ascribed to its ability to generate free radicals and reactive oxygen species (ROS) in the intracellular medium (Hsu and Guo, 2002; Ates et al., 2008), and to deplete antioxidant non-enzymatic and enzymatic defenses, such as GSH and superoxide dismutase or glutathione peroxidase (GPx), respectively (Kasperczyk et al., 2012, 2013), due to its high affinity for sulfhydryl (SH) groups (Dai et al., 2012). Because of this, Pb exposure, when not ameliorated, leads to several deleterious effects, such as lipid peroxidation, cell membrane damage, and, consequently, cyto-and genotoxicity (Hsu and Guo, 2002). In the present study, the Se:Pb molar ratio was 64:1, indicating a likely protective effect of Se against Pb in the Amazonian *Ginglymostoma cirratum* population investigated herein.

Although a non-significant correlation was noted between Se and Rb, the high contamination levels observed for Rb herein make this relationship noteworthy of discussion. Rubidium is not considered an essential element, and is metabolically interchangeable with potassium (K), due to similar physico-chemical characteristics as a Group 1 alkali metal (Relman, 1956; Behne et al., 1988). Scarce studies concerning its relationship with Se are available, and none in elasmobranchs. In one assessment, a positive correlation between these elements was detected in human seminal plasma (Behne et al., 1988), although the authors pose the question of whether the presence of Rb in seminal plasma is of any biological significance or if its levels are only maintained by the mechanisms responsible for K metabolism. In another assessment, a significantly higher Se:Rb ratio was noted in chondrosarcoma tissue compared to intact bone, which may be suggestive of a protective effect of Se against Rb, although no hypotheses are given by the authors (Zaichick and Zaichick, 2015). On the other hand, Rb has also been reported as interacting or impairing Se function in thyroid physiology (Kohrle et al., 2005). As significantly lower values were noted for both Rb and Se in *G. cirratum* specimens in the rainy season, it is possible that a toxic threshold may have been surpassed for Rb due to lowered Se concentrations, potentially initiating toxic effects and invalidating any potential protective Se effects. However, further assessments are required to either confirm or deny these hypotheses.

Due to the greater attention given to the harmful effects of mercury, the protective effect of Se was briefly assessed. The Se in high concentrations was not enough to further a protective function to Hg in nurse sharks (molar ratio Se:Hg 0.7), corroborating reports for pelagic species such as the mako shark (*Isurus oxyrinchus* - molar ratio Se:Hg 0.5) (Kaneko and Ralston, 2007), the blue shark (*Prionace glauca* - molar ratio Se:Hg 0.3) (Escobar-Sánchez et al., 2011), and the pelagic thresher (*Alopias pelagicus* – molar ratio Se:Hg 0.2) (Lara et al., 2020). Interestingly, the same was not observed for the lemon shark (*Negaprion brevirostris* - moral ratio Se:Hg 3.51) (Nam et al., 2011), a large-sized coastal species, and for coastal-pelagic, semi-oceanic sharks, such as the great white shark (*Carcharodon carcharias* - molar ratio Se:Hg > 1) (Merly et al., 2019), and the scalloped hammerhead (*Sphyrna lewini* - molar ratio Se:Hg 5.6) (Bergés-Tiznado et al., 2015). Such results indicate that the protective effect of Se to Hg is species-specific rather than habitat-related, as the nurse shark, a coastal benthic shark, lacks the protective effects of Se, as observed for pelagic sharks. Branco et al. (2012) show *in vivo* assay studies with Zebra-seabreams fish (*Diplodus cervinus)* that Hg accumulation is much lower when exposed along with Se as both elements compete for the thiol groups (−SH) of protein reducing Hg absorption. Another mechanism of action is through Se binding to Hg, facilitating excretion. Moreover, due to the importance of Se for shark physiology, an unfavorable Hg balance can also induce physiological alterations through the reduction in Se bioavailability and alterations in antioxidant function (Kaneko and Ralston, 2007; Yang et al., 2008; Nam et al., 2011). In the present study, even high Se concentrations were not enough to reduce Hg accumulation, as noted by the calculated molar ratio. In fact, the Hg concentrations detected herein are the highest ever reported for any shark species. Thus, it is plausible to infer that, other mechanisms rather than Se protection are taking place, although, interestingly, MT synthesis and binding to Hg does not seem have taken place, even at such high Hg concentrations, and oxidative stress effects in the form of GSH, the main antioxidant cellular defense, were not observed.

As for the potential sources that resulted in the accumulation of toxic elements detected in the analyzed sharks, some alarming situations must be considered. The Canal do Navio runs between Maranhão and Alcantara Islands, which is home to the Brazilian Air Force Rocket Launch Center. Due to the proximity of Alcantara to the Equator, fuel consumption is lower compared to bases at higher latitudes and for that reason, Alcantara is considered a promising launching site. In 2003, three days before the VLS-1 V03 rocket was due to launch, a technical failure led to a major fire, followed by several explosions that caused the death of 21 workers and destroyed the rocket. Along with the destruction, significant amounts of space fuel may have leaked to the outskirts of the island. Lead and Rb are among the components used in the manufacture of space fuel, which could explain the high concentrations detected in all analyzed sharks. In addition to the potential abrupt contact at the time of the leak and in the following days, since Rb tends to sink (Collins, 1963; Ertan and Erdoğan, 2016), it is plausible to infer that due to their benthic habit, site fidelity, and resident behavior, the nurse sharks continued to be exposed to this element years after the accident. In fact, Rb concentrations in the muscle of the nurse sharks evaluated in the present study were 7-fold higher (5.44 ± 2.59 mg kg^{-1} d.w.) than in both arctic sharks for which data is currently available (McMeans et al., 2007), indicating that the BAC and, more specifically, the Canal do Navio), is a hotspot for Rb accumulation, with unknown effects on the overall health of affected sharks. As concentrations of all elements were significant, it is also possible that apart from being assimilated through bottom-feeding, Cd, Hg, Pb, and Rb are also made bioavailable due to local dredging activities performed at the Canal do Navio every four months, to ensure the adequate depth for cargo ships traffic. Moreover, port activities and local industries cannot be ruled out as constant sources of contamination in the region either. Therefore, along with this one-off disaster and coastal urbanization, the constant dredging in the region are chronic stressors to the assessed nurse shark population and should be considered a threat in future risk assessments and potentially detrimental to the conservation of the species in this global hotspot.

5. Conclusions

The nurse shark population evaluated herein exhibits alarming Cd, Hg, and Pb concentrations, potentially linked to the disaster that occurred at the space base located close to resident nurse shark area, along with the constant dredging performed in the region. Furthermore, despite high Se concentrations, potentially protective effects were detected only for Pb. However, as oxidative stress responses were not observed, it is possible that the species is benefiting from alternative detoxification mechanisms not evaluated. Either way, the nurse shark population situation is alarming, as this species is listed as Vulnerable and severe population fragmentation along with regional extinctions have already been reported. In northeastern Brazil, population remnants suffer the effects of capture by the artisanal fleet, with a significant reduction in parental biomass. Such dynamics directly affect population recruitment, and any additional pressure on reproductive potential can further aggravate their situation. Given the deleterious effects of the toxic metals evaluated on vertebrate reproduction, attention must be given to this population, as bioaccumulation impacts may be already in place. Another aggravating factor are the high Rb concentrations detected in this population. Little is known about this metal, but considering its potential for accumulation and magnification, along with previously described impacts on certain reproductive traits of vertebrates, future studies should focus on assessing possible Rb effects on the resident nurse shark population at the Canal do Navio in more detail.

Author contributions

NW and APC were responsible for the material collection and analysis. RHD, TDS and IQW were responsible for data structuring and elemental and biomarker determinations. RDL was responsible for the statistical design. JLSN provided logistical and financial support for the study. NW and RHD were responsible for the structuring and extensive revision of the manuscript. All authors contributed intellectually to the manuscript.

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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