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Ecological and biological factors associated to mercury accumulation in batoids (Chondrichthyes: Batoidea) from northeastern Brazil

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ABSTRACT

The present study aims to understand how ecological and biological factors affect the Hg levels in stingrays occurring in the Northeastern Brazilian coast. Total mercury (Hg), methylmercury (MeHg) and stable isotope ($\delta^{13}C$ and $\delta^{15}N$) analyses were performed in five species. *Hypanus americanus* and *Gymnura micrura* showed the highest total Hg concentrations (300 and 176 $ng.g^{-1}$, respectively). *Hypanus guttatus* exhibited a significant correlation between total Hg and size. Both species of the genus *Hypanus* presented the highest percentage of MeHg, around 100%, whereas the other species showed median percentages below 50%. The $\delta^{13}C$ and $\delta^{15}N$ signatures suggest that all studied species present the same foraging habitat but different trophic positions. Trophic position and animal size were the main factors influencing total Hg and MeHg concentrations in batoid species. The genus *Hypanus*, present in the Brazilian fish markets, showed concentrations above the accepted limits for human consumption.

Mercury (Hg) is a trace metal naturally occurring in the environment, but anthropogenic activities have changed its distribution and concentrations in coastal ecosystems (Fitzgerald et al. 2007). In that context, aquatic organisms can reflect changes in environmental concentrations because Hg suffers high bioaccumulation and in its organic form, methyl-mercury (MeHg) it biomagnifies along food chains. Consequently, higher trophic level species, including human populations, are potentially exposed to Hg contamination and its negative effects (Streets et al. 2019).

Predatory fishes, like elasmobranchs, often present higher Hg concentrations compared to other fish species (Wang and Wang 2019). Slow growth, late maturation, high trophic level and other characteristics particular to this group contributes to the more efficient bioaccumulation of contaminants (Gelsleichter and Walker 2010). Among elasmobranchs, batoids (e.g. stingrays, skates, and guitar fishes) are potentially more exposed to contaminants by inhabiting benthic habitats (Frisk 2010), where Hg concentrations (Lamborg et al. 2014) and bioavailability (Signa et al. 2017) are generally higher compared to adjacent water column. In a recent review, Bezerra et al. (2019) discussed Hg accumulation trends in batoid species worldwide and highlighted the scarcity of contamination assessments, especially for the South Atlantic Ocean, despite their importance as benthic predators and high occurrence in fisheries production as by-catch.

To date, for the South Atlantic Ocean, there is only a few studies reporting total Hg levels in batoid species, *Hypanus guttatus* (Lacerda et al. 2016); *Sympterygia bonapartei* and *Myliobatis goodei* (Marcovecchio et al. 1988); *Zapteryx brevirostris* (Muto et al. 2014); and *Dasyatis margarita* (Ntow et al. 1989). Therefore, making difficult the understanding of the biological and ecological factors influencing Hg uptake and accumulation. Also, based on such few species and locations reported on those studies, it is still impossible to compare the situation of batoid in the South Atlantic Ocean with other areas of the world's oceans. To help improve this situation, the present study aims to understand how ecological and biological factors (e.g. size, weight, sex, diet, δ13C, and δ15N) affect the Hg levels in five batoid species occurring in the continental shelf of the Northeastern Brazilian coast. The results allowed

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the evaluation of the potential exposure risk to humans that consume these batoid species and to contribute to the limited literature of contamination assessments in batoids worldwide.

Batoid samples were obtained from artisanal fishery vessels at Icaraí beach, Ceará State, Northeastern Brazil (Lat. 03°67′S; Long. 38°67′W) between March and July 2015. Ceará state is part of the East Brazilian Shelf, a region with meso-tide regime under the influence of the North Brazil current. This region has annual average surface temperature of 28 °C and oligotrophic waters with a low primary productivity (Sherman and Hempel 2009). Local climate varies from semi-arid to sub-humid regimes with annual precipitation of 700 mm to 1200 mm (Lima et al. 2000). Fishery production is predominantly artisanal corresponding to 78.2% of all fishing fleets, and responsible for 64.7% of fish captured in the Ceará state (IBAMA 2002).

Batoid species were captured using bottom long line and gill-net. Taxonomic identification, life stage and diet were based on the literature for each species: *Aetobatus narinari* - Whitespotted eagle ray (Bassos-Hull et al. 2014; Schluessel et al. 2010), *Rhinoptera bonasus* - Cownose ray (Collins 2005; Smith and Merriner 2006), *Hypanus americanus* - Southern stingray (Nunes 2015; Silva 2005), *Hypanus guttatus* - Longnose stingray (Silva et al. 2001; Silva 2005) and *Gymnura micrura* - Smooth butterfly ray (Yokota et al. 2013; Yokota and Carvalho, 2017). Individual weight and disk width (DW) were determined by digital scale (0.1 g precision), and measure tape (0.1 cm precision), respectively. Sex was determined by the presence of clasper.

The muscle samples from the pectoral fins of each individual were freeze-dried upon arrival in the lab. Moisture content was calculated for each species and used to report the results on a wet weight basis. All species showed average moisture content of 77%, except *Aetobatus narinari* that presented a slightly but significantly lower average (75%) (F = 31.2, $p < 0.05$).

Total Hg concentrations were quantified by cold vapor atomic absorption spectrophotometry (CV-AAS). The average limit of detection (LOD) was 0.7 ng.g^{-1}. Validation of the methods was obtained by simultaneous analysis, in duplicate, of certified reference material (Mussel Tissue ERM-CE 278K) with recovery of 113 ± 35%.

MeHg and stable isotopes (δ^{13}C and δ^{15}N) were determined in selected samples, based on size and total Hg concentration as criteria. We selected sub-samples of the large, medium and small animals (Table 1). In species with a low range of size, animals with the highest and lowest total Hg concentrations were selected.

To quantify MeHg, approximately 100 mg of muscle samples were placed in an oven at 68 °C for 3–4 h with 3.0 mL of 25% KOH/methanol (degree HPLC) in the dark to avoid possible degradation of MeHg (EPA, 2001; Liang et al., 1994). The subsequent ethylation was made using 200 µL of 2 M acetate buffer (pH 4.5) followed by 30 µL of the sample and 50 µL of tetraethyl sodium borate (1%) (Taylor et al. 2011). The quantification was obtained with a GC-AFS (MERXTM Automated Methyl Mercury Analytical System, Brooks Rand, USA). Certified standard (DOLT-2) was included in every sample batch yielding a recovery of 104 ± 6%. The limit of detection was 0.5 ng.g^{-1} (Ellison et al. 2002).

The stable isotopes ratios (δ^{13}C and δ^{15}N) were quantified in a continuous-flow isotope-ratio mass spectrometer (Delta V Advantage, Thermo Scientific, Germany) coupled to an elemental analyzer (Flash 2000) using 1 mg of lyophized sub-samples. Isotopes concentrations are expressed in the conventional delta (δ) notation relative to Pee Dee Belemnite for δ^{13}C and atmospheric N_2 for δ^{15}N (Peterson and Fry 1987).

To estimate the risk of human exposure to MeHg, we use Eq. (1) described by Vieira et al. (2015), where C corresponding to batoids Hg concentrations (ng.g^{-1} wet weight), I is the human ingestion per capita, where we adopt the rate of fish consumption of the region's population (35.6 g.day^{-1}) reported by Sartori and Amancio (2012), and W is the adult average body weight (70 kg).

$$E = \frac{C \times I}{W} \tag{1}$$

The calculated exposure levels (E) were derived from Eq. (2) to assess the Hazard Quotient (HQ) for each species (Newman and Unger 2002).

$$HQ = \frac{E}{RfD} \tag{2}$$

where, RfD is the reference dose from the World Health Organization (0.1 $\mu g_{MeHg}.kg_{body\ weight}{}^{-1}.day^{-1}$; UNEP/WHO, 2008). Outliers data were identified and removed from subsequent statistical analysis. Shapiro-Wilk test was employed to test normality assumptions. Non-parametric Kruskal-Wallis test was used to compare DW, weight, Hg concentrations among species and Man-Whitney test was employed to compare differences between males and females. Scatterplots of Hg/DW and δ^{13}C/δ^{15}N plots were used to observe the relationship between size and Hg concentrations and the influence of feeding ecology of the sampled species on their Hg content. All significance tests were

Table 1

Median and range of total Hg concentrations, diet composition, δ ^{13}C and δ ^{15}N stable isotope ratios, life stage, and biometric information (disc width (DW)) of five batoid species from Ceará coast, NE Brazil.

Common name (Scientific name)	Feeding habit[a]	Diet	Life Stage[b]	DW (cm)	Weight (kg)	Total Hg (ng.g^{-1})	δ^{13}C (‰)	δ^{15}N (‰)
Southern stingray (*H. americanus*)	C	Molluscs Crustacean Fishes	A	74 52–91 (*n* = 12)	10 4–22 (n = 12)	300 200–1197 (n = 12)	−14.4 −15.4 to −13.9 (*n* = 4)	11.4 8.7–13.6 (n = 4)
Longnose singray (*H. guttatus*)	C	Molluscs Crustacean Fishes	J/A	32 11–72 (*n* = 17)	0.7 0.1–11 (*n* = 16)	56 1–1089 (n = 17)	−14.7 −15.3 to −13.7 (*n* = 7)	11.1 8.7–13.9 (n = 7)
Smooth butterfly ray (*G. micrura*)	C	Crustacean Fishes	J/A	48 24–58 (*n* = 6)	1 1.1 – 1.8 (n = 6)	176 42–417 (n = 6)	−14.3 −14.7 to −13.8 (*n* = 3)	13.0 11.8–15.8 (n = 3)
Cownose ray (*R. bonasus*)	D	Bivalves	NB/A	35 30–90 (*n* = 9)	0.5 0.3–9 (n = 9)	10 4–32 (n = 9)	−14.8 −15.5 to −14.0 (n = 3)	9.5 9.2–13.5 (n = 3)
Spotted eagle ray (*A. narinari*)	D	Bivalves	J	41 33–48 (n = 12)	0.8 0.4–1.2 (n = 12)	6 1–22 (n = 12)	−15.1 −16.1 to −14.4 (n = 4)	9.7 9.4–10.3 (n = 4)

[a] D – Durophagous; C – Carnivorous.

[b] NB – Newborn; J – Juvenile; A – Adult.

Table 2
MeHg percentages (w.w. basis), exposure level (E) and hazard quotient (HQ) of five batoid species from Ceará coast, NE Brazil.

Common name (Scientific name)	MeHg (%)	E (μg.kg^{-1}.day^{-1})	HQ
Southern stingray (*H. americanus*)	103 92–116 (*n* = 5)	162 475–162	1.6 1–4.8
Longnose singray (*H. guttatus*)	128 88–151 (n = 6)	280 25–663	2.8 0.2–6.6
Smooth butterfly ray (*G. micrura*)	47 41–97 (n = 3)	21 11–97	0.2 0.1–0.9
Cownose ray (*R. bonasus*)	20 17–44 (n = 3)	1.4 1–4	0.01 0.01–0.04
Spotted eagle ray (*A. narinari*)	45 15–75 (n = 4)	3 0.8–5.1	0.02 0.009–0.05

conducted using an alpha value of 0.05 (95% confidence). Graphs and statistical tests were performed using Microsoft® Office 2016 (Microsoft Corporation, 2016) and Copyright© StatSoft. Inc. (1984–2011).

A total of 56 individuals from the five species (*Hypanus americanus*, *H. guttatus*, *Aetobatus narinari*, *Rhinoptera bonasus* and *Gymnura micrura*) were assessed. Species feeding habit, life stage, disc width, weight, total Hg and MeHg concentrations, and stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) are presented in Table 1.

Total Hg concentrations were highest in *H. americanus* (300 ± 112 ng.g^{-1}), but not significantly different than *G. micrura* (median of 176 ± 68 ng.g^{-1}) ($U = 15$, $p > 0.05$), followed by *H. guttatus* (56 ± 90 ng.g^{-1}), *R. bonasus* (10 ± 3 ng.g^{-1}) and *A. narinari* (6 ± 2 ng.g^{-1}) (Table 1).

The genus *Hypanus*, in contrast to the other species, presented MeHg concentrations above the safety limits for human consumption (Table 2). All individuals of *H. americanus* and three of *H. guttatus* showed MeHg concentrations above the maximum level allowed by the World Health Organization (100 ng.g^{-1}), however only one individual of each species presented Hg concentrations above the safety limits established by Brazilian legislation for predatory species (1000 ng.g^{-1}). Correlations between Hg levels and DW were only significant for *H. guttatus*. Concentrations showed an accentuated increase with animal size for this species (Fig. 1).

The genus *Hypanus* displayed a higher MeHg/Hg ratio among the batoid species studied. Virtually all Hg measured in muscle tissues of *H. americanus* and *H. guttatus* was MeHg (Table 2). In contrast, *G. micrura*, *A. narinari* and *R. bonasus* exhibited 47%, 44% and 22% MeHg/Hg ratio, respectively (Table 2). Only one specimen of *G. micrura* showed a higher proportion (97%). MeHg, similar to total Hg concentrations, presented significant and strong correlation with DW in *H. guttatus*. However, no significant correlation was found between MeHg concentrations and size in the other species.

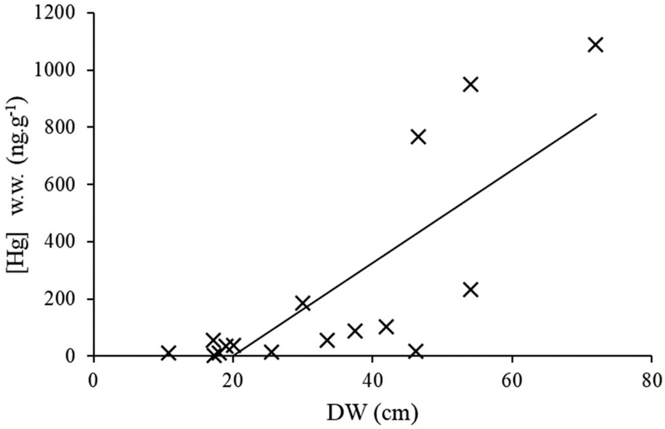

Fig. 1. Total Hg bioaccumulation curve for *H. guttatus* from the Ceará coast, NE Brazil.

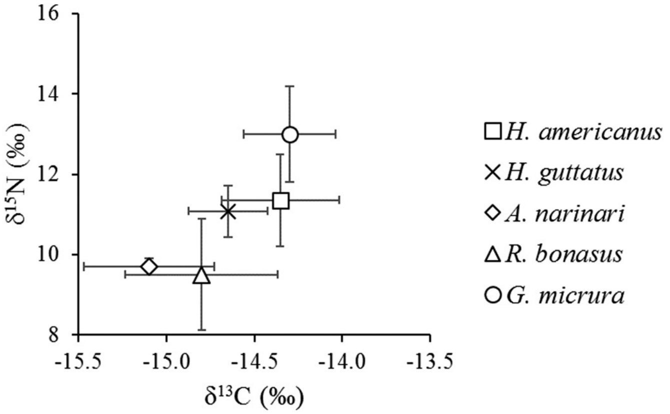

Fig. 2. Medianf (± Standard error) isotopic ratios ($\delta^{13}C$ and $\delta^{15}N$) for stingrays from the northeastern Brazilian coast.

Differences in Hg concentration and size between male and female were not statistically significant for *H. guttatus* ($U = 10$, $p > 0.05$; Size: $U = 15$, $p > 0.05$), *A. narinari* ($U = 15$, $p > 0.05$; Size: $U = 14$, $p > 0.05$) and *R. bonasus* ($U = 5$, $p > 0.05$; Size: $U = 7$, $p > 0.05$). The species *H. americanus* and *G. micrura* were composed mostly of male and female specimens, respectively, which prevented us to test for sex differences in Hg concentrations.

Highest $\delta^{13}C$ and $\delta^{15}N$ ratios averages were observed in *G. micrura*, followed by *H. americanus* and *H. guttatus*, whereas *R. bonasus* and *A. narinari* presented the lowest (Fig. 2). However, these observed differences were not statistically significant ($\delta^{13}C$: $H = 4.4$, $p > 0.05$; $\delta^{15}N$: $H = 5.6$, $p > 0.05$). Considering the relationship between stable isotopes and Hg concentrations it was possible to observe a general increase in concentrations with increases in $\delta^{15}N$ ratios for all species, except *H. americanus*, which presented the highest median Hg concentrations (Fig. 3). *H. guttatus* was the only species that presented a significant correlation between Hg concentrations and $\delta^{15}N$ ($r = 0.83$, $p < 0.05$) (Fig. 4). The other species showed no significant correlation, between Hg concentrations and $\delta^{15}N$.

The median values of HQ calculated for genus Hypanus surpass the reference level of exposure (RfD) (Table 2). Three individuals of the *H. guttatus* and all individuals of *H. americanus* showed HQ > 1.0 relative to the RfD, that it is considered the level to cause adverse effects to consumers.

Diet is an important factor that drives Hg and MeHg concentrations

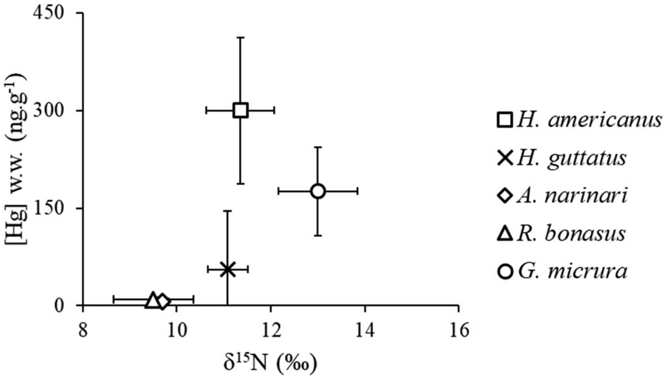

Fig. 3. Median concentrations of Hg (± Standard error) concentration in the function of δ15N variation for each stingray species.

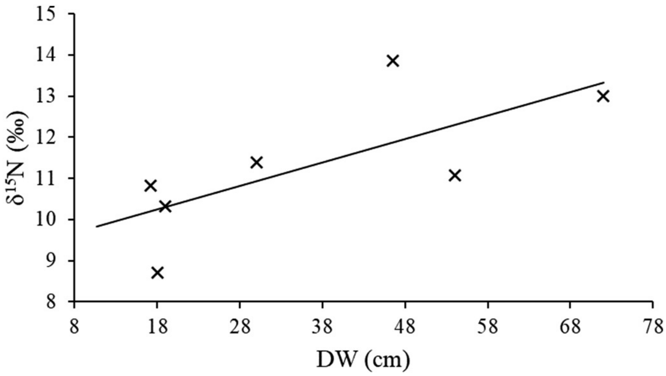

Fig. 4. Variation in the diet, expressed as δ15N ratios, for *H. guttatus* according to size.

in fish species, due to its capacity to biomagnify along the food web (Chumchal et al. 2010; Clarkson and Magos 2006; Li et al. 2009; Moura and Lacerda, 2018). In elasmobranches, Pinho et al. (2002) found relationship between the Hg concentrations and feeding habits when analyzing sharks from Brazilian offshore waters. For batoids, Bezerra et al. (2019) shown similar results, where Hg concentrations are generally higher in crustacean feeders compared to zooplankton feeders. In the present study, the highest Hg concentrations were found in *H. americanus*, wich presents diets mainly composed of crustaceans and some fishes, and *G. micrura*, a strictly piscivorous species (Figueiredo 1977; Tilley 2011). In contrast, *A. narinari* and *R. bonasus* presented the lowest Hg concentrations likely because their a diet is mainly composed by molluscs (Figueiredo 1977). Except for *H. guttatus*, which presented Hg concentrations above that found by Lacerda et al. (2016) for the same species, none of the sampled species have been previously reported regarding Hg levels which hamper direct comparisons with our results.

Another important aspect of Hg accumulation is its relationship with size, in which older/larger fishes generally present higher Hg content. That is the case for many predatory bone fish (Junqué et al. 2018; Stafford and Haines 2001), as well as elasmobranch species (Murillo-Cisneros et al. 2018; Taylor et al. 2014; Sandoval-Herrera et al. 2016). We found a significant positive correlation between Hg concentrations and size in *H. guttatus* ($r_{spearman} = 0.79$), which was attributed to differences in diet between juveniles and adults. Ontogenetic shifts in diet was previously described for this species (Silva et al. 2001) with juveniles presenting higher frequency of invertebrates in the stomach, while adults presented some fish species. A positive relationship between $\delta^{15}N$ and size found in this same species is an important evidence of ontogenetic shifts. Nielsen et al. (2019) and Park et al. (2018) comparing the stomach content and isotopic signature in Greenland sharks and Walleye pollock, respectively, found differences in $\delta^{15}N$ signatures between individuals with distinct diet composition.

The same author also described a similar diet shift for *H. americanus*, but because we only analyzed adult specimens a clear relationship between Hg concentrations and size was not observed.

Although the increase in size is not related to a rise in Hg concentrations. When the growth rate is higher than bioaccumulation, it is possible to see a reduction in Hg concentrations (Pinho et al. 2002). These factors are possible explanations for the absence of a significant correlation between Hg concentrations and length in the studied species, except for *H. guttatus*.

Among the Hg chemical compounds, MeHg deserves a special attention because of the high toxicity and longer residence time in the organism (UNEP/WHO, 1990; Bisinoti and Jardim 2004). In our study, only the genus Hypanus presented the majority of their Hg content as MeHg (> 100%), while the other species showed lower proportions, varying from 20% to 47%. The relative contents of MeHg found in *A. narinari* and *R. bonasus* are in agreement with their diet, which is composed mostly by invertebrates (Collins 2005; Schluessel et al. 2010). A similar result was found by Pinho et al. (2002) in shark species from the Brazilian coast, in which the piscivorous *Squalus mitsukurii* presented higher MeHg content relative to the total Hg concertation than *Mustelus canis,* an invertebrate feeder.

The $\delta^{13}C$ ratios observed in all the studied species corroborate a coastal water feeding habitat (Ben-David et al. 1997; Hobson et al. 1997; Peterson and Fry 1987). We found no statistically significant differences in $\delta^{15}N$ and $\delta^{13}C$ among species, however, a clear increase in Hg concentration was observed in species enriched in $\delta^{15}N$. This pattern was previously reported in sharks from the Western Atlantic Ocean (Taylor et al. 2014) and the North Atlantic (Newman et al. 2011), where increases in Hg concentrations were positively correlated with $\delta^{15}N$.

A. narinari, *R. bonasus*, and *G. micrura*, which present low MeHg percentage, showed calculated HQ values below the reference dose, which means the level of exposure does not cause a threat to consumers, but these species are not frequently consumed because of the low quality of their meat (Figueiredo 1977). Although the genus Hypanus showed high median HQ value with concentrations above the safety limits for human consumption. These species, differently from another one, presented a high MeHg percentage and are commonly commercialized in the northeastern Brazilian fish market (Spanopoulos-Zarco et al. 2014).

Our results contribute to the scarce number of studies on the Hg distribution in batoid species in the South Atlantic, and it is the first study simultaneously presenting MeHg content and stable isotopes signatures in stingrays from this basin. Animal size and feeding ecology were the main factors influencing variation in Hg concentrations. However, due to a small sample size for some species we urge future studies to collect a higher number of individuals, and including juvenile and adult specimens, in order to assess any eventual variation in Hg levels due to ontogenetic shifts. Moreover, the genus Hypanus presented high MeHg levels and high risk of exposure to humans consuming them. We found three individuals of *H. guttatus* and all individuals of *H. americanus* containing Hg levels above the consumption limit. That is especially concerning for local coastal populations that present a high consumption rate of these fisheries and may be impacted from potential adverse effects from Hg concentration.

CRediT authorship contribution statement

V.L. Moura: Investigation, Formal analysis, Writing - original draft. **J.N. Rabelo:** Investigation, Formal analysis, Methodology. **M.F. Bezerra:** Writing - review & editing. **G.B. Silva:** Writing - review & editing, Methodology. **V.F. Faria:** Writing - review & editing. **C.E. Rezende:** Methodology, Resources, Writing - review & editing. **W.R. Bastos:** Methodology, Resources, Writing - review & editing. **L.D. Lacerda:** Conceptualization, Resources, Supervision, Writing - review & editing.

Declaration of competing interest

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

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