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ANIMAL SCIENCE

Balancing conservation, traditional cuisine and pollution: case study of the stingray *Hypanus berthalutzae* in Pernambuco, Brazil

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Abstract: Fish is an important food item in the diet of humans, whereas it is also a major source of exposure to toxic chemicals. Persistent organic pollutants (POPs) and mercury (Hg) are known to have hotspots of contamination across the coast of Pernambuco, Brazil. This study investigated organochlorines and Hg in muscle samples from respectively 17 and 14 individuals of the stingray *Hypanus berthalutzae* – a fishing resource used to prepare a traditional dish in the local cuisine. DDTs, HCHs and mirex were not detected in the samples. Mean concentrations of PCBs, CHLs and total Hg were 0.032, 0.004 and 60 ng g^{-1} wet weight (ww), respectively. Ecological and biological parameters are important factors in the bioaccumulation of pollutants in fish. Although the concentrations of POPs and Hg do not present a health risk to consumers in general, they may pose a health risk to certain groups such as children and people who eat *H. berthalutzae* daily. This paper is relevant for the conservation of an endemic stingray species widely consumed in northeastern Brazil and classified as vulnerable in terms of risk of extinction.

Key words: Elasmobranch, endemic, food safety, mercury, pesticides, POPs.

INTRODUCTION

In the 1950s, it was discovered that fish consumption could contaminate domestic animals and humans, triggering illnesses and eventually leading to death. This episode is known as the Minamata disease that was triggered by mercury poisoning (Igata 1993, Eto 2000) and sparked a growing interest in understanding the risk of exposure to toxic pollutants such as trace metals (Hg) and persistent organic pollutants (POPs) (Kiviranta et al. 2004, Darnerud et al. 2006, Baptista et al. 2013, Malarvannan et al. 2014, Lacerda et al. 2016). As fish consumption is a potential source of exposure to these compounds, many studies have been evaluating levels of contaminants in fish (Rodríguez et al. 2015, Bezerra et al. 2019), especially those purchased in fish markets since they are easily available to humans (Lacerda et al. 2016).

In its various forms, mercury (Hg) is recognized as a potentially dangerous pollutant to the marine environment (Dias et al. 2008). Similarly, POPs such as polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane and its metabolites (DDTs), hexachlorocyclohexanes (HCHs), chlordane-related compounds (CHLs) and other chlorinated pesticides are highly toxic to humans and wild animals since they are persistent in the environment, resistant to biodegradation and susceptible to long-range atmospheric transport (Gramatica & Papa 2007, Alves et al. 2010). POPs along with the organic form of Hg (methylmercury) have a strong bioaccumulative nature due to their lipophilic characteristics. Both tend to biomagnify across the food chain so that animals that occupy higher trophic positions such as rays and sharks are particularly exposed to toxic chemicals (Ferreira et al. 2004, Storelli et al. 2011a, b, Good et al. 2014).

Fish is important for human nutrition since it provides proteins, vitamins, minerals and polyunsaturated fatty acids such as omega-3 (Domingo 2007, Pal et al. 2018). Some species of rays are consumed by human populations (Barreto et al. 2017, Bornatowski et al. 2018, Marques et al. 2019). In Brazil, for instance, there is a traditional dish known as *moqueca* that it is made from stingrays' meat (e.g. *Hypanus berthalutzae*) in the north shore of Pernambuco State. Rays of the genus *Hypanus* along with *Aetobatus narinari* are widely consumed in Pernambuco (Lessa et al. 2005), and eight species of rays are commonly observed in local artisanal fishing landings: *Hypanus marianae*, *Hypanus guttatus*, *Hypanus berthalutzae*, *Gymnura micrura*, *Aetobatus narinari*, *Rhinoptera brasiliensis*, *Rhinoptera bonasus* and *Urotrygon microphthalmun* (Melo 2016, Santander-Neto et al. 2016, Queiroz et al. 2019, 2023, Araújo et al. 2022). *H. berthalutzae* is particularly abundant in local fisheries (Lessa et al. 1999) in addition to being an endemic species to Brazil (Petean et al. 2020). Thus, it is a potential route of human exposure to Hg and POPs considering the historical local sources (Yogui et al. 2018, Julio et al. 2022). The average consumption of finfish in Brazil was 4 kg per year in 2008/2009 while the average in northeastern states (including Pernambuco) was 12.8 kg per year (IBGE 2010).

This study quantified organochlorines (OCs) and mercury in the muscle of *H. berthalutzae* stingrays caught on the north coast of Pernambuco (Brazil). It is hypothesized that levels of such contaminants can pose a threat to local seafood safety due to historical contamination of the study area and its vicinities. Parameters influencing contamination of *H. berthalutzae* in the study area are discussed, and safety for human consumption is also assessed. Finally, recommendations are provided for future studies of chemical contaminants in fish.

MATERIALS AND METHODS

Study area

Pernambuco is located on the northeastern coast of Brazil. The fishing ground of *H. berthalutzae* in the study area is the continental shelf off Itamaracá Island (7°42' S, 34° 48' W) in the north shore of Pernambuco. Catches take place about 5 to 18 km from the coastline (Figure 1) at depths up to 50 m (Queiroz et al. 2023). The local shelf has a sandy bottom interspaced with sandreefs and rhodoliths (Magalhães et al. 1997, Cocentino et al. 2004, Viana 2005). The northern portion of the Santa Cruz Channel (Barra de Catuama) and surrounding areas (e.g. Ponta de Pedras) is a site historically contaminated by Hg due to past input of Hg-rich effluents from a chlor-alkali industrial plant (Julio et al. 2022). High concentrations of Hg have been found in local sediments, oysters and suspended particulate matter (Meyer & Medeiros 2017).

In addition, the Capibaribe River estuary is the main hot spot of contamination across the coast of Pernambuco, and it is located about 30 km south of Itamaracá Island (Macedo et al. 2007, Yogui et al. 2018, Gomes et al. 2022). It is sediments exhibit contamination of DDTs at levels that might trigger adverse biological effects (Yogui et al. 2018). Shore drift along the coast of Pernambuco is mainly northward, carrying contaminants to the study area.

Sampling

Currently known as *Hypanus berthalutzae*, the species has undergone some recent taxonomic revisions (Last et al. 2016, Petean et al. 2020). Previously, it was referred to as *Hypanus*

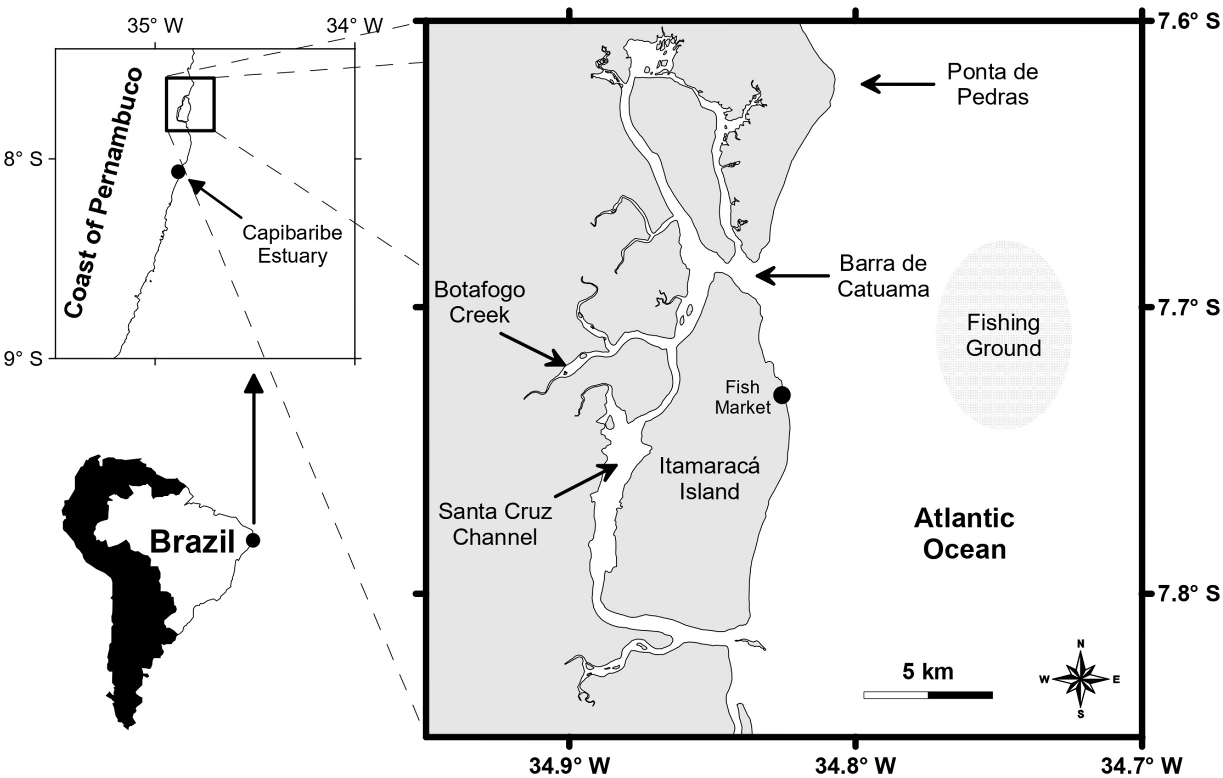

Figure 1. Geographical setting of the study area. The right panel shows the northern coast of Pernambuco (Brazil), including fishing grounds of the stingray *Hypanus berthalutzae* and the fish market where samples were purchased. Location of the Capibaribe Estuary, a hot spot of pollution along the coast of Pernambuco, is depicted in the upper left panel.

americanus (2016 to 2020) and *Dasyatis americana* (before 2016). Even older scientific names include *Pastinaca hastata*, *Dasibatis hastata*, *Dasyatis hastata* and *Dasybatus hastatus* (Petean et al. 2020).

H. berthalutzae is typically caught by either gill net or bottom longline at Itamaracá Island (Melo 2016, Queiroz et al. 2023). The latter is selective and catches only adult individuals of *H. berthalutzae* (Queiroz et al. 2023). Samples were collected during fisheries landing in October, 2020. Stingrays were landed eviscerated and with no head. Species identification was based on the stingray carcass and researcher's background. Ray disk width was not measured but it was clear that they were all adults since their width was over 75 cm - size at which females are sexually mature (Henningsen 2000, Ramírez-Mosqueda et al. 2012). A total of 17 muscle fillets from the dorsal pectoral fin (near spine) were taken for contaminant analysis. Samples were wrapped in clean aluminum foil and transported to the laboratory in coolers at 4 °C.

In the laboratory, scalpel and tweezers were used for removing skin from muscle. Subsamples were weighed on an analytical balance, placed in an oven (60 °C) for 24 h and weighed again for gravimetric determination of dry weight. Subsequently, samples were freeze-dried for several days at -60 °C, macerated using porcelain pestle and mortar, homogenized, transferred to clean glass jars and stored in freezer at -20 °C until chemical analysis.

Persistent organic pollutants

Analysis of POPs was carried out in all muscle samples according to procedures described in Miranda & Yogui (2016). In brief, approximately 3.0 g of freeze-dried tissues were Soxhlet-extracted using 80 mL of n-hexane and methylene chloride (1:1, v/v) for 8 h. Prior to extraction, 100 ng of internal standards (DBOFB, PCB-103 and PCB-198) were added to the samples. An aliquot of the organic extracts (10%) was taken out for gravimetric determination of lipids using a microanalytical balance. The remaining extracts were sequentially cleaned up with sulfuric acid (95-98%) and organics-free distilled water. The cleaned up extracts were concentrated down under a gentle nitrogen flow and recovery standard (TCMX) was added to the final extracts.

Extracts were injected into a gas chromatograph coupled to a mass spectrometer (GC-MS, Agilent Technologies, models 7820A and 5975C). Splitless injections of 1 µL were done with an autosampler. Helium was the carrier gas at a constant flow rate of 1.2 mL min^{-1} into the capillary column (HP-5ms: 30 m length × 0.25 mm inner diameter × 0.25 µm film thickness). Detailed description of temperatures and oven programs in the PCBs and OCPs runs are described elsewhere (e.g. Miranda & Yogui 2016). The mass spectrometer was operated with an electron ionization (EI) source at 70 eV. The full list of organochlorines investigated can be checked in the Supplementary Material - Tables SI, SII. Identification of analytes in the GC-MS was based on retention times and relative abundances of major *m/z* ions, whereas quantification was based on internal standardization. The analytical curves were based on 6-point linear regression with acceptable coefficient of determination (R^2) equal to or greater than 0.995.

Less than 5% of target analytes were detected in laboratory blanks. These traces of blank contamination were subtracted from the samples in each analytical batch. The average recovery of internal standards was 64.2 ± 2.4% (95% confidence interval). NIST certified reference material SRM 2974a (organics in freeze-dried mussel tissue) was analyzed to verify the accuracy and precision of the method. Recovery of certified analytes in the SRM averaged 114 ± 8% (confidence interval 95%). The average coefficient of variation (CV) of SRM replicates was 6.5%. Instrumental limits of quantification (LQ) were calculated by dividing the lowest calibration level of a target analyte by the amount of sample extracted (Lauenstein & Cantillo 1998). LQs for PCBs, DDTs, HCHs, CHLs and mirex ranged from 0.071 to 0.089 ng g^{-1} wet weight (ww), with a mean of 0.073 and a standard deviation of 0.008 ng g^{-1} ww. Individual LQs are reported in the Tables SI and SII).

Mercury

Due to sample constraints, Hg was analyzed in just 14 out of 17 samples. Analysis of total Hg was done in triplicate samples of ~100 mg of dry muscle tissues. Samples were digested with 2 mL of nitric acid (65%) previously purified in a sub-boiling distillation system. Digestion was carried out in a conventional microwave (model MEO44, Electrolux) with irradiation at 780 W for 5 min followed by further 5 min for cooling. The digested extracts were completed to 5 mL with ultrapure water. Total Hg was determined using an inductively coupled plasma optical emission spectroscopy system (ICP-OES, Spectro Analytical Instruments, model Cirus CCD) equipped with a quartz torch (2.5 mm inner diameter) injector tube. A continuous flow chemical steam generation system was coupled to ICP-OES. Solutions were conducted to a gas/liquid separator and argon was used for transporting vaporized Hg species directly to the ICP-OES plasma. The wavelength used for quantification was 184.950 nm. The average

LQ was 10 ng g^{-1} ww. Certified reference material (DOLT 4 – dogfish liver) was analyzed with the analytical batch for checking method accuracy. Recovery of Hg in DOLT 4 ranged from 95 to 105% of the certified concentration.

Exposure assessment estimation

Discussion of Hg and POPs data is done on wet basis. For POPs, the estimated concentrations were compared with maximum limits recommended by U.S. EPA (United States Environmental Protection Agency; USEPA 2000). Hg food safety calculation was performed as described below. Health risk associated with fish consumption was assessed using the following equation proposed by Newman & Unger (2002):

$$HQ = E \, / \, RfD \quad (1)$$

where HQ is the health risk coefficient, E is the level of exposure or intake of Hg (ng kg^{-1} body weight day^{-1}) and RfD is the Hg reference dose (470 ng kg^{-1} body weight day^{-1}) (BCS 2007). The exposure level (E) was calculated as follows:

$$E = C * I \, / \, W \quad (2)$$

where C is the concentration of Hg (ng g^{-1} ww), I is the *per capita* intake rate (35.06 g day^{-1}; IBGE 2010) and W is the average weight of the local adult population (70 kg). If HQ < 1, the exposure level is lower than the reference dose. It means that daily exposure at this level is unlikely to cause adverse effects to consumers.

Additionally, the estimated daily intake (EDI – expressed as mg kg_{BW}^{-1} day $^{-1}$) was calculated for Hg using two finfish consumption rates: 35 g day^{-1} (IBGE 2010) and 142 g day^{-1} (Bezerra et al. 2023). The former represents the general *per capita* fish consumption for the population of northeastern Brazil, whereas the latter represents the specific *per capita* consumption of traditional communities that eat fish for subsistence. EDI for Hg (EDI_{Hg}) can be calculated as follows:

$$EDI_{Hg} = \frac{C_{ray} \, X \, CR_{local}}{BW}$$

where C_{ray} (mg kg^{-1}) is the Hg concentration in *H. berthalutzae*, CR_{local} (g day^{-1}) is the fish consumption rate, and BW is the consumer body weight (70 kg for adults and 15 kg for children) (USEPA 2000).

Target risk quotient (THQ) was estimated since it represents the chronic non-carcinogenic health risk of exposure to Hg consumed via finfish. Values below 1 represent no expected health effect while values above 1 represent a potential for adverse health effects. Calculation was made according to the following equation:

$$THQ = \frac{EF \times CR_{local} \times ED \times C_{ray}}{RfD \, X \, BW \times AT}$$

where EF is the exposure frequency (365 days per year), ED is the exposure duration (77 years for adults and 6 years for children), RfD is the reference dose of Hg (0.0001 mg kg_{BW}^{-1} day^{-1}), and AT is the average exposure time (EF × ED) (USEPA 2001, 2022).

The local safety level (FSL_{local}) was also calculated (USEPA 2001) according to the equation below. It consists of an upper limit for Hg concentration (ng g^{-1} ww) in consumed fish. Such limit should not be exceeded due to risk of developing health issues in the consumer.

$$FSL_{Local} = \frac{BW \times RfD}{CR_{local}}$$

RESULTS

Concentration of contaminants in muscle of *H. berthalutzae* is summarized in Table I. DDTs, HCHs and mirex were not detected in samples while PCBs and CHLs were detected below LQ.

Table I. **Concentration (ng g^{-1}) of organochlorine compounds and mercury in muscle samples of stingray *Hypanus berthalutzae* caught on the northern coast of Pernambuco, Brazil. Legend: ww = wet weight; dw = dry weight; lw = lipid weight.**

	ww	dw	lw	ww	dw	lw	ww	dw	lw
Contaminant	**Average**			**Minimum**			**Maximum**		
PCBs*	0.032	0.147	5.28	nd	nd	nd	< 0.089	< 0.410	< 14.7
DDTs	nd	nd	nd	nd	nd	nd	nd	nd	nd
HCHs	nd	nd	nd	nd	nd	nd	nd	nd	nd
CHLs*	0.004	0.019	0.696	nd	nd	nd	< 0.071	< 0.330	< 11.8
Mirex	nd	nd	nd	nd	nd	nd	nd	nd	nd
Total Hg	60	280	10,000	26	120	4,300	102	470	16,800

*** DDTs, HCHs and mirex were not detected in samples while PCBs and CHLs were detected below LQ, thus, average concentrations were calculated assuming that each sample concentration is half of the respective LQ.**

In this case, average concentrations for PCBs and CHLs were calculated assuming that each sample concentration is half of its respective LQ. This approach is known as the middle-bound principle, and it has been commonly used in dietary intake studies (De Mul et al. 2008, Llobet et al. 2008, Wang et al. 2009). Hg concentrations in muscle of *H. berthalutzae* ranged from 30 to 100 ng g^{-1} ww (mean ± SD = 60 ± 5 ng g^{-1} ww) (Figure 2). Such levels are below the local safety level (FSL_{Local}) for general adult population (200 ng g^{-1} ww). In contrast, mean Hg levels found in *H. berthalutzae* are similar to the safety level for general children population (43 ng g^{-1} ww), and above both adults (49 ng g^{-1} ww) and children (11 ng g^{-1} ww) who consume fish for subsistence.

The health risk coefficient (HQ) ranged from 0.03 to 0.11 (mean ± SD = 0.06 ± 0.03). This is below the reference level of exposure (E) that ranged from 0.01 to 0.05 (mean ± SD = 0.03 ± 0.01). The target risk quotient (THQ) for the general adult population ranged from 0.1 to 0.5 (mean ± SD = 0.3 ± 0.1), and for general children ranged from 0.6 to 2.4 (mean ± SD = 1.4 ± 0.6). THQ calculated for traditional communities that consume fish for subsistence ranged from 0.5 to 2.1 (mean ± SD = 1.2 ± 0.5) in adults, and from 2.4 to 9.8 (mean ± SD = 5.7 ± 2.3) in children.

The estimated daily intake (EDI) ranged from 0.00001 to 0.00005 (mean ± SD = 0.00003 ± 0.00001) for the general adult population, and from 0.00006 to 0.00024 (mean ± SD = 0.00014 ± 0.00006) for children. EDI calculated for traditional communities ranged from 0.00005 to 0.00021 (mean ± SD = 0.00012 ± 0.00005) for adults and from 0.00024 to 0.00098 (mean ± SD = 0.00057 ± 0.00023) for children (Table II).

DISCUSSION

The low concentration of POPs in *H. berthalutzae* could be attributed to low contamination in the stingray foraging area. Despite no data is available for POPs in sediments of the northern continental shelf of Pernambuco, Silva (2015) analyzed sediment samples in the Santa Cruz Channel and found concentrations of PCBs ranging from < LQ to 5.62 ng g^{-1} dry weight (dw). The author concluded that sediments of the Santa Cruz Channel have low concentrations of

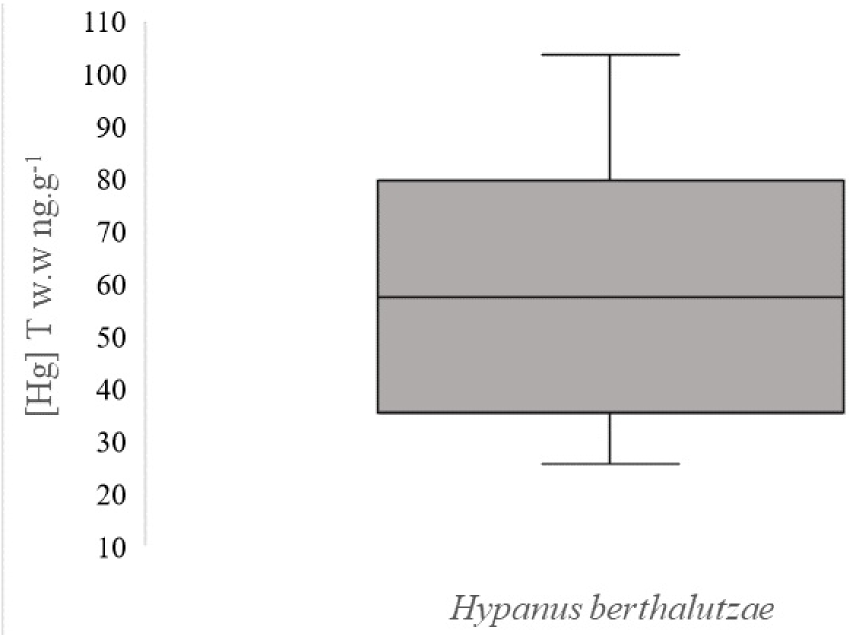

Figure 2. Box-and-whisker plots of Hg concentration in *Hypanus berthalutzae* from the coast of Pernambuco, northeastern Brazil.

PCBs, typical of poorly industrialized estuaries. The Santa Cruz Channel is over 22 km long and has a width varying from 0.6 to 1.5 km (Paiva et al. 2017). It borders the west side of Itamaracá Island and its northern connection to the ocean (Barra de Catuama) is right away from *H. berthalutzae* fishing ground (Figure 1).

In Todos os Santos Bay (Bahia State, Brazil), Santos et al. (2020) found average PCBs levels of 0.68 ng g^{-1} ww in *H. berthalutzae* muscle (Table III) (LQ = 0.38 in ng g^{-1} dry weight). This is an order of magnitude greater than the mean concentrations calculated for this study. Todos os Santos Bay is known to be more industrialized than the northern coast of Pernambuco. However, Sotão-Neto et al. (2020) found PCBs concentrations in sediments of the bay ranging from < LQ to 4.66 ng g^{-1} dw. This range is similar to the one observed at Santa Cruz Channel, evidencing that local sediment contamination is not enough for explaining the low levels of PCBs detected in *H. berthalutzae* in the northern coast of Pernambuco.

Miranda & Yogui (2016) investigated POPs in *Scomberomorus cavalla* caught in the coast of Pernambuco. Average concentration of PCBs in muscle was 8.57 ng g^{-1} ww (LQ = 0.50 ± 0.15 ng g^{-1} dry weight). *S. cavalla* is a carnivorous bony fish frequently employed in biomonitoring of contaminants due to its commercial importance (Grady et al. 1989, Adams & McMichael 2007, Ploetz et al. 2007, Costa & Lacerda 2009, Lacerda et al. 2016, Miranda & Yogui 2016, Silva et al. 2021). PCBs levels found in *S. cavalla* are 50 times higher than those in *H. berthalutzae* caught in the present study. In comparison to other fish species, low levels of PCBs were also found in muscle of the stingray *Hypanus americanus* in Florida, USA (Johnson-Restrepo et al. 2005). According to the authors, absorption of organochlorines seems to be associated with the feeding behavior and habitat use of each species. They also argued that accumulation of PCBs is a tissue-specific process controlled by both metabolic and physiological characteristics of each organism. Cascaes et al. (2014) also found significant differences in accumulation of PCBs and DDTs when comparing the demersal shark *Rhizoprionodon lalandii* with other oceanic species. Based on the discussion above, the low

Table II. Parameters calculated for assessment of human exposure to mercury (Hg) contamination in muscle of the stingray *Hypanus berthalutzae* caught on the northern coast of Pernambuco, Brazil. Legend: EDI = estimated daily intake (expressed as mg kg_{BW}^{-1} day^{-1}); THQ = target risk quotient; GA = general adult population; SA = subsistence adult population; GC = general children population; SC = subsistence children population; SD = standard deviation.

Sample	EDI GA	EDI SA	EDI GC	EDI SC	THQ GA	THQ GC	THQ SA	THQ SC
R1	0.00002	0.00007	0.00008	0.00031	0.16	0.77	0.67	3.11
R4	0.00002	0.00010	0.00012	0.00047	0.25	1.17	1.01	4.73
R6	0.00004	0.00017	0.00020	0.00080	0.42	1.96	1.71	7.97
R7	0.00004	0.00016	0.00018	0.00073	0.38	1.79	1.56	7.28
R8	0.00004	0.00016	0.00018	0.00074	0.39	1.82	1.58	7.39
R9	0.00003	0.00011	0.00012	0.00049	0.26	1.22	1.06	4.94
R10	0.00001	0.00005	0.00006	0.00024	0.13	0.60	0.52	2.42
R11	0.00001	0.00006	0.00007	0.00026	0.14	0.65	0.57	2.65
R12	0.00005	0.00020	0.00023	0.00095	0.50	2.34	2.04	9.51
R13	0.00003	0.00011	0.00013	0.00052	0.27	1.28	1.11	5.18
R14	0.00005	0.00021	0.00024	0.00098	0.52	2.42	2.10	9.82
R15	0.00002	0.00007	0.00008	0.00034	0.18	0.85	0.74	3.44
R16	0.00003	0.00012	0.00014	0.00057	0.30	1.40	1.22	5.69
R17	0.00003	0.00013	0.00015	0.00060	0.32	1.49	1.29	6.04
Mean	**0.00003**	**0.00012**	**0.00014**	**0.00057**	**0.30**	**1.41**	**1.23**	**5.73**
SD	0.00001	0.00005	0.00006	0.00023	0.12	0.57	0.50	2.33

concentration of POPs found in *H. berthalutzae* in this study might be associated with diet of the local population and/or biochemical composition of tissues over ontogeny of individuals. For example, lipid content varies throughout the species' life cycle, reaching minimum values during the ovulation period (Marshall et al. 1999, Ababouch 2005).

H. berthalutzae belongs to the infraorder Batoidea. Lower levels of POPs in the species might also be related to biochemical characteristics of Batoidea since individuals of the group exhibit low lipid content in muscle (Krzynowek & Murphy 1987, Ackman 1990). This would make accumulation of POPs more difficult in muscle of stingrays and might even justify the incipient number of studies of POPs in rays worldwide (Bezerra et al. 2019, Fuentes et al. 2023), making comparisons more difficult. Other issues also contribute to such a difficult in comparing different studies: targeted biological tissues and concentration basis for reporting contamination.

In the first case, some studies have investigated either muscle (Santos et al. 2020) or liver contamination (Rosenfelder et al. 2012, Cascaes et al. 2014, Paiva et al. 2021, Corrêa et al. 2022). Overall, muscle is more relevant when addressing food safety since it is the way fish is consumed by humans. In contrast, liver plays a

Table III. **Average concentration (ng g^{-1} wet weight) of persistent organic pollutants in muscle of fishes from Brazil.**

Species	Site	PCBs	CHLs	Reference
Hypanus berthalutzae	Pernambuco (Brazil)	0.032	0.004	This study
Scomberomorus cavalla	Pernambuco (Brazil)	8.57	0.07	Miranda & Yogui (2016)
Hypanus berthalutzae	Bahia (Brazil)	0.68		Santos et al. (2020)
Mycteroperca bonaci		0.03		
Caranx crysos		0.24		
Micropogonias furnieri		0.12		
Scomberomorus brasiliensis		0.07		

key role in the animal's metabolism, and it is more important from an ecological point of view. The second issue is the lack of uniformity among papers since different bases have been used for reporting concentration of contaminants. Some studies on fish present results on a wet weight basis (e.g. Silva et al. 2007, 2009, Lavandier et al. 2013) while others use dry weight (e.g. Miranda & Yogui 2016) or lipid weight basis (e.g. Rosenfelder et al. 2012, Cascaes et al. 2014, Paiva et al. 2021, Corrêa et al. 2022). Unfortunately, full data for converting concentrations between distinct bases are not always available for facilitating comparisons. In fact, this reflects the lack of consensus on the basis contaminant data sets should be presented and discussed. It is an issued that remains to be addressed by the scientific community. The easiest way to solve it would be publication of full data sets (see Tables SI, SII) containing individual concentration of contaminants along with wet, dry and lipid weights of each sample tissue. Alternatively, a summary table containing concentrations expressed on the three possible bases (wet, dry and lipid) would be very useful (see Table I) although not common to see in the literature (Miranda & Yogui 2016). Lack of standardization when reporting units of concentration is a minor issue but it would be convenient whether all studies could present data expressed in the smallest unit detectable (i.e. ng g^{-1}). These recommendations are important for supporting public policies toward conservation of fishing resources and human food safety.

A recent review on contaminants in chondrichthyans pointed to lack of data on effects of POPs in Batoidea (Fuentes et al. 2023), highlighting the need for more research in Brazil and elsewhere. The same authors also concluded that variations in the concentration of POPs depend on feeding habits, sex and maturity stage of the specimens. Influence of these variables should be addressed in future studies in order to better understand their role in contaminant accumulation.

Total Hg averaged 60 ng g^{-1} ww in *H. berthalutzae* from the northern coast of Pernambuco. This is about an order of magnitude lower than levels found by Moura et al. (2020) in the coast of Ceará, Brazil (Table IV). The same authors investigated several species of rays that exhibited distinct mean concentrations ranging from 6 ng g^{-1} ww for *Aetobatus narinari* to 300 ng g^{-1} ww for *H. berthalutzae*. Differences of up to three orders of magnitude were attributed to the distinct diets (Moura et al. 2020).

In Pernambuco, Julio et al. (2022) found mean Hg levels of 919 ng g^{-1} ww in the stingray *H. guttatus*. This is 15 times higher than average Hg found in *H. berthalutzae* in this study. Both

Table IV. Average total mercury concentration (ng g^{-1} wet weight) and health risk coefficient (HQ) in muscle of rays from Brazil. Numbers in bracket denote range.

Species	Site	Concentration	HQ	Reference
Hypanus berthalutzae	Pernambuco - Brazil	60 (30 – 100)	0.06 (0.03 - 0.11)	This study
Hypanus guttatus	Pernambuco - Brazil	919 (129 – 2,130)	1.07 (0.14 - 2.3)	Julio et al. (2022)
Hypanus berthalutzae	Ceará - Brazil	300 (200 – 1,197)	1.6 (1–4.8)	Moura et al. (2020)
Hypanus guttatus		56 (1 – 1,089)	2.8 (0.2–6.6)	
Gymnura micrura		176 (42 – 417)	0.2 (0.1–0.9)	
Rhinoptera bonasus		10 (4 – 32)	0.01 (0.01–0.04)	
Aetobatus narinari		6 (1 – 22)	0.02 (0.009–0.05)	
Hypanus guttatus	Ceará - Brazil	83	0.09	Lacerda et al. (2016)

species have the same foraging habitats but occupy distinct trophic positions. Despite the higher trophic level (Moura et al. 2020, Queiroz et al. 2023), *H. berthalutzae* exhibited much lower concentrations than *H. guttatus* in the coast of Pernambuco. Thus, another factor must explain such differences. In this case, higher concentrations of Hg in *H. guttatus* might reflect ontogenetic changes in the diet since juveniles feed mainly on invertebrates while adults forage benthic fish (Silva et al. 2001, Moura et al. 2020). Furthermore, *H. guttatus* is a demersal stingray that lives on sandy and muddy estuarine bottoms of the Santa Cruz Channel (Julio et al. 2022, Queiroz et al. 2023), an ecosystem historically contaminated by Hg. There are significant amounts of Hg complexed with organic matter in local estuarine sediments, including methyl-Hg (Lacerda et al. 2020) that turns the metal more available to biota. Juveniles of *H. guttatus* live in high salinity environments such as the continental shelf while adults prefer environments with lower salinity such as estuarine intertidal zones (Figueiredo 1977, Menni & Lessa 1998, Yokota & Lessa 2007, Gomes et al. 2010, Gianeti 2011, Melo 2016). Therefore, adults of *H. guttatus* are more exposed to Hg contamination in the Santa Cruz Channel than *H. berthalutzae* that prefers to inhabit coastal marine habitats (Petean et al. 2020). *H. berthalutzae* presents ontogenetic segregation between life stages since juveniles live in coastal areas close to beaches and adults live seaward close to the shelf break (Freitas et al. 2019, Queiroz et al. 2023). Queiroz et al. (2023) did not detect statistically significant differences in the diet between life stages of *H. berthalutzae*. However, younger stages tend to prefer crustaceans while adults have a preference for feeding on cephalopods (Queiroz et al. 2023). Both stingrays (*H. guttatus* and *H. berthalutzae*) have a demersal habit but occupy distinct niches and trophic positions in the ecosystem they inhabit. Although a generalist feeder, *H. berthalutzae* exhibits a less diverse diet when compared to *H. gutattus* (Queiroz et al. 2023). So foraging habits might also explain distinct accumulation of Hg in both species.

Although disc width of rays was not measured in this study, it is known that all sampled individuals were adults. So it is worth highlighting that Hg tends to exhibit a positive correlation with body size, age and trophic position (Boening 2000, Lacerda et al. 2000). Moura et al. (2020) found a significant correlation between the size of *H. guttatus* and level of Hg in muscle. Julio et al. (2022) also found a significant positive correlation between Hg concentration in muscle and body size of both stingray *H. guttatus* and shark *Rhizoprionodon porosus*.

Environmental contaminants such as POPs and Hg can contribute to the loss of biodiversity (Rolland 2000) since they have the ability to disrupt animals' endocrine, reproductive and immune systems (Colborn & Clement 1992, Guillette et al. 1994, Guillette 1995, Rolland et al. 1995). The reproductive effects of PCBs include changes in sex steroid hormones, gonadotropins, gonad growth and vitellogenin production (Monosson 2000). The reported developmental effects of the same contaminants include embryonic and larval growth as well as survival (Monosson 2000). PCBs also have a potential long-term effect on the survival of offspring (Monosson 2000). Mercury is an endocrine disruptor that can promote, block, or reduce androgenic and estrogenic activity in fish (Wiener & Spry 1996, Friedmann et al. 2002). Thus, it hinders the reproductive success of species even when found at low concentrations. Furthermore, there is maternal transfer of both PCBs and Hg to embryos (Lyons & Lowe 2013, Lyons 2018). This is alarming because offspring is born contaminated and will likely be exposed to more contaminants throughout their lives.

Rays typically exhibit low fecundity, late sexual maturation and internal fertilization when compared to bony fish (Last & Stevens 1994), making them more susceptible to the risk of extinction as a consequence of anthropogenic factors such as pollution and overfishing (Holden 1974). In relation to sharks, rays are less studied and present deficient data for assessing their risk of extinction although some species can already be considered threatened (Oliveira et al. 2019). About 38% of ray species that occur in Brazil lie on a state of concern (ICMBio 2016, Oliveira et al. 2019) while others have not yet been evaluated or have insufficient knowledge for making decisions toward their conservation (Oliveira et al. 2019). *H. berthalutzae* is currently classified by the IUCN as a vulnerable species (VU) (ICMBio/MMA 2022, Charvet et al. 2020). This is of great concern since it is endemic to Brazil. In the past decades, several species of elasmobranchs have been threatened and some populations have experienced declines of up to 90% at certain regions (Dent & Clarke 2015). Therefore, they are under a real risk of extinction without public policies for their conservation.

Besides contamination of the species, fishing pressure also contributes to its risk of extinction because it is widely consumed in northeastern Brazil, making fisheries another factor to be addressed for conservation. Studies like this one are important for generating knowledge that supports science-based decisions and avoids loss of biodiversity.

Major factors influencing concentrations of both POPs and Hg in muscle of *H. berthalutzae* can be attributed to biological variables such as diet. Proximity to known hot spots of contamination such as the Capibaribe Estuary (PCBs and DDTs) and the Santa Cruz Channel (Hg) apparently does not play a relevant role in contamination of the species. This is also supported by a global review on contaminants in rays (Bezerra et al. 2019). According to these authors, ecological characteristics such as bottom feeding and higher trophic position are potential factors that contribute to absorption and accumulation of contaminants along with metabolism of the species and individual physiology. Van der Oost et al. (2003) also highlighted ecological and biological parameters (e.g. habitat use, diet, trophic position, age, sex, body size, season, lipid content and mobility) as important factors in the bioaccumulation of pollutants in fish.

Bezerra et al. (2019) and Fuentes et al. (2023) emphasized the need for more studies of contaminants in rays, particularly POPs. For instance, just 11 studies had been published worldwide on POPs in rays up to 2019 (Bezerra et al. 2019). In South America, Fuentes et al. (2023) reported just five studies in their review considering both Pacific and Atlantic coasts.

Regarding POPs, *H. berthalutzae* caught on the northern coast of Pernambuco can be considered safe for human consumption. USEPA (2000) recommends maximum limits of 380 ng g^{-1} ww for PCBs, 904 ng g^{-1} ww for DDTs and 9400 ng g^{-1} ww for CHLs. Levels found in this paper are much lower than the safe limits set by the U.S. EPA. Overall, values of HQ were below 1 and do not represent risk of exposure to human consumers but assessing the THQ approach for chronic non-carcinogenic health risk reveals different scenarios when considering four populational groups. Such groups are the general population and the traditional communities that rely on seafood caught by themselves for subsistence. Both were subdivided as children and adults. The general adult population exhibited THQ below 1, confirming no health risk from consumption of *H. berthalutzae* meat (Table II). Conversely, the general children population and the traditional community population (both children and adults) exhibited mean THQ near to or well above 1 (Table II), suggesting that Hg poses a risk for non-carcinogenic effects over time.

Considering the EDI approach, the average value was higher than the Hg reference dose (RfD = 0.0001 mg kg_{BW}^{-1} day^{-1}) only for children in the traditional community group (Table II), suggesting potential negative effects due to chronic exposure. Bezerra et al. (2023) argued that THQ and EDI are good proxies for evaluating exposure to Hg via seafood consumption. For reducing such risk, it is recommended that children of traditional communities at the northern coast of Pernambuco feed on a variety of seafood protein sources, including vegetables and land animals that are likely to exhibit lower Hg contamination.

The calculated local safety level (FSL_{Local}) was compared to values estimated by Bezerra et al. (2023) from several regulatory agencies worldwide. In this study, FSL_{Local} for the general adult population (i.e. those who sporadically eat *H. berthalutzae* meat) was 200 ng g^{-1} ww. This is below the recommended values for health benefits of fish consumption worldwide (210 ng g^{-1} ww) and for USA recreational fishermen (290 ng g^{-1} ww) set by FAO/WHO (2011) and USEPA (2001), respectively. It is also below values set for the general Brazilian population (260 ng g^{-1} ww) according to MPA (2012) and for the general northeastern population (280 ng g^{-1} ww) according to IBGE (2021). In this study, FSL_{Local} value calculated for the general children population was generally in accordance with those set by Brazilian authorities (60 ng g^{-1} ww) (MPA 2012, IBGE 2021), USA (90 ng g^{-1} ww) agency (USEPA 2001) and worldwide (50 ng g^{-1} ww) (FAO/WHO 2011). In Brazil, the regulatory agency does not have issued FSL_{Local} values for traditional communities that have a high intake of seafood. So, values calculated in this study were compared to those set for subsistence fishermen in the USA. Such FSL_{Local} reference values are 50 ng g^{-1} ww for adults and 10 ng g^{-1} ww for children (USEPA 2001). Adults who feed for subsistence are below the value (49 ng g^{-1} ww) while children are at risk of Hg contamination, not within safe limits (11 ng g^{-1} ww). Setting these limits is important for avoiding the risk of excessive exposure to Hg through consumption of seafood (Bezerra et al. 2023).

CONCLUSIONS

This study is relevant for both conservation and food safety purposes. It was the first one investigating levels of both POPs and Hg in an endemic stingray that inhabits demersal marine environments at northeastern Brazil. POPs were not detected or detected below the limit of quantification while Hg exhibited low concentrations in muscle of the stingray *H. bethalutzae*. Therefore, contamination by POPs and Hg is not an issue for the conservation of the species on the north coast of Pernambuco. *H. bethalutzae* also has cultural importance to the local cuisine since it has been used in the preparation of traditional dishes such as stew and *moqueca*. The food safety assessment showed that the adult population feeding on this stingray is not at risk. Conversely, *H. bethalutzae* may pose a health threat to certain populational groups such as children and traditional communities that rely exclusively on the species for subsistence (e.g. local fishermen and their families). Considering that *H. bethalutzae* is listed as a vulnerable species by the IUCN, monitoring fisheries and level of pollutants over time should be warranted in order to support public policies toward its conservation.

Depending on the methodology used and the most vulnerable subgroups within the human population, the concentration found in the ray's muscles may or may not present a health risk due to exposure to Hg. Therefore, it is recommended to use different methodologies and population subgroups in addition to considering multiple consumption scenarios when assessing the risk of exposure to Hg and other pollutants.

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SUPPLEMENTARY MATERIAL

Tables SI, SII.

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