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## ANIMAL SCIENCE

# Mercury (Hg) concentration in fish commercialized in the São Luís fish market (MA) and potential exposure of consumers

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Abstract: Fish consumption is the main path of human exposure to Hg and may represent a risk to public health, even with low Hg concentrations in fish, if consumption rates are high. This study quantifies, for the first time, the Hg concentrations in nine most commercialized species in the São Luís (MA) fish market, where fish consumption is high, and estimates human exposure. Average Hg concentrations were highest in carnivorous species, yellow hake (Cynoscion acoupa) (0.296 mg kg<sup>-1</sup>), the Atlantic croaker (Micropogonias undulatus) (0.263 mg kg<sup>-1</sup>), whereas lowest concentrations were recorded in iliophagous Mullets (*Mugil curema*) (0.021 mg kg<sup>-1</sup>) and the Shorthead drum Larimus breviceps (0.025 mg kg<sup>-1</sup>). Significant correlations were observed between Hg concentrations and fish length in two species: the Coco-Sea catfish (Bagre bagre) and the Atlantic bumper (Chloroscombrus crysurus), but not in the other species, since they presented relatively uniform size of individuals and/or a small number of samples. Risk coefficients, despite the relatively low Hg concentrations, suggest that consumers should limit their consumption of Yellow hake and Atlantic croaker, as they can present some risk to human health (EDI > RfD and THQ > 1), depending on the frequency of their consumption and the consumer's body weight.

Key words: Contamination, fish, human exposure, risk assessment.

# INTRODUCTION

Health and well-being result from the interactions of different determining factors of social, economic, cultural and political nature, but mostly on environmental health, including access to sanitation and exposure to pollutants. A systemic and inter-sectorial approach to environmental and social impacts is required to grasp the causal relationships between pollutant exposure and their effects on human health. This approach breaks with the classical model aimed at an understanding of the health-disease process proper (Netto et al. 2006, Gurgel et al. 2009, Barbosa et al. 2011).

A relevant issue to be addressed in this new approach is the finding that the concentration of metals and organic substances has increased considerably due to a combination of anthropogenic releases from urban wastes and industrial and mining effluents (Santos et al. 2019) and the remobilization of the deposited pollutants accumulated during the past century, the so-called "legacy of contamination". Among these pollutants, Hg stands out as being of significant environmental importance due to its ubiquity and toxicity. The mobility, bioavailability and consequently toxicity of this Hg legacy will depend not only on the bulk quantity of the pollutant released, but on the effect of environmental changes, which are highly influence changes in physical-chemical and microbiological conditions of aquatic ecosystems, resulting from direct human interventions (e.g. eutrophication) or indirectly as a response to global climate change (Lacerda et al. 2020).

Consumption of fish and shellfish is the main pathway of Hg and its highly toxic methyl-Hg to humans (BCS 2007, Barbosa & Dorea 1998, Castilho et al. 1998, Vieira et al. 2013, 2015). In addition, fish is the basis of the livelihood of riverine populations of many areas in the world, a fact which consequently expose them to high levels of Methyl-Hg associated with fish consumption (Oliveira et al. 2010).

São Marcos Bay (Supplementary Material Figure S1), where the second largest harbor complex in Latin America is located, and its adjacent continental shelf supply a diversified fluvial and estuarine fish to humans. Large pelagic and small schooling species from coastal waters, support moderate-to high commercial and artisan fisheries that are of substantial economic value, as well as a significant portion of the local diet of local consumers. Fish and shellfish respond to 69% of the total protein consumption of the local population, from which 85% corresponds to fishes, 52% of consumers eat fish 2-3 times per week and average week consumption varies from 1 to 2 kg, average of 0.142 kg day<sup>-1</sup> in this high consumption group (Silva et al. 2012). Therefore, Hg concentrations in these fisheries raise awareness about the health of this estuarine-marine ecosystem and eventual human exposure. Mercury accumulates in tissues of aquatic organisms and can be biomagnified through the food chain and may attain concentrations that are potentially harmful to human health (Doney 2010, Bisi et al. 2012). Recent studies on trace metal concentrations in São Marcos Bay, reported mobilization of

metals associated with environmental changes (Santos et al. 2019), suggesting an increase in their bioavailability and bioaccumulation, probably explaining previously reported high concentrations in the Bay's local biota (Carvalho et al. 2000, Rojas et al. 2014).

Taking these previous results into consideration, this study emphasizes the concentration of Hg in commercialized fish in the São Luis fish market, which is still unreported, notwithstanding the large proportion of fish on the local population's diet. The observed concentrations of Hg in commercialized fish associated with answers of a questionnaire applied to local consumers on their food habits, were used to assess Hg exposure risk to humans due to fish consumption using different risk indexes.

# MATERIALS AND METHODS

One hundred and twenty-five individuals (125) of the nine (9) fish species were selected from the most frequent items sold in the São Luis fish market and the local consumer's diet, based on questionnaires (n = 574) completed by local consumers between 2017 and 2019. The questionnaires consisted of questions about individual weight of consumer, number of fish meals per week and the preferred fish species. Taxonomic identification, trophic status and diet of the selected species were obtained from FishBase (2022). Individual weight and length were determined by digital scale (0.1 g precision) and measuring tape (0.1 cm precision), respectively (Table I).

Samples were prepared according to Adair & Cobb (1999). Muscle samples individually freezedried upon arrival in the laboratory. Moisture content was calculated for each species and used to report the results on a wet weight basis. All species showed average moisture

Table I. Common and scientific names and number of individuals analyzed of the most commercialized fish species
in the São Luis Market, northern Brazil. Trophic position according to FishBase (2022). Preference in diet from
answers to local questionaries (n = 1.212).

Common name (n)	Species	Preference (% of diet)	Trophic position	Length (cm)	Weight (g)
Coco Sea catfish (16)	Bagre bagre	6.8	4.0	45.8 ± 3.2	540 ± 110
Whitemouth croaker (5)	Micropogonias furnieri	7.5	3.1	60.0 ± 6.9	1,560 ± 470
Atlantic croaker (4)	Micropogonias undulatus	1.5	4.0	43.8 ± 4.6	700 ± 200
Atlantic bumper (13)	Chloroscombrus chrysurus	5.0	3.5	22.3 ± 2.2	110 ± 20
Peixe Cero, Serra (8)	Scomberomorus regalis	22.5	4.5	58.4 ± 2.1	890 ± 200
Shorthead drum (12)	Larimus breviceps	12.9	3.5	33.6 ± 1.7	320 ± 50
Mullet (12)	Mugil curema	8.7	2.0	26.4 ± 2.2	170 ± 50
Crucifex catfish (5)	Arius proops	6.8	4.4	65.2 ± 3.6	2,180 ± 530
Yellow hake (50)	Cynoscion acoupa	28.1	4.1	73.1 ± 3.3	4,680 ± 510

content of 74 ± 7.2%. Duplicate dry samples (0.5 g) were digested in Teflon<sup>®</sup> tubes containing 10 mL of analytical grade MERCK<sup>®</sup> HNO<sub>3</sub> (65%), at room temperature for one hour and then placed in a MARS CEM microwave digester at 200 °C for 30 minutes. After digestion, 1 mL of H<sub>2</sub>O<sub>2</sub> was added to each tube, transferred, and diluted in volumetric flasks to 100 mL. Total Hg concentrations were quantified by cold vapor atomic absorption spectrophotometry (CV-AAS) in a NIC RA-3 (NIPPON®) spectrophotometer. The average linearity coefficient of the calibration curves (R2) obtained using a MERCK<sup>®</sup> Hg calibration solution (1,000 ng L<sup>-1</sup>), was 0.9998 ± 0.0001. The average limit of detection (LOD) was 0.001 mg kg<sup>-1</sup>, calculated as three times the standard deviation of the reagent blanks divided by the slope of the calibration curve. Validation of the methods was obtained by simultaneous analysis, in duplicate, of certified reference material (Mussel Tissue ERM-CE 278K) with recovery of 95.1  $\pm$  5.8% (n = 14) (Supplementary Material Table SI).

### Human health assessment

We estimated the local fish safe level (FSL<sub>local</sub>) which is the maximum Hg concentration (mg kg<sup>-1</sup> wet weight) in edible fish tissue that is safe to consume considering the local fish consumption rate (CR<sub>local</sub>) and the Hg reference dose (RfD). The local fish safe level (FSL<sub>local</sub>) was estimated using the equation (1) according to USEPA (2001), where BW is the consumer's body weight in kg, CR<sub>local</sub> is the local daily fish consumption rate (kg day<sup>-1</sup>) and RfD is the reference dose of 0.0001 mg kg<sub>bw</sub><sup>-1</sup> day<sup>-1</sup>.

$$FSL_{local} = \frac{BW \times RfD}{CR_{local}}$$
(1)

To estimate the maximum monthly number of meals ( $CR_{mm}$ ) of each fish species that can be consumed without risk of deleterious health effects, we first modified the equation (1) to solve for the maximum safe daily fish consumption rate ( $CR_{max}$ ) as follows, equation 1.2. where  $CR_{max}$ is expressed in kg day <sup>-1</sup>, and  $C_{fish}$  (mg kg<sup>-1</sup>) is the Hg concentration in the seafood product.

$$CR_{max} = \frac{BW \times RfD}{C_{fish}}$$
(1.2)

After these steps,  $CR_{mm}$  (meals per month) was calculated using equation 2 where  $T_{ap}$  is the

averaging time period (365.25 days per 12 months or 30.44 days month<sup>-1</sup>), and MS is the average meal size (kg meal<sup>-1</sup>) (USEPA 2000). For our calculation, we assumed an average fish meal size of 150 g of raw fish for adults, which roughly provides the daily calories recommendation of 190 kcal per fish portion according to the Brazilian Health Ministry (BRASIL 2014). We assumed an average fish meal size of 75 g for children, defined here as under 6 years old.

$$CR_{mm} = \frac{CR_{max} \times T_{ap}}{MS}$$
(2)

Equation (3), was used to estimated the daily Hg intake (EDI<sub>Hg</sub> mg kg<sub>BW</sub><sup>-1</sup> day<sup>-1</sup>) for two scenarios: a) Adults and children consuming fish at a daily consumption rates (CR<sub>tocal</sub> kg day<sup>-1</sup>) of 0.142 kg day <sup>-1</sup>, which is the local fish intake estimated through questionnaire application (Silva et al. 2012), and b) adults and children consuming fish at a daily consumption rate of 0.030 kg day<sup>-1</sup>, which is the per capita consumption rate for the Brazilian population (IBGE 2020), where C<sub>fish</sub> (mg kg<sup>-1</sup>) is the Hg concentration in the seafood product, and BW is the consumer body weight (65.5 kg for adults (locally obtained by the questionnaires) and 15 kg for children).

$$EDI_{Ha} = \frac{C_{fish} \times CR_{local}}{BW}$$
(3)

We also estimated the target hazard quotient (THQ) using equation (4) according to the United States Environmental Protection Agency (US EPA) methodology based on the Region III risk-based concentration table (USEPA 2022). The THQ represents the chronic non-carcinogenic health risk posed by exposure to Hg from ingested seafood. A THQ lower than 1 represents no expected health effects while a THQ higher than 1 represents a potential for adverse health effects, where EF is the exposure frequency (365 days.yr<sup>-1</sup>), ED is the exposure duration (77 years for adults, and 6 years for children), and AT is the averaging exposure time (EF × ED).

$$THQ = \frac{EF \times CR_{local} \times ED \times C_{fish}}{RfD \times BW \times AT}$$
(4)

### Statistical analysis

Outliers were identified and removed from subsequent statistical analysis. The Shapiro-Wilk test was employed to test normality assumptions. Non-parametric tests were used to compare length, weight and Hg concentrations among species (Kruskal-Wallis) and Mann-Whitney test was employed to compare differences between males and females of *Cvnoscion acoupa*. Bioaccumulation curves were used to observe the relationship between size and Hg concentrations and the influence of feeding ecology of the sampled species (with n≥10) on their Hg content. All significant tests were conducted using an alpha value of 0.05 (95% confidence). Graphs and statistical tests were performed using Microsoft® Office 2016 and Copyright© StatSoft. Inc. (1984-2011).

### **RESULTS & DISCUSSION**

The most important commercialized fish species in the São Luis market were sampled in this study, based on the extensive survey of Silva et al. (2012), this survey suggests a relatively large fish consumption *per capita* of about 2 kg per week, about 0.142 kg per day. This consumption rate is 5 times greater than the average per capita consumption for Brazilian population (0.027 kg day<sup>-1</sup>), and three times greater than the average fish consumption rate for the northeast region (Sartori & Amancio 2012). The nine (9) major species and their relative preference in the local consumers' diet, and their trophic position are shown in Table I. Total Hg concentrations and the human health risk assessment parameters are shown in Tables II and III.

No species presented Hg concentrations higher than the Brazilian safe limits for human

consumption (ANVISA 2013). Even considering more restrict limits, such as 0.3 mg kg<sup>-1</sup> established by USEPA (2000), for example, average Hg concentrations did not exceed it, guaranteeing commercialization even to more strongly regulated market. In addition, we found no significant difference between sex among the species studied. Highest average Hg concentrations were measured in the Yellow hake (Cynoscion acoupa)  $(0.296 \pm 0.241 \text{ mg kg}^{-1})$ with a relative participation in the total fisheries consumption by the local population, also highest among all species, of about 28.1%, depending on season. The Atlantic croaker (Micropogonias undulatus) presented the second highest Hg concentrations  $(0.263 \pm 0.089 \text{ mg kg}^{-1})$  with about 1.5% participation only in the consumers diet, whereas Scomberomorus regalis (0.107 ± 0.039 mg kg<sup>-1</sup>) responded to about 22.5% participation, the second in the consumers preference. On the other hand, the lowest concentrations were recorded in iliophagous Mullets (Mugil curema)  $(0.021 \pm 0.010 \text{ mg kg}^{-1})$ , 8.7% of preference, and the Shorthead drum (Larimus breviceps) (0.025 ± 0.009 mg kg<sup>-1</sup>), with 12.9% preference. The other for species with intermediate Hg concentrations respond together to less than 26% participation in the local consumers' diet (Silva et al. 2012).

Apart from fish preferences, the questionnaires also showed a mean adult individual body weight of the local consumers of 65.5 kg. Of the total 574 questionnaires, 501 responded to the quantity of fish meals per week. Of those 50.5% has at least one fish meal per week, 27.1% two meals, and 12.4% three fish meals per week, 10% of the answers indicated four to 7 fish meal per week and 14.4 responded "anytime" and could not made it more precise. This reflects the relatively high consumption rates suggested by Silva et al. (2012).

Significant correlations were observed between Hg concentrations and individual length

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Table II. Mean (standard deviation), and 95<sup>th</sup> percentiles of Hg (mg kg<sup>-1</sup>), and EDI (mg kg<sub>BW</sub><sup>-1</sup> day<sup>-1</sup>) for seafood species. The reference dose (RfD) for Hg

Hg		EDI <sub>adult_MA</sub>		EDI children_MA		EDI adult_mercad		EDI <sub>children_</sub> mercado	
Aean ± SD	95 <sup>th</sup>	Mean ± SD	95 <sup>th</sup>	Mean ± SD	95 <sup>th</sup>	Mean ± SD	95 <sup>th</sup>	Mean ± SD	95 <sup>th</sup>
296 ± 0.241	0.751	0.00014±0.00011	0.0003	0.0006 ± 0.0005	0.0015	0.00064±0.00052	0.0016	0.0028 ± 0.0023	0.0071
263 ± 0.089	0.356	0.00012 ± 0.0001	0.0002	0.0007 ± 0.0004	0.0011	0.0008 ± 0.0004	0.0012	0.0030 ± 0.002	0.0054
112 ± 0.024	0.141	< RfD	< RfD	0.0002 ± 0.00005	0.0003	0.00024±0.00005	0.0003	0.0010 ± 0.00023	0.0013
107 ± 0.038	0.158	< RfD	< RfD	0.0002 ± 0.0001	0.0003	0.00023±0.00008	0.0003	0.0010 ± 0.00036	0.0015
071 ± 0.032	0.114	< RfD	< RfD	0.0001 ± 0.00006	0.0003	0.00015±0.00007	0.0002	0.0007 ± 0.00031	0.0011
063 ± 0.019	0.088	< RfD	< RfD	0.0001 ± 0.00004	0.0002	0.00014±0.00004	0.0002	0.0006 ± 0.0002	0.0008
J38 ± 0.008	0.053	< RfD	< RfD	< RfD	0.0001	< RfD	0.0001	0.0004 ± 0.00008	0.0005
025 ± 0.009	0.039	< RfD	< RfD	< RfD	0.0001	< RfD	0.0001	0.0002 ± 0.00008	0.0004
020 ± 0.009	0.035	< RfD	< RfD	< RfD	< RfD	< RfD	< RfD	0.0002 ± 0.00009	0.0003
	Hg lean ± SD 296 ± 0.241 263 ± 0.089 112 ± 0.024 107 ± 0.038 371 ± 0.038 371 ± 0.032 363 ± 0.019 363 ± 0.009 325 ± 0.009	Hg         95 <sup>th</sup> lean ± SD         95 <sup>th</sup> 296 ± 0.241         0.751           253 ± 0.089         0.356           112 ± 0.024         0.141           107 ± 0.038         0.158           371 ± 0.038         0.158           371 ± 0.038         0.168           371 ± 0.032         0.114           353 ± 0.019         0.088           325 ± 0.009         0.039           320 ± 0.009         0.035	Hg         ED1 <sub>adut.Ma</sub> lean $\pm$ SD         95 <sup>th</sup> Mean $\pm$ SD           296 $\pm$ 0.241         0.751         0.00014 $\pm$ 0.00011           235 $\pm$ 0.089         0.356         0.00012 $\pm$ 0.0001           261 $\pm$ 0.024         0.141         < RfD	Hg         EDl <sub>adut.MA</sub> lean $\pm$ SD $95^{th}$ $1000014 \pm 0.00014$ $95^{th}$ lean $\pm$ SD $95^{th}$ Mean $\pm$ SD $95^{th}$ $296 \pm 0.241$ $0.751$ $0.00014 \pm 0.00011$ 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in three species: the Coco Sea catfish (Baare bagre), the mullet (M. curema) and the Atlantic bumper (Chloroscombrus crysurus) (Figure S2). All present low to intermediate Hg concentrations and sum up a relatively small proportion of the local consumers' diet. In addition, given the relatively uniform size of individuals of most other species and/or the small number of samples, the relationship between Hg and size was not found. Therefore, to estimate the exposure risk, fish size was not taken onto consideration nor any standardization by size was performed to the original Hg concentrations found. Similarly, no statistical differences were observed in Hg concentrations between sexes of the studied species and thus sex, was not taken into consideration, also.

The results show the importance of feeding habits as a relevant element that drives Hg concentrations in organisms. Figure 1 shows the relationship between trophic status and Hg concentrations in the studied fish species. Mercury concentrations related exponentially with trophic position. Relatively large carnivorous species with the highest trophic levels ( $\geq$  4.0) presented the highest Hg concentrations, whereas relatively small iliophagous and omnivores (*M. curema* and *L. breviceps*) presented the lowest Hg concentrations. This trend in the distribution of Hg concentrations with the highest found in carnivorous and the lowest in iliophagous or herbivores has been extensively reported in literature on fish Hg content in north and northeastern Brazil fish species (Bastos et al. 2015, Lacerda et al. 2016, Moura et al. 2018, Moura & Lacerda 2022).

All collected fishes, except M. curema, are carnivorous species, some however, small fish predating mostly on invertebrates, principally polychaetes, crustaceans, and mollusks C. acoupa is a large, mostly piscivore species, Ferreira et al. (2015) found a fish occurrence frequency of 48.3% (meals month <sup>-1</sup>) for each seafood species. Number of meals greater than 16 meals per month indicates no obvious human health risk by consuming the respective fish species (USEPA 2000). THQ smaller than 1 indicates no Table III. Mean, standard deviation, and 95 $^{
m th}$  percentile values of THQ and CR  $_{
m mm}$  (

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Snorios (n)	THO	MA	THQ <sub>children_</sub>	MA	THQ <sub>adult_mer</sub>	cado	THQ <sub>children_me</sub>	ircado	CR	ı_adult	CR	children
	Mean ± SD	95 <sup>th</sup>	Mean <u>±</u> SD	95 <sup>th</sup>	Mean ± SD	95 <sup>th</sup>	Mean ± SD	95 <sup>th</sup>	Mean	95 <sup>th</sup>	Mean	95 <sup>th</sup>
Cynoscion acoupa (50)	1.4 ± 1.1	3.4	5.9 ± 4.8	15	6.4 ± 5.2	16.3	28.1 ± 22.8	71.1	4.5	1.8	1.0	0.4
Micropogonias undulatus (4)	1.2 ± 0.4	1.6	5.3 ± 1.8	7.3	5.7 ± 1.9	7.7	24.9 ± 8.4	33.8	5.1	3.7	1.2	0.9
Arius proops (5)	~	~	2.3 ± 0.5	2.8	2.4 ± 0.5	ю	10.5 ± 2.3	13.3	11.9	9.4	2.7	2.2
Scomberomorus regalis (8)	~	~	2.1 ± 0.8	3.1	2.3 ± 0.8	3.4	10.1 ± 3.6	14.9	12.4	8.4	2.8	1.9
Bagre bagre (16)	~	~	1.4 ± 0.6	2.3	1.5 ± 0.7	2.5	6.7 ± 3	10.8	> 16	11.6	4.3	2.7
Micropogonias furnieri (5)	~	۲ ۲	1.3 ± 0.4	1.8	1.4 ± 0.4	1.9	6.0 ± 1.8	8.4	> 16	15.1	4.8	3.4
Chloroscombrus chrysurus (13)	~	۲ ۲	~	1.1	~	1.2	3.6 ± 0.8	5.1	> 16	> 16	8.0	5.7
Larimus breviceps (12)	~	۲ ۲	~	~	~	~	2.4 ± 0.8	3.7	> 16	> 16	12.0	7.8
Mugil Curema (12)	~	~	~	v v	~	v	1.9 ± 0.9	3.3	> 16	> 16	14.8	8.6
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**Figure 1.** Exponential relationship between Hg concentrations and trophic position of fish species most commercialized in the São Luís fish market, Maranhão, Northern Brazil.

in the adult individuals' stomachs of *C. acoupa* and this ecological characteristic explains the high Hg concentrations. *Micropogonias undulatus* and *Scomberomorus regalis*, also large carnivores with diet like *C. acoupa* (Mendes & Barthem 2010), also presented relatively high Hg concentrations. This pattern of larger fishes usually presenting higher Hg concentrations than smaller ones (Gorski et al. 2003, Gewurtz et al. 2011, Verdouw et al. 2011). The strong and positive correlation between Hg and fish length observed in *B. bagre, C. chrysurus*, and *M. curema* (Figure S2) shows the importance of size in affecting Hg concentrations.

The exposure risk for Hg through seafood consumption varied with species and consumer type. We estimated THQ, EDI<sub>Hg</sub>, CR<sub>max</sub>, and CR<sub>mm</sub>, for adults and children, using mean and 95<sup>th</sup> percentile values of Hg concentrations for each fish species, for two consumption scenarios: a) A per capita fish consumption of 30 g day<sup>-1</sup>; and b) A fish consumption rate of 142 g day<sup>-1</sup> (Tables II, III). The 95<sup>th</sup> percentiles are the high-end exposure estimates and represent the plausible worst-case scenario (USEPA 2000). Under scenario "a", THQ was slightly greater than 1 for *C. acoupa* and *M. undulatus* for adult consumers, while for

children consumers a THQ greater than 1 was observed for C acoupa, M. undulatus, A. props, S. regalis, B. bagre, and M. furnieri (Table III). When considering the 95<sup>th</sup> percentile values, the same result was observed (Table III). Under scenario "b", THQ was greater than 1 for C acoupa, M. undulatus, A. props, S. regalis, B. bagre, and M. furnieri for adult consumers, while for children a THQ greater than 1 was observed for all species (Table III). When considering the 95<sup>th</sup> percentile values, the same result was also observed (Table III). Similarly, under scenario "a", the estimated daily intake (EDI) for adult consumers exceeded slightly the Hg reference dose (RfD) of 0.0001 mg kg<sub>RW</sub> day<sup>-1</sup> for *C. acoupa* and *M. undulatus* (Table III). For children, EDI values greater than RfD were observed for C. acoupa, M. undulatus, A. props, S. regalis, B. bagre, and M. furnieri. When considering the 95<sup>th</sup> percentile values, a similar result was observed (Table II). Under scenario "b", EDI values exceeded the RfD for C. acoupa, M. undulatus, A. props, S. regalis, B. bagre, and M. furnieri (Table II). For children under scenario "b", the EDI for all sampled species exceeded the reference dose (Table II). A similar result was also observed when considering the 95<sup>th</sup> percentile values of Hg concentrations (Table II).

We calculated the number of seafood meals per month that can be safely consumed by children and adult consumers (Figure 2 and Table III). For adult consumers (average weight of 65.5 kg), four out of the nine studied species require some restriction of consumption. These species are *M. undulatus, C. acoupa, A. proops,* and *S. regatta,* with a maximum of 5.5, 10.6, 12.4, and 14 meals per month, respectively. For children consumers (average weight of 15 kg), seven out of nine studied species require some restriction of consumption. These species are *M. undulatus, C. acoupa, A. proops, S. regatta, M. undulatus, C. acoupa, A. proops, S. regatta, M. furnieri, B. bagre,* and *C. chrysurus,* with a



Figure 2. Estimated number of meals per month that can be safely consumed by Adult (Top) and Children (Bottom) in Maranhão State.

maximum of 2.5, 4.8, 5.7, 6.4, 10.2, 10.3, and 14.6 meals per month, respectively.

The estimated fish screening levels (FSL, mg kg<sup>-1</sup>) for adults and children consuming seafood under different scenarios are shown in Table IV. For adults and children consuming fish at a rate of 30 g per day (per capita consumption for Maranhão State), the Hg concentration of fish should not exceed 0.22 and 0.05 mg kg<sup>-1</sup>, respectively. These protective Hg levels are even lower if we assume a consumption rate of 142 g per day (São Luiz fish market consumption rate), 0.05 and 0.01 mg kg<sup>-1</sup>, for adults and children respectively. We observed that the higher the seafood consumption rate the lower the FSL, as shown by the FSL from different Brazilian fish-eating populations (Table IV), where the lowest FSL are observed in the Amazon riverine inhabitants, characterized by extremely high fish consumption rate.

It becomes clear that the Hg exposure risk in humans, from seafood consumption, is strongly dependent of consumption rate, and consumer body weight, as well as the Hg burden in seafood. This is why relying solely on

indices, such as THQ and EDI, is not enough to estimate the multiple scenarios of exposure in any given seafood consumer's population. The maximum Hg levels established by regulatory agencies are also not protective of consumers, since fish with Hg concentrations much lower than these maximum limits still represent risks of Hg exposure to consumers, depending on consumption rates (Figure 2, Table III). In addition, to better inform about Hg exposure risks, it is important to provide consumers with the maximum number of meals that can be safely consumed for each one of the most consumed seafood types. As shown in Figure 3, we argue that it is more effective to provide consumers with species specific information to allow them to an informed decision as it relates to how much seafood they will consume.

### CONCLUSIONS

The results confirm the importance of fish in the São Luís population, well above the average Brazilian fish consumption and as high as those observed in the Amazon region. The most

# **Table IV.** Fish screening levels for adults (65.5 kg of average body weight) and children (15 kg of average body weight) considering multiple fish consumption rates.

Consumption rate	Site	FSL [Hg] <sub>fis</sub>	<sub>h</sub> (mg kg⁻¹)*	Reference	
(g day <sup>-</sup> ')		Adults	Children	Kererenee	
30.0	State of Maranhao, Brazil	0.22	0.05	This study	
142	Sao Luiz fish market	0.05	0.01	This study	
27.4	Brazil – General population	0.24	0.06	(MPA 2012)	
42.7	Jaguaribe River Estuary	0.15	0.04	(Costa 2014)	
32.9	Recommended seafood consumption	0.19	0.05	(FAO/WHO 2011)	
416.4	Amazon River	0.02	0.004	(Isaac 2015)	

\*Hg RfD = 0.0001 mg kg<sup>-1</sup>day<sup>-1</sup>.



Figure 3. Safe fish maximum amount consumed per month at different body weigths for *Cynoscion acoupa* from São Luiz fish market.

consumed species, yellow hake (*C. acoupa*) and croakers (*Micropogonias* spp.) are those with the highest Hg concentrations, but still below the maximum limits of 0.5 mg kg<sup>-1</sup>. However, we found that seafood from the São Luiz fish market can be an important source Hg exposure to consumers depending on the consumption rate and the type of consumer. Particularly for children, consuming seafood at a rate of 142 g day<sup>-1</sup>, we estimated EDI values greater than the reference dose for all species. On the other hand, at a lower consumption rate (30 g day<sup>-1</sup>, the average for MA state), we found EDI values to be lower than the reference dose for the following species: *Bagre bagre, Micropogonias furnieri*, Chloroscombrus chrysurus, Larimus breviceps, and Mugil Curema. Therefore, the rate of seafood consumption and the type of consumer (adult and children) are important metrics when assessing exposure risk to contaminants. This is why we found that most studied species could potentially pose some risk of Hg exposure to human consumers, even with Hg levels below the established safety limit of 0.5 mg kg<sup>-1</sup>.

It is important to note that these exposure estimates are for those consuming seafood consistently at moderate to high rates over a long period of time (12 consecutive months) and, thus, should be seen as the worst-case scenario rather than the norm. The estimated Fish Screening Level (FSL) for adults and children are 0.22 and 0.05 mg kg<sup>-1</sup>, respectively, which can be interpreted as the upper limit of Hg contamination in fish that does not require consumption restrictions.

An important observation is the apparent lack of relationship between size and Hg concentrations. Apart from the small number of samples, this my result form the uniform size of commercialized fish. A part of the population, mostly fishers and those buying catches directly from them, may consume smaller or large sizes, which may eventually alter their risk assessment. This calls for urgent evaluation of the variability of Hg concentrations in a broader range of size to increase precaution regarding Hg exposure through fish consumption.

Finally, we recommend using multiple exposure metrics and scenarios when assessing Hg exposure risk from seafood consumption and informing consumers of the maximum number of meals per month estimated to inform and protect seafood consumers.

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### SUPPLEMENTARY MATERIAL

Figures S1, S2. Table I.

### How to cite

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### **Author contributions**

LD Lacerda designed the study, interpreted the results, wrote and edited the manuscript and provided funding support; VL Moura interpreted the results and wrote the manuscript; RWS Oliveira, collected samples, applied and interpreted the questionnaires and performed chemical analysis; KLCF Carmo performed chemical analysis and analysis of questionnaires, JLS Nunes collected samples, applied and interpreted the questionnaires, AS Freitas, collected samples, applied and interpreted the questionaries, MF Bezerra designed the study, interpreted the results, wrote and edited the manuscript.

