ARQUIVOS CIENTÍFICOS (IMMES)

Bioaccumulation of mercury in predatory fish from Amapá State, Brazil

Bioacumulação de mercúrio em peixes predadores do estado do Amapá, Brazil

Cecile de Souza Gama[1], *Luís Mauricio Abdon da Silva*[2]

[1] *Instituto de Pesquisas Científicas e Tecnológicas do Amapá, Núcleo de Biodiversidade, Rod, JK, Kn 10 s/n. Fazendinha, Macapá – AP, Brasil. https://orcid.org/0000-0002-5128-6120 E-mail: cecilegama@hotmail.com. *Corresponding author*

[2] *Instituto de Pesquisas Científicas e Tecnológicas do Amapá, Núcleo de Biodiversidade, Rod, JK, Kn 10 s/n. Fazendinha, Macapá – AP, Brasil. https://orcid.org/0000-0001-9510-0933 E-mail: luis.abdon13@gmail.com*

Key-words

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Bioaccumulation of Hg in aquatic systems varies considerably according to the food chain structure and age of fish. As fishes are an important food resource in the Amazon region, common species of carnivorous and piscivorous fish in Amapá State were analyzed for contamination by Mercury. Specifically, the influence of standard length and weight of individuals on the observed Hg concentrations was investigated and used to evaluate whether bioaccumulation rates vary among different species. The fishes were collected from five locations in Amapá State between August 2017 and May 2018. We measured the total mercury content in 204 specimens representing nine carnivorous/piscivorous species of fishes: Serrasalmus rhombeus, Plagioscion squamosissimus, Megalops atlanticus, Hoplias malabaricus, Cichla monoculus, Brachyplatystoma vaillantii, Boulengerella cuvieri, Ageneiosus inermis and Hoplias aimara. All fishes had some level of Hg contamination and the potential for bioaccumulation of this contaminant varied among species. A positive relationship between weight and/or length and the total body Hg concentration was observed only for Serrasalmus rhombeus, Hoplias malabaricus, Cichla monoculus and Brachyplatystoma vaillantii.

Palavras chave

Contaminação de Mercúrio
Bioacumulação de Hg
Amapá

A bioacumulação de mercúrio em sistemas aquáticos varia consideravelmente de acordo com a estrutura da cadeia alimentar e com a idade do peixe. Como os peixes são um recurso alimentar importante na região Amazônica, espécies comuns de peixes carnívoros e piscívoros no estado do Amapá foram analisadas quanto à sua contaminação por mercúrio. Especificamente, foi observada a influência do comprimento padrão e do peso dos indivíduos na concentração de mercúrio encontrada e usada para avaliar se as taxas de bioacumulação variam entre as diferentes espécies. Os peixes foram coletados em cinco localidades no estado do Amapá entre agosto de 2017 e maio de 2018. Foram medidos o mercúrio total em 204 espécimes representando nove diferentes espécies de peixes carnívoros/piscívoros: Serrasalmus rhombeus, Plagioscion squamosissimus, Megalops atlanticus, Hoplias malabaricus, Cichla monoculus, Brachyplatystoma vaillantii, Boulengerella cuvieri, Ageneiosus inermis e Hoplias aimara. Todos os peixes apresentaram algum nível de contaminação por mercúrio e o potencial para bioacumulação deste contaminate variou entre as espécies. Uma relação positiva entre peso e/ou comprimento e a concentração total de mercúrio corporal foi observada apenas para Serrasalmus rhombeus, Hoplias malabaricus, Cichla monoculus and Brachyplatystoma vaillantii.

INTRODUCTION

Studies in the Amazon Basin show numerous examples of aquatic systems contaminated by mercury (Hg). For the fishes that inhabit these systems, the concentration of mercury is closely linked to their feeding regime and position in the trophic web (FRÉRY et al., 2001). Species at higher trophic levels have a higher mercury concentration when compared to species at the base of the food chain, such as herbivores (ZHOU; WONG, 2007; AZEVEDO-SILVA et al., 2016). This is because mercury, in the form of methylmercury, is easily absorbed by fish and other aquatic animals, which leads to its deposition in the tissues. The mercury accumulates in the biological chain over time (*i.e.*, bioaccumulation), reaching concentrations much higher than those found in waters and sediments (AZEVEDO, 2003).

In general, the bioaccumulation of Hg in aquatic systems varies considerably according to the food chain structure and age of fish. In a simplified way, plankton is found at the base, followed by herbivores, then small to large piscivorous fish where the highest Hg concentrations are expected (BARBOSA, 2003). In fact, mercury concentrations in muscle tissue are approximately 10 times higher in carnivorous fish compared to herbivorous fish (BRUGGEMAN, 1982; CASTILHOS; BIDONE, 2000). For this reason, the top fishes in the food chain are widely used as bioindicators of mercurial contamination in aquatic ecosystems (RODDRIGUES et al., 2010). Fish age, measured indirectly by standard length, may

(CARRASCO et al., 2011) or may not influence the accumulated levels of Hg (ROULET; MAURY-BRACHET, 2001; SAMPAIO DA SILVA et al., 2006; BASTOS et al., 2008) as a consequence of the exposure time.

Fishes are an important food resource in the Amazon region and a few studies have examined the bioaccumulation of mercury in specific parts of the basin (DOREA; BARBOSA, 2007; SOUZA-ARAUJO et al., 2016; LINO et al., 2018). Those studies determined that fishes are the main route of mercury contamination in the population, especially in villages along rivers where contaminated fishes are the main source of protein (MALM et al., 1995; BASTOS et al., 2007).

Common species of carnivorous and piscivorous fish in Amapá State were analyzed for contamination by Mercury. Specifically, the influence of standard length and weight of individuals on the observed Hg concentrations was investigated and used to evaluate whether bioaccumulation rates vary among different species.

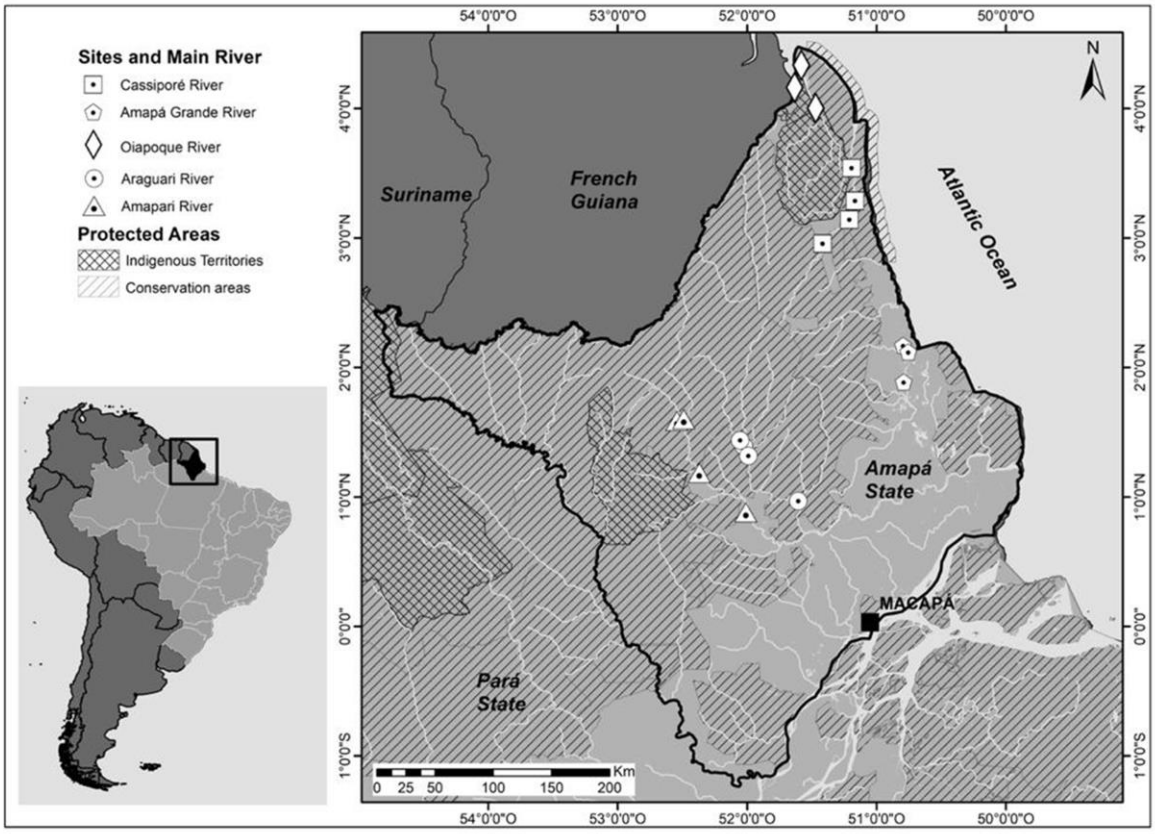
METHODOLOGY

The fishes were collected from five locations in Amapá State between August 2017 and May 2018: Rio Cassiporé, near the Vila Velha community; Amapá Grande River, coastal region near its mouth and that of the Flexal River; Oiapoque River, close to its mouth and in part of the Uaçá River; Araguari River, main channel and some major tributaries within the Flona do Amapá and PARNA Mountains of Tumucumaque; Amapari River, region close to its confluence with the Anacuí River in the PARNA Mountains of Tumucumaque (Figure 01)

Fish were caught by local fishermen hired to support the research project. According to the local fishermen, each fish species was captured using a particular gear such as gillnets, hand lines with hooks, fishing rods and long lines. Each specimen was identified to species level, weighed and measured. A minimum of 70g of muscle tissue free of skin was removed from the dorsal region of the body. All dissection equipment was sterilized between samples to avoid contamination. Samples were stored on ice for transportation to laboratory freezers.

Total mercury analysis was carried out in the Analytical Chemistry Laboratory of the the Pontific University of Rio de Janeiro, using Cold Vapor Atomic Fluorescence Spectrometry (EPA, 2002). The Analytical quality was determined by control strick blank with duplicate analysis and compared to analytical results of certified reference materials (DORM-2, Dogfish Muscle Certified Reference Material for trace element, National Research Council, Canada). Total mercury analysis from wet weight was adopted.

Figure 1. Study area showing sampling sites (GERCO 2020)



The tolerance limit recommended for consumption by the WHO is 0.50 $mg.kg^{-1}$ for carnivorous and 0.30 $mg.kg^{-1}$ for non-carnivorous fishes (IPCS, 1990). In Brazil, the Hg limits was set at 0.50 $mg.kg^{-1}$ for nor-carnivorous and 1.00 $mg.kg^{-1}$ for carnivorous fishes (MS, 1998; ANVISA, 2013).

Using a more conservative approach, we used the values established by WHO as a reference, since the daily consumption of fishes in the Amazon Basin is much higher than in other regions of Brazil.

The total weight / standard length relationship was determined after graphical inspection of the log data and then adjusted by the least squares method according to the expression:

$$Wt = a\ Sl^{b}$$

Where, **Wt** is the Total Weight (g), **Sl** is the Standard Length (cm), **a** is the linear coefficient and **b** is the slope of the regression.

The exponent b must have values between 2.5 and 3.5 (usually 3). When b = 3, the growth in weight is isometric; when different from 3, the growth is said to be allometric, which can be positive (b > 3) or negative (b < 3) (KING, 1996). Relations were tested between the standard value of 3 using Student's t test (ZAR, 1996).

The length and weight data were used to determine the well-being or nutritional status of the fish, by calculating the relative condition factor (Kr) according to the equation (LE CREN, 1951):

$$Kr=Mt/Me$$

Where:

Mt = Empirically recorded mass in grams;

Me = Theoretically expected mass in grams.

To calculate the theoretically expected values of mass (Me), the following equation was used:

$$Me = (Sl^{b}).a$$

Where, **Me** is the expected mass, **Sl** is the standard length; **a** the intercept or condition factor and **b**, the angular coefficient of the potential curve or the allometry coefficient (LE CREN, 1951). **Kr** was calculated and tested with the Kr = 1.00 standard (LE CREN, 1951), using Student's t test, ($p < 0.05$) (ZAR, 1996).

To check for a relationship between standard length (cm), weight (g) and mercury concentration (Hg), a regression analysis was applied. The dependent variable was the concentration of mercury while the independent variables were lengths and weights. According to Jacques-Callegari (2007) when the **r** (Pearson correlation coefficient) value is between 0 and 0.29, the correlation between variables is weak, between 0.30 and 0.59 it is regular, between 0.6 and 0.89 it is strong and between 0.9 and 1 it is very strong.

RESULTS

Analyses were completed for total of 204 specimens representing nine carnivorous/piscivorous species of fishes: *Serrasalmus rhombeus*, *Plagioscion squamosissimus*, *Megalops atlanticus*, *Hoplias malabaricus*, *Cichla monoculus*, *Brachyplatystoma vaillantii*, *Boulengerella cuvieri*, *Ageneiosus inermis* and *Hoplias aimara*. The maximum, minimum and average values for the standard length and weight, as well as the total number of specimens analyzed by species, are shown in Table 01. Figure 02 shows the values for total Hg contamination ($mg.kg^{-1}$) for each species. All of the fish analyzed showed some contamination by Hg and 39.7% of the specimens had levels above 0.5mgkKg^{-1} (Table 01), the limit established by WHO for the consumption of carnivorous fish.

The regression analysis to check for a relationship between standard length (cm), weight (g) and mercury concentration (Hg), are shown in Tables 03 and 04, respectively, and in Figures 03 and 04 when the results were significant.

DISCUSSION

All fishes had some level of Hg contamination, showing that this heavy metal is present in the environment. However, the potential for bioaccumulation of this contaminant varied among species. All species had piscivorous or carnivorous eating habits, and such species tend to have higher concentrations of Hg than omnivorous, detritivorous and / or herbivorous fishes (BASTOS, 2006; DOREA et al., 2006).

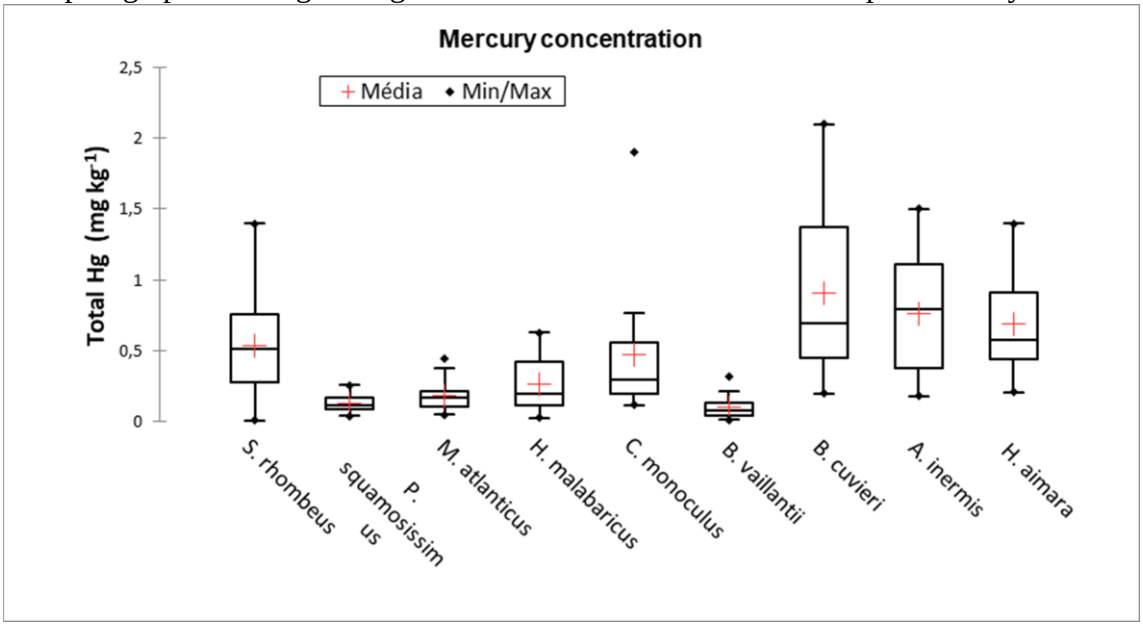
According to Cabana and Rasmussen (1994), trophic level and mean weight explain a large proportion of variation in Hg levels in fishes. Besides that, since the length of the fishes can be associated with age, a positive relationship between length and mercury concentration means an increase of concentration with time and that the fishes accumulated mercury throughout their lives. Thus, species that showed a positive relationship between body weight and length and the concentration of mercury in the muscle tissue constitute species with greater potential for bioaccumulation of this heavy metal. Based on this study, those species are: *Serrasalmus rhombeus, Hoplias aimara, Brachyplatystoma vaillantii, Cichla monoculus* and *Hoplias malabaricus*.

Plagioscion squamosissimus had low mercury

Table 1. Maximum, minimum and average values for the standard length (Lp) and weight (Wt) of specimens analyzed by species

Species	n	% above 0.5 mg/Kg	Lp (cm)			Wt (g)		
			Min.	Max.	Av.	Min.	Max.	Av.
Serrasalmus rhombeus	35	51.4	18.7	35.0	25.1	170.0	1500.0	596.0
Plagioscion squamosissimus	14	0	28.9	39.8	33.6	435.0	1140.0	713.2
Megalops atlanticus	23	0	33.0	55.7	41.6	550.0	3010.0	1288.2
Hoplias malabaricus	16	18.8	22.8	34.5	26.8	235.0	940.0	412.2
Cichla monoculus	12	33	21.0	53.5	31.3	245.0	5090.0	1177.9
Brachyplatystoma vaillantii	17	0	33.6	50.7	40.8	500.0	2420.0	1144.1
Boulengerella cuvieri	14	71.4	32.1	56.0	45.8	220.0	1800.0	1007.1
Ageneiosus inermis	10	60	24.3	38.7	31.8	195.0	960.0	472.5
Hoplias aimara	63	63.5	23.8	65.5	40.8	310.0	6110.0	1900.6

Figure 2. Boxplot graph showing the Hg concentration values for each fish species analyzed.



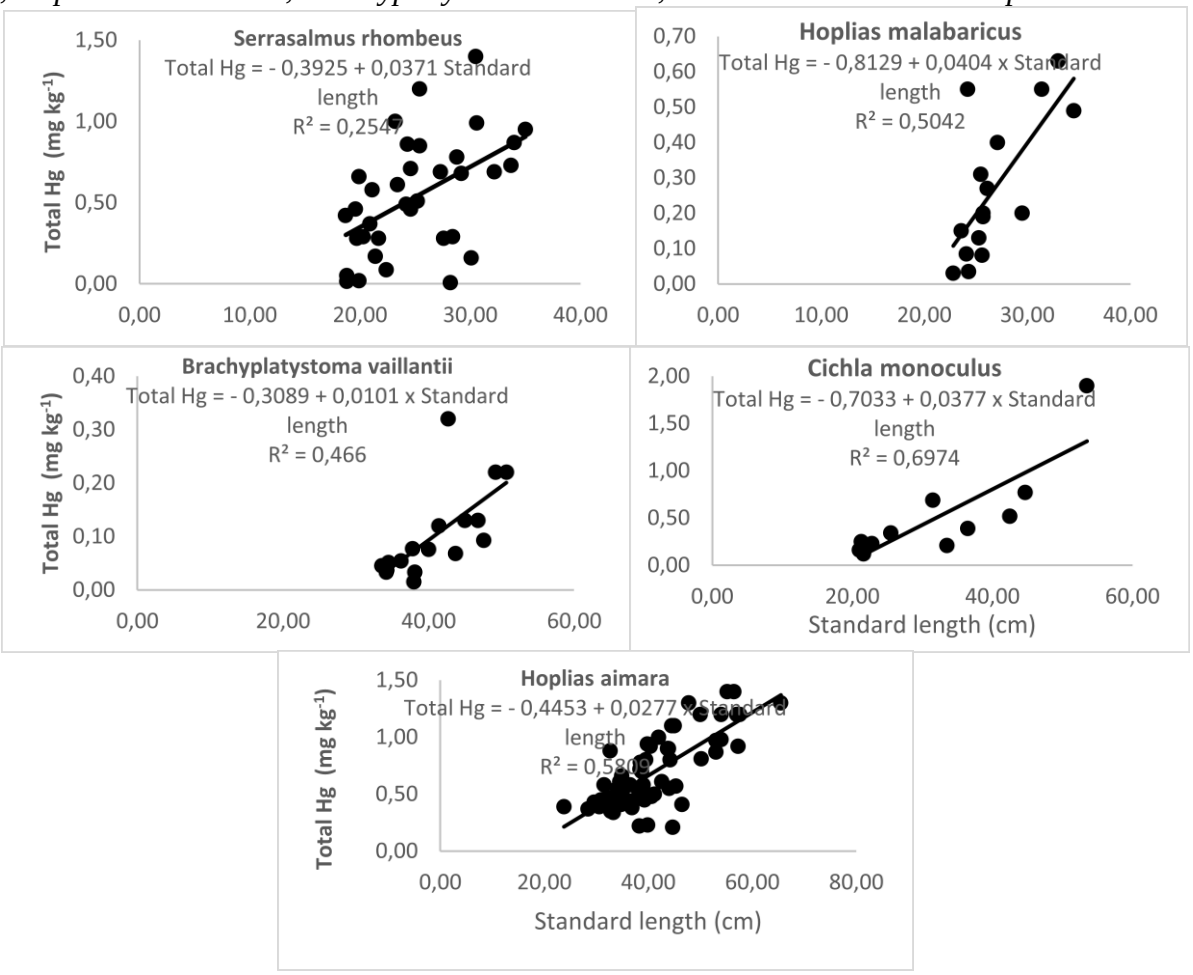
contamination, and no individual had a level above 0.5 mg.kg-1, the limit recommended by WHO for consumption of carnivorous fish. These results differ from those of Beltran-Pedreros (2011) in Lago Grande de Manacapuru (Solimões River) where this species showed levels above 0.5 mg.kg-1 of Hg. *Plagioscion squamosissimus* showed isometric growth and no relationship was found between the levels of contamination by Hg and its weight or length. Similarly, Sampaio da Silva et al. (2006) found no such relationship in the Tapajós River, despite the carnivorous / piscivorous diet reported for *P. squamosissimus* (BELTRAN-PEDREROS, 2011; MÉRONA; RANKIN-DE-MÉRONA, 2004; DARY et al., 2017). When young, this species has a more general diet, feeding on crustaceans and insects (STEFANI; ROCHA, 2009). This may allow smaller individuals to avoid high Hg values, resulting in a non-significant relationship between these values and weight and length.

Serrasalmus rhombeus and *Hoplias malabaricus* showed high levels of Hg contamination and a positive correlation between Hg and body length and weight, results similarly found by Soares et al. (2016) in the Negro River basin. In the Tapajós river, Sampaio da Silva et al. (2006) found a positive relationship between Hg and the length of *Serrasalmus rhombeus* and *Cichla monoculus*, among other carnivorous / piscivorous species studied in the region. In the present study, *C. monoculus* showed high concentrations of Hg, and a positive relationship between this metal and its weight and length. Similar observations were reported for this species by Vera et al. (2008) in the Tapajós river and by Bastos et al. (2008) in the Madeira River. Soares et al. (2016) also found a

Table 2. Length/Weight relationship analysis and Condition factor values (Kr) os al fishes analysed and conclusion of their growth type

Species	n	Lenght/Weight relationship	b-value	Condition fator Kr	Growth	Growth t-test
Serrasalmus rhombeus	35	F<0.0001	3.49	1.001 (=1)	Positive allometric	Confirmed
Plagioscion squamosissimus	14	F<0.0001	2.6	1.01 (=1)	Negative allometric	Not confirmed Isometric
Megalops atlanticus	23	F<0.0001	3.04	1.01 (=1)	Positive allometric	Not confirmed Isometric
Hoplias malabaricus	16	F<0.0001	3.24	1.01 (=1)	Positive allometric	Not confirmed Isometric
Cichla monoculus	12	F<0.0001	3.07	1.01 (=1)	Positive allometric	Not confirmed Isometric
Brachyplatystoma vaillantii	17	F<0.0001	3.36	1.005 (=1)	Positive allometric	Not confirmed Isometric
Boulengerella cuvieri	14	F<0.0001	3.42	1.003 (=1)	Positive allometric	Confirmed
Ageneiosus inermis	10	F<0.0001	3.16	1.003 (=1)	Positive allometric	Not confirmed Isometric
Hoplias aimara	63	F<0.0001	3.44	1.01 (=1)	Positive allometric	Confirmed

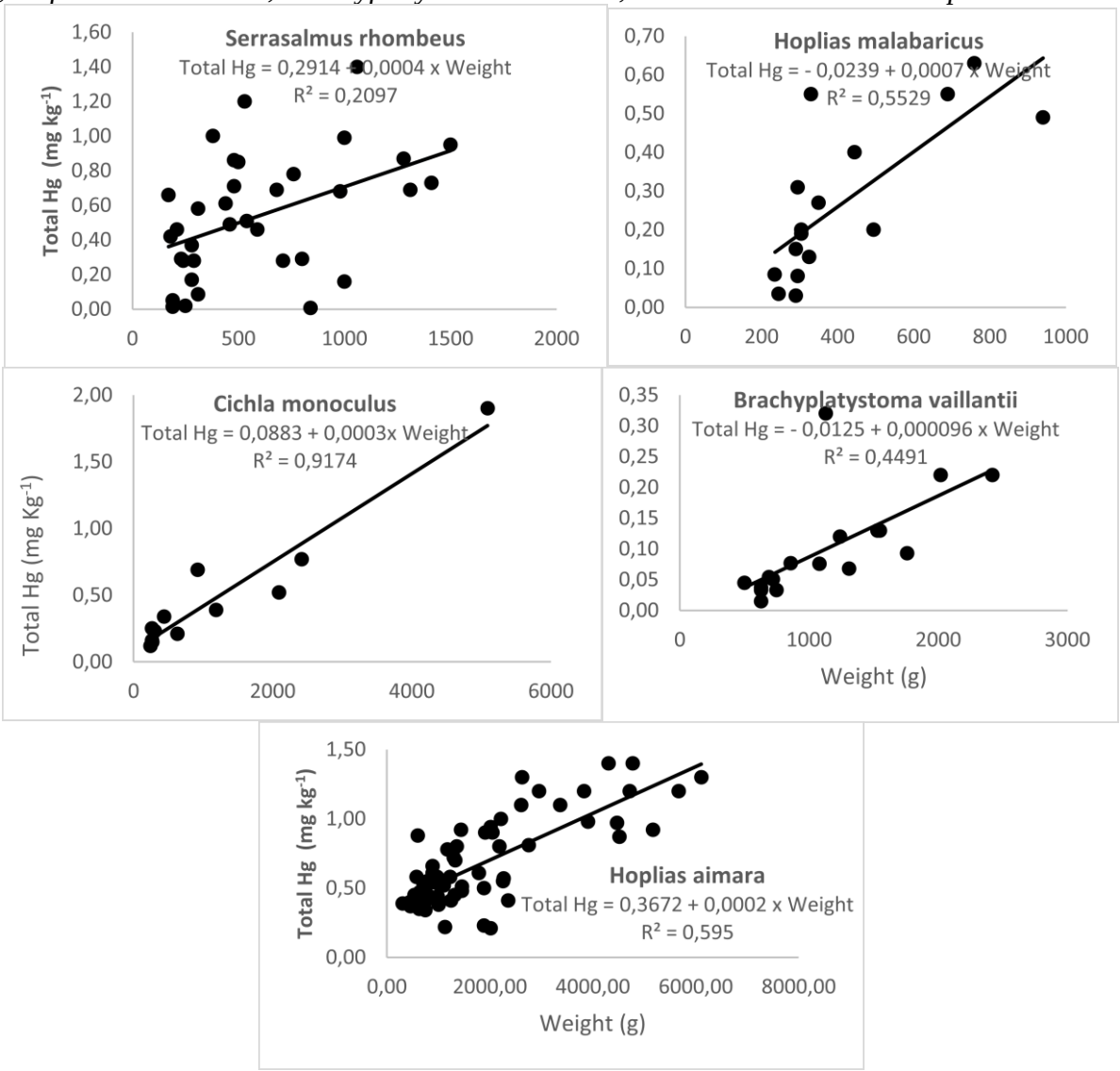
Figure 3. Relationship between the Hg Concentration (mg Kg^{-1}) and the Standard Length of *Serrasalmus rhombeus*, *Hoplias malabaricus*, *Brachyplatystoma vaillantii*, *Cichla monoculus* and *Hoplias aimara*.



positive relationship between Hg and standard length (but not weight) for *Cichla temensis* in the Negro river.

Ageneiosus inermis and *Boulengerella cuvieri* showed high concentrations of mercury, and Venturieri et al. (2017)

Figure 4. Relationship between the Hg Concentration (mg Kg^{-1}) and the Weight of *Serrasalmus rhombeus*, *Hoplias malabaricus*, *Brachyplatystoma vaillantii*, *Cichla monoculus* and *Hoplias aimara*.



reported similarly high levels in rivers in Amapá. However, no relationship was observed between this metal and body weight or length. *Ageneiosus inermis* is considered a carnivorous species (DARY et al., 2017), but the diet of a related species in Amapá's Araguari River, *A. ucayalensis*, includes other important items such as crustaceans (SÁ-OLIVEIRA et al., 2014 (b)). If similarly true of *A. inermis*, this plasticity in diet could explain the fact that there is no positive relationship between mercury and the body weight and length of this species. *Boulengerella cuvieri* is considered piscivorous (SÁ-OLIVEIRA et al., 2014 (a); DARY et al., 2017), but the diet of this species when young is too poorly known to understand the relationship between mercury contamination and body weight and length.

Megalops atlanticus showed some mercury concentration in all samples. But, those values were low and no relationship was found between Hg concentration and the body weight and length in this species. *Megalops atlanticus* generally feeds on fish, but also consumes insects and vegetable matter. The proportion of these items in its diet can change throughout the year according to their availability in the environment, as noted by Cataño and Garzón-Ferreira (1994) in the coastal region of Colombia.

The catfish *Brachyplatystoma vaillantii* did not exhibit a high concentration of Hg, but showed a positive relationship between Hg and body weight and length, in contrast to the results of Bastos et al. (2008) for fishes of the same genera from the Madeira River.

Hoplias aimara showed high concentrations of Hg and a positive relationship between this contaminant and body weight and length. High concentrations of Hg for this species also were found by Venturieri et al. (2017) in different rivers in Amapá, by Fréry et al. (2001) in the Maroni river, French Guiana and by Lima et al. 2015 for the Cassiporé River

(Amapá). Fréry et al. (2001) also found a positive relationship between Hg and the body weight of *Hoplias aimara* in French Guiana.

CONCLUSIONS

All of the carnivorous fish species analyzed in this study showed some level of mercury contamination, but did not necessarily exhibit levels higher than that considered safe for consumption by WHO. The bioaccumulation potential of Hg was observed for *Serrasalmus rhombeus*, *Hoplias malabaricus*, *Cichla monoculus* and *Brachyplatystoma vaillantii*, all of which showed a positive relationship between weight and / or length and the total body Hg concentration. Environmental conditions and dietary plasticity can interfere with the degree of contamination and bioaccumulation of mercury among carnivorous / piscivorous species of fishes.

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