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RESEARCH ARTICLE

Organotropism of total mercury (THg) in *Cichla pinima*, ecological aspects and human consumption in fish from Amazon region, Brazil

Juliana de Souza Azevedo[1] · **Marcos Antonio Hortellani**[2] · **Jorge Eduardo de Souza Sarkis**[2]

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Abstract

Specimens of the *Cichla pinima* are of ecological and economic importance in the Amazon region and are a good bioindicator species of Hg bioaccumulation. Adult specimens of *Cichla pinima* were obtained by fisheries in the Tapajós river region's impacted gold mining area. Tissues such as muscle, liver, skin, and gill were submitted for total mercury (THg) analysis. For hepatic bioavailability, assays were carried out in the whole liver and in the sub-cellular hepatic fraction. The weight–length relationship showed an equation of $W = 2E - 06L^{3.3002}$ ($R^2 = 0.856$) indicating an allometric growth. The mean THg values found in the muscle tissue of 676 ± 258 μg kg^{-1} were below the maximum limit established for human consumption, but were similar to *Cichla* fish from other areas impacted by Hg in the Amazon region. The trends of levels in the tissues were as follows: liver>muscle>gonads>skin>gill, with no significant differences between the gills and skin. A significant and positive correlation as $r_s = 0.65$ was obtained between the THg contents in cytosolic fraction and the total hepatic THg (liver homogenate). However, only 7% of the THg were found and were available to the sub-cellular hepatic fraction. This profile can be an indicative of a hepatic cellular transference in fish exposed to high Hg levels in the Amazon region now that low concentrations of Hg have been found in the sub-cellular fraction. However, data of differential THg partition in the subcellular compartments should be considered, since others' hepatic fractions can act in the Hg linkage into the cell.

Keywords Amazon fish · Tucunare · Cytosolic mercury · Bioavailability · Tapajós River · Brazil

Introduction

Mercury is a very toxic element (Clarkson 1997), and in the natural environment, it may occur in the oxidation form Hg^0, Hg^{+1}, and Hg^{+2}. However, various anthropic activities, such as the burning of fossil fuels, gold mining, sewage incineration, and disposal of manufactured goods such as batteries, fluorescent lamps, thermometers, thermostats, dental amalgam, paints, and pesticides release this metal into the aquatic system, causing a significant increase in its concentration therein (Smith et al. 1991; Jackson 1997; UNEP 2013).

The methylation process is very important to mercury transit into the aquatic food chain (Barbosa et al. 2003; Nevado et al. 2010) as in its organic form ($CH_3CH_2Hg^+$, CH_3Hg^+) it tends to cross cell barriers more easily. Although most of the mercury in biological systems is found in the form of methyl mercury (MeHg), which is highly absorbable by diet (95–100%), the inorganic form (Hg^{+2}), is absorbed to a lesser extent (5–10%), which may also bioaccumulate and have a toxic effect on aquatic organisms (Heath 1990; Dias et al. 2008).

Many countries have used gold mining activities to improve their economy, for instance, in the Amazon region where reports of mercury contamination in the Amazon basin as a consequence of gold mining has been described since the 1980s (Smith et al. 1991; Hacon et al. 2008). Beyond the input of mercury caused by gold mining due to gravel treatment, for

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✉ Juliana de Souza Azevedo
juliana_azevedo@msn.com; juliana.azevedo@unifesp.br

[1] Instituto de Ciências Ambientais, Químicas e Farmacêuticas, Departamento de Ciências Ambientais, Universidade Federal de São Paulo, Rua São Nicolau, 210, Centro, Diadema, Brazil

[2] Centro de Lasers e Aplicações, Instituto de Pesquisas Energéticas e Nucleares, Av. Prof. Lineu Prestes, 2242, Cidade Universitária, São Paulo, Brazil

example, other activities act to increase the mercury content in the Amazon basin, such as the high natural mercury content in Amazonian soils (Roulet and Lucotte 1995; Fadini and Jardim 2001; Wasserman et al. 2003) and atmospheric transport. Human activities, such as deforestation and the intensification of agriculture practices, also increase the erosion and lixiviation of materials and chemical compounds, such as mercury, along tributaries of the Tapajós River (Oliveira et al. 2001).

In the Amazon region, the human population and aquatic organisms are exposed to the three inorganic forms (Hg^0, Hg^{+1}, Hg^{+2}), as well as to the organic forms of mercury (Frery et al. 2001). Most of the gold mining activities are found along the central area of the Tapajós river, the largest tributary of the Amazon (Nevado et al. 2010), with a length of 843 km (DNIT 2017).

Organisms, such as fish, have an important role in the distribution of mercury between the different biotic compartments, as disparate species tend to present differentiated levels of mercury incorporation according to their position in the trophic chain, also undergoing the biomagnification process (Vieira et al. 2011; Azevedo-Silva et al. 2016). In addition, the mercury uptake in fish may be a consequence of exposure by diet or water, in which, with fish of demersal habits, there is still an additional route of incorporation through sediments.

Most of the Hg occurring in the Brazilian Amazon is of natural origin, since this element is a natural component of the soil (Fadini and Jardim 2001; Oliveira et al. 2001). However, in many cases, inputs by human actives such as gold mining have increased these levels to toxic concentrations. In a previous study, we clarified some questions regarding Hg and its effects on icthyofauna species such as the Cichlidae family in the Brazilian Amazon as specified that "the intense gold mining activity in the region has increased the levels of this toxic metal, especially in the middle section of the Tapajós River." Specimens of the *Cichla* genus have great economic, ecological, and environmental importance, as they are the most consumed fish in the Brazilian Amazon region, are at the top of the Amazon food chain, and are used as bioindicator species of contamination due to their carnivorous feeding habits.

Cichlids have high levels of mercury and are one of the most consumed fish in the Brazilian Amazon region (Santos et al. 2000; Nevado et al. 2010). Therefore, they are important food constituents for the local population. Ecologically, they are at the top of the regional aquatic food chain, being a good indicator for Hg bioaccumulation because the concentrations of Hg in their tissues tend to reflect the accumulation process of successive exposure (Barbosa et al. 2001; Castilhos et al. 2001).

Fish of the Cichlidae family (Actinopterygii) are distributed around South America in Suriname and French Guiana and also in Guyana (Kullander and Ferreira 2006), with great abundance in the north of Brazil in the Amazon basin, where they are named Tucunare. They are sedentary and territorial species and do not perform migration, living mainly in waters with low flooding (Kullander and Ferreira 2006). As a common characteristic of the species, these fish have spheric ocelli in the peduncle and rayed dorsal fin spines (Kullander 2003). In accordance with Arcifa and Meschiatti (1993), the *Cichla* species have modifications in their feeding habits due to their age, with juvenile fish feeding mainly on aquatic insects. On the other hand, adult specimens are strictly carnivorous, feeding primarily on other fish and less frequently on the debris of organisms and crustaceans (Súarez et al. 2001).

Concerning fish biology, aspects such as metabolism, length, weight, age, and growth can be affected by exposure to several chemical substances, for instance, mercury (Hg). Therefore, knowledge about the weight–length relationship in fish species submitted to these compounds is ecologically important, along with obtaining information about bioavailability and the uptake rate in target detoxification organs, such as the liver.

There is little information regarding the bioavailability of THg concerning the fractionation of this metal in fish (Araújo et al. 2015). Mercury toxicity can be influenced by subcellular storage and partitioning because of the detoxification mechanism in target organs, for instance the liver. In recent years, some studies have contemplated the determination of sub-cellular, and consequentially intracellular, metals to reveal and understand aspects of the detoxification and bioavailability of toxic metals. However, an absence of information still exists with respect to Amazon fish that are submitted to high Hg levels in aquatic systems, such as *Cichla pinima* from the Tapajos River.

Previously, we had shown partial data concerning the THg transference rate between the cytosolic and total hepatic tissue in *Cichla sp*. (Azevedo et al. 2014). Therefore, to improve these results, the aim of this study was to investigate the Hg fate in Amazon fish from the Tapajós River assessing biological and ecological drivers to Hg fate in *Cichla pinima*. Additionally, the bioavailability of total mercury (THg) by sub-cellular evaluation in the liver to improve the knowledge concerning the transference rate between the total hepatic contents and cytosolic THg was made, and addressed a relatively novel approach to mercury distribution in fish.

Material and methods

Sample collection and biometric data acquisition

Twenty-six specimens of adults *Cichla pinima* were collected close to the Itaituba municipality (Fig. 1). Gold mining along the central Amazon was very common, particularly around the tributaries of the Tapajós river. Fish were sampled in July of 2011 in accordance with the permissible Brazilian period for

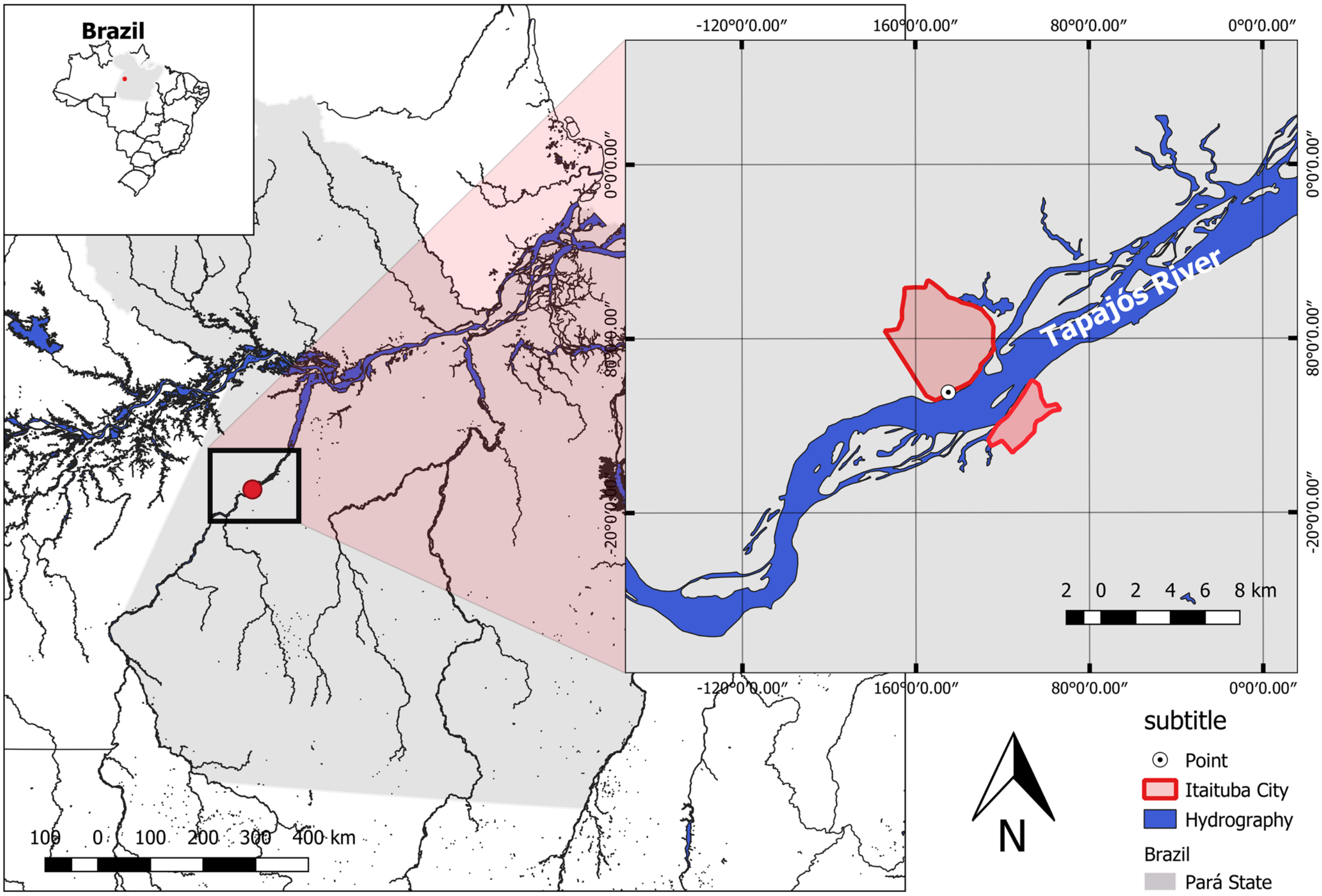

Fig. 1 Sampling sites showing the Tapajós River, close to Itaituba city, in the Brazilian Amazon region instead (adapted from wikmapia.org)

acquisition of this species, with the help of local fishermen using gill nets and harpoons. Fish species were identified in accordance with Kullander and Ferreira (2006). After collection, fish were kept in a box at −4 °C and transported to the laboratory of chemical and isotopic characterization of the CQMA-IPEN for further analysis. Fish were also identified, and morphometric data were recorded for total length (L), body weight (W), and sex identification using macroscopic observation. Later, fish were dissected by the removal of the epaxial muscle from the dorsal surface, liver, gonad, gill, and skin, washed with distilled water, packed in polyethylene bags, labeled, and then stored at −20 °C until subsequent analysis.

Total mercury determination in tissues and in the sub-cellular hepatic fraction

The mercury content was measured in insoluble and soluble (cytosol) fractions of liver. A total of 130 tissue samples and 26 hepatic cytosolic fractions were submitted to THg determination. All mercury determinations were performed using the flow injection cold vapor atomic absorption spectrometry (FI-CV-AAS) technique. For THg analysis in the total tissues, the procedure concerning the amount of individual tissues followed the described in Azevedo et al. (2012). Hepatic cytosolic fractions were obtained by homogenization followed by ultracentrifugation as described in Azevedo et al. (2009) in which samples of liver were weighed and homogenized in the Tris-HCl buffer (0.02 M) at pH 8.6, 10% BHT, and centrifuged at 30,000*g* for 45 min at 4 °C; the aliquots obtained were used for Hg determination.

The analytical procedure for Hg quantifications followed the methods described by Hortellani et al. (2005) with modifications to biological samples, in which different samples were digested in a concentrated solution of HNO_3, H_2SO_4, and HClO (1:2:1) using glass flasks. Mercury concentrations were reported in wet weight.

Analytical control procedures

Detection and quantification limits were calculated in accordance with the National Institute of Metrology, Quality and Technology (INMETRO 2010), as DL = mean blank +3 × blank's and QL = mean blank +5 × blank's standard deviation × dilution. The obtained DL and QL were 0.527 μg kg^{-1} and 2.585 μg kg^{-1}, respectively. Quality control of the analysis was measured with Hg concentrations in certified reference material (Dogfish muscle DORM-2, National Research Canada Council, NRCC). The mean data obtained was 4.63 μg g^{-1} ± 0.04 (n = 3) with a recovery of 99% at a 0.05 significance level within the confidence interval of the certified level, exhibiting, therefore, good precision and accuracy. The technique used in this study for total Hg (FIA–CV-AAS) is accredited by the "Coordenação Geral de Acreditação do INMETRO" (CGCRE/INMETRO 2013).

Statistical analysis

The normality of the data prior to analysis was checked, and the Spearman correlation was used, since the data were not normally distributed. Therefore, the Matrix correlation was made through the use of the Spearman correlation (r_s) and significance at the 0.01 and 0.05 levels (two-tailed) was considered. For the box plot of the metal concentrations in different tissues, the whisker length of one sigma with a rounding quartile method was used.

Results

Biometric data of the *Cichla pinima* specimens showed a mean length and weight of 395 ± 51 mm and 840 ± 418 g, respectively. In general, the individuals showed more homogeneous biological aspects with only eight fish showing a length higher than 400 mm (Fig. S1); however, regarding the standard deviation, these organisms are within the range of variation. Individuals of *C. pinima*, where 53% were males and 47% were females, the weight–length relationship observed for the sampled specimens (Fig. S1) showed an equation of $W = 2E - 06L^{3.3002}$ (R^2 = 0.856), revealing an allometric growth.

Due to the low sample number, sexual differences were not considered. However, there was a similar proportion of males (53%) and females (47%). Furthermore, homogeneous biometric aspects of the individuals such as length and weight indicate no significant biological influence on the metal data.

Although the THg values found in the muscle tissue of *C. pinima* are close to the maximum limit established for Hg in carnivorous fish limits of 1.0 μg g^{-1} (BRASIL 1998; USEPA 1997), only two specimens exceeded this limit. With respect to the THg content in other tissues, in general, a larger variation in the individual THg concentrations was observed in the liver, muscle tissue, and skin, respectively, with a greater homogeneity in the gills. Concerning comparisons with other fish from the Amazon region (Table S1), in general, the obtained THg levels for *C. pinima* in this work are in accordance with values found in *C. pinima* from the Tapajós river, near Itaituba city. Additionally, the obtained THg values were similar to the concentrations in *C. pinima* fish from other areas impacted by Hg in the Amazon region.

Figure 2 shows a box plot of the THg data with the median, 25th and 75th percentiles, and minimum and maximum THg levels in tissues as muscle, liver, gills, skin, and gonad in the *C. pinima*. The trends of concentrations in the tissues were as follows: liver>muscle>gonads>skin>gill. Two outliers were observed in the liver and one in the gills. Statically significant differences among the tissues were found only with respect to the liver ($p < 0.05$).

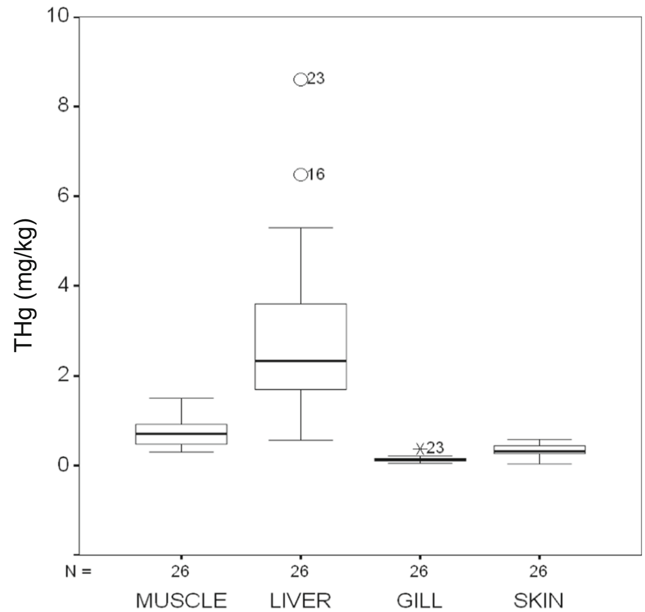

Fig. 2 Boxplot with median values of the total mercury (THg) data in different tissues of *C. pinima* sampled in the Tapajós river close to Itaituba city. Outliers are indicated as circles above the boxplot

Although the gills had higher THg values than the skin, the range of concentrations was not so different (gill 0.047–0.373 μg g^{-1}; skin 0.037–0.582 μg g^{-1}). The THg contents found in gonads had a minimum value of 0.162 μg g^{-1} and a maximum of 0.709 μg g^{-1}, with a mean value very close to that observed in the skin. The levels found in muscle had a range of 0.300 to 1.172 μg g^{-1}. Finally, the THg concentrations obtained in the liver ranged from 0.557 to 5.305 μg g^{-1} with two outliers of 6.480 and 8.604 μg g^{-1}.

Table 1 shows the correlation matrix of biometric variables, such as length and weight and THg contents in the liver, muscle, gill, gonad, and skin of *C. pinima*. Regarding the biological variables, a positive and significant correlation was found between W versus L ($r_s = 0.893$) and W versus muscle ($r_s = 0.442$) and liver ($r_s = 0.463$). Furthermore, high levels and significant correlation was also observed of Hg contents in muscle versus liver ($r_s = 0.817$), muscle versus gill ($r_s = 0.747$) and liver versus skin ($r_s = 0.464$). Gonad tissue was the only one without significant correlation to other tissues.

With respect to the hepatic bioavailability by the subcellular THg evaluation, a significant and positive correlation ($r_s = 0.650$) was found between the cytosol and the full hepatic tissue (Fig. 3). Furthermore, high levels were obtained in the total liver ranging from 1.7 to 3.8 mg kg^{-1}, but only circa of 7% of the THg were available to the cytosol hepatic fraction (Fig. 4).

Table 1 Spearman correlation matrix (r_s) of total mercury (THg) in different tissues (muscle, liver, gill, and skin) and biological parameters as length (L) and weight (W) of *C. pinima* from Tapajos River. Analyzed samples = 182

		Muscle	Liver	Gonad	Gill	Skin	L	W
Muscle	r_s	1						
	p							
Liver	r_s	0.817**	1					
	p	0.0001						
Gonad	r_s	− 0.083	0.059	1				
	p	0.769	0.836					
Gill	r_s	0.747**	0.549**	− 0.170	1			
	p	0.0001	0.004	0.544				
Skin	r_s	0.436*	0.464*	− 0.426	0.380	1		
	p	0.026	0.017	0.114	0.055			
L	r_s	0.339	0.319	0.255	− 0.081	0.158	1	
	p	0.09	0,112	0.359	0.694	0,441		
W	r_s	0.442*	0.463*	0.255	− 0.062	0.158	0.893**	1
	p	0.024	0.017	0.359	0.763	0.442	0.0001	

**Correlation is significant at the 0.01 level

*Correlation is significant at the 0.05 level

Discussion

We aim to improve our understanding of Amazon fish with economic and ecological importance; mercury contamination is due to several different anthropogenic activities along tributaries such as the Tapajós river due to gold mining and land use changes, such as agriculture practices and lixiviation of materials as a consequence of deforestation (Oliveira et al. 2001). In this study, some ecological knowledge of *C. pinima*, as well as Hg fate including subcellular fractionation and the human impact related to the consumption of this species, are shown.

Regarding the human consumption data of THg in muscle tissue, most individuals had concentrations below the national and international established limits for human consumption. In fact, the international maximum limit of 1.0 μg g^{-1} standard was developed to protect interstate commerce in the USA, in addition to human health. Thus, it has been rejected by federal agencies in the USA (EPA and FDA) and Canada for use in studies designed to protect human health, and, therefore, international standards for protecting human health are typically in the order of 0.5 μg g^{-1} (Health Canada 2007). Despite the low sample size, about 70% of the analyzed fish showed THg contents greater than 0.5 μg g^{-1} in the muscle tissue, which could be a sign of health risk. Besides, it is important to promote a better management and encourage strategies and programs for environmental monitoring and diagnosis in the Amazon region, as mining activities cause several disturbances in the aquatic system and in the integrity of the biota.

Concerning the biological and ecological aspects, in general, the length of the first maturation (LFM) for fish from the genus *Cichla* fluctuates depending on several factors such as intraspecific changes, locality, being native or introduced, and with the adaptation of the species to the new environments of others. In fact, some authors consider that aspects such as food abundance and absence or reduction in the natural predators

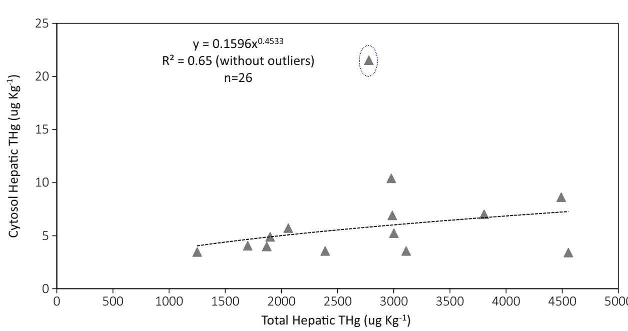

Fig. 3 Metal contents in the total liver versus cytosolic hepatic fraction of *C. pinima* from the Tapajós river

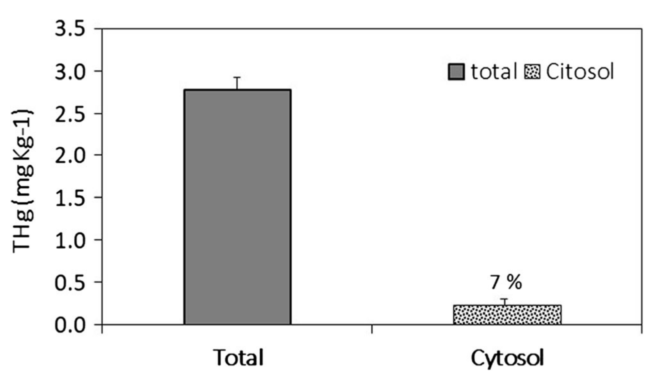

Fig. 4 THg available level (%) between the total tissue and cytosolic hepatic fraction of *C. pinima* from the Tapajós river

are important factors that can influence reducing the length of the first gonadal maturation (Gomiero et al. 2009). Luiz et al. (2011) found 225 mm with LFM to males and females of *Cichla piquiti* populations in the Cachoeira Dourada reservoir. From the species *Cichla ocellaris* and *Cichla monoculus* from southeast Brazil, a LFM of 200 mm and 215 mm were obtained, respectively (Gomiero and Braga 2004). On the other hand, in studies with *C. ocelaris* from Lake Gatun, Panamá, 322 and 332 mm LFM were obtained (Zaret 1980). In fact, regardless of variations among species, all cichlids analyzed in this work were adult, who certainly had reached the LFM, as the smallest individual captured had 325 mm and 356 mm for male and female, respectively.

Another important biological aspect that can be influenced by metal exposure in natural environments, for instance Hg, is the growth of the organism. Therefore, it is important to check possible changes in the growth profile of individuals in association with the bioaccumulation and bioavailability studies of toxic elements. Fish species may show isometric or allometric growth, and from the eq. $Y = ax^b$, it is possible to estimate the growth constant of species (b). This constant should vary between 2.5 and 4.0 and values equal or close to 3.0 are considered ideal, showing isometric growth. On the other hand, values higher than 3.0 are considered to be negative allometric growth and less than 3.0, they are considered to be positive allometric growth (Vazoller 1996).

No studies were found in relation to biological variables in *C. pinima* in natural environments in the Amazon region, such as the Tapajos River, as shown in this study regarding the length–weight relationship and type of growth, i.e., differences between allometric or isometric growth conditions. Therefore, in this study, through observation, these biological aspects were compared to other *Cichla* species. In a study conducted with farmed *Cichla temensis*, the authors obtained the equation to describe the weight–length relationship as $W = 0.0073Lt^{3.1435}$, showing a relative allometric growth (Tavares-Dias et al. 2011). Therefore, the data of weight–length observed in specimens of *Cichla sp.* from Itaituba (Brazil) shown in this work is in accordance with species such as *C. ocellaris* and *C. monoculus* (farmed and wild) as the *Cichla sp.* with allometric growth (Gomiero and Braga 2003), showing that it is a characteristic of the genus. Due to the intra- and interspecific competition and predation in natural conditions, it is possible that the weight–length relationship should be lower than that observed in farmed fish. However, in situations using farmed species, as in stressful conditions, a reduction in the weight–length relationship can also be observed (Tavares-Dias et al. 2011).

Through the analysis of THg in a sample bank of 2538 specimens of freshwater Amazon fish from the Tapajos River, Bastos et al. (2016) verified that there is a limited influence of sex on fish THg bioaccumulation. Therefore, in the case of Amazon fish, such as *Cichla sp.*, biological variables such as sex are not determinants to drivers of the patterns of THg. Although, in this study, the biological data in *Cichla sp.* with homogeneous biometric aspects of the individuals indicate no significant biological influence on the metal data, it is reported in the literature that there is a significant correlation between fish size and total Hg concentrations in carnivorous fish such as *Cichla sp.* (Bastos et al. 2015). These patterns are probably not observed in this study due the relative low sample number necessary to make this biological inference.

Regarding the organotropism, it is important to consider the differences between the species, sites, and sampling periods once these aspects interfere in the Hg uptake in the tissues of the fish. The individual variability observed in the Hg contents for the bioaccumulation pattern, for instance, with outliers for Hg levels on the liver and gills, may be influenced by biological factors, such as the age of individuals, since no significant differences in length, weight, and sex were observed. However, to corroborate this hypothesis, it is necessary to increase the sample number to make populational inferences.

Mercury concentrations in gills probably cannot indicate an input by changes and adsorption process in the branchial lamellas. In fact, species from the *Cichla* genus are carnivorous, feeding mainly on other fish. Thus, the main input route of mercury in the analyzed fish in this study can be via feeding and trophic transfer, and not the gills, as corroborated by the other studies in which the mercury exposure and uptake in aquatic organisms occurs mainly by trophic pathways (Harris and Bodaly 1998). When compared to the THg concentrations in the gills, similar THg levels in the skin, with the absence of differences being statistically significant ($p > 0.05$), suggest that the skin does not have an important contribution of mercury uptake in *C. pinima*. The fish's skin has mucus that has some functions, such as the reduction of friction with water, protection against bacterial and fungal infections, and some studies still report that in association with the skin, these barrier systems have a great contribution to make against the exposure of xenobiotics, such as trace metals (Tao et al. 2000; Al-Weher 2008; Jovičić et al. 2016), containing enzymes and antibodies that can act in defense of the organisms. In fact, chemical compounds with the ability to remove or weaken the mucus/skin layer have a toxic potential to the cell, as, along with the gills and food, these are the first input routes of xenobiotics, such as mercury into the organism.

There is still insufficient knowledge with respect to the subcellular determination and linkage with the total mercury contents of cytosolic mercury concentrations in Amazon fish. An evaluation of THg concentrations in liver homogenate and in sub-cellular compartments such as nuclei, plus cellular debris (N + D), granules (Gran), mitochondria (Mit), lysosomes plus microsomes (Lys + Mic), heat stable protein (HSP), and heat denaturable proteins (HDP), in the wild fish *Liza aurata* is found in a recent study about THg hepatic bioavailability

(Araújo et al. 2015). The authors indicated that when mercury is high in the liver, it is partitioned into Mit and HDP fractions, with an absence of evidence for the saturation of mercury detoxification mechanisms, as the fractions HSP and granules had low mercury levels (below the detection limit).

Araújo et al. (2015) found a positive and significant correlation between the sub-cellular THg contents as granules (Gran), mitochondria (Mit), lysosomes plus microsomes (Lys + Mic), heat denaturable proteins (HDP), and the whole liver (homogenate). In this study, a corresponding correlation was obtained between the THg contents in cytosolic fraction and the total hepatic THg (liver homogenate) as $r = 0.65$. Additionally, a similarity concerning the range of THg variation in the sub-cellular fractions was observed with THg in cytosol of *C. pinima*. In fact, the sub-cellular fraction reflects the concentration of soluble SH groups, since the protocol of ultracentrifugation and thermocoagulation excludes high-weight proteins, ensuring the permanence of low-weight, cysteine-rich proteins (with SH groups).

Bioavailability in the hepatic tissue, considering the transference between the total liver and the cytosolic fraction, has a great contribution to make towards understanding some aspects of the transference of THg into the hepatic compartments in a bioindicator species from the Amazon region, for instance, Tucunare. For the *C. pinima* from the Tapajós river analyzed in this study, a difference of 7% of THg found in the sub-cellular fraction (cytosolic) was observed when compared to the THg concentrations in the whole liver. This profile is very interesting as the low concentration obtained in the sub-cellular fraction suggests an effective hepatic cellular transference in fish exposed to high mercury levels in the Amazon region. On the other hand, this is an initial point that can be reinforced by data of differential THg partitions in subcellular compartments, mainly the HSP fraction that was not determined in this study, but is responsible for major metal ligands, such as metallothionein.

Finally, these data are interesting to comprise some aspects of the transference of THg in different tissues of *C. pinima*, an important fish for consumption and ecological relevance, being the species at the top of the food chain. Therefore, this study is an important key to understanding the fate of Hg in such an important fish group in the Amazon, addressing a relatively novel approach to mercury distribution in fish.

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Compliance with ethical standard

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. This article does not contain any studies with human participants performed by any of the authors.

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