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ORIGINAL ARTICLE

Mercury in muscle and liver of *Plagioscion squamosissimus* (Acanthuriformes: Sciaenidae) from the Machado River, Brazilian Amazon

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ABSTRACT

The Machado River is the main tributary of the Madeira River in the state of Rondônia, a region that has been impacted by deforestation, livestock, urban development, alluvial gold mining and urban and industrial effluents that likely contribute to the introduction of mercury (Hg) in the aquatic system. We aimed to determine the concentrations of total Hg (THg) in muscle and liver of *Plagioscion squamosissimus*, a main fishing resource in the region. Fish were sampled for two years at five sites, along a 90-km stretch of the middle Machado River. THg concentrations were analyzed in 64 muscle and 54 liver samples by cold vapor atomic absorption spectrophotometry (FIMS-400). THg levels were related with fish size and sex and with periods of the hydrological cycle. THg concentrations in both organs were positively and significantly related with fish body size. There was no significant variation in THg among periods of the hydrological cycle. Mean THg concentrations in muscle (1.09 ± 0.72 mg kg^{-1}) and liver (1.28 ± 1.23 mg kg^{-1}) were higher than the limit established by the WHO. Our results suggest that residual mercury from former alluvial gold extraction and lixiviation of mercury naturally occurring in the soil due to large-scale deforestaion is accumulating in the aquatic food chain in the Machado River. We conclude that it is necessary to monitor the levels of mercury in commercially important fish species in the region.

KEYWORDS: fish, heavy metal, Rondônia

Mercúrio no músculo e fígado de *Plagioscion squamosissimus* (Acanthuriformes: Sciaenidae) do Rio Machado, Amazônia brasileira

RESUMO

O Rio Machado é o principal afluente do Rio Madeira no estado de Rondônia, uma região impactada por desmatamento, pecuária, desenvolvimento urbano, mineração de ouro e efluentes urbanos e industriais, que provavelmente contribuem para a introdução de mercúrio (Hg) no sistema aquático. Nosso objetivo foi determinar as concentrações de Hg total (THg) em músculo e fígado de *Plagioscion squamosissimus*, um importante recurso pesqueiro na região. Os peixes foram amostrados durante dois anos em cinco locais ao longo de 90 km no médio Rio Machado. As concentrações de THg foram analisadas em 64 amostras de músculos e 54 de fígado por espectrofotometria de absorção atômica com vapor frio (FIMS-400). As concentrações de mercúrio foram relacionadas ao tamanho e sexo dos peixes, e com períodos do ciclo hidrológico. As concentrações de

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THg em ambos os órgãos foram relacionadas positiva e significativamente com o tamanho corporal dos peixes. Não houve variação significativa do THg entre os períodos do ciclo hidrológico. As concentrações médias de THg no músculo (1,09 ± 0,72 mg kg^{-1}) e no fígado (1,28 ± 1,23 mg kg^{-1}) foram superiores ao limite estabelecido pela OMS. Os resultados sugerem que o mercúrio residual da extração de ouro e lixiviação de mercúrio que ocorre naturalmente no solo desmatado está se acumulando na cadeia alimentar aquática do Rio Machado. Concluímos que é necessário monitorar os níveis de mercúrio em espécies de peixes comerciais na região.

PALAVRAS-CHAVE: peixe, metal pesado, Rondônia

INTRODUCTION

Mercury concentrations found in different environmental matrices in the Amazon region, can be attributed to natural (Lacerda and Pfeiffer 1992) and anthropic origin, the main source being gold mining (Lacerda and Pfeiffer 1992; Bastos *et al.* 2006). During the burning of gold-mercury amalgam, Hg is volatilized and is transported via the atmosphere, contaminating rivers and soils in other regions (Lacerda and Salomons 1992; Hancon 1995). In the Machado River, a tributary of the Madeira River, which forms a major right-margin subbasin of the Amazonas River in the southwestern Brazilian Amazon, alluvial gold extraction has also been recorded (CNEC 1985). The Madeira River is known for intensive gold extraction, maily in the 1970s and 1980s (Lacerda and Pfeiffer 1992; Bastos *et al.* 2006). The Machado River flows for 972 km from the central Brazilian Shield, through a region that has suffered high rates of slash and burn deforestation (Ferraz *et al.* 2005), along one of the main highways into the Amazon region (BR-364). Over 50% of the Machado River basin was deforested until 2019 (INPE/Prodes 2019). Soil, unprotected as a result of desforestation, can contribute to entry of mercury into Amazonian aquatic ecosystems (Nascimento *et al.* 2020). At present, alluvial gold extraction is declining in the basin and has become rare (Rodrigues and Marta 2017). However, even where sediments contaminated with mercury have been removed to prevent methylation, the mercury used in gold amalgamation can still be found in high levels in rivers throughout the Amazon Basin (Mailman *et al.* 2006).

The middle section of the Machado River passes through areas with medium to very high alteration, mainly pasture and soybean croplands and associated urban centers and industrial plants, such as tanneries, meat packing plants and dairy plants. The main sources of water contamination are domestic sewage and industrial wastewater, irregular trash dumps, and runoff of agricultural chemicals (Vasanthi *et al.* 2019), causing pollution by toxic substances, such as oil, grease, nitrogen, phosphorus and heavy metals, as well as pathogenic bacteria (Nunes and Jesus 2019). Among the heavy metals, mercury is found in the water, air, soil, sediments, plants and animals in organic forms (methylmercury, dimethylmercury), metallic form and as ionic mercury salts (Vasile *et al.* 2019). Mercury is a highly toxic pollutant due to its ability for bioaccumulation (Ferreira *et al.* 2015), mainly in aquatic ecosystems (Vasile *et al.* 2019). The toxic status of mercury depends on the chemical form that is absorbed by the organism, the organic forms being the most toxic (Ferreira *et al.* 2015; Milhome *et al.* 2018). In general, fish have an average methylmercury percentage relative to total mercury ranging from 97.3% in carnívorous fish to 96.4% in omnívoros fish (Kehrig and Malm 1999).

Some species of carnivorous fish are bioindicators of pollution in aquatic habitats and a source of food for other animals, as well as economically important fishing resources (Tacon and Metian 2009; Silva *et al.* 2019). Thus, it is important to determine concentrations of potentially toxic elements in their tissues (Milačič *et al.* 2019). The South American silver croaker, *Plagioscion squamosissimus* (Heckel, 1840) is a sedentary fish native to the Amazon region (Santos *et al.* 2006). It is economically important in the region as a major protein source for local communities (Montes *et al.* 2011) and in recreational fishing (Barros *et al.* 2012). The species has a wide feeding spectrum (Hahn *et al.* 1999; Bennemann *et al.* 2000; Bennemann *et al.* 2006; Santos *et al.* 2016), although it is mostly piscivorous (Hahn *et al.* 1997) or a generalist carnivore (Bennemann *et al.* 2011; Rocha *et al.* 2015; Neves *et al.* 2015). Due to its wide geographical distribution in the Amazon, abundance and tolerance to environmental stress in dammed river systems, and the relevance of studyng species on the top of the aquatic food chain (Custódio *et al.* 2020), total mercury analysis of *P. squamosissimus* can help reveal important environmental features in tropical aquatic environments (Wunderlich *et al.* 2015).

The objective of this study was to analyze the total mercury concentration in *P. squamosissimus* in the Machado River, considering that, as a carnivorous species, it can accumulate high concentrations of mercury, wich can be transferred to humans through consumption. Mercury concentrations in muscle and liver were related to fish size and sex, and to periods of the hydrological cycle.

MATERIAL AND METHODS

Study area

The study was carried out in the Machado River (also known as Ji-Paraná River). The Machado River Basin covers 75,400 km^2 in the state of Rondônia, Brazil (Figure 1). The river is formed by the Pimenta Bueno and Comemoração rivers (Krusche *et al.* 2005) and runs through the states of

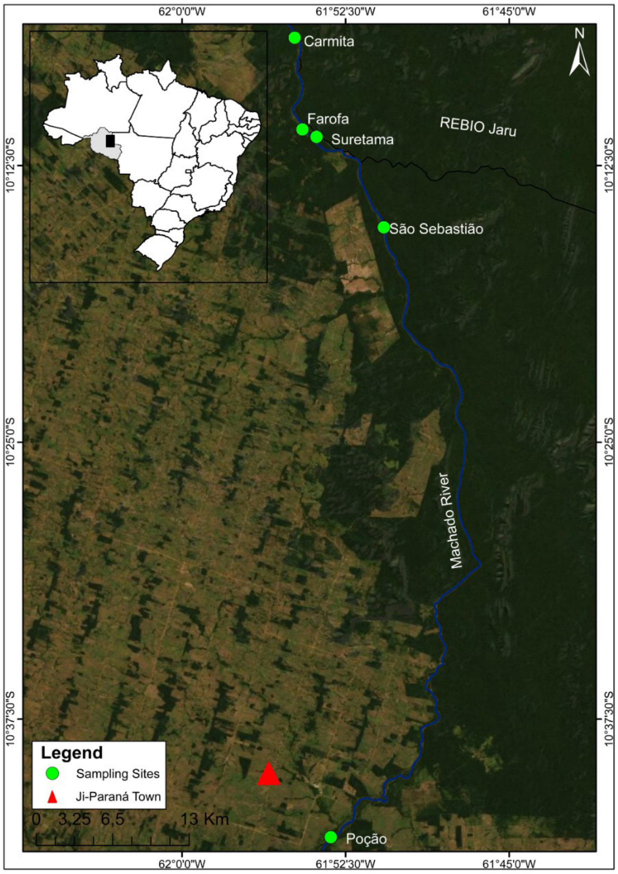

Figure 1. Location of the study area in Rondônia state, northwestern Brazil and sampled stretch of the Machado River showing the sampling sites (see details in Figure S1). This figure is in color in the electronic version.

Rondônia and Amazonas, Brazil. The hydrological regime is characterized by a flood peak in March and minimum ebb level in September (ANA 2020). It runs through the Jaru Biological Reserve (ReBio Jaru, Figure 1), which has a total area of 47,733 km^2 (ICMBIO 2010), with a conserved riparian zone covered by mostly open ombrophilous forest with low floristic variation (IBGE 1992). The preserved area along ReBio Jaru has a riverine zone constituted of shrubby and woody angiosperms (Supplementary Material, Figure S1a,b) that compose the primary forest (Supplementary Material, Figure S1c), which provides reproduction and feeding ground for fish and where fishing is prohibited (ICMBIO 2010). The unpreserved area outside ReBio Jaru is composed of pasture, with a riverine zone on the right bank composed of grasses, and a narrow strip with a few woody angiosperms and stretches of bushes on the left bank (Supplementary Material, Figure S1f). In this area, there is fishing activity (artisanal and recreational and constant sand dredging (Supplementary Material, Figure S1d,e).

Fish sampling and biometric data

Samplings were carried out bimonthly from June 2013 to April 2015 simultaneously at five sites along the Machado River. Three sites were located in ReBio Jaru (Carmita, Farofa and Suretama) and two sites (São Sebastião and Poção) outside ReBio Jaru (Figure 1). A total of 12 samples were taken (four samples in 2013, six in 2014 and two in 2015. Of these two samplings occurred during the rising water season, four during high water, two during subsiding water and four during low water. The hydrological periods were categorized based on ANA (2020). Average river depth is 11.4 m in high water season and 6.5 m in the low water season. Poção and São Sebastião are located, respectively, 50 and 5 km upstream of ReBio Jaru, while Carmita, Farofa and Suretama are at approximately 4 km from each other, making a total sampling distance of 90 km. At each site, eight sets of gillnets (2 x 20 m with mesh sizes varying from 30 to 100 mm) were used. The sampling effort was standardized, and scientific capture was carried out for 24 hours continuously at each sampling site. The living specimens found at the end of the 24-hour period were sacrificed in a solution of clove oil (Eugenol, two drops per liter; according to the American Veterinary Medical Association 2001). One specimen was fixed in 10% formalin, subsequently preserved in 70% ethanol and deposited in the ichthyology collection at Rondônia Federal University (voucher nr. UFRO-ICT 023107).

For each specimen captured, the standard length (SL) (in cm) was measured using an ichthyometer with 0.1 cm accuracy, and total wet weight (TW) (in g) was obtained with a digital scale with 0.01 g accuracy. The sex was determined through macroscopic gonad inspection (Vazzoler 1996; Nuñez and Duponchelle 2009), only adult individuals were analyzed. Samplings was authorized by Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio (SISBIO licenses nr. 47345-1 and 40663-2).

Chemical extraction for mercury quantification

All samples were transported on ice to the Environmental Biogeochemistry Laboratory at Universidade Federal de Rondônia (UNIR), where they were catalogued and stored in freezers until analysis. Total mercury was extracted according to Bastos *et al.* (1998). About 200 mg of muscle and liver tissue (ww) of each specimen were weighed separately in glass tubes and inserted into a block digester (Tecnal, TE-040/25) for 30 min at 70 ºC using 1.0 mL of H_2O_2 (Merck) and 4.0 mL of H_2SO_4:$HN0_3$ (1:1, Merck). Thereafter, 6.0 mL of $KMnO_4$ (5%, Merck) were added to each sample, which were than inserted into the bock digester at 70º·C for a further 20 min. After cooling the samples, droplets of $HONH_2$.HCl (12%, Merck) were added and the final volume was completed to 15 ml with ultra-pure water in Falcon tubes, where total Hg was measured by cold vapor atomic absorption spectrophotometry (CV-AAS, PerkinElmer FIMS-400 flow injection mercury system, Germany). All measurements were performed in triplicate and analyzed in parallel with internationally certified material (DORM-2, NRC-Canada) to ensure satisfactory quality control. The THg data were expressed relative to wet weight. Recovery rates were 102 ± 2% and detection and quantification limits were 0.018 mg kg^{-1} and 0.054 mg kg^{-1}, respectively.

Data analysis

The Shapiro-Wilks and the Levene test were used to analyze the normality and homoscedasticity of the data and determine the application of parametric tests (ANOVA and t-test) (Zar 1996). Length and weight were compared between males and females using t-tests. Mercury concentration in muscle and liver tissue was compared between males and females, and between hydrological periods of the Machado River using two-way ANOVA. The relation between mercury concentration in muscle and liver and the length (cm) and weight (g) of the *P. squamosissimus* individuals was evaluated with linear regression. All statistical analyses were performed in R version 3.3.3. (R Core Team 2017). In all analyses, results were considered significant at $p \leq 0.05$.

RESULTS

A total of 65 *P. squamosissimus* individuals ($\bar{X}_{SL}$ = 55.0 ± 8.3, SL_{Min} = 16.5 cm, SL_{Max} = 68.5 cm; $\bar{X}_{tW}$ = 2626 ± 879 g, TW_{Min} = 85 g, TW_{Max} = 4200 g) were used for THg measurement in muscle tissue and 55 for liver tissue. Some liver samples were lost due to logistical problems. The average concentration of THg was 1.09 ± 0.72 mg kg^{-1} in muscle and 1.28 ± 1.23 mg kg^{-1} in liver.

Average length and weight of the males was 47.5 ± 7.3 cm (SL_{Min} = 16.5 cm, SL_{Max} = 68.5 cm) and 2618 ± 893 g (TW_{Min} = 85 g, TW_{Max} = 4200 g), respectively. For females, average length was 49.0 ± 5.0 cm (SL_{Min} = 38.0 cm, SL_{Max} = 55.0 cm), and weight was 2658 ± 856 g (TW_{Min} = 1200 g, TW_{Max} = 4135 g). No significant differences occurred in length (t = 1.77; df = 1; p = 0.10), and weight (t = 1.27; df = 1; p = 0.22) between males and females, as well as in THg concentration in muscle and liver (ANOVA, F = 0.48; df = 3; p = 0.48) (Figure 2).

Ther was a positive and significant regression of THg in muscle (r^2 = 0.37; p = 0.008; n = 64) (Figure 3a) and liver (r^2 = 0.18; p = 0.04; n = 55) (Figure 3c) on total length, and also of THg in muscle (r^2 = 0.29; p = 0.04; n = 64) (Figure 3b) and liver (r^2 = 0.33; p = 0.04; n = 54) (Figure 3d) on total weight.

The regression THg concentration in muscle and liver was positive and significant (r = 0.83; p < 0.0001; n = 52) (Figure 4). The THg concentration in muscle and liver did not vary significantly between the periods of the hydrological cycle (ANOVA, F = 1.43; df = 3; p = 0.23) (Table 1).

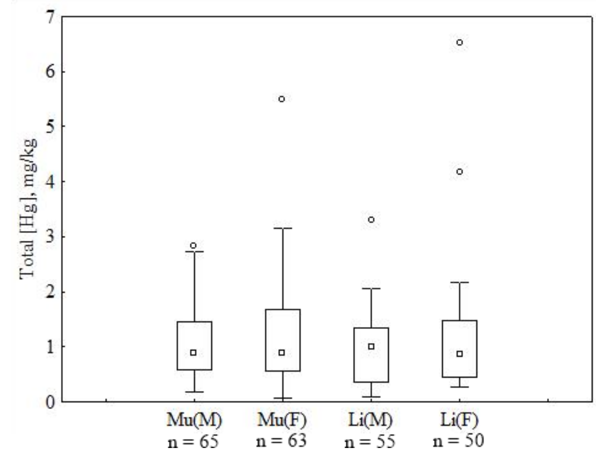

Figure 2. THg concentration in muscle (Mu) and liver (Li) of *Plagioscion squamosissimus* males (M) and females (F) sampled in the Machado River (Brazilian Amazon). The square indicates the median, the box the 25 and 75 percentiles, the bars the range, and the circles represent data points considered to be outliers.

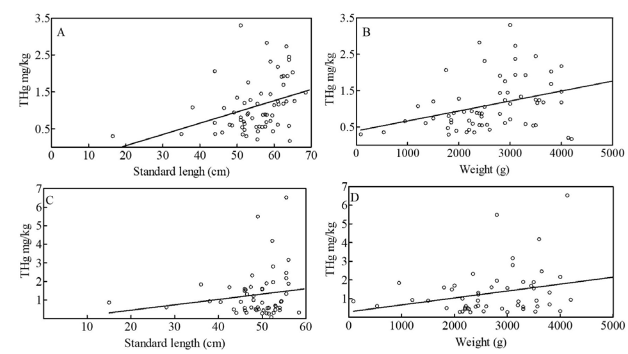

Figure 3. Linear regression of total Hg concentration in muscle (A, B) and liver (C, D) on standard length (A, C) and weight (B, D) of *Plagioscion squamosissimus* sampled in the Machado River (Brazilian Amazon).

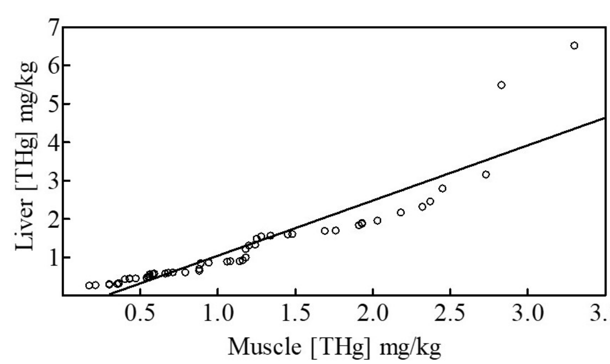

Figure 4. Regression analysis of total Hg concentration in muscle on total Hg concentration in liver in 52 individuals of *Plagioscion squamosissimus* sampled in the Machado River (Brazilian Amazon).

DISCUSSION

Average THg concentration in muscle and liver of *P. squamosissimus* was above the limit of 0.50 mg.kg^{-1} established for human consumption (WHO 2019) for both sexes and throughout the hydrological cycle. Similar results were obtained in other studies in the Amazon region (e.g. Bastos *et al*. 2008; Sampaio da Silva *et al*. 2013; Azevedo-Silva 2016), yet our sample size (n = 65) was more representative than the average sample size in the mentioned studies (30.4 ± 29.8). The mercury concentrations found in our study were higher than in other studies carried out in the Amazon basin (Table 2).

As in the studies of Bastos *et al*. (2015, 2016) and Pecoraro *et al*. (2019), there were no significant differences in the THg concentration of males and females in our study. THg accumulation in fish depends on the trophic level of the focal species (Romanuk *et al*. 2011). The trophic level of *P. squamosissimus* is between 3.4 and 3.6 (Costa and Angelini 2020). There are indications of the existence of only small-scale artisanal mining of gold and cassiterite outside the Jaru Biological Reserve (J.P.O. Gomes, JBR management, pers. comm.). Thus, it is likely that the Hg found in the fish is mainly of natural origin, i.e., from the Hg naturally present in Amazonian soils (Bastos *et al*. 2007; Rua-Ibarz *et al*. 2019), which reaches the rivers through leaching from the soil in deforested areas.

The measurement of THg concentration in body tissues provides important information for biological monitoring programs, but the analyses are expensive and laborious. Muscle is usually the target tissue to detect accumulation of methylmercury (MeHg) and the reservoir of highly toxic mercury species, but is not a good tissue to measure deposition of inorganic Hg (iHg) (Régine *et al*. 2006). Muscle tissue has a slower response to changes in Hg concentrations in the environment since it reflects the accumulation of Hg during a long exposure period, making it less sensitive to short-term changes of environmental Hg levels (Xu *et al*. 2015). In

Table 1. Biometrical data and total mercury (THg) concentration in muscle and liver of *Plagioscion squamosissimus* in different periods of the hydrological cycle in the Machado River, Rondônia state, Brazil. Values are the mean ± standard deviation followed by the range (in parentheses).

Hydrological period	N	Standard length (cm)	Total weight (kg)	N	THg muscle (mg kg^{-1})	N	THg liver (mg kg^{-1})
Rising water	34	49.3 ± 0.9 (48.7 - 50.0)	2.41 ± 0.12 (2.27 - 2.50)	19	1.32 ± 0.72 (0.09 - 2.73)	15	1.78 ± 1.55 (0.07 - 5.48)
High water	27	45.7 ± 5.4 (36.0 - 55.5)	2.06 ± 0.79 (0.95 - 0.41)	17	0.67 ± 0.40 (0.20 – 1.20)	10	2.03 ± 2.25 (0.44 - 6.52)
Subsiding water	19	46.1 ± 8.9 (15.2 - 54.0)	2.57 ± 0.89 (0.08 - 4.20)	9	0.69 ± 0.31 (0.35 - 1.13)	10	0.41 ± 0.12 (0.27 - 0.60)
Low water	40	50.1 ± 3.9 (41.1 - 55.5)	2 .90 ± 0.64 (1.80 - 4.00)	20	0.87 ± 0.52 (0.17 - 1.92)	20	1.17 ± 0.69 (0.30 - 2.45)
Overall	120	52.1 ± 8.4 (15.2 – 55.5)	2.66 ± 0.87 (0.08 - 4.20)	65	1.09 ± 0.71 (0.09 – 2.73)	55	1.27 ± 1.24 (0.07 - 6.52)

Table 2. Data on mercury concentration in muscle (relative to wet weight) of *Plagioscion squamosissimus* sampled in several rivers of the Amazon basin over 14 years. N = sample size (values in parentheses indicate number of males/females, if available). Length (standard length) and weight values are the median (minimum - maximum). Hydrological period: R = rising water; H = high water; S = subsiding water; L = low water. Biometrical and mercury values are the mean followed by the range in parentheses, when available. ND = not determined.

River	N (M/F)	Length (cm)	Weight (kg)	Hydrological period	THg (mg kg^{-1})	Reference
Machado	65 (51/14)	57.0 (16.5 - 68.5)	2.62 (0.08 - 4.20)	R, H, S, L	1.09 (0.09 - 3.30)	this study
Madeira	2	ND	ND	R, H, S, L	0.62 (0.15 - 1.10)	Oliveira *et al*. (2010)
Madeira	5	29.6 (27.0 - 33.0)	ND	ND	0.41 (ND)	Azevedo-Silva (2016)
Madeira	72	180.0 (ND)	ND	R, H, S, L	0.24 (0.01 - 1.32)	Bastos *et al*. (2015)
Madeira	ND	ND	ND	ND	>0.50	Bastos *et al*. (2006)
Madeira	81 (39/42)	ND	ND	R, H, S, L	<0.50	Bastos *et al*. (2016)
Madeira and Jamari	41	ND	ND	R, H, S, L	0.44 (0.00 - 1.10)	Bastos *et al*. (2008)
Madeira and Roosevelt	2	48.5 (ND)	2.32 (ND)	ND	<0.50	Anjos *et al*. (2016)
Manacapuru Lake	12	ND	ND	R, H, S, L	0.54 (0.17 - 0.97)	Beltran-Pedreros *et al*. (2011)
Tapajós	50	ND	ND	R, S	0.59 (ND)	Sampaio da Silva *et al*. (2009)
Purus	3	ND	ND	L	0.61 (0.31 - 0.86)	Castro *et al*. (2016)
Madeira	41	ND	ND	S	0.33 (ND)	Sousa *et al*. (2015)
Tapajós	69	(12.1 - 40.0)	ND	R, L	0.57 (0.13 - 2.94)	Da Silva *et al*. (2013)
Cassiporé	14	40.0 (ND)	0.84 (ND)	H, L	0.50 (ND)	Lima *et al*. (2015)
Beni	4	ND	ND	ND	0.66 (ND)	Rivera *et al*. (2016)
Amazon Region	ND	ND	ND	ND	1.10 (ND)	Bittarello *et al*. (2019)
Amazon and Tapajós	ND	ND	ND	ND	1.81 (ND)	Bourdineaud *et al*. (2015)

contrast, liver tissue is highly responsive to exposure to iHg and is the best target tissue for detection of short-term changes in contamination, because the liver is able to accumulate higher Hg concentrations than other tissues (Rua-Ibarz *et al*. 2019). The liver has an important function in the process of detoxification and metabolism of Hg, and is one of the main organs responsible for demethylation of MeHg and subsequent redistribution of the two main forms of Hg (Rua-Ibarz *et al*. 2019). Hence, the iHg in the liver can originate from direct capture by the organism and/or demethylation of MeHg (Rua-Ibarz *et al*. 2019). Changes in Hg exposure are generally reflected in the concentration in liver tissue before other organs and tissues, such as muscle (Xu *et al*. 2015).

The Hg concentration in our study showed no seasonal variation. The hydrological cycle and levels of anthropic activity in the basin can affect the transport and availability of Hg in aquatic environments (Squadrone *et al*. 2013). The increased rainfall during the rising water and high water periods can be an additional factor for the increase of Hg in the region, since this trace element can be released into the atmosphere and fall back to earth in rainwater, reaching various areas of the basin (Veiga and Hinton 2002). Rain can therefore contribute to the remobilization of Hg in the substrate of aquatic habitats (Moreno-Brush *et al.* 2016), increasing its availability in the environment.

The hydrological cycle of the rivers also influences the dynamics of gold mining in the Amazon basin, because the gold is alluvial. Low water level makes gold mining more difficult, so miners tend to interrupt their activity in the low water season, thus reducing the discharge of Hg and exposure to fish (Soares *et al*. 2018). No significant differences in the concentration of Hg in carnivorous fish species (*Serrasalmus* spp, *Hoplias malabaricus* (Bloch 1794) and *Cichla* spp) were found between the low water and high water seasons in the Negro River (Dorea *et al*. 2006), nor in carnivorous/piscivorous species (*Serrasalmus* spp, *Pinirampus pirinampu* (Spix & Agassiz, 1829) and *Cichla* spp) in the Madeira River (Bastos *et al*. 2007). In places that have high Hg levels in the soil and high potential for methylation and deterioration of biomass, as found in the Amazon region, the interactions between species occur in all periods of the hydrological cycle, but do not alter the general state of bioaccumulation of Hg by fish (Bastos *et al*. 2007).

The complex trophic interactions (trophic plasticity, feeding selectivity, amplitude and overlap of food niches) among species, as well as their variability in Hg bioaccumulation patterns in high and low water periods (Bastos *et al*. 2007), can explain the absence of significant differences in THg concentrations between the periods of the hydrological cycle in our study. In the rising water and high water seasons, when average THg concentrations were also above the recommended limit (WHO 2019), spawning activity occurs in *P. squamosissimus*. The exposure of fish to Hg can compromise the reproduction, growth and immunity (Graves *et al*. 2017). Embryos and fingerlings exposed to Hg can develop abnormalities, changes in behavior and delayed development, reducing the chances of survival (Weis 2009).

The THg concentrations in most of the *P. squamosissimus* specimens were above the level allowed for the consumption of meat from predatory fish by humans by Brazilian legislation (1.0 mg kg^{-1}) (Brasil 1998) and above the limit established for fish consumption by the WHO (0.50 mg kg^{-1}) (WHO 2019). Mercury intoxication can cause various disturbances in humans, especially in vulnerable groups such as babies, children and pregnant women (Fuentes-Gandara *et al*. 2018). Mercury can affect humans at different biological levels, causing considerable damage to the central nervous system and kidneys (Beckers and Rinklebe 2017). *Plagioscion squamosissimus* is widely consumed by the urban and rural population of the central region of Rondônia state, with relevant economic importance in regional trade. Therefore, it is necessary to monitor the Hg levels in this fish species on a regular basis.

CONCLUSIONS

The average mercury concentrations in the muscle and liver tissues of *Plagioscion squamosissimus* sampled in the Machado River were above the limits established by the WHO for human consumption. Since muscle tissue is the main part consumed, the results of this study are of great interest for analysis of regional food safety, mainly with regard to the different chemical species of Hg. However, we emphasize that no significant differences were found in the concentration of mercury between muscle and liver, between sexes and among the annual hydrological periods. We highlight that elemental analyses are necessary, involving species and isotopes of Hg (1) in different fish species in the watershed, (2) in the same species in different places, (3) at different trophic levels, and particularly (4) in different tissues of the same species, to configure a robust approach to understand this important threat to the enviornment and human health.

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SUPPLEMENTARY MATERIAL (only available in the electronic version)

Costa *et al*. Mercury in muscle and liver of *Plagioscion squamosissimus* (Acanthuriformes: Sciaenidae) from the Machado River, Brazilian Amazon

Figure S1. Sampling sites in the Machado River, Rondônia state, Brazil: A – Carmita; B – Farofa; C – Suretama (preserved area); D – São Sebastião, sand dredging activity; E and F – (S1F) Poção, pasture, few woody angiosperms and stretches of bushes, degraded local banks (unpreserved area).