



Variation in Hg accumulation between demersal and pelagic fish from Puruzinho Lake, Brazilian Amazon

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Accepted: 1 October 2019 / Published online: 16 October 2019
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Abstract

Aquatic ecosystems in the Amazon are exposed to mercury, mostly from natural sources. Hg accumulation in fish tissues poses a risk to the local population since fish is one of the main sources of protein in the region. The aim of this study was to evaluate Hg distribution in demersal and pelagic carnivorous fish between seasons in Puruzinho Lake in the Brazilian Amazon. Total Hg was quantified in 221 individuals of 8 species obtained during the high water and low water seasons. Two-way ANOVA indicated an interaction between foraging habitat and season. During high water, total Hg concentrations were similar between demersal and pelagic fish, while in low water, total Hg levels were higher in demersal fish. Pelagic and demersal fishes' Hg levels were similar between the two seasons.

Keywords Mercury · Feeding habits · Amazon · Demersal · Pelagic

Introduction

Mercury (Hg) is a toxic metal released in the environment by anthropogenic sources (e.g., fossil fuel and coal combustion) and natural sources (e.g., atmospheric deposition, weathering). Although anthropogenic emission of Hg in the Brazilian Amazon has been as high as 2000 metric tons due to gold mining (Malm 1998), the study of Roulet et al. (1998) confirmed that natural sources of Hg are more important because Amazonian soils are enriched with this toxic metal on a geological scale.

Human exposure to Hg occurs mainly through food intake, especially of fish. Most of the Hg accumulated in the muscle of fish is in its most toxic form, methylmercury, which increases the risk to humans and biota in general (Hong et al. 2012). Due to its slow excretion rate and high affinity for thiol groups of proteins in fish muscle,

methylmercury represents more than 85% of accumulated Hg species (Bloom 1992; Redmayne et al. 2000; Kehrig et al. 2008; Martorell et al. 2011; Burger et al. 2013; Paiva et al. 2017). Freshwater fish are one of the most important sources of animal protein in the Amazon (Freitas and Rivas 2006). For instance, fish consumption by the Puruzinho Lake riparian community (Madeira River Basin) is 406 g per capita day⁻¹ (Oliveira et al. 2010), which is one order of magnitude higher than the national average (11 g per capita day⁻¹) (Avegliano et al. 2015). The freshwater fish catch in the Amazon is the highest in the country, with an average of 280,000 tons per year (MPA 2009).

Hg accumulation in fish is influenced by several factors, like water pH, dissolved organic carbon and primary productivity (Sorensen et al. 2005; Brown et al. 2010; Poste et al. 2015). Vieira et al. (2018) reported that in Amazonian black water ecosystems, which have acid pH and high concentration of dissolved organic matter, methylmercury levels are higher in biotic and abiotic matrices in comparison with other aquatic ecosystems due to higher methylation rates. Several studies have indicated that higher levels of methylation of inorganic Hg are related to acidic environments (Ramlal et al. 1986; Bloom et al. 1991; Gilmour and Henry 1991; Spry and Wiener 1991; Ullrich et al. 2001). In contrast, high primary productivity has been related to low concentrations of Hg in aquatic biota due to algal biodilution, a process well described by other authors

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(Chen and Folt 2005; Pickhardt et al. 2005; Chen et al. 2008). The foraging habitat (demersal or pelagic) also plays a role in Hg accumulation (Eagles-Smith et al. 2008). Li and Xie (2016) and Jiang et al. (2018) reported that total Hg concentrations were higher in demersal than in pelagic fish. However, Rocha et al. (2015) observed similar Hg concentrations between the two foraging habitats while Lavoie et al. (2010) reported a stronger biomagnification in the pelagic food chain. From a human health safety standpoint, understanding the influence of foraging habitat on Hg levels is also important because commercial and subsistence fishing may focus on a particular food chain. For instance, commercial fishing in Western Amazonia is focused on the capture of demersal catfishes like *Brachyplatystoma vaillantii* and *Pseudoplatystoma filamentosum* (Freitas and Rivas 2006).

Fish capture in the Amazon is highly influenced by seasonality. During the high water season (HW), the fish catch is higher in comparison with the low water season (LW) (Freitas et al. 2002). Additionally, consumption of piscivorous fish is higher during the high water season among the riparian population from the upper Madeira River Basin (Maurice-Bourgoin et al. 2000). Individuals from the Amazon region that have a lower intake of fish during the low water season showed lower Hg concentrations in hair (Maurice-Bourgoin et al. 2000). There is evidence that seasonal variation in Hg levels can influence Hg accumulation in humans.

Understanding how Hg levels in fish fluctuate among seasons and foraging habitats in a region where the human population has high fish consumption is crucial. In this context, the aim of this study was to evaluate Hg distribution between pelagic and demersal carnivorous fish in two seasons (high water and low water) from an Amazonian lake.

Material and methods

The study area is Puruzinho Lake (63°6'0"W; 7°24'0"S), located 20 km from the urban region of the municipality of Humaitá, in the Madeira River Basin of Western Amazonia (Amazonas state, Brazil). Puruzinho Lake is a black water lake, which means that it is rich in dissolved organic matter and has acid pH, low primary productivity, and low concentrations of both dissolved elements and suspended particulate matter (Wissmar et al. 1981; Almeida et al. 2014). This lake is heavily influenced by the hydrological regime. During the high water (HW) season, the water column can reach 12.5 m, but in the low water (LW) season this value can drop to 0.30 m (Almeida et al. 2014). The following parameters also vary between seasons: pH (HW: 4.97 ± 0.23; LW: 5.48 ± 0.32); dissolved oxygen (HW: 3.40 ±

0.31 mg L⁻¹; LW: 5.42 ± 0.49 mg L⁻¹); water temperature (HW: 29.80 ± 1.30 °C; LW: 30.90 ± 1.60 °C); and electrical conductivity (HW: 8.36 ± 0.68 mS cm⁻¹; LW: 14.27 ± 0.63 mS cm⁻¹) (Almeida et al. 2014). Total Hg concentrations and organic matter content of bottom sediment in HW, reported by Almeida et al. (2014), were 84.20 ng g⁻¹ dry wt and 7.88%, respectively, while during LW the reported results were 71.20 ng g⁻¹ dry wt and 8.40%.

Sampling campaigns in the HW season occurred in December 2016, February 2017 and April 2017, while in the LW season they occurred in June 2017 and October 2017. Samples were obtained with help of local fisherman. Since the aim of the study was to evaluate the influence of foraging habitats, we considered only species with the same feeding habit. The eight sampled species are carnivorous. Demersal species obtained were *Plasgioscion squamosissimus*, *Calophysus macropterus*, *Cichla pleiozona* and *Hoplias malabaricus*, while pelagic species obtained were *Ageneiosus inermis*, *Pellona flavipinnis*, *Pellona castelnaeana* and *Rhaphiodon vulpinus* (Table 1). Information about foraging habitat was retrieved from Froese and Pauly (2019). A total of 221 ($N = 221$) specimens were caught. Total length (TL) and weight (TW) of specimens were measured after capture. Skinless white dorsal muscle was removed, frozen and transported to the laboratory. Although other tissues like gills, liver and kidney accumulate Hg (Mella et al. 2007; Azevedo et al. 2018), we did not consider these tissues. Keva et al. (2017) reported that, although liver has a higher turnover rate than muscle due to intense metabolic activity, THg concentration in both tissues varies significantly between seasons. Therefore, including liver in our analysis, or other tissues with fast turnover rates like kidney, would be redundant since muscle and liver vary similarly.

We do not measure Puruzinho Lake water level, however the Madeira River flow and water level, measured at the Humaitá station, were available from ANA (2019). As expected, both parameters showed higher values in the HW season (December 2016, February and April 2017) than in LW (June and October 2017). The summarized data about Madeira River flow and water level during December 2016 to October 2017 are presented in Azevedo et al. (2019).

Digestion of aliquots (0.2 g wet wt) of fish muscle samples for total Hg (THg) determination followed the method described by Bastos et al. (1998): (i) addition of 1 mL of 30% H₂O₂ and 4 mL of 65% HNO₃: 98% H₂SO₄ (1:1); (ii) heating in a Tecnal digestion block (model: TE04/25) for 30 min at 70 °C; (iii) cooling at room temperature; (iv) addition of 5% KMnO₄; heating for 15 min; (v) cooling at room temperature overnight; (vi) addition of 12% NH₂OH.HCl; and (vii) addition of ultrapure water to a final volume of 12 mL. Measurements were performed by cold vapor atomic absorption spectrometry (CVAAS),

Table 1 Non-normalized and length normalized THg concentration (mg kg^{-1} wet wt) in demersal and pelagic fish from Puruzinho Lake

| | Demersal fish | | | |
|----------------|------------------------------------|-------------------------------|-----------------------------|----------------------------|
| | <i>Plasgioscion squamosissimus</i> | <i>Calophysus macropterus</i> | <i>Cichla pleiozona</i> | <i>Hoplias malabaricus</i> |
| High water | | | | |
| Non-Normalized | 0.86 ± 0.34 | 0.87 ± 0.22 | 0.94 ± 0.43 | 0.82 ± 0.16 |
| Normalized | 0.026 ± 0.010 | 0.032 ± 0.008 | 0.035 ± 0.016 | 0.020 ± 0.005 |
| Low water | | | | |
| Non-Normalized | 0.89 ± 0.001 | 1.95 ± 0.86 | 0.96 ± 0.40 | 1.10 ± 0.72 |
| Normalized | 0.033 ± 0.0001 | 0.067 ± 0.033 | 0.033 ± 0.014 | 0.029 ± 0.019 |
| | Pelagic fish | | | |
| | <i>Ageneiosus inermis</i> | <i>Pellona flavipinnis</i> | <i>Pellona castelnaeana</i> | <i>Rhaphiodon vulpinus</i> |
| High water | | | | |
| Non-Normalized | 0.97 ± 0.001 | 1.17 ± 0.45 | 0.80 ± 0.32 | 0.78 ± 0.30 |
| Normalized | 0.027 ± 0.0001 | 0.047 ± 0.018 | 0.032 ± 0.014 | 0.020 ± 0.009 |
| Low water | | | | |
| Non-Normalized | 0.31 | – | 0.58 ± 0.21 | 1.00 ± 0.30 |
| Normalized | 0.011 | – | 0.021 ± 0.010 | 0.021 ± 0.008 |

using a flow injection mercury system (FIMS-400) from PerkinElmer (Germany). Blanks were used to control the quality of the reaction medium. Samples were analyzed in duplicate to evaluate the precision of the method. Certified material (DORM-2) was analyzed in triplicate at each 30 samples (recuperation: $99 \pm 2.5\%$). Detection limit was 0.007 mg kg^{-1} .

Statistical analyses were performed using the R environment (R Core Team 2018). To compare THg concentrations in pelagic and demersal fish between HW and LW, we conducted a two-way ANOVA. In the first step of this statistical analysis, the interaction term was evaluated. The interaction term, when significant ($p < 0.05$), indicates that seasonality influences Hg concentrations in fish of different feeding habits differently (e.g., during the transition from LW to HW period, Hg concentrations increase in demersal fish while decreasing in pelagic fish). In this situation, the Tukey post hoc test was performed to compare each combination of seasonality and feeding habits, since a comparison of each of the variables (seasonality and feeding habits) cannot be done individually. Data were transformed to square root to meet ANOVA requirements (linearity, normality and homoscedasticity of the residuals). We normalized concentrations by length, as suggested by Scuder-Eikenberry et al. (2015), to remove the influence of size, following the formula: $\text{THg normalized} = \text{THg (concentration/total length)}$. Total length is a better proxy for fish age than weight (Yi and Zhang 2012; Perugini et al. 2014). Therefore, by normalizing data by length the

influence of age is also removed. The comparisons were carried out with the normalized data.

Results and discussion

In general, demersal fish were bigger and heavier than pelagic fish (Table 2). The two-way ANOVA indicated interaction between seasons and the foraging habitat (interaction season \times foraging habitat: $F_{1,216} = 9.61$; $p = 0.002$). This result suggests that seasonality influences THg_{Norm} accumulation differently between demersal and pelagic fish. THg_{Norm} concentration in demersal and pelagic fish was statistically similar between LW and HW (Tukey post hoc; Demersal: Effect size = 0.01, $p = 0.22$; Pelagic: Effect size = -0.02 , $p = 0.07$) (Fig. 1). The comparison between demersal and pelagic fish showed that in HW season, THg_{Norm} concentration was similar (Tukey post hoc; Effect size = -0.003 , $p = 0.97$) while during LW season, THg_{Norm} was significantly higher in demersal fish (Tukey post hoc; Effect size = -0.04 , $p < 0.0001$) (Fig. 1).

Our results disagree with those of Azevedo et al. (2018), who observed higher concentration of THg_{Norm} in fish from the lower Paraiba do Sul River Basin (southeastern Brazil) during a prolonged low water season. However, the hydrological regime in the Amazonian region is drastically different from the one found in southeastern Brazil. Additionally, the increased concentrations reported by Azevedo et al. (2018) were observed during an atypical low water

Table 2 Number of samples (*N*) and mean \pm SD of total length and total weight of demersal and pelagic in both seasons

| | Total Length (cm) | | Total weight (g) | | <i>N</i> | |
|------------------------------------|-------------------|-----------------|--------------------|-------------------|----------|----|
| | HW | LW | HW | LW | HW | LW |
| Demersal | | | | | | |
| <i>Plasgioscion squamosissimus</i> | 33.0 \pm 4.5 | 27 | 690.0 \pm 320.4 | 400 | 26 | 2 |
| <i>Calophysus macropterus</i> | 26.7 \pm 3.1 | 29.5 \pm 2.1 | 391.0 \pm 135.3 | 275.0 \pm 68.9 | 10 | 6 |
| <i>Cichla pleiozona</i> | 26.5 \pm 3.1 | 28.9 \pm 3.5 | 386.8 \pm 114.4 | 498.6 \pm 305.2 | 25 | 61 |
| <i>Hoplias malabaricus</i> | 40.0 \pm 3.1 | 35.0 \pm 7.4 | 1133.3 \pm 294.3 | 700.0 \pm 509.1 | 6 | 7 |
| Pelagic | | | | | | |
| <i>Ageneiosus inermis</i> | 35 | 27 | 700 | 200 | 2 | 1 |
| <i>Pellona flavipinnis</i> | 24.5 \pm 0.7 | – | 176.5 \pm 54.4 | – | 4 | – |
| <i>Pellona castelnaeana</i> | 24.5 \pm 3.0 | 28.6 \pm 3.6 | 232.5 \pm 72.1 | 370 \pm 120.41 | 23 | 6 |
| <i>Rhaphiodon vulpinus</i> | 40.2 \pm 5.1 | 54.0 \pm 39.5 | 474.0 \pm 163.6 | 586.5 \pm 426.0 | 15 | 27 |

HW high water, LW low water

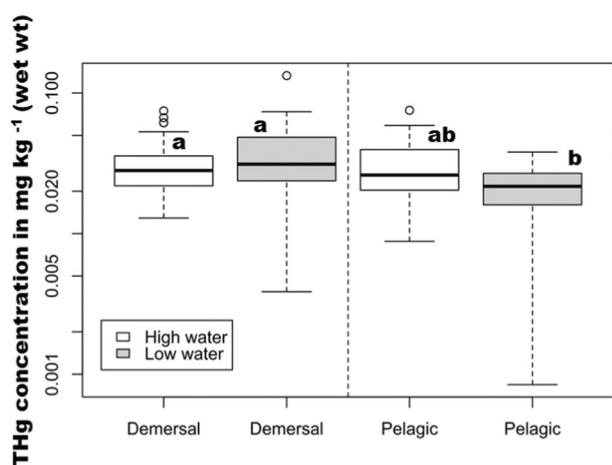


Fig. 1 Comparison of THg_{Norm} concentrations among different demersal and pelagic fish during high water and low water seasons. Different letters indicate statistical differences ($\alpha = 0.05$). Two-way ANOVA indicated an interaction between season and foraging habitat (interaction season \times foraging habitat: $F_{1,216} = 9.61$; $p = 0.002$). There were no statistical differences in THg_{Norm} in demersal and pelagic fish between seasons. THg_{Norm} concentrations were higher in demersal fish compared to Pelagic fish only during low water ($p < 0.0001$)

season. In the Amazon region, Marshall et al. (2016) observed higher THg concentration in *Paracheirodon axelrodi* (Characidae) collected during LW season in the Negro River and Amaro et al. (2014) also observed higher concentration in *Brachyplatystoma rousseauxii* (Pimelodidae) from a fish market in Belem (Pará state) during LW. Although our results differed from these ones, other authors like Brito et al. (2017) reported that Hg concentration in zooplankton did not vary significantly along the seasons in Janaucá Lake, Solimões River, Amazon.

Seasonal variation of fish Hg concentration can be considered a controversial issue, since many authors have reported the occurrence of this process (Farkas et al. 2003; Murphy et al. 2007; Zhang et al. 2012; Moreno et al. 2015; Azevedo et al. 2018) and others have observed no seasonal

variation (Farkas et al. 2000; Foster et al. 2000; Burger et al. 2009). Mills et al. (2018) reported in a meta-analysis that seasonal variation in Hg concentration in fish is not related to waterbody type (e.g., lakes, rivers, reservoirs, etc.), fish size and trophic status. These authors observed that seasonal variation occurs when mean Hg concentration of the fish population increases. In 90% of the studies evaluated by Mills et al. (2018), significant seasonal variation was observed when the mean Hg concentration of the population exceeded 0.30 mg kg^{-1} (wet weight, non-normalized by fish size). The mean Hg concentrations of the demersal and pelagic fish populations in this study were 0.97 and 0.87 mg kg^{-1} (wet weight, non-normalized by fish size), respectively, which is almost 3 times higher than the mean 0.30 mg kg^{-1} suggested by Mills et al. (2018) as an indication of significant seasonal variation. Therefore, our results disagree with their meta-analysis.

It is important to mention that non-significant seasonal variation was observed at the guild level (i.e., pelagic carnivorous and demersal carnivorous). Species were pooled into guilds due to their similar feeding habitat and to increase representativeness. At the species level, only *Cichla pleiozona* (demersal) and *Rhaphiodon vulpinus* (pelagic) had enough samples in both seasons to test for seasonal variation. The results showed, for both species, non-significant variation (*Cichla pleiozona*: Tukey post hoc; Effect size = -0.003 , $p = 0.69$; *Rhaphiodon vulpinus*: Tukey post hoc; Effect size = 0.002 , $p = 0.81$), which is consistent with the guild level results. However, more data are necessary to test if seasonal variation in THg concentration is species-specific.

The lower THg concentration in pelagic fish during the LW period may be derived from higher competition with demersal fish for food resources. Several studies have reported that competition for resources among fish is more intense during the low water season (Matthews 1998; Schlosser et al. 2000; Wantzen et al. 2002; Costa-Pereira

et al. 2017). It is well established that feeding is the main pathway of fish exposure to THg. Therefore, an increase in competition for food could reduce the uptake of THg for the weakest competitor. During the LW, pelagic fish have to compete for resources with an environmentally more fit group (i.e., demersal fish), which may explain the lower THg concentration (Fig. 1).

During HW period, when the water column reaches approximately 8–12.5 m (Almeida et al. 2014), the inflow of resources to the Puruzinho Lake is higher, which may reduce the interspecific competition pressure (Costa-Pereira et al. 2017). Under these conditions, THg concentrations between pelagic and demersal fish were statistically similar (Tukey post hoc; Effect size = -0.003 , $p = 0.97$) (Fig. 1). In contrast, during the LW, when the water column decreases to approximately 0.30–2.3 m (Almeida et al. 2014), interspecific pressure increased and THg concentrations were statistically higher (Tukey post hoc; Effect size = -0.04 , $p < 0.0001$) (Fig. 1) in demersal than in pelagic fish, which suggests that the former group, in shallow water column conditions, is more exposed to the contaminant.

Besides ecological factors, geochemical features, like water chemistry, sediment resuspension and methylation rate, may also have contributed to the observed results. In black water ecosystems, dissolved organic carbon (DOC) plays an important role in Hg mobility and speciation (Silva et al. 2009). For instance, in LW season, when there is no input of fresh organic carbon from the drainage basin, black water ecosystems create a sunlight oxidative barrier that prevents the formation of dissolved gaseous mercury (DGM), thus reducing Hg export to the atmosphere, and degrades part of the dissolved methylmercury (i.e., photodemethylation) (Silva et al. 2009). Although methylmercury photodemethylation is higher during LW season, Bisinoti et al. (2007) observed higher levels of dissolved THg and methylmercury in the Negro River Basin during the LW season. Additionally, Bisinoti et al. (2007) also observed a negative relationship between THg and methylmercury and water level, indicating that the concentrations of these contaminants are lower in HW season due to dilution. Based on our results, the increase in THg and methylmercury availability during LW influences demersal fish more than pelagic fish.

Although Hg methylation and accumulation are distinct processes, increases in the methylation rate can influence THg accumulation in fish (Gabriel et al. 2014). For instance, Maruszczak et al. (2011) attributed the low concentrations of Hg in arctic charr to low methylation rates. Higher methylation rates increase the concentration of bioavailable Hg in aquatic environments (Marvin-DiPasquale et al. 2003; Hammerschmidt and Fitzgerald 2004; Hollweg et al. 2010), which in turn influence all the links of the food chain

(Lacerda and Malm 2008). It is possible that increases in mercury methylation rates during LW season cannot explain higher levels of THg in demersal fish. Almeida et al. (2014) observed that during this season in Puruzinho Lake, pH became less acid (HW: 4.97 ± 0.23 ; LW: 5.48 ± 0.32) and dissolved oxygen levels increased (HW: $3.40 \pm 0.31 \text{ mg L}^{-1}$; LW: $5.42 \pm 0.49 \text{ mg L}^{-1}$). Therefore, the better conditions for higher methylation rate are present in HW instead of LW season.

By definition, demersal fish have close contact with bottom sediment, and Almeida et al. (2014) observed higher remobilization of THg associated with the bottom sediment during the LW season. Additionally, Mason et al. (2003) and Kalnejais et al. (2007) reported that sediment resuspension is an important source of metals in the water column. Therefore, the higher THg concentrations in demersal than in pelagic fish during LW may be related to this process.

Conclusions

Although the Amazon is considered a hotspot for Hg and several studies have evaluated the element's concentrations in fish, this is to our knowledge the first study to investigate the variation in Hg accumulation between foraging habitats. Our results indicate that the interaction between seasonality and foraging habitat can influence THg concentration in demersal and pelagic fish. During LW, demersal species showed higher levels of THg than pelagic species. In the HW season, THg concentrations were similar between the two foraging habitats. Significant seasonal variation in THg was not observed. This study provides evidence that foraging habitat is a factor able to influence Hg accumulation in carnivorous fish during the low water season.

Acknowledgements This work was supported by the CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) through CNPq/CT-Universal project (Grant no. 458977/2014-4). We are grateful to IBAMA (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis) for the fish collection license (DIFAP/IBAMA no. 091). Special thanks go to the fisherman Raimundo Nonato dos Santos ("Leleco"). This study was also financed in part by Coordenação de Aperfeiçoamento de Pessoa de Nível Superior—Brazil (CAPES)—Finance Code 001.

Compliance with ethical standards

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

Ethical approval All applicable national guidelines for the care and use of animals were followed. Fish sampling was approved by Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA) (license: DIFAP/IBAMA no. 091).

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