

Influence of the flood pulse on mercury accumulation in detritivorous, herbivorous and omnivorous fish in Brazilian Amazonia

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Abstract

Hg accumulation in fish is influenced by several factors including seasonality. In the Amazon, ecosystems are marked by strong seasonal variation in precipitation, which leads to drastic changes in the water level of lakes and rivers. The aim of this study was to evaluate Hg levels in muscle of detritivorous, herbivorous and omnivorous fish from an Amazon lake (Madeira River Basin, Amazonas, Brazil) over four seasons (rising water, high water, falling water and low water). We hypothesized that total Hg concentration varies during the seasons. The results indicate that total Hg levels in detritivorous fish were higher in rising and low water seasons while in herbivorous and omnivorous fish the total Hg concentration was higher during the rising water season. The hypothesis was supported by the results. Additionally, the study provides evidence that Hg levels in fish with different feeding habits are influenced by the flood pulse of the Amazon region.

Keywords Mercury · Amazon · Flood pulse · Detritivorous fish · Herbivorous fish · Omnivorous fish

Introduction

Mercury (Hg) is the third most dangerous contaminant according to the Agency for Toxic Substances and Disease Registry (ATSDR [2017](#page-6-0)), due to its high toxicity, widespread distribution and biomagnification potential. Several studies have demonstrated that, due to biomagnification, species that occupy higher trophic levels in food webs, like fish, have higher Hg levels in their organs in comparison with low trophic level organisms (e.g., plants and invertebrates) (Watras and Bloom [1992](#page-7-0); Signa et al. [2017\)](#page-7-0).

Hg accumulation in fish can vary geographically, as demonstrated by the meta-analysis of Lavoie et al. [\(2013](#page-7-0)). Those authors observed more intense Hg biomagnification in temperate aquatic environments in comparison with tropical ones. However, they considered only a small number of studies in the Amazon, a region known for high Hg concentrations in fish. In the Amazon, Hg has been extensively used in artisanal gold mining. Malm ([1998\)](#page-7-0) estimated that approximately 2000 tons of Hg from gold mining had been released in the Brazilian Amazon over the period from 1979 to 1994. Although anthropogenic emissions of Hg are significant, the studies of Roulet et al. [\(1988](#page-7-0)) and Roulet et al. [\(2001](#page-7-0)) showed that Amazon soils are naturally enriched with this metal. In agreement with this finding, Fadini and Jardim ([2001\)](#page-6-0) observed Hg concentration of 172 mg kg -1 in soils far from anthropogenic influence. Therefore, Amazonian soils act as reservoirs of Hg. However, during the rainy season, the transport of Hg accumulated in soils to adjacent aquatic environments is more intense due to the influence of the rain (Roulet et al. [2001](#page-7-0)), which could affect Hg accumulation in fish.

Amazonian environments are marked by strong seasonal variations in the rainfall which in turn results in alterations in water level of aquatic ecosystems (Sahoo et al. [2016](#page-7-0)). In the central Brazilian Amazon, during rising water and high water seasons, which can be considered as the rainy seasons, rainfall is more intense. In the high water season rivers

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overflow and interconnect with lakes, providing habitat and food for many fish species (Santos and Santos [2005;](#page-7-0) Melack et al. [2009;](#page-7-0) Correa et al. [2008;](#page-6-0) Queiroz et al. [2011](#page-7-0)). During the falling water and the low water seasons, which are considered as the dry seasons, rainfall reduces drastically and water level of aquatic ecosystems also reduces.

Seasonal variation in rainfall can also influence Hg bioavailability by changing Hg partition between particulate and dissolved phase. Maia et al. ([2018\)](#page-7-0) observed higher total Hg and methylmercury concentrations in the dissolved and particulate phase during the rainy seasons in white water lakes from the Amazon river basin. Dissolved Hg, especially methylmercury, is easily absorbed by fish due to its high bioavailability (Batley [1983\)](#page-6-0). Maia et al. ([2018\)](#page-7-0) also observed that methylmercury is more prone to desorption of particles in black water lakes, due to low pH, which increase the concentration of methylmercury in the dissolved phase. Other studies in Amazon corroborate the pattern observed by Maia et al. ([2018\)](#page-7-0) like Kasper et al. [\(2017](#page-6-0)) that reported higher dissolved methylmercury concentration during the rainy season in Solimões/Amazon and Negro rivers. However, Pestana et al. [\(2018](#page-7-0)) observed different results with no relationship between seasonality and dissolved methylmercury levels in the Cuniã lake. Regarding biota, Roulet et al. [\(2000](#page-7-0)) observed influence of the seasonality on methylmercury accumulation in zooplankton in Tapajós river while Brito et al. [\(2017](#page-6-0)) found no influence of this parameter on Hg levels in plankton of Janaucá lake, Solimões river basin. The contrasting results reported by the aforementioned authors indicate the complexity of the Hg dynamics in Amazon aquatic ecosystems. It is important to keep searching for patterns for a better understanding of how the flood pulse can influence the Hg distribution in both abiotic and biotic compartments.

The increase of Hg bioavailability can impact the whole aquatic food chain. Roulet et al. [\(2000\)](#page-7-0) attributed the increase in methylmercury concentrations in zooplankton from the dry season to the end of the rainy season to an increase in bioavailability during the rainy season. The primary producers like phytoplankton have great affinity for dissolved Hg, especially organic mercury, and the concentration in its cells can be 10,000 higher than in the water (Pickhardt and Fisher [2007;](#page-7-0) Carroll et al. [2011](#page-6-0)). Since Hg is slowly excreted (Bernhoft [2011\)](#page-6-0), primary consumers (i.e., herbivores) that feed on phytoplankton would be exposed to high levels of Hg and secondary consumers (i.e., carnivores) would be exposed to even higher levels of Hg. On the other hand, during the rainy seasons (rising water and high water), the influx of Hg bound soil particles in the aquatic ecosystem is higher (Maia et al. [2018](#page-7-0)) and particulate Hg is less bioavailable to the biota (Batley [1983\)](#page-6-0). Additionally, dissolved Hg can be diluted by intense rainfall (Maia et al. [2009\)](#page-7-0), resulting in lower concentrations in this phase.

In this context, the aim of this study was to evaluate Hg concentration in fish with different feeding habits (detritivorous, herbivorous and omnivorous) in Puruzinho Lake (Madeira River Basin) over the four characteristics seasons of Amazon (rising, high, falling and low waters). Since fluctuation in rainfall among the seasons can influence Hg geochemistry, we expect differences in Hg concentrations in fish over the seasons.

Materials and methods

The study area was Puruzinho Lake, in the Madeira River Basin, western Amazonia, Brazil (63°6'0"W; 7°24'0"S). Puruzinho Lake is a black water ecosystem characterized by high concentration of dissolved organic matter, acid pH, low primary productivity and low suspended particulate material concentrations (Wissmar et al. [1981](#page-7-0); Almeida et al. [2014](#page-6-0)). Black water ecosystems are suitable environments for Hg methylation due to their acid pH and high levels of dissolved organic matter (King et al. [1999\)](#page-6-0). Puruzinho Lake is strongly influenced by seasonal hydrology. During the high water season, the Madeira River overflows and interconnects with Puruzinho Lake. Water levels change drastically between high water (12.5 m) and low water seasons (0.30 m) (Almeida et al. [2014\)](#page-6-0) due to the Madeira River's influence, which makes Puruzinho Lake a suitable environment to evaluate the influence of seasonality on Hg concentration in fish.

Sampling campaigns were carried out in the four seasons: rising water (RW) in December 2016 and February 2017; high water (HW) in April 2017; falling water (FW) in June 2017; and low water (LW) in October 2017. These seasons are mainly characterized by differences in rainfall intensity, and consequently water level of Puruzinho Lake. Figure [1](#page-2-0) depicts the monthly rainfall measured at the Humaitá pluviometric station in Amazonas state (Puruzinho Lake is located 20 km from Humaitá) (INMET [2018\)](#page-6-0). During the sampling campaigns, the rainfall followed the regular pattern in the region, with higher values in the RW and HW seasons (December 2016 to April 2017) and lower values in the FW and LW seasons (June 2017 to October 2017). Additionally, the Madeira river water level and flow (Table [1](#page-2-0)), measured at the Humaitá station (ANA [2019\)](#page-6-0), reflected the rainfall pattern with higher values of both parameters during RW and FW seasons and lower values in FW and LW seasons (Table [1](#page-2-0)). Therefore, RW and HW can be considered as the rainy seasons and FW and LW as the dry seasons. We did not have the water level values of Puruzinho Lake, but based on the regular rainfall regime and the Madeira river water level and flow during the sampling (Fig. [1](#page-2-0); Table 1), we assume that the lake's water level is higher during the RW and HW and lower during

Fig. 1 Rainfall (mm) in Humaitá pluviometric station along December/ 2016 to October/2017. Red boxes highlight the sampling months (December in 2016 and February, April, June and October in 2017). Puruzinho Lake is located 20 km from Humaitá

FW and LW (Almeida et al. [2014](#page-6-0)). It is important to note that the sampling in HW (April 2017) was at the the peak of the rainy season because of the high rainfall values of the previous months, and the sampling in LW (October 2017) was at the peak of the dry season because of the low rainfall values in May, June, July, August and September (Fig. 1).

A total of 391 specimens ($N = 391$) were caught with the help of local fisherman. Specimens were grouped based on their feeding habits: detritivorous, herbivorous and omnivorous. Detritivorous fish consisted of Potamorhina altamazonica, Potamorhina latior, Prochilodus nigricans, Psectrogaster amazonica, Semaprochilodus insignis, Hemiodus unimaculatus and Psectrogaster rutiloides; herbivorous fish consisted of Mylossoma duriventre and Schizodon fasciatus; and omnivorous fish consisted of Pimelodus blochii Triportheus albus and Leporinus friderici. Although food diversity and availability to fish can change seasonally, trophic ecology studies can provide a pattern of fish diets. We defined the food habits of the studied species based on Cella-Ribeiro et al. ([2017](#page-6-0)) that compiled detailed data about stomach content and biology of several fish species from the Madeira River Basin. The only species whose feeding habit was not determined based on Cella-Ribeiro et al. [\(2017\)](#page-6-0) was Leporinus friderici, due to the low number of stomachs analyzed by those authors $(N = 9)$. Leporinus friderici feeding habit was classified as omnivorous based on Santos [\(1982\)](#page-7-0), Durães et al. [\(2001\)](#page-6-0) and Marçal-Simabuku and Peret [\(2002\)](#page-7-0). Specimens were measured to obtain total length (cm) and total weight (g) (Table [2](#page-3-0)).

Total Hg (THg) determination in muscle (0.2 g wet wt) followed the protocol described by Bastos et al. [\(1998](#page-6-0)):

Table 1 Water level (m) and Flow $(m^3 s^{-1})$ of madeira river in humaitá station

Month	Water level (m)	Flow $(m^3 s^{-1})$
December/2016	13.62 ± 0.89	12002.2 ± 2261.2
January/2017	17.69 ± 0.95	24053.5 ± 3218.9
February/2017	19.15 ± 0.75	29370.0 ± 2920.7
March/2017	21.68 ± 0.32	39796.6 ± 1449.1
April/2017	21.72 ± 0.13	39987.4 ± 615.4
May/2017	20.39 ± 0.61	34272.8 ± 2575.2
June/2017	18.10 ± 0.82	25425.54 ± 2933.1
July/2017	14.34 ± 1.17	13902.35 ± 3108.2
August/2017	11.24 ± 0.52	6815.4 ± 995.4
September/2017	10.66 ± 0.41	5767.1 ± 705.8
October/2017	10.22 ± 0.16	5015.8 ± 267.6

Humaita Station is close to Puruzinho lake. Data extracted from ANA ([2019\)](#page-6-0)

addition of 1 mL of 30% H₂O₂ and 4 mL of 70% HNO₃:98% $H₂SO₄$ (1:1); heating in a digestion block (model TE04/25) for 30 min in 70 °C; cooling to room temperature; addition of 5% KMnO4; heating for 15 min; cooling to room temperature overnight; addition of 12% NH₂OH.HCl; and addition of ultrapure water to a final volume of 12 mL (MilliQ Plus, Millipore, Bedford, MA, USA). Determination was conducted by CV-AAS with a FIMS 400 Flow Injection Mercury System (PerkinElmer, Germany). Detection limit of the method was 0.007 mg kg⁻¹. Certified material (DORM-2) was analyzed in triplicate at each 30 samples (recuperation: $99 \pm 2.5\%$). Additionally, each sample was analyzed two times to test precision. Blanks were used for quality control.

To compare THg levels among seasons, we normalized concentrations by length, as suggested by Sccuder-Eikenberry et al. [\(2015\)](#page-7-0), to remove the influence of size, following the formula: THg normalized $=$ THg concentration / total length. Sccuder-Eikenberry et al. [\(2015\)](#page-7-0) recommended multiplying THg normalized by the mean total length of the species. However, the statistical analysis conducted with the data multiplied by the mean total length and not multiplied showed the same patterns. Therefore, we present in this paper the normalization without the multiplication by the mean total length. Comparisons were conducted with ANOVA and the Tukey post-hoc test (α = 0.05). When necessary, data were transformed with Box-Cox (Venables and Ripley [2002](#page-7-0)) to fit ANOVA premises. Two-way ANOVA $(\alpha = 0.05)$ was used to test for interactions between seasons and feeding habits. Linear regressions ($\alpha = 0.05$) were used to test for relationship between THg concentration and size (total length and total weight). Statistical analyses were performed using the R software (R Core Team [2018\)](#page-7-0).

It is well known that Hg, especially methylmercury, forms strong bonds with thiol compounds in muscle proteins, which

in turn leads to a long half-life in the organism. Also, due to this strong bond, methylmercury is slowly excreted and is the major Hg chemical species accumulated in fish muscle (Ikingura and Akagi [2003](#page-6-0)). This high half-life of Hg could influence the interpretation of our results, since we compared individuals in different seasons. However, the environmental conditions of the study area and the size of the fish can mitigate this issue because methylmercury excretion rate is positively correlated with water temperature and negatively with body size (Trudel and Rasmussen [1997](#page-7-0)). In the Amazon region, water temperature is high the whole year (INMET [2018\)](#page-6-0), which can reduce Hg half-life. Additionally, the weight of the sampled individuals was similar to the ones that presented the highest Hg excretion rates in the long-term experiment of Trudel and Rasmussen [\(1997\)](#page-7-0). Those authors also observed that fish chronically exposed to methylmercury excrete this contaminant 2.1 times faster than acutely exposed fish. Since there is no point source of Hg in the study area, it is more likely that fish in Puruzinho Lake are chronically exposed to the contaminant, thus showing a high excretion rate. Therefore, we assume that the Hg concentration observed in a fish sample in a given season represents that season and not past periods.

Results and discussion

THg concentrations in the detritivorous guild were statistically higher during the RW and LW in comparison with HW and FW $(p = 0.01)$, while herbivorous guild THg levels were highest in RW ($p = 0.001$) and THg levels in the omnivorous guild were statistically higher in the RW (p) $= 0.0001$) (Fig. [2;](#page-4-0) Table [3](#page-4-0)). The results indicate that THg levels in all guilds vary along the seasons.

Most of the detritivorous fish sampled in this study belong to the order Characiforme (families Prochilodontidae and Curimatidae). These species have phytoplankton as their primary source of carbon (Araujo-Lima et al. [1986](#page-6-0); Forsberg et al. [1993\)](#page-6-0). Nascimento et al. [\(2006\)](#page-7-0) reported that THg concentration in plankton from Puruzinho Lake was higher during the RW (337.0 ng g^{-1}), which explains the higher levels observed in detritivorous fish during this seasons (Fig. [1](#page-2-0)). The two species herbivorous species, Mylossoma duriventre and Schizodon fasciatus, feeds mainly on C3 and C4 plants during RW (Oliveira et al. [2010\)](#page-7-0) while phytoplankton constitutes an insignificant part of its diet. Despite this, THg levels in herbivorous was also higher during RW. The same pattern was observed in omnivorous fish that have Triportheus albus as its most sampled specie in RW. Triportheus albus feeding habit is a mix of insects and plants (Cella-Ribeiro et al. [2017](#page-6-0)) and phytoplankton is not often consumed by this specie. Therefore, higher THg levels in phytoplankton during RW can explain the higher levels of THg in detritivorous fish but not in herbivorous and omnivorous fish. Other factors like water physico-chemical parameters may have influenced THg accumulation in fish. Black water ecosystems, such as Puruzinho Lake, provide optimum conditions to increase Hg methylation and bioavailability, like low pH and high concentration of dissolved organic matter (Watras et al. [1995](#page-7-0); Vieira et al. [2018\)](#page-7-0).

Fig. 2 Total mercury concentrations normalized by size (mg kg⁻¹ wet wt) in detritivorous, herbivorous and omnivorous fish from Puruzinho Lake (Madeira River Basin). Different letters indicate statistical differences ($p < 0.05$)

Table 3 Total mercury concentrations (mg kg[−]¹ wet wt) in fish species in the four seasons (Puruzinho Lake)

During the HW season, the Madeira River's main course overflows and invades Puruzinho Lake (Queiroz et al. [2011\)](#page-7-0), resulting in an inflow of white waters. This is one of the three water groups in the Amazon and is characterized by high levels of suspended particulate matter (SPM) and neutral pH (Vieira et al. [2018](#page-7-0)). Therefore, the inflow of white waters in Puruzinho Lake may reduce Hg bioavailability to biota due to the high affinity of Hg for SPM (Maia et al. [2018\)](#page-7-0). THg bound to SPM is less available to biota (Batley [1983](#page-6-0)) while dissolved Hg has strong affinity to phytoplankton biomass (Carroll et al. [2011\)](#page-6-0). Since phytoplankton are the primary source of carbon for detritivorous fish (Araujo-Lima et al. [1986](#page-6-0); Forsberg et al. [1993](#page-6-0)), the reduction of Hg bioavailability to phytoplankton

during HW certainly influenced THg concentration in fish (Driscoll et al. [2007;](#page-6-0) Chen et al. [2012\)](#page-6-0). Additionally, the high inflow of SPM during the HW may reduce phytoplankton growth due to increase in water turbidity (Northcote et al. [2009](#page-7-0)).

The influence the Madeira River's main course on Puruzinho Lake was weaker during the FW. In this season, THg concentration in detritivorous and herbivorous fish were similar to that in the HW, possibly due to the presence of white waters from the Madeira River. During the LW, when there was no influence of the river, the THg concentration in detritivorous and omnivorous fish showed different patterns. In detritivorous fish, THg levels increased significantly and were statistically similar to those in the RW season, while in omnivorous fish, THg concentrations decreased significantly. The observed pattern in detritivorous fish was expected since there was no more influence of white waters in the lake and the optimum condition for increased Hg methylation was reestablished (Vieira et al. [2018\)](#page-7-0). However, omnivorous fish's THg levels did not increase in LW. This result may be related to the omnivorous fish diet rather than Hg bioavailability. The most sampled omnivorous species in LW was Triportheus albus, which may have switched to a more plant based diet in LW due to a lower input of terrestrial insects in the lake. In this season, rainfall is low (Fig. [1](#page-2-0)) which negatively impacts the influx of allochthonous food items (Rezende and Mazzoni [2005;](#page-7-0) Lamberti and Gregory [2007\)](#page-7-0). This certainly influences the diet of Triportheus albus, since Cella-Ribeiro et al. ([2017\)](#page-6-0) reported that terrestrial arthropods correspond to 56.39% of the animal protein for this species. Therefore, a plant-based diet can result in lower THg levels in omnivorous fish.

To test if the relationship between Hg and size varies over the seasons, we performed linear regressions with the data from the four seasons (Table 4). Weak positive relationships between size and THg (Table 4) were observed in the detritivorous guild (RW and LW) and a stronger positive relationship was observed in the herbivorous guild during RW and FW. Omnivorous and herbivorous guilds showed negative relationships in RW and HW, respectively (Table 4). The results showed no clear trend for relationship between size and THg levels among seasons. However, only during RW was the relationship significant for all guilds. Although it is well established that THg concentration increases with fish size (Kraepiel et al. [2003](#page-7-0); Campbell et al. [2010;](#page-6-0) Bosch et al. [2016](#page-6-0)), Roulet and Maury-Brachet ([2001\)](#page-7-0) observed that significant relationships between size and THg concentration in Amazonian fish is rare. In agreement with this author Bastos et al. [\(2008](#page-6-0)) reported a non-significant relationship between size and THg in 86 species from the Madeira River Basin during the dry and rainy seasons.

Table 4 Linear regression ($\alpha = 0.05$) between total mercury levels and size

	$THg \times TL$	$THg \times TW$
Rising water (RW)		
Detritivorous	0.11(0.001)	0.08(0.006)
Herbivorous	0.61(0.0001)	0.35(0.0001)
Omnivorous	0.41(0.003)	0.43(0.002)
High water (HW)		
Detritivorous	0.01(0.49)	0.0001(0.98)
Herbivorous	0.20(0.05)	0.10(0.18)
Omnivorous		
Falling water (FW)		
Detritivorous	0.004(0.65)	0.01(0.41)
Herbivorous	0.24(0.004)	0.04(0.24)
Omnivorous		
Low water (LW)		
Detritivorous	0.15(0.01)	0.09(0.05)
Herbivorous		
Omnivorous	0.05(0.24)	0.07(0.16)

 R^2 (p) in fish from Puruzinho Lake

Italic coefficients: negative trend

In general, the capture of migratory species in the Amazon region is higher during the RW and HW (Freitas et al. [2002\)](#page-6-0). Therefore, increases in THg level during these seasons may result in a higher risk of exposure to fish consumers. In the Brazilian Amazon, preference for fish among the population is 70.7%, against 14.7% and 12.2% for beef and chicken, respectively (Lopes et al. [2016\)](#page-7-0). The study of Oliveira et al. ([2010\)](#page-7-0) showed that the fish eating among the people living near Puruzinho Lake was 406 g day[−]¹ , which is very high. It is important to advise consumers, especially those who consume fish often, to have a more mixed diet during the rainy seasons due to higher levels of THg.

We recognize that Hg exposure to consumers can be influenced by which species is consumed. However, THg concentration among most species (Table [3\)](#page-4-0) was in same order of magnitude. The only exception was the herbivore Mylossoma duriventre, which showed lower mean THg levels (Table [3](#page-4-0)). This suggests that risk of exposure to THg by intake of most of the detritivorous, omnivorous and herbivorous species of Puruzinho Lake is similar.

Conclusions

THg concentration in detritivorous, herbivorous and omnivorous fish was influenced by the flood pulse. In detritivorous fish, THg levels were higher during Rising Water, decreased in high water and falling water and increased again in low water. Herbivorous fish showed a similar pattern, with higher concentration of THg in Rising Water and lower concentration in high water and falling water. We suggest that this pattern is explained by the strong influence of the "white waters" from the Madeira River that invade Puruzinho Lake during high water and falling water. Also, phytoplankton THg concentration is higher during the Rising Water season in comparison with other seasons. When the seasons were grouped in rainy (Rising Water and high water) and dry (falling water and low water) seasons, THg concentration were higher during the rainy season in detritivorous, herbivorous and omnivorous fish.

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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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