Mercury biomagnification and the trophic structure of the ichthyofauna from a remote lake in the Brazilian Amazon

Claudio Eduardo Azevedo-Silva a,*, Ronaldo Almeida b, Dario P. Carvalho a, Jean P.H.B. Ometto c, Plínio B. de Camargo d, Paulo R. Dorneles a, Antonio Ázeredo e, Wanderley R. Bastos f, Olaf Malm a, João P.M. Torres a

a Laboratório de Radioisótopos Eduardo Penna, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, Cidade Universitária, Av. Carlos Chagas Filho s/n, bloco G, Sala 60, Subsolo, Ilha do Fundão, Rio de Janeiro, RJ, Brazil
b Instituto Natureza e Cultura, Universidade Federal do Amazonas, Rua 1 de Maio. Colegiado de Ciências Agrárias, Benjamin Constant, Colônia, AM, Brazil
c Instituto Nacional de Pesquisas Espaciais, Centro de Ciências do Sistema Terrestre, Avenida dos Astronautas, 1758, Jardim da Granja, São José dos Campos, SP, Brazil
d Núcleo de Estudos de Saúde Coletiva, Universidade Federal do Amazonas, Avenida Horácio Macedo, S/N, Ilha do Fundão, Rio de Janeiro, RJ, Brazil
e Instituto Natureza e Cultura, Universidade Federal do Amazonas, Rua 1 de Maio. Colegiado de Ciências Agrárias, Benjamin Constant, Colônia, AM, Brazil
f Laboratório de Ecologia Isotópica, Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, Avenida Centenário, 303, São Dimas, Piracicaba, SP, Brazil
g Laboratório de Biogeoquímica Ambiental – Universidade Federal de Rondônia, Br 364km 9.5, Sentido Acre, Porto Velho, RO, Brazil

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The present study assesses mercury biomagnification and the trophic structure of the ichthyofauna from the Puruzinho Lake, Brazilian Amazon. In addition to mercury determination, the investigation comprised the calculation of Trophic Magnification Factor (TMF) and Trophic Magnification Slope (TMS), through the measurements of stable isotopes of carbon (δ13C) and nitrogen (δ15N) in fish samples. These assessments were executed in two different scenarios, i.e., considering (1) all fish species or (2) only the resident fish (excluding the migratory species). Bottom litter, superficial sediment and seston were the sources used for generating the trophic position (TP) data used in the calculation of the TMF. Samples from 84 fish were analysed, comprising 13 species, which were categorized into four trophic guilds: iliophagous, planktivorous, omnivorous and piscivorous fish. The δ13C values pointed to the separation of the ichthyofauna into two groups. One group comprised iliophagous and planktivorous species, which are linked to the food chains of phytoplankton and detritus. The other group was composed by omnivorous and piscivorous fish, which are associated to the trophic webs of phytoplankton, bottom litter, detritus, periphyton, as well as to food chains of igapó (blackwater-flooded Amazonian forests). The TP values suggest that the ichthyofauna from the Puruzinho Lake is part of a short food web, with three well-characterized trophic levels. Mercury concentrations and δ13C values point to multiple sources for Hg input and transfer. The similarity in Hg levels and TP values between piscivorous and planktivorous fish suggests a comparable efficiency for the transfer of this metal through pelagic and littoral food chains. Regarding the two abovementioned scenarios, i.e., considering (1) the entire ichthyofauna and (2) only the resident species, the TMF values were 5.25 and 4.49, as well as the TMS values were 0.21 and 0.19, respectively. These findings confirm that Hg biomagnifies through the food web of Puruzinho Lake ichthyofauna. The migratory species did not significantly change mercury biomagnification rate in Puruzinho Lake; however, they may play a relevant role in Hg transport. The biomagnification rate (TMS value) in Puruzinho Lake was higher than the average values for its latitude, being comparable to TMS values of temperate and polar systems (marine and freshwater environments).

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1. Introduction

Mercury (Hg) is a toxic metal whose effects comprise neurotoxicity, nephrotoxicity and genotoxicity, among others (JPHA, 2001; WHO, 2003). This metal presents a complex biogeochemical cycle, which includes biomagnification (Fitzgerald and Mason, 1997; Morel et al., 1998; Barbosa et al., 2003). Newman and Unger (2002) define biomagnification as an increase in concentration of a contaminant from a trophic level to the superior one through feeding, i.e., between prey and predator. Nevertheless, biomagnification is generally associated to the trophic transfer of a contaminant, with a concentration increase through successively

* Corresponding author.
E-mail addresses: ceass@biof.ufrj.br, azevedo.silva@gmail.com (C.E. Azevedo-Silva).
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higher trophic levels (Kidd et al., 1995; Meili, 1997; Watras et al., 1998). As a result, the highest concentrations are found in the species occupying the highest trophic positions, including human beings (Beek et al., 2000). Mercury biomagnification has been verified by a number of studies comprising different aquatic environments (Lavoie et al., 2013). However, much need to be understood about the subject in Amazon, due to the complexity of its aquatic environments. The generation of more knowledge on pollutant biomagnification in Amazonian environments would be important from the human health point of view as well, given the high importance of fish for the nutrition of Amazonian riparians (Dorea, 2003).

The Amazon basin presents a wide diversity of freshwater ecosystems, with different geomorphological, physicochemical and biological features, including river-floodplain systems (Purch & Junk, 1997; Junk et al., 1989). The floodplain constitutes a mosaic of open waters (lakes and rivers), flooded forests and floating meadows that have their areas and proportions modified according to the water level seasonal variation (Junk et al., 1989, Junk, 1997b). Methylmercury formation and accumulation have been verified in tropical river-floodplain systems (Guimarães et al., 2000a, 2000b; Roulet et al., 2001). These aspects favour Hg biomagnification and the contamination of riparian Amazonian populations, for whom fish constitute the main protein source (Malm et al., 1995; Boisio and Hensel, 2000; Bastos et al., 2006).

Regarding anthropogenic input of Hg, the Amazon region has been the main gold producer in Brazil since the 1970–1980s (Porto et al., 2002). The gold mining activities in Amazon resulted in the release of 87 t of mercury (Hg) to the environment between 1979 and 1990 (Lacerda et al., 1989). Investigations performed in the region during 1980–1990 s attributed the high environmental levels of Hg to these activities (e.g., Martinelli et al., 1988; Lacerda et al., 1989; Pfeiffer et al., 1991). At the end of the 1990s however, investigations carried out in the Amazon region demonstrated the high soil Hg concentrations to be of natural origin (Roulet et al., 1998; Lechler et al., 2000; Fadini and Jardim, 2001). A number of studies on mercury have been performed in the region in order to understand both the biogeochemical processes and the toxic effects on riparian populations (e.g., Pfeiffer and Lacerda, 1988; Malm et al., 1990; Malm, 1998; Malm et al., 1995; Guimarães et al., 2000a, 2000b; Maurice-bourgoin et al., 2000; Dorea et al., 2006; Bastos et al., 2007; Almeida et al., 2014). Despite being a part of Hg biogeochemical cycle that contributes to the human exposure to this toxic metal, little is known on the trophic flow of Hg through the aquatic Amazonian food webs (Barbosa et al., 2003).

Considering the demonstrated importance of searching for a better understanding of Hg biogeochemical cycle in the region, we propose the following hypothesis (H1): Hg biomagnifies through the food web of Puruzinho Lake ichthyofauna. In addition, we have tested the importance of migratory species to the calculation of biomagnification rates. The latter testing is based on the fact that Borgá et al. (2012) highlighted that the presence of migratory species may influence the calculation of biomagnification rates. In addition, the fish assemblage of the Amazon region presents a high diversity (Junk, 1997a) with migratory species playing an significant role in energy flow and matter cycling (Hoeninghaus et al., 2006) and hence being important for trace element cycling as well.

2. Study area

This study was performed in Puruzinho Lake, which is part of the Puruzinho River drainage basin (Fig. 1). The Puruzinho River is located at the Lower Plateau of the Western Amazon, covering an area of “cerrado” vegetation downstream to the middle section of its course. Subsequently, the rivers cover an area of dense ombrophylous forest and rain forest within its area of seasonal flooding (IBGE, 2003; Almeida, 2006). The study area is part of a region characterized by Junk et al. (1989) as a river-floodplain system and presents a monomodal flooding. Puruzinho Lake has an area of 4.84 Km² during the dry season and is located between the latitudes 07° 20’ 05”S and 07° 22’ 38”S and between the longitudes 63° 05’ 05” W and 63° 00’ 57” W. The lake has been classified as a blackwater system, with anoxic and hypoxic periods in water column (Almeida, 2006; Nascimento et al., 2006; Azevedo-silva, 2011).

3. Material and methods

3.1. Sampling

Sampling was performed in two phases of the Puruzinho Lake hydrological cycle. The first sampling procedure was carried out at the end of the dry season, in September 2006. The second sampling period was in February 2007, during the high water period.

3.1.1. Sampling for the calculation of the baseline

Seston, bottom litter and superficial sediment were used as energy sources (baseline) for the calculation of the trophic position (TP). Seston and bottom litter integrate the isotopic signatures of pelagic and littoral food chains, respectively, corresponding to the input of allochthonous matter to the lake (Post, 2002). Additionally, in tropical river-floodplain systems, bottom litter also integrates the pelagic food chain associated to the igapó (blackwater-flooded Amazonian forests). According to Layman et al. (2005a), the allochthonous production may play an important role for tropical river-floodplain systems. The choice for the superficial sediment was a consequence of the fact that detritivorous/illio-phagous fish are key species in tropical riverine environments, being able to predominate the ichthyobiomass of these systems (Bowen, 1983). This turns the superficial sediment into an important source for the benthic food chain (Jepsen and Winemiller, 2002).

Seston sampling was performed through horizontal surface hauls using a 20 μm mesh-size net. In order to reduce the interference from zooplankton, samples were subsequently filtered with a 68 μm mesh-size net. Six seston samples were collected, comprising three samples from each hydrological phase. Bottom
litter samples were collected in marginal areas of the lake, with five and three samples representing dry and high water seasons, respectively. Superficial sediment samples were collected using an Ekman dredge, comprising five samples from each hydrological phase. All samples were stored in transparent polyethylene bags and kept frozen until the analyses.

3.1.2. Fish sampling

Adult fish sampling was performed with the use of 30-, 40-, 50- and 70-mm mesh-size gill nets. After species and gender identification, each individual had its weight (g) and its total length (cm) measured. Muscle samples were stored in transparent polyethylene bags and kept frozen until the analyses. In the present study, 84 fish were sampled, comprising 13 species, which were sorted out into four trophic guilds: iliophagous, planktivorous, omnivorous and piscivorous fish. This classification was based on feeding habit data from published studies that dealt with stomach content analyses and stable isotope measurement. The iliophagous species analysed were Potamorhina altamazonica; Potamorhina latior and Potamorhina pristigaster (Carvalho, 1984; Soares et al., 1986; Aranquire, 2002; Mérona and Mémona, 2004; Pouilly et al., 2004), two out of the three species of the genus Potamorhina, i.e., P. latior and P. altamazonica, present a migratory behaviour (Fernandes, 1997; Granado-lorêncio et al., 2005). The omnivorous fish were Mesonauta festivus, Satanoperca jurupari and Triportheus elongatus. The species T. elongatus constituted the only migrating omnivorous fish (Almeida, 1984; Diaz-sarmiento and Alvarez-león, 2003, Granado-lorêncio et al., 2005) in the present study. The two planktivorous fish analysed, Anodus elongatus and Hypophthalmus edentatus, were migratory species (Diaz-sarmiento and Alvarez-león, 2003; Agostinho et al., 2004; Granado-lorêncio et al., 2005). The piscivorous fish studied were Acaronia nassa, Acestrorhynchus falcirostris, Cichla monoculus, Hoplias malabaricus and Plagioscion squamosissimus. All Piscivorous fish are resident species.

3.2. Determination of total Hg concentrations

The analytical procedure for total mercury (THg) determination has been detailed elsewhere (Bastos et al., 1998). Briefly, sample and blank solutions were analysed in duplicates. Aliquots of approximately 0.4 g of fresh muscle were digested for THg determination, which was performed using an Atomic Absorption Spectrophotometer with Flow Injection System (FIMS-400) and autosampler (AS-90), both from Perkin-Elmer. The Hg measurements were performed at the Radioisotope Eduardo Penna Franca Laboratory, Biophysics Institute, Federal University of Rio de Janeiro (UFRJ). The detection limit of the method (DLm) was 1.96 ng g⁻¹. DLm was calculated by multiplying the detection limit of the instrument (DLi, in ng/mL) by the final volume of the extracts (in mL) and dividing this result by the mean value of the sample mass (in grams). DLi was determined based on three times the standard deviation of 20 runs of the blank solution, divided by the slope (a) of the calibration curve (DLi=SD³/a). The Certified Reference Material DORM-2 (Dogfish muscle) from the National Research Council of Canada (NRCC) was used for the determination of accuracy and precision of the method. The accuracy was calculated through the formula [A=(M/C)¹00], where M was Hg concentration determined by our method in DORM-2 and C was Hg concentration certified by NRCC. The precision was assessed through the coefficient of variation of concentrations. The analyses of DORM-2 were carried out in all analytical batches, comprising 20 replicas. The accuracy and precision values were 100.3% ± 6.75%, respectively.

3.3. Stable isotope measurements

3.3.1. Sample treatment

Sediment and fish samples were freeze-dried and macerated with mortar and pestle. The bottom litter samples were washed with MilliQ water, macerated in a grinder and oven-dried (50 °C). Sediment, fish and bottom litter samples were sieved (pore size 0.07 mm) to ensure their homogeneity. Septon samples were concentrated in precombusted (500 °C for 5 h) 47 mm-diameter glass fibre filters (pore size 0.7 μm, GF/F Whatman) and weighed.

3.3.2. Determination of δ¹³C and δ¹⁵N values

Homogenized powdered material from the above mentioned samples were weighed into tin cups and their respective isotope compositions were determined through combustion in an elemental analyser (Carlo Erba, CHN-1110) coupled to an isotope ratio mass spectrometer (Thermo Finnigan Delta Plus). The stable isotope measurements were performed at the Isotopic Ecology Laboratory, Centre of Nuclear Energy in Agriculture (CENA), University of São Paulo (USP). The standards used for the carbon and nitrogen ratios were PeeDee Belemnite (PDB) and atmospheric nitrogen, respectively. The assessment of the precision of the method was executed through the analyses of nine replicates of the used standards. The coefficients of variation of δ¹³C and δ¹⁵N were 0.674% and 0.403%, respectively. The calculations of the delta notation values of stable isotopes of carbon (δ¹³C) and nitrogen (δ¹⁵N) are expressed according to the following:

\[ \delta X = \left( \frac{R_{sample}}{R_{standard}} - 1 \right) \times 1000 \]  

where X is ¹³C or ¹⁵N and R is the corresponding ratio ¹³C/¹²C or ¹⁵N/¹⁴N.

3.3.3. Arithmetic lipid normalization

Samples from A. elongatus and H. edentatus were corrected (δ¹³C) using arithmetic lipid normalization as described by Post et al. (2007), as the species presented values greater than 3.5 for the C/N ratio, which turned the lipid extraction into a necessary procedure. The arithmetic lipid normalization was executed according to the following:

\[ \delta^{13}C = \delta^{13}C - 3.32 + 0.999C/N \]  

3.3.4. Assessment of the trophic position

The trophic position (TP) of the consumers was estimated according to the following: 

TP=\left[\left(\delta^{15}N_{consumer} - \delta^{15}N_{source}\right)/3.4\right] + \lambda \quad \text{[Jepsen and Winemiller, 2002; Winemiller et al., 2007]}

The denominator value (3.4) was obtained from investigations on isotopic enrichment of ¹⁵N (Minagawa and Wada, 1984; Post, 2002) and λ stands for the reference value ‘1’, which corresponds to the source for the ecosystem (Vander Zanden et al., 1997; Jepsen and Winemiller, 2002). The δ¹⁵Nsource constitutes the mean δ¹⁵N value of the energy source matrices (seston, bottom litter or superficial sediment), i.e., it corresponds to an adjustment for the δ¹⁵N baseline signature. In addition, the TP estimation followed the parameters suggested by Vander Zanden et al. (1997), where primary consumer, omnivorous and secondary consumer species presents the values of 2.0, 2.5 and 3.0, respectively.

3.4. Biomagnification estimation

Total mercury (THg) biomagnification was assessed through the Trophic Magnification Factor (TMF) and slope (b) of a log-linear regression between pollutant concentration and δ¹⁵N. TMF is the anti-log of the slope of a log-linear regression between pollutant concentration and TP. A TMF value higher than 1.0 would indicate
bomagnification, whereas a TMF < 1.0 would point out to trophic dilution of the contaminant (Fisk et al., 2001; Kelly et al., 2008; Jæger et al., 2009; Borgå et al., 2012). Therefore, the adopted formula is TMF = 10^b, which comes from the equation Log10[Hg] = a + b(TP), where a is the dimension at the point of intersection of the axis of ordinates and b is the slope of the regression line.

A significant and positive slope (b > 0) of a log-linear regression between pollutant concentration and δ15N (Log10[Pollutant] = a + b(δ15N)) indicates pollutant biomagnification in a food web (Lavoie et al., 2013). The slope (b) of regression, that can be called Trophic Magnification Slope (TMS), have been used to estimate contaminant biomagnification in different food webs (Kidd et al., 2001; Campbell et al., 2005; Lavoie et al., 2013, Poste et al., 2015), facilitating the comparison between ecosystems.

3.5. Statistical treatment

The statistical software used comprised the Statistica 5.0 and the Bioestat 5.0. The significance level was defined at p < 0.05. The Shapiro-Wilk's test was applied for verifying the distribution of the data, while the Levene's test was used for investigating the homogeneity of the variance. The Mann-Whitney and the T tests were used for investigating the significance of the difference between two series of data, while the Kruskal-Wallis test followed by Dunn’s multiple-comparison test were applied for verifying the significance of the difference among k series of data.

4. Results and discussion

4.1. Stable isotopes

4.1.1. Carbon and nitrogen sources to the ichthyofauna

Despite the apparent lower δ13C values in seston (Fig. 2), due to the presence of phytoplankton (Hamilton and Lewis, 1992), there was no significant difference among the sources for this variable (K.Wallis, p = 0.1010). Differently from the δ13C, for which the sources presented overlapping values, there were significant differences for the δ15N values (K.Wallis, p = 0.001). These differences were found between bottom litter and superficial sediment, as well as between bottom litter and seston (Dunn, p < 0.01 in both cases), with lower δ15N values for bottom litter in both cases (Table 1).

4.1.2. Ichthyofauna

Data on δ13C, δ15N and Hg of the ichthyofauna from the two hydrological periods were grouped in the present study. The rationale for this grouping is given to the fact that: (1) none of the species exhibited significant difference between dry season and high water period for Hg concentrations; (2) only one species has shown significant differences between the two periods for δ13C and δ15N (p < 0.05); and (3) it was not possible to sample all species in both hydrological periods. In this context, it is important to highlight that this grouping contributed to the robustness of the statistical testing, as well as to the biomagnification assessment.

![Fig. 2. Carbon (δ13C) and nitrogen (δ15N) isotopic ratio (in ‰) of energy sources and fish species from Puruzinho Lake, exhibiting median, minimum and maximum values. Sources: Bottom litter (×), superficial sediment (■) and seston (Δ). 2.A – Iliophagous: P. altamazonica (●), P. latior (□) and P. pristigaster (●). 2.B – Omnivorous: T. elongatus (●), M. festivus (□) and S. jurupari (●). 2.C – Planktivorous: A. elongatus (Δ) and H. edentatus (●). 2.D – Piscivorous: A. nassa (●), C. monoculus (◇), H. malabaricus (●), P. squamosissimus (●) and A. falcirostris (○).]
Footnote: The superscript letters A and B indicate significant differences.

4.1.2.1. Iliophagous fish. The species Potamorhina altamazonica, P. latior and P. pristigaster do not present significant differences for the δ13C values (K.Wallis, p = 0.5466). However, the same species exhibited a significant difference for the δ15N values (K. Wallis, p = 0.0054), which was found between P. altamazonica and P. pristigaster (Dunn, p < 0.01) (Table 1). Therefore, there was no significant difference in δ13C between resident and migratory iliophagous fish. Although iliophagous fish consume substrate-associated microorganisms and organic matter flocculated on sediment (Bowen, 1983; Pouilly et al., 2004; Santos et al., 2006), the δ13C values of the three Potamorhina species were closer to values from estuaries than to those from sediment, linked to the phytoplanktonic food chain. Studies evaluating stomach contents and δ13C values in these species consider phytoplankton to be the main carbon source (Carvalho, 1984; Arawo-li-ma et al., 1986; Forsberg et al., 1993; Araguren, 2002; Pouilly et al., 2004). Lepsen and Winemiller (2007) considered phytoplankton to be the main carbon source for iliophagous fish from river-floodplain systems.

However, it was found lower δ13C values for the three Potamorhina species than for estuary samples (Fig. 2A). This could be explained by the fact that estuaries do not represent the phytoplankton carbon signature properly. Hamilton and Lewis (1992) state that estuary δ13C values are in general higher than those from phytoplankton due to the presence of particulate organic matter from other sources adsorbed to the former matrix.

It is important to highlight also that the low δ13C values found in Potamorhina spp could reflect the carbon from methanotrophic bacteria as well. The anoxic sediment from the lake is characterized by methane biosynthesis (with δ13C values between ~52 and ~80‰) by methanogenic bacteria, which in turn influences the δ13C values of methanotrophic bacteria and the aquatic food web, up to the fish level (Woltemate, 1984; Wantzen et al., 2002; Sanseverino et al., 2012). Water bodies rich in humic compounds are characterized by high heterotrophic activity, which may result in a higher production than that from phytoplankton (Thomaz, 1999). The Puruzinho Lake is a humic body of water, with anoxic and hypoxic periods in water column (Almeida, 2006; Azevedo-Silva, 2011). This feature turns the lake into a suitable environment for heterotrophic production, including the one from methanogenic and methanotrophic bacteria. Conrad et al. (2011) observed methane production in the sediment of Lake Puruzinho with values of ~80% δ13C.

According to Pouilly et al. (2004), curimatid fish (such as the Potamorhina species) are widely recognized as microphytobenthic animals. However, P. altamazonica, P. latior and others curimatid fish also consume sediment-associated microorganisms (Santos et al., 2006), including methanotrophic bacteria. Studies performed on the Brazilian Pantanal suggest the association of some iliophagous/detrivorous fish (including a Potamorhina species, P. squamoralveis) to the detrital food chain through methanotrophic bacteria (CALHEIROS, 2003). Sanseverino et al. (2012) found a 50% contribution of carbon from methanotrophic bacteria for P. squamoralveis from Brazilian Pantanal. Pouilly et al. (2013) also suggest the contribution of carbon from methanotrophic bacteria to the ichthyofauna of River Iréne (Madeira River Basin). Therefore, the more 13C-depleted biomass of both phytoplankton and methanotrophic bacteria would explain the δ13C values of the iliophagous fish from the present study.

Among the Potamorhina species, only P. pristigaster exhibited TP close to 2.0, the value suggested by Vander Zanden et al. (1997) for the primary consumers. Although some individuals of the species P. latior and Potamorhina presented TP close to 2.0, their median TP values were close to the one (2.5) suggested by Vander Zanden et al. (1997) for the omnivorous fish (Table 1). No published information was found on the trophic position of these three Potamorhina species; however, Forsberg et al. (1993) placed P. latior and Potamorhina on the primary consumer TP. Layman et al. (2005a,b) also classify the algivorous/detrivorous species, which includes the iliophagous fish, as primary consumers. The higher trophic positions found for P. latior and Potamorhina may be a consequence of their migratory behavior. Migratory animals may present different δ15N values from the resident species (MacAvoy et al., 2001), in response to δ15N variation of the bottom of the food webs among distinct ecosystems (Post, 2002).

4.1.2.2. Omnivorous fish. There was no significant difference in δ13C values among S. jurupari, M. festivus and T. elongatus (K. Wallis, p = 0.0544). Oppositely, there were significant differences in δ15N values among the same three species (K. Wallis, p = 0.0035), with significantly higher values in T. elongatus than in both M. festivus and S. jurupari (Dunn p < 0.05 in both cases) (Table 1). The high δ15N values placed T. elongatus in a superior trophic position in comparison to the other two omnivorous fish.
Differently from *M. festivus*, whose $\delta^{13}$C values overlapped only those from seston; *S. jurupari* and *T. elongatus* presented $\delta^{13}$C values that overlapped those from the other sources (Fig. 2. B). *Triprotophyes elongatus* is an omnivorous species that uses resources from the flooded forests as the main energy source (Claro-júnior et al., 2004). Therefore, $\delta^{13}$C values close to those from the bottom litter would be expected for *T. elongatus*. However, the $\delta^{13}$C values found in this fish were closer to those from seston, which suggest an important contribution of this carbon source for the species (Fig. 2. B). It should be taken into account though that *T. elongatus* presents a migratory behaviour, which may influence its $\delta^{13}$C values. Post (2002) reports variation among ecosystems in the $\delta^{13}$C values of the base of the food web, which can influence the $\delta^{13}$C of migratory species. Among the omnivorous fish, *S. jurupari* was the species that presented the widest overlapping with the three sources regarding the $\delta^{13}$C values (Fig. 2. B). *Satana operca jurupari* is a littoral species that presents the habit of revolving the bottom in search for food (Machado, 1983), which suggests an association with sediment. Nevertheless, the carbon isotopic signature of the species do not allow strong conclusion on which energy source contributes the most to this fish (Fig. 2B). Although the $\delta^{13}$C values of *M. festivus* suggest a contribution from seston (Fig. 2. B), Lowe-McConnell (1969), Machado (1983) and Santos et al., (2009) observed an important participation of filamentous algae (periphyton) in the feeding of this littoral species. This apparent discord between these three previous investigations and our study may be explained by the fact that periphyton and seston may present overlapping $\delta^{13}$C values in river-floodplain systems (Hamilton et al., 1992; Hamilton and Lewis, 1992; Wantzen et al., 2002; Jepsen and Winemiller, 2007). However, stable isotope measurements were not performed in periphyton in the present investigation. The $\delta^{13}$C values from both the scientific literature and the present study suggest that the omnivorous fish assimilates carbon from sediment, flooded forest and periphyton food chains. The resident species *S. jurupari* and *M. festivus* exhibited median TP values, which agrees with the proposed TP value (2.5) for omnivorous fish by Vander Zanden et al. (1997). *T. elongatus* did not present a TP value in agreement with its feeding habit, since the species is well known as an omnivorous fish (Almeida, 1984; Braga, 1990; Mérona et al., 2001; Claro-júnior et al., 2004; Mérona and Mérona, 2004). Therefore, it seems that the migratory behaviour of this fish influenced the highest $\delta^{15}$N values found for the species, and hence the trophic position. *T. elongatus* exhibited median TP values, which agrees with the TP value (3.0) proposed for secondary consumers fish by Vander Zanden et al. (1997).

4.1.2.3. Planktivorous fish. The Planktivorous fish analysed (*H. edentatus* and *A. elongatus*) did not exhibit significant differences on $\delta^{13}$C (test t, $p=0.6450$) or $\delta^{15}$N values (test t, $p=0.4009$) (Table 1). Although both species are well known as planktivorous fish (Abujanra and Agostinho, 2002; Pouilly et al., 2003, 2004), practically all $\delta^{13}$C values from both species were lower than those from seston (Fig. 2. C), which may be explained by the fact that the C isotopic signature of seston does not represent phytoplankton (Hamilton and Lewis, 1992; Bastviken et al., 2003) suggested that the carbon from methanotrophic bacteria is transferred directly or indirectly to zooplankton. In other words, zooplankton organisms can feed directly on these bacteria or they can feed on protozoans that rely on methanotrophic bacteria. As mentioned, methane biogenesis is high in anoxic sediment from freshwater environments (Woltemate, 1984). Since Puruzinho is a shallow lake, zooplankton could also access food items from surface sediments (Sanseverino et al., 2012), which are frequently resuspended in this type of environment (Hamilton and Mitchell, 1998). Furthermore, zooplankton is found in anoxic water due to adaptations to these environments (Bastviken et al., 2003). The Puruzinho Lake is a blackwater (heterotrophic) system that presents important seasonal differences in depth, which may vary from 1 to 12 m within a one-year interval. During the high water season, the period of highest depth, most of the water column of Puruzinho Lake is anoxic, which generates adequate conditions for methane biogenesis. As mentioned, methane synthesis has been verified in the sediment of Puruzinho Lake generating $\delta^{13}$C values of $\sim80\%$ (Conrad et al., 2011). During the seasonal periods of lower depths on the other hand, the environment favors the resuspension of surface sediments. Both situations contribute to the association between zooplankton and the food web of methanotrophic bacteria and hence between this food web and planktivorous fish. Calheiros (2003) also proposed that methanotrophic bacteria constitute an important carbon source to zooplankton in the flood season of Brazilian Pantanal. More recently, Sanseverino et al. (2012) have verified in zooplankton from a lake in Brazilian Pantanal a 45% contribution of carbon from methanotrophic bacteria. Pouilly et al. (2013) have also raised the hypothesis of an important contribution of carbon from methanotrophic bacteria to the ichthyobiomass from River Iténez (Madeira River Basin). Therefore, the more $\delta^{13}$C-depleted biomass of *H. edentatus* and *A. elongatus* found in the present study, as well as data from literature, suggest that these planktivorous fish may be associated to the phytoplanktonic and detrital food chains, including methanotrophic bacteria.

Forberg et al. (1993) concluded that *A. elongatus* and *H. edentatus* occupied the trophic levels of primary and secondary consumers, respectively. This conclusion was subsequently corroborated by stomach content studies, since *A. elongatus* was considered phytoplanktivorous (Aranguren, 2002; Pouilly et al., 2003, 2004) and *H. edentatus* was classified as zooplanktivorous (Carvalho, 1980; Abujanra and Agostinho, 2002). In the present study their trophic position values were around 3.0, then suggesting for secondary consumers (Vander zanden et al., 1997), placing *H. edentatus* on the expected position and *A. elongatus* on one TP above the predicted one (Table 1). It should be taken into account that both species are migratory fish that may therefore be carrying isotopic signatures from another region. Phyto- and zooplankton species may present different $\delta^{15}$N values among ecosystems (Kling et al., 1992; Matthews and Mazunder, 2003). It is important to mention as well that both species exhibited the secondary consumer TP despite the fact that zooplankton food webs may present a large number of trophic levels (Sprules and Bowerman, 1988). However, Molina et al. (2010) observed short plankton trophic webs in an Amazon river-floodplain system, with one or two trophic levels. Vander Zanden and Rasmussen (1996) suggest that despite the complexity of the plankton food webs, the production that reaches planktivorous fish can be directly transferred from primary consumers due to high trophic transfer and feeding in low trophic levels. The aspects suggested by the latter authors would help to explain the TP value exhibited by *H. edentatus*.

4.1.2.4. Piscivorous fish. A significant difference in $\delta^{13}$C values was observed within this trophic guild (K. Wallis, $p=0.017$); specifically, due to the difference found between *C. monoculus* and *H. malabaricus* (Dunn, $p<0.01$). Conversely, no significant difference was found for $\delta^{15}$N values (K. Wallis, $p=0.095$) (Table 1). The TP values of piscivorous fish were close to the one (3.0) suggested by Vander Zanden et al. (1997) for secondary consumers. The piscivorous species presented similar $\delta^{13}$C values than those from sediment, bottom litter and seston, with *C. monoculus* exhibiting the more $\delta^{13}$C-depleted biomass among the species from this trophic guild. The $\delta^{13}$C values of *C. monoculus* suggest that microphagous species such as the iliophagous fish may be important in its diet. The presence of iliophagous fish among the food items was
evidenced for *C. monoculus* (Rabelo and Araujo-Lima, 2002).

The δ¹⁵N values from previous investigations on the diet of these piscivorous fish suggest that these species are associated to the trophic webs of phytoplankton, bottom litter and detritus (Fig. 2. D). Nevertheless, representatives of this trophic guild may be associated to other food chains. Borgå et al. (2012) noted that high trophic level species might be associated to diverse food chains from the ecosystem.

The similar δ¹⁵N values of all piscivorous species reflected on TP values close to 3.0, the one suggested for secondary consumers by Vander Zanden et al. (1997). These findings corroborate the investigation performed by Layman et al. (2005a), who analysed 31 piscivorous species from the floodplain of Cinaruco River (Orinoco River Basin), including four species in common with the present study. The latter authors observed that the piscivorous fish occupied a relatively low trophic position (secondary consumers) by exploiting a short and productive food web, due to the diversity in size, morphology and affinity for habitats of primary consumers (herbivorous and algivorous/detrivorous species).

4.2. Mercury

4.2.1. Mercury biomagnifications

The calculations of the Trophic Magnification Factors (TMF) and Trophic Magnification Slopes (TMS) were performed in two different scenarios, i.e., considering either (1) the total ichthyofauna or (2) the resident species only (resident ichthyofauna of Puruzinho Lake).

Regarding TMF, the slopes of the log-linear regressions between THg concentration and TP in these two scenarios, i.e., for total ichthyofauna and resident ichthyofauna, were higher than zero (Test *t*, *p* < 0.0001), with the values of 0.72 (R²adj = 0.55) and 0.65 (R²adj = 0.49), respectively (Fig. 3. A; B). The TMF values were 5.3 and 4.5 for total ichthyofauna and resident ichthyofauna, respectively. Therefore, in both scenarios the TMFs were higher than 2.0, the value suggested by Borgå et al. (2012) for augmenting the reliability of the TMF results.

Regarding TMS, the slopes of the log-linear regressions between THg concentration and δ¹⁵N in these two scenarios, i.e., for total ichthyofauna and resident ichthyofauna, were higher than zero (Test *t*, *p* < 0.0001), with the values of 0.21 (R²adj = 0.55) and 0.19 (R²adj = 0.49), respectively (Fig. 4. A; B). The slope values suggest total mercury (THg) biomagnification in Puruzinho Lake, considering both, the total and the resident ichthyofauna. Therefore, THg biomagnification could be verified in Puruzinho Lake ichthyofauna by both methods for calculating the biomagnification rates, i.e., TMF and TMS, as well as considering both total ichthyofauna and resident ichthyofauna.

Total mercury (THg) biomagnification in ichthyofauna was verified in other Amazon aquatic environments, i.e., in Negro (Barbosa et al., 2003), Tapajós (Castilhos and Bidone, 2000; Da Silva et al., 2005) and Iténez (Pouilly et al., 2013) river basins. However, with the exception of the study performed by Pouilly et al. (2013), these assessments were accomplished through different methods. In addition to the latter study, comparison was possible to the data generated by Kwon et al., (2012) in Orinoco River Basin as well. Pouilly et al. (2013) found slope values of 0.43, 0.34 and 0.22 for the relation between Log[Hg] and trophic position (calculated from δ¹⁵N) in Iténez, Blanco and San Martin rivers (Iténez River Basin), respectively. Kwon et al., (2012) verified a TMS value of 0.10 for the ichthyofauna of Las Marías River. Both investigations presented lower values than those verified in the present study. It is important to highlight the marked differences in TMS and slope values between Puruzinho Lake (TMS value=0.21, slope=0.72) and both Las Marías River (TMS value=0.10) and Iténez River Basin (slope=0.22). These values suggest a wide variation in Hg biomagnification rates among the studied ecosystems, which may be explained by differences in geomorphological, physicochemical and biological features among these bodies of water. The Puruzinho Lake is a blackwater lentic system from the Madeira River Basin (Almeida, 2006; Nascimento et al., 2006; Azevedo-Silva, 2011); the Las Marías River is a clearwater lotic system from the Orinoco River Basin (Kwon et al., 2012); and the rivers that belong to the Iténez River Basin constitute a lotic system that varies between clearwater and whitewater (Pouilly et al., 2013). The meta-analysis performed by Lavoie et al. (2013) has shown that the mean TMS value for THg is higher in lentic (0.16) than in lotic (0.12) systems, which helps explaining the observed differences between Puruzinho Lake (lentic system) and Las Marías River (lotic system), as well as between the former body of water and the Iténez River Basin (lotic system). Moreover, the waters of Amazon and Orinoco river basins have been classified into three main types according to their colour, physicochemical features and specific conditions of their drainage basins. Therefore, the mentioned
waters were classified into: clearwaters, which vary from transparent to greenish waters drained from areas with little erosion; blackwaters, which are mainly formed in podzolic soil and owe their colour to the high concentrations of dissolved humic substances; and whitewaters, which possess a clay colour due to the high concentrations of dissolved humic substances; and whitewaters, which possess a clay colour due to the high concentrations of dissolved humic substances. These environments presented different trophic statuses (oligotrophic, mesotrophic and eutrophic) and physico-chemical features (Clayden et al., 2013). Other authors have also observed the influence of the geochemistry of the body of water on Hg biomagnification rate, as correlation between this variable and both productivity (Poste et al., 2015) and physico-chemical features (Clayden et al., 2013) have been found in African and North American lakes, respectively. Poste et al. (2015) calculated Hg TMF of food webs from a number of tropical African lakes assuming a 3.4% enrichment in δ¹⁵N per trophic level (Post, 2002). For these calculations, Poste et al. (2015) used their own data as well as data from other authors who had used the TMS for assessing Hg biomagnification. The calculated TMF values varied from 2.8 in Ziway Lake (Tadiso et al., 2001) to 9.0 in Thurston Bay at Victoria Lake (Campbell et al., 2004). The food webs that presented the lowest TMF values to those found in Puruzinho Lake (4.5 and 5.3) were the benthic web of Bosomtwe Lake (5.3) (Poste et al., 2008), the littoral web of Tanganjika Lake (5.6) (Campbell et al., 2008), Chad Lake (5.2) (Kidd et al., 2004), Lake Albert (5.6), Napoleon Gulf at Victoria Lake (4.8) and Nkuruba Lake (5.6) (Poste et al., 2015). These environments presented different trophic statuses (oligotrophic, mesotrophic and eutrophic) and surface areas (Poste et al., 2013, 2015), which did not allow an association to these factors.

Lavoie et al. (2013) found higher mean TMS values in polar (0.21 and 0.19) and temperate (0.17 and 0.16) than in tropical (0.13 and 0.12) systems, considering both marine (first TMS value between parentheses) and freshwater (second TMS value between parentheses) environments. The authors suggested that the latitudinal difference would be correlated to a biodilution in warm ecosystems, to a reduced excretion of MeHg in cold environments, to physico-chemical aspects, as well as to the fact that trophic webs from cold regions present lower diversity. However, the TMS values observed in Puruzinho Lake (0.19 and 0.21) were similar to those from marine and freshwater polar ecosystems. Some tropical ecosystems presented high TMS values, which was the case of Thurston Bay at Lake Victoria (0.28) (Campbell et al., 2004), Lake Tanganyika (0.22) (Campbell et al., 2008) and Lake Malawi (0.25 e 0.23) (Kidd et al., 2003). Therefore, further studies are necessary for better understanding the latitudinal influence on Hg biomagnification.

The similarity in trophic positions and Hg levels (Dunn, p > 0.05) between planktivorous and piscivorous fish suggests comparable efficiencies of Hg biomagnification in two distinct (pelagic and littoral) food webs. This probably occurs due to the high trophic transfer and the feeding in low trophic levels featured by these two guilds (Layman et al., 2005a; Vander Zanden et al., 1996), as well as to the similar THg transfer rates found between pelagic and littoral food webs. Uryu et al. (2001) observed similar Hg concentrations between some piscivorous species and a planktivorous fish (taxonomic genus Hypopthalmus) in Tapajós river basin (Amazon river basin). Bastos et al. (2008) verified the same scenario while comparing Hg concentrations between planktivorous (Hypopthalmus sp.) and piscivorous (H. malabaricus, P. squamosissimus and Cichlaspp.) fish from Madeira river basin (Amazon river basin).

4.2.2. Influence of migratory species

In the present study, there was no significant difference in the slopes of the log-linear regressions (THg concentration versus TP and THg concentration versus δ¹⁵N) between total (including the migratory species) and resident ichthyofauna (Test t, p = 0.5880;
The migrating iliophagous and omnivorous species exhibited multiple Hg sources integrated by a three-trophic-level food web. Krümmel et al. (2003) also noted that the salmon (Oncorhynchus nerka) acts as a contaminant biovector, transporting polychlorinated biphenyls bioaccumulated in the ocean to the lakes of origin. Fish from tropical river-floodplain systems perform longitudinal (upstream and downstream) and lateral (between river and floodplain systems) migrations, comprising a wide variety of distances (Fernandes, 1997; Agostinho et al., 2003; Diaz-Sarmiento and Alvarez-león, 2003; Harvey and Carolsfeld, 2003; Miranda-Chumacero et al., 2015). During their migrations, the species interact with the trophic web of the environment they occupy, promoting nutrient and energy transfer among different ecosystems (Winemiller and Jepsen, 1998; Miranda-Chumacero et al., 2015). It is important to highlight herbivorous and detritivorous migrating species may represent up to 70% of the freshwater ichthyobiomass of South America, transferring part of the secondary production from one region to the other (Harvey and Carolsfeld, 2003). In oligotrophic environments such as the blackwater rivers, migrating iliophagous fish provide nutrition support capable of augmenting the productivity of piscivorous species above the level they would reach if they relied only on the production of their own ecosystem (Winemiller and Jepsen, 1998). Migratory species play an important role in the distribution of energy and nutrients in tropical river-floodplain systems; therefore, it is plausible that a similar role ends up being played for pollutants as well. Nevertheless, new studies should be performed for dimensioning pollutant transport by migrating fish in this ecosystem and other tropical river-floodplain systems.

5. Conclusion

Regarding Hg input and transfer, δ³¹C and δ¹⁵N values suggest multiple Hg sources integrated by a three-trophic-level food web. The migrating iliophagous and omnivorous species exhibited higher δ¹⁵N values than the iliophagous and omnivorous resident fish, respectively, which resulted in higher trophic positions (TPs) than those predicted for their guilds. This suggests that migration may influence the trophic structure assessment performed with stable isotopes of nitrogen. However, no significant difference in δ¹³C values has been found between migrating and resident iliophagous fish. Mercury biomagnified in Purusinho Lake, with highest Hg concentrations in highest TP species, i.e., piscivorous and planktivorous, which suggests a similar efficiency for Hg transfer through pelagic and littoral food chains. The biomagnification rate in Purusinho Lake was higher than the average values for its latitude, being comparable to values of temperate and polar systems (marine and freshwater environments in both cases), not corroborating thus the general latitudinal pattern. The migrating species did not significantly alter the biomagnification calculation, but they may play a role in Hg transport in Amazon River Basin. Nevertheless, future studies are necessary for shedding further light on the influence of migrating species on Hg transport and biomagnification in tropical river-floodplain systems.

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