Bioacumulação e Biomagnificação de Mercúrio em Peixes do lago Puruzinho (Amazônia Ocidental): influência do regime hidrológico e do nicho isotópico

LUCAS SILVA AZEVEDO

UNIVERSIDADE ESTADUAL DO NORTE FLUMINENSE DARCY RIBEIRO

CAMPOS DOS GOYTACAZES - RJ

MARÇO - 2021

Bioacumulação e Biomagnificação de Mercúrio em Peixes do lago Puruzinho (Amazônia Ocidental): influência do regime hidrológico e do nicho isotópico

LUCAS SILVA AZEVEDO

Tese apresentada ao Centro de Biociências E Biotecnologia da Universidade Estadual do Norte Fluminense Darcy Ribeiro, como parte das exigências para obtenção do título de doutor em Ecologia e Recursos Naturais

Orientador: Prof. Dr. Wanderley Rodrigues Bastos

Coorientador: Dr. Inácio Abreu Pestana

CAMPOS DOS GOYTACAZES - RJ

MARÇO – 2021

FICHA CATALOGRÁFICA

UENF - Bibliotecas
Elaborada com os dados fornecidos pelo autor.

A994 Azevedo, Lucas Silva.

BIOACUMULAÇÃO E BIOMAGNIFICAÇÃO DE MERCÚRIO EM PEIXES DO LAGO PURUZINHO (AMAZÔNIA OCIDENTAL) : INFLUÊNCIA DO REGIME HIDROLÓGICO E DO NICHO ISOTÓPICO / Lucas Silva Azevedo. - Campos dos Goytacazes, RJ, 2021.

149 f. : il.
Inclui bibliografia.

Tese (Doutorado em Ecologia e Recursos Naturais) - Universidade Estadual do Norte Fluminense Darcy Ribeiro, Centro de Biociências e Biotecnologia, 2021.
Orientador: Wanderley Rodrigues Bastos.
Coorientador: Inácio Abreu Pestana.

1. Mercúrio. 2. Amazônia. 3. Nicho Isotópico. 4. Variação Sazonal. 5. Pulso de Inundação. I. Universidade Estadual do Norte Fluminense Darcy Ribeiro. II. Título.

CDD - 577

Bioacumulação e Biomagnificação de Mercúrio em Peixes do lago Puruzinho (Amazônia Ocidental): influência do regime hidrológico e do nicho isotópico

LUCAS SILVA AZEVEDO

Tese apresentada ao Centro de Biociências e Biotecnologia da Universidade Estadual do Norte Fluminense Darcy Ribeiro, como parte das exigências para a obtenção do título de doutor em Ecologia e Recursos Naturais.

Aprovada em: **19/03/2021**

Comissão Examinadora:

Daniele Kasper

Dr.ª Daniele Kasper (Dr.ª em Ecologia) – UFRJ

Prof. Carlos Eduardo Veiga de Carvalho (Dr. em Geoquímica) – UENF.

Marcelo Gomes de Almeida

Dr. Marcelo Gomes Almeida (Dr.ª em Biociências e Biotecnlogia) – UENF

Inácio Abreu Pestana

Dr. Inácio Abreu Pestana (Dr. em Ecologia e Recursos Naturais) - UENF (Coorientador)

Prof. Wanderley Rodrigues Bastos (Dr. em Biofísica) – UNIR (Orientador)

Governo do Estado do Rio de Janeiro
Universidade Estadual do Norte Fluminense Darcy Ribeiro
Pró-Reitoria de Pesquisa e Pós-Graduação

DECLARAÇÃO

Eu, Marina Satika Suzuki, coordenadora do Programa de Pós-Graduação em Ecologia e Recursos Naturais (PPG-ERN) da Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF), seguindo a Resolução CPPG nº2 de 2021, declaro validadas as assinaturas constantes da Folha de Assinaturas da Tese intitulada **“Bioacumulação e Biomagnificação de Mercúrio em Peixes do lago Puruzinho (Amazônia Ocidental): influência do regime hidrológico e do nicho isotópico”** de autoria de Lucas Silva Azevedo, defendida no dia 19 de março de 2021.

Campos dos Goytacazes, 19 maio de 2021

Marina Satika Suzuki
Coordenadora PPG-ERN / UENF
ID. Funcional 641333-1

Documento assinado eletronicamente por **Marina Satika Suzuki**, **Coordenadora**, em 19/05/2021, às 15:25, conforme horário oficial de Brasília, com fundamento nos art. 21º e 22º do Decreto nº 46.730, de 9 de agosto de 2019.

A autenticidade deste documento pode ser conferida no site http://sei.fazenda.rj.gov.br/sei/controlador_externo.php?acao=documento_conferir&id_orgao_acesso_externo=6, informando o código verificador **17212827** e o código CRC **299FAE4A**.

Referência: Processo nº SEI-260009/002124/2021 SEI nº 16736771

“Deixe tudo acontecer a você
Beleza e Terror
Apenas Continue
Nenhum Sentimento é Final”

Rainer Maria Rilke

Dedico este trabalho à memória da Professora Cristal,

Agradecimentos

Agradeço a Professora Cristal que me acolheu como seu aluno no Laboratório de Ciências Ambietnais da UENF em 2013. Desde então enfrentei muitos desafios, mas sempre pude contar com seu apoio profissional e pessoal. Apesar de ter nos deixado em 2020, ela não será esquecida pois sua presença transformou a minha vida e a de muitos outros.

Agradeço ao Professor Wanderley Rodrigues Bastos, que assumiu minha orientação e por sempre me apoiar no que fosse preciso.

Agradeço a todos os membros do grupo de pesquisa da Professora Cristal, Inácio, Annaliza, Clara, Adélia, Dayana, Marcela, Luiza, Layra, Daniel, Ralph, Daniele, Paula e Bruno. Faço questão de mencionar os nomes de todos estes que passaram pela minha vida acadêmica e tornaram-na mais fácil e prazerosa. Dedico um agradecimento especial ao meu amigo Inácio Pestana, pela conversas pessoais e profissionais enriquecedoras, pela parceria e, principalmente, pelo tanto que aprendi com ele sobre os mais variados assuntos.

Agradeço a minha companheira Julia Gazzoni Jardim por estes sete anos maravilhosos que passaram em um piscar de olhos. Enfrentar a vida é mais fácil com você por perto.

Por fim, agradeço a todos os professores e técnicos do LCA, funcionários da UENF, as agêcias de fomento e a todos que tornaram possível chegar até aqui.

SUMÁRIO

Lista de Abreviações

Hg – mercúrio

RW – Rising Water (Enchente)

HW – High Water (Águas Altas)

FW – Falling Water (Vazante)

LW – Low Water (Águas Baixas)

TMS – “Trophic Magnification Slope”

CV–AAS – Espectrometria de Absorção Atômica com Geração de Vapor Frio

p – Probabilidade de ocorrência de um dado, assumindo como verdadeira a hipótese nula

R^2 – Coeficiente de determinaçãomúltiplo de um modelo geral ou generalizado linear

‰ – Partes por mil

(C:N)*a* – Razão atômica de carbono e nitrogênio

$\delta^{13}C$ – Resultado de ($^{13}C/^{12}C_{\text{amostra}}$ / $^{13}C/^{12}C_{\text{da formação Pee Dee}}$ -1) * 1000

$\delta^{15}N$ – Resultado de ($^{15}N/^{14}N_{\text{amostra}}$ / $^{15}N/^{14}N_{\text{nitrogênio atmosférico}}$ -1) * 1000

IRMS – Espectrometria de massa de razões isotópicas

FAPERJ – Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro

CNPq – Conselho Nacional de Desenvolvimento Científico eTecnológico

UENF – Universidade Estadual do Norte Fluminense Darcy Ribeiro

UNIR – Universidade Federal de Rondônia

Lista de Tabelas

Capítulo 1

Capítulo 2

Capítulo 3

Lista de Figuras

Capítulo 1

Capítulo 2

Capítulo 3

Resumo

Esta pesquisa teve como objetivos: (Capítulo 1) avaliar a variação sazonal das concentrações de mercúrio em guildas tróficas (carnívoros, piscívoros, detrítivoros, herbívoros, onívoros e planctívoros) de peixes de um lago de várzea (lago Puruzinho) na Amazônia Ocidetal ao longo das quatro estações do regime hidrológico da região (enchente, águas altas, vazante e águas baixas); (Capítulo 2) avaliar a variação sazonal na biomagnificação de mercúrio em uma cadeia trófica composta por uma espécie herbívora (*Mylossoma duriventre*), uma detritívora (*Prochilodus nigricans*) e duas espécies piscívoras (*Serrasalmus rhombeus* e *Cichla pleiozona*); (Capítulo 3) avaliar a variação na ecologia alimentar das quatro espécies mencionadas anteriormente, utilizando isótopos estáveis de carbono e nitrogênio, ao longo das quatro estações do regime hidrológico do lago Puruzinho. Os principais resultados obtidos foram: (Capítulo 1) um padrão semelhante de variação sazonal nas concentrações de Hg (concentrações mais elevadas na enchente e nas águas baixas e menores nas águas altas e vazante) foi observado nas guildas herbívora, detritívora, carnívora e piscívora; (Capítulo 2) não foi observada variação sazonal no "Trophic magnification slope", métrica utilizada para estimar a biomagnificação, entre regimes hidrológicos. Contudo, o TMS variou significativamente com a composição da cadeia trófica, o que significa que a escolha dos hábitos alimentares que comporão a cadeia trófica tem influência sobre o resultado; (Capítulo 3) o padrão esperado de nichos isotópicos mais amplos nos períodos chuvosos (enchente e águas altas) não foi observado na maior parte das espécies. De uma maneira geral os nichos foram mais amplos na vazante e nas águas baixas. Os resultados apresentados nos capítulos 1, 2 e 3 acrescentam novas informações sobre a relação da acumulação do Hg e do nicho isotópico com o regime hidrológico dos lagos de várzea da Amazônia.

Palavras-chave: Mercúrio; Amazônia; Nicho Isotópico; Variação Sazonal; Pulso de Inundação

Abastract

This research had three aims: (Chapter 1) evaluate the seasonal variation in the mercury concentrations in fish trophic guilds (carnivorous, piscivorous, detritivorous, omnivorous and planctivorous) in floodplain lake (Puruzinho lake) in Western Amazon along the four hydrological seasons (rising water, high water, falling water and low water); (Chapter 2) evalute the seasonal variation in mercury biomagnification in a food chain composed of an herbivorous (*Mylossoma duriventre*), an detritivorous (*Prochilodus nigricans*) and two piscivorous (*Serrasalmus rhombeus* e *Cichla pleiozona*); (Chapter 3) evaluate the variation in fish trophic ecology, using carbon and nitrogen stable isotopes, along the four seasons of the hydrological regime of Puruzinho lake (Chapter 3). The main results obtainded in this research were: (Chapter 1) a similar pattern in seasonal variation of mercury concentrations (higher levels during rising and low water and lower concentrations during high and falling water) was observed among herbivorous, detritivorous, carnivorous and piscivorous trophic guilds; (Chapter 2) seasonal variation in “Trophic magnification slope”, metric used to estimate biomgafnifiication, was not observed among hydrological seasons. However, TMS varied significantly with the food chain structure, which indicated that the selected feeding habits to compose food chain can influence TMS results; (Chapter 3) the expected pattern in isotopic niche width variation (wider niches during rising and high water seasons) was not observed in most of the species. In general, isotopic niches were wider in falling and low water seasons. The results presented in chapter 1, 2 and 3 added new information about the relationship between mercury accumulation and isotopic niche with the hydrological regime of Amazon floodplain lakes.

Keywords: Mercury; Amazon; Isotopic Niche; Seasonal Variation; Flood Pulse.

SOBRE A ESTRUTURA DESSA TESE

Essa tese é composta de:

(1) **Uma introdução geral:** apresenta os temas que serão discutidos nos capítulos 1, 2 e 3;

(2) **Três artigos científicos:** os capítulos da tese foram estruturados na forma de três artigos científicos. Os capítulos 1 e 2 foram publicados e o capítulo 3 está no processo de revisão;

(3) **Uma discussão geral:** nesta seção serão sintetizados os principais resultados descritos nos capítulos 1, 2 e 3;

(4) **Considerações finais**: nesta seção serão apontandas as principais lacunas que foram observadas nos capítulos 1, 2 e 3.

1. Introdução Geral

Os efeitos adversos associados à contaminação por mercúrio (Hg) são conhecidos e envolvem principalmente danos ao sistema nervoso, comprometimento da coordenação motora e, em casos extremos, óbito (Pacheco-Ferreira, 2001; Cordier *et al.,* 2002). Embora seja um elemento ubíquo na natureza, sua concentração entre as diversas matrizes bióticas (animais, plantas, bactérias e etc) e abióticas (sedimento, água, material particulado e etc) varia consideravelmente. Em relação às matrizes bióticas, os peixes, principalmente os predadores, estão entre os maiores acumuladores do elemento devido ao seu elevado potencial de biomagnificação (Lino *et al*., 2019). A biomagnificação, ou seja, o aumento das concentrações de um determinado poluente ao longo dos níveis da cadeia trófica, não é apenas um problema para a biota aquática, mas para os seres humanos que, ao ocuparem o topo da cadeia, estão expostos à concentrações elevadas de contaminantes (Renzoni *et al.,* 1998). É por esta razão que a alimentação, mais especificamente o consumo de peixes, é considerada a principal via de exposição a poluentes que biomagnificam.

A contaminação dos peixes representa um problema sério para a região amazônica, pois as populações humanas desta região são as que mais consomem pescado no Brasil (Lopes *et al.,* 2016). Por isso, diversos estudos se dedicaram a avaliar o grau da exposição humana e da biota ao Hg (Silva *et al.,* 2019; Azevedo *et al.,* 2020). Tanto a população que vive nos grandes centros urbanos, como Manaus e Porto Velho, quanto os ribeirinhos têm preferência elevada pelo pescado como item alimentar, mas os ribeirinhos têm uma dependência maior, pois sua situação econômica precária não permite a diversificação dietética (Oliveira *et al.,* 2010).

A acumulação de poluentes em peixes depende de uma série de fatores ambientais que podem potencializa-la ou reduzi-la. O pH da água, por exemplo, pode influenciar a biodisponibilidade do Hg para a biota (Bisinoti *et al.,* 2007; Vieira *et al.,* 2018). Variações nos parâmetros físico-químicos dos ecossistemas aquáticos amazônicos são marcantes e estão associados às cores de suas águas, de forma geral. Por causa disso, Sioli (1967) classificou os ecossistemas aquáticos amazônicos da seguinte maneira: águas brancas, pretas e claras. Os rios de águas brancas (e.g., Madeira, Amazonas, Purus) nascem nos Andes e arrastam grande quantidade de material particulado em suspensão e sedimentos, o que confere uma cor barrenta para estes rios. Os rios e lagos de águas pretas (e.g., Negro, Jutaí, Tefé) têm cor escura devido à grande quantidade de matéria orgânica dissolvida e, também, baixo pH. Os rios de águas claras (e.g., Tapajós e Xingu) nascem nas regiões dos Escudos do Brasil Central e das Guianas, drenam solos menos erodidos e, por isso, as concentrações de material particulado em suspensão e de matéria orgânica são muito baixas. Dentre os três tipos mencionados, Vieira *et al.* (2018) reportaram uma tendência de maiores concentrações de Hg na biota associada a ecossistemas de águas pretas, o que pode ser explicado pelo pH ácido que aumenta a solubilidade do metal facilitando sua absorção.

A hidrodinâmica dos lagos de várzea da Amazônia (i.e., formação geológica comum na região, caracterizada por um seção lêntica, que corresponde ao lago propriamente dito, e um canal que conecta a seção lêntica a um rio) está sujeita à influência do pulso de inundação (i.e., processo de inundação seguido por seca, que ocorre anualmente em períodos relativamente definidos) (Junk, 1997) que promove, durante o período chuvoso, um aumento considerável na área dos lagos resultando na inundação das áreas adjacentes. A inundação traz consigo uma série de alterações ecológicas. Entre elas está o fato de as novas áreas inundadas oferecerem novos recursos alimentares para os peixes, diversificando sua dieta. Em contrapartida, durante o período seco a tendência é que a diversificação da dieta seja reduzida, pois os peixes ficam confinados em um espaço menor e a entrada de recursos alimentares terrestres é limitada. Portanto, presume-se que a amplitude do nicho trófico dos peixes nos lagos de várzea seja influenciada pelo pulso de inundação. Recursos alimentares não são os únicos materiais que são arrastados para os lagos de várzea durante o período chuvoso: o Hg adsorvido às partículas do solo também é transportado para os lagos. O aporte de Hg não significa necessariamente que as suas concentrações na biota aumentarão, pois, o metal pode ser diluído no grande volume de água. As variações sazonais na bioacumulação Hg em peixes de lagos de várzea e rios da região Amazônica foram reportadas por outros autores (Amaro *et al.,* 2014; *Silva et al.,* 2019), entretanto ainda não existe um padrão consistente nesta variação.

As inundações e secas periódicas, características do pulso de inundação, alteram a disponibilidade de recursos alimentares para os pexes (Meroná e Rankin-de-Meroná, 2004; Oliveira *et al.,* 2006), o que influencia a ecologia trófica das espécies, resultando em nichos mais ou menos amplos (Pool *et al.,* 2017). Durante o período de inundação, os peixes têm acesso a recursos alimentares terresteres, como frutas, sementes, folhas, insetos e etc, enquanto durante a estação seca as fontes autótctones predominam (Junk *et al.,* 1997). Na região Amazônica, estudos sobre a influência das inundações e secas periódicas sobre a ecologia trófica de peixes de lagos de várzea ainda são escassos. Neste contexto, as análises isotópicas de carbono e nitrogênio representam uma alternativa eficaz pois eles têm sido utilizados com sucesso para elucidar a ecologia trófica de comunidades de peixes e de outros animais (Herlevi *et al.,* 2018).

O presente estudo visa abordar em três capítulos os seguintes temas: no capítulo 1 será abordada a variação na bioacumulação de Hg em peixes de diversas guildas tróficas em um lago de várzea (lago Puruzinho, Humaitá-AM) ao longo dos quadro períodos do ciclo hidrológico da Amazônia (enchente, águas altas, vazante, águas baixas). No capítulo 2 será aborada a variação sazonal na biomagnificação de Hg em uma cadeia trófica composta por peixes do lago Puruzinho. Finalmente, no capítulo 3 será aborada a variação sazonal na amplitude de nicho trófico de espécies herbívoras, detritívoras e piscívoras. Com os resultados desta tese espera-se definir os padrões da variação sazonal da bioacumulação, biomagnificação e amplitude de nicho trófico em peixes de um lago de várzea amazonense.

CAPÍTULO 1: **Mercury concentration in six fish guilds from a floodplain lake in western Amazonia: Interaction between seasonality and feeding habits**

Publicado na revista *Ecological Indicators* (Qualis A2 – Biodiversidade), Fator de Impacto (4,22). DOI:10.1016/j.ecolind.2019.106056

Mercury concentration in six fish guilds from a floodplain lake in western Amazonia: Interaction between seasonality and feeding habits

Lucas Silva Azevedo[a,*], Inácio Abreu Pestana[a], Adriely Ferreira da Costa Nery[b], Wanderley Rodrigues Bastos[b], Cristina Maria Magalhães Souza[a]

[a]Laboratório de Ciências Ambientais, Centro de Biociências e Biotecnologia, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, Rio de Janeiro, RJ, CEP: 28013-602, Brazil.
[b]Laboratório de Biogeoquímica Ambiental, Universidade Federal de Rondônia, Porto Velho, Rondônia, RO, CEP: 76815-800, Brazil.

ABSTRACT

Floodplain systems are widespread in the Amazon and several studies have evaluated mercury biogeochemistry in these systems. The Amazon region can be considered a “hotspot” for the study of mercury (Hg), due to the naturally high concentration of the pollutant in its old soils and the anthropogenic emissions from small-scale gold mining activities. The periodic flooding influences the availability and distribution of Hg contamination. Therefore, it is possible that flooding can influence accumulation of Hg in fish. The aim of this study was to evaluate total Hg (THg) concentration in four seasons (rising water, high water, falling water and low water) in six fish guilds in a lentic part of a floodplain system (Puruzinho Lake). We hypothesized that fish guilds would show different seasonal patterns of THg concentration variation. A total of 2016 fish specimens were sampled over the four seasons. Two-way ANOVA indicated an interaction between guilds and seasonality (F= 7.3; $p < 0.001$), suggesting different seasonal patterns of THg concentration variation among the guilds. Two major patterns were identified. Herbivorous, detritivorous, carnivorous and piscivorous fish guilds showed higher concentrations during the rising and low water seasons, while planktivorous and omnivorous guilds showed lower THg levels during the low water season. There were no significant differences in THg levels between the early and late periods of each season.

Keywords: Mercury, Fish, Amazon, Seasonal Variation, Floodplain

1. Introduction

Floodplain systems in the Amazon are composed of a lentic section (also known as “várzea” lakes), which are connected to a river by a channel (locally called “igarapés”), a formation that is widespread across Amazonia (Affonso et al., 2015; Feitosa et al., 2019). A particular characteristic of these systems is that they are periodically flooded by the river during the high water season (known as the “flood pulse” effect), while in the low water season the water level drops significantly (Junk, 1997; Pestana et al., 2019). For instance, in the floodplain system of the Japurá and Solimões rivers, the water level in the high water period was 38.2 m while in the low water period it was 26.7 m (Affonso et al., 2015).

Limnological variables of lentic and lotic sections vary significantly between seasons: during high water, the parameters are more homogeneous among lake, channel and river due to the large volume of water that interconnects all three sections, while during the low water season the differences in limnological parameters between the lotic and lentic sections are more pronounced due to the loss of connectivity (Forsberg et al., 1988; Barbosa et al., 2010; Almeida and Melo, 2009; Affonso et al., 2011; Pedro et al., 2013; Brito et al., 2014). In the Puruzinho lake system, the coefficient of variation of Secchi depth, maximum depth, total nitrogen, total phosphorus and soluble reactive phosphorus concentrations were almost two times higher in the low water than high water season (Feitosa et al., 2019), indicating the homogeneity of the parameters in the latter.

Amazonian water bodies are classified according to three water types: “black water”, which have acid pH and high concentration of dissolved organic matter; “white water”, which have neutral-basic pH and high content of particulate matter; and “clear water”, with very low content of particulate matter (Sioli, 1967).

Water types differ in availability of contaminants, like mercury (Hg) (Vieira et al.., 2018). The fractions of reactive Hg (i.e., fraction available for biogeochemical reactions) in black and white waters are 24% and 8%, respectively (Bisinoti et al., 2007). There is a trend of black > clear > white for total Hg concentration in sediment and biota (plankton and macroinvertebrates) (Vieira et al., 2018).

Among Hg chemical species, methylmercury (MeHg) is the most toxic and bioaccumulative. In the Madeira River Basin, MeHg represents 70-92 % of the total Hg accumulated in fish muscle (Bastos et al., 2015). MeHg production in aquatic environments is increased by acid pH, high dissolved organic matter concentration and low dissolved oxygen concentration, and mostly results from the action of sulfate- and iron-reducing bacteria (Benoit et al., 2002; Si et al., 2015). Due to their particular water chemistry, black water ecosystems have higher methylation rates of Hg in comparison with white and clear water systems (Watras et al., 1995; Maurice-Bourgoin et al., 2003; Vieira et al., 2018). In addition to the type of water, the methylation rate is also influenced by seasons (Guimarães et al., 2000; Coelho-Souza et al., 2011; Lazaro et al., 2016). In four floodplain lakes in the Tapajos River Basin, net MeHg methylation increased from 6.2 ± 2.5% at the start of the dry season to 9.0 ± 7.2 % in the peak flood period (Coelho-Souza et al., 2011).

The periodic flood pulse in floodplain systems changes the water chemistry (Melack and Forsberg, 2001) and can influence Hg geochemistry (Bisinoti et al., 2007). There is a strong negative correlation between the water level of the Tarumã

River and total dissolved Hg concentrations (Bisinoti et al., 2007). Total Hg levels were three times higher during low water season in comparison with high water (Bisinoti et al., 2007). Also, Jardim et al. (2010) reported that in black water ecosystem during the dry period, the oxidative water conditions are higher, which promotes the conversion of Hg^0 to Hg^{2+}, inhibiting the water/atmosphere flux and increasing accumulation. Nutrient inputs are higher during flooding (Agostinho et al., 2004), which increases primary productivity. There is evidence that Hg concentration in fish and other consumers decreases in more productive ecosystems due to the biodilution effect caused by the algal bloom (Pickhardt et al., 2005; Chen and Folt, 2005; Brito et al., 2017). The changes in Hg biogeochemistry during the seasons suggest that fish Hg accumulation is also susceptible to seasonal variation.

It is well known that the major pathway of exposure to Hg of fish is their food. Due to the process of biomagnification, plant resources generally accumulate less Hg than animal food resources (Arcagni et al., 2017; Arcagni et al., 2018; Lino et al., 2019). Hence, there is a tendency for lower concentrations of Hg in herbivorous and detritivorous fish in comparison with carnivorous and piscivorous ones. The flood pulse can influence the availability of food resources to fish (Junk and Wantzen, 2004; Fernandes et al., 2009). Some fish guilds, like carnivorous and piscivorous, have more availability of prey during the low water season when prey are confined in shallow environments (Vazzoler, 1996; Winemiller, 2004; Luz-Agostinho et al., 2009). For herbivorous, omnivorous and detritivorous guilds, the high water season is when more food is available (Almeida et al., 1993; Gomes and Agostinho, 1997; Cunico et al., 2002; Winemiller, 2004). The planktivorous guild has an omnivorous diet since the fish feed on both phyto and zooplankton, so the high water season can provide better feeding conditions for this guild as well.

The aim of this study was to evaluate seasonal variation of THg concentration in carnivorous, herbivorous, omnivorous, planktivorous, detritivorous and piscivorous fish from Puruzinho Lake, western Amazonia, during the rising water (RW), high water (HW), falling water (FW) and low water (LW) seasons. We expected: (1) an interaction between seasons and feeding habits; (2) higher concentrations of THg in herbivorous, detritivorous, omnivorous and planktivorous guilds during high water season, when food is more available; and (3) higher concentrations of THg in carnivorous and piscivorous guilds during the low water season.

2. Material and Methods

2.1 Study Area

Puruzinho Lake is located in the municipality of Humaitá, state of Amazonas, Brazil, in the Madeira River Basin (63°6’0’’W; 7°24’0’’S) (Figure S1). It is connected with the Madeira River by an 8 km channel forming a floodplain system. The lake is the lentic part of this system and has an area of 8.2 km^2. The Madeira River is a whitewater ecosystem with basic pH that is rich in particulate matter (Sioli, 1967), while the lake is a black water ecosystem with acid pH and high concentrations of dissolved organic matter (Wissmar et al., 1981). The local hydrological cycle is marked by four seasons: rising water (RW), high water (HW), falling water (FW) and low water (LW). The RW season starts in October (early RW) and reaches its peak in February (late RW). The HW begins in February (early HW) and ends in May (late

HW). In this season, the Madeira River overflows and mixes with the lake waters for weeks to months. The water level starts to drop in May (early FW) and reaches the low point in September (late FW). The LW season starts in September (early LW) and ends in October (late LW). The RW and HW can be considered as the rainy season and FW and LW as the dry season. There are significant differences in lake depth between seasons. In the rainy season, depth reaches 11.00 ± 0.90 m while during the dry season the value drops to 0.90 ± 0.60 m (Almeida et al., 2014). The variations in lake depth follow the variation in the Madeira River depth (Feitosa et al., 2019).

2.2 Sampling

Sampling campaigns occurred in December 2016 and 2018 (early RW) and February 2017 and 2018 (late RW); April 2017 and 2018 (late HW); June 2017 and 2018 (early FW); August 2018 (late FW) and October 2018 (late FW); and October 2017 (early LW) and December 2017 (late LW) (Table S1). The terms "early" and "late" indicate the beginning and the end of the season, respectively. Therefore, the sampling of December 2016 and 2018 was done in the beginning of the RW season while the sampling in December 2017 was done at the end of the LW season. Although we do not have data about Puruzinho Lake's water level during the sampling months, data about flow and water level of the Madeira River (ANA, 2019), measured at the Humaitá station (Puruzinho Lake is located 20 km from the urban area of Humaitá) (Fig. 1) followed the regular hydrological cycle of the region. The water flow and level from December 2016 to December 2018 are presented in Table S1.

The total number of samples was 2,016, seasonally divided among RW 565, HW 277, FW 857 and LW 317 (Table S2). A total of 28 species of 6 guilds were sampled: Carnivorous - *Ageneiosus inermis*, *Calophysus macropterus*, *Cichla pleiozona*, *Hoplias malabaricus*, *Pellona flavipinnis*, and *Plasgioscion squamosissimus*; Detritivorous - *Potamorhina altamazonica*, *Potamorhina latior*, *Prochilodus nigricans*, *Psectrogaster amazonica*, *Semaprochilodus insignis*, *Hemiodus unimaculatus* and *Psectrogaster rutiloides*; Herbivorous - *Mylossoma*

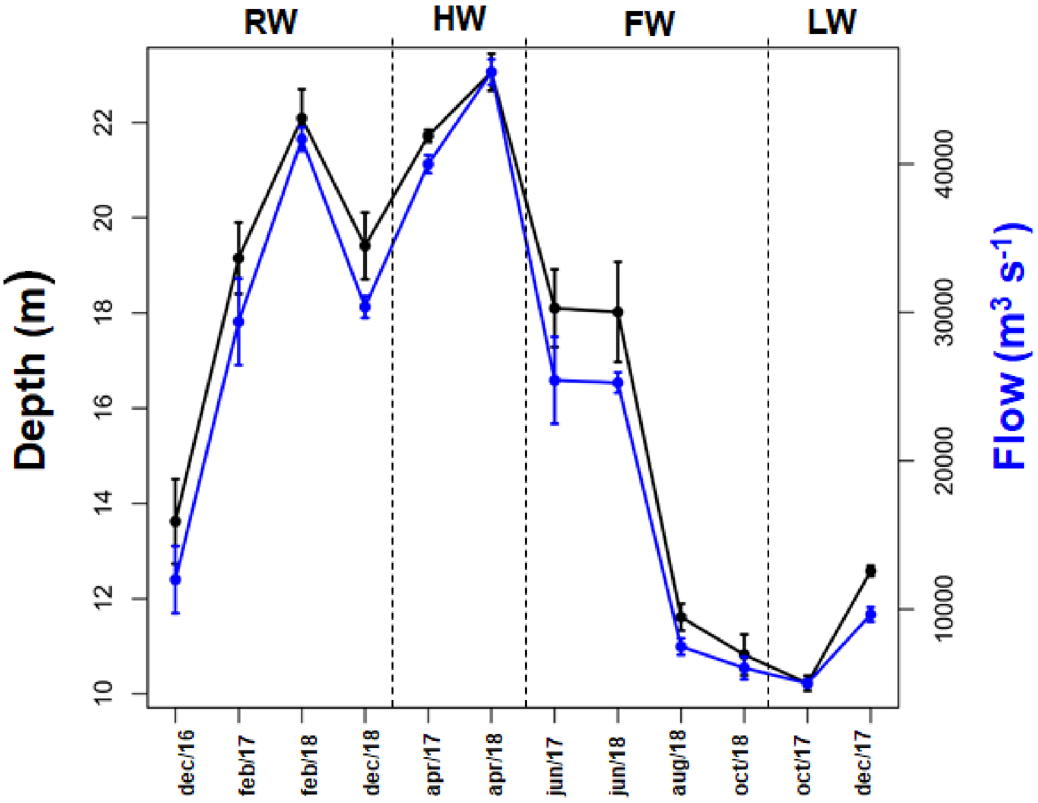


Figure 1. Depth (m) and flow (m^3 s^{-1}) of Madeira River measured at Humaitá station during the sampling months. Puruzinho Lake is located 20 km from the urban area of Humaitá. Data retrieved from ANA (2019). RW: Rising Water; HW: High Water; FW: Falling Water; LW: Low Water.

duriventre and *Schizodon fasciatus*; Piscivorous - *Pellona castelnaeana*, *Rhaphiodon vulpinus*, *Serrasalmus rhombeus*, *Acestrorhynchus falcirostris* and *Pinirampus pirinampu*; Omnivorous - *Pimelodus blochii*, *Triportheus albus*, *Leporinus friderici*, *Phractocephalus hemioliopterus* and *Brycon* sp.; and Planktivorous - *Anodus elongates*, *Hypophthalmus marginatus* and *Hypophthalmus edentates*. These six guilds were chosen because they are often cited in studies of Amazonian ecosystems and represent the feeding habits of regional ichthyofauna (Bastos et al., 2008). Therefore, the seasonal patterns of Hg concentration encompass the most common feeding behavior in Amazon freshwater ecosystems. Samples were obtained using nets with mesh sizes of 30, 45 and 60 mm. The sampling was performed in the lentic section of floodplain system to maximize fish capture. No fish were captured in the channel or in the lotic section. Adult fish species were stored in boxes with ice and taken to the Environmental Biogeochemistry Laboratory Rondônia Federal University (UNIR). Skinless white dorsal muscle was extracted using scalpel and scissors. Samples were stored in plastic containers and placed in a freezer until chemical analysis.

2.3 Chemical Analysis

A total of 1625 samples were analyzed for total Hg (THg) in fish muscle (0.2 g wet wt) following the protocol described by Bastos et al. (1998). We added 391 data of THg levels in fish from Puruzinho lake published by Azevedo et al. (2019), resulting in N = 2,016. Digestion of fish muscle (0.2 g wet wt) followed the method described by Bastos et al. (1998): (i) 1 mL of 30% H_2O_2 and 4 mL of 65% HNO_3: 98% H_2SO_4 (1:1); (ii) heating in a Tecnal digestion block (model: TE04/25) for 30 min at 70 ºC; (iii) cooling at room temperature; (iv) 5% $KMnO_4$; heating for 15 min; (v) cooling at room temperature overnight; (vi) 12% $NH_2OH.HCl$; and (vii) addition of ultrapure water to a final volume of 12 mL. Determination was performed by cold vapor atomic absorption spectrometry (CVAAS), with a flow injection mercury system (FIMS-400) from PerkinElmer (Germany). Blanks were used to control the quality of the reaction medium. The method's detection limit was 0.007 mg kg^{-1}. Certified material (DORM-2) was analyzed in triplicate for each 30 samples (recuperation: 99±2.5%). Each sample was analyzed two times to test precision. Results are shown in mg kg^{-1} wet weight.

2.4 Data Analysis

Two-way ANOVA was applied to test for interaction between feeding habits and seasonality. The Tukey test was used to compare THg concentrations between the combinations of feeding habits and seasonality ($\alpha = 0.05$). THg concentrations were not standardized by size because the majority of guilds did not show significant relationships between THg levels and length or weight (Table 1). Only 39.0% of the correlation coefficients of the THg-length relationship were significant, and among these more than half (55.0%) showed weak correlations (i.e., 0.07-0.22). The relationship between THg-weight followed the same pattern as the THg-length (Table 1). Therefore, the influence of size in the analysis was negligible. Mean and standard deviation of total length are summarized in Table S2. Linear regressions were used to evaluate the influence of lake water level on fish Hg concentration. The models, confidence intervals and prediction intervals, both calculated with 95% certainty, were graphically presented with their determination coefficients (R^2) and p values. The p values were corrected using the Bonferroni method to maintain the overall error level type I at α = 0.05. When necessary, a maximum likelihood function

Table 1. Linear regression between THg levels and length and weight (α = 0.05)

Guild		**Length**			**Weight**		
		R^2	***p***	**slope**	**R^2**	***p***	**slope**
Carnivorous	RW	0.03	0.19	-0.002	0.01	0.31	-0.0005
	HW	<0.01	0.81	-0.001	0.01	0.37	-0.0001
	FW	<0.01	0.29	0.009	<0.01	0.80	-0.00003
	LW	<0.01	0.86	0.0003	0.06	0.06	0.001
Piscivorous	RW	0.03	0.05	0.009	<0.04	0.47	0.0002
	HW	0.02	0.13	-0.01	0.07	0.05	-0.0005
	FW	**0.22**	**<0.01**	**0.02**	**0.12**	**<0.01**	**0.0009**
	LW	<0.01	0.50	-0.001	0.01	0.19	0.0002
Detritivorous	RW	**0.13**	**<0.01**	**0.01**	**0.06**	**<0.05**	**0.0003**
	HW	**0.07**	**0.01**	**0.004**	**0.06**	**0.03**	**0.0002**
	FW	**0.11**	**<0.01**	**0.008**	**0.34**	**<0.01**	**0.003**
	LW	0.01	0.39	0.002	0.01	0.39	0.0001
Herbivorous	RW	**0.68**	**<0.01**	**0.03**	**0.41**	**<0.01**	**0.002**
	HW	**0.33**	**<0.01**	**0.01**	**0.78**	**<0.01**	**0.001**
	FW	**0.47**	**<0.01**	**0.01**	**0.38**	**<0.01**	**0.001**
	LW	0.26	0.13	0.009	0.02	0.65	0.0003
Planktivorous	RW	<0.01	0.98	-0.0001	0.06	0.05	0.0006
	HW	0.44	0.06	0.02	<0.05	0.85	0.0001
	FW	0.05	0.05	0.01	0.03	0.10	0.0009
	LW	0.01	0.29	0.004	0.03	0.15	0.0002
Omnivorous	RW	**0.46**	**<0.04**	**-0.03**	**0.48**	**<0.03**	**-0.001**
	HW	-	-	-	-	-	-
	FW	**0.17**	**<0.01**	**0.007**	**0.18**	**<0.05**	**0.0007**
	LW	0.01	0.50	0.006	0.04	0.27	0.0004

Bold: significant relationships. RW: Rising Water; HW: High Water; FW: Falling Water; LW: Low Water.

(Box-Cox, MASS Package, Venables and Ripley 2002) was used to transform the variables and meet the ANOVA and linear regressions requirements (normality and homoscedasticity of residuals). All statistical analysis and graphing were carried out with the R software (R Core Team, 2019).

3. Results

The two-way ANOVA showed a significant interaction between seasonality and feeding habit ($F_{15,1989}$= 7.3; $p < 0.001$), which indicates that these variables cannot be interpreted independently from each other. THg concentration varied seasonally among guilds in two different patterns (Fig. 2). In the first pattern, which was observed in herbivorous, detritivorous, carnivorous and piscivorous guilds (Fig. 2), there was a tendency for slightly higher mean concentrations during the RW and LW and lower mean concentrations during HW and FW. In the second pattern, which was observed in the omnivorous and planktivorous guilds, there was a tendency for decreasing mean concentrations during FW and LW (Fig. 2). Additionally, THg concentrations in planktivorous and omnivorous guild during RW were similar to FW (Fig. 2).

The water level of the lotic fraction of floodplain systems is a reliable proxy for the lentic fraction water level due to the connectivity (Feitosa et al., 2019). Linear regression analysis between Madeira River water level and THg concentrations in fish guilds (Figure S2) showed non-significant negative trends in carnivorous (Fig.

S2 A) detritivorous (Fig. S2 B), herbivorous (Fig. S2 C), omnivorous (Fig. S2 D), and piscivorous (Fig. S2 E) guilds.

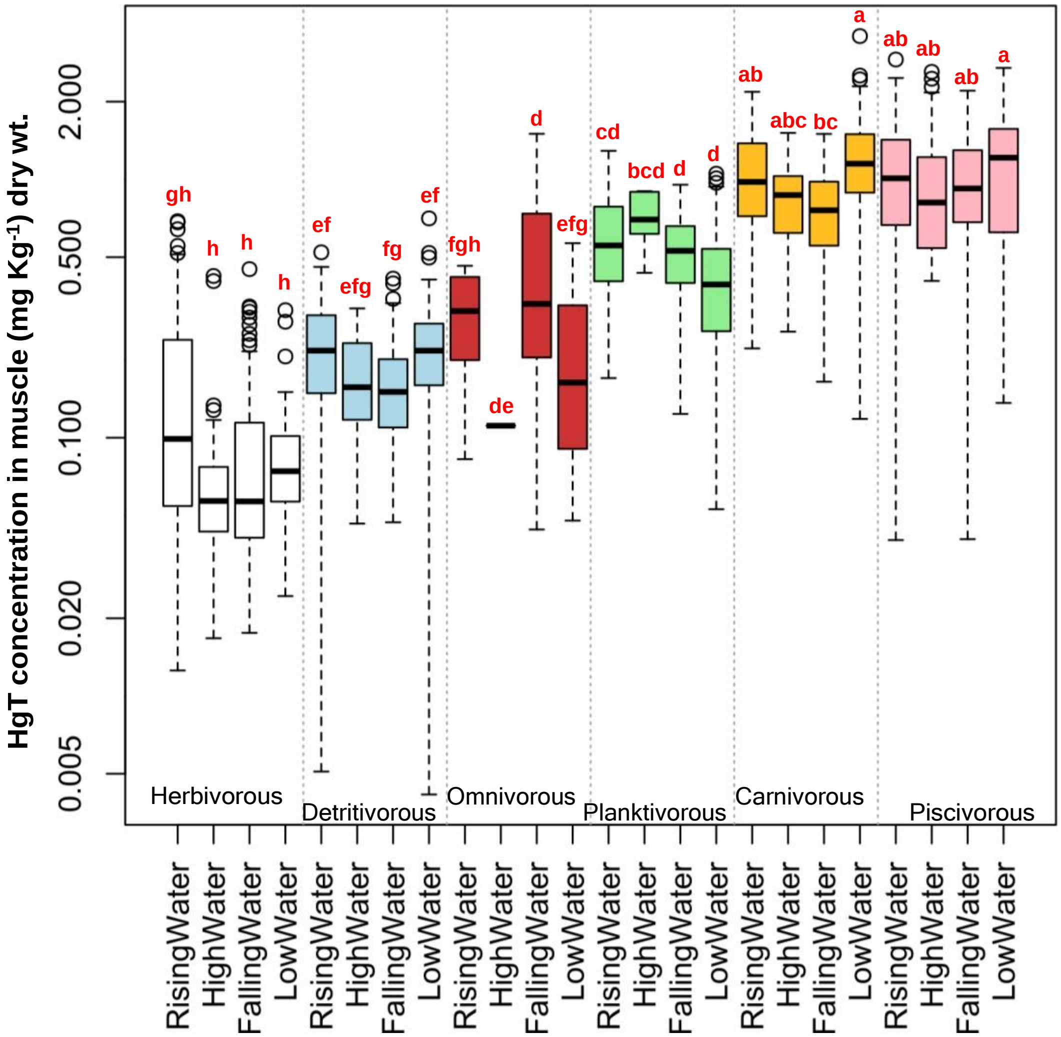


Figure 2. THg concentrations (mg kg^{-1} wet wt) in six guilds (herbivorous, detritivorous, omnivorous, planktivorous, carnivorous and piscivorous) from Puruzinho Lake during the four seasons. Different letters indicate statistical differences (α = 0.05). Two-way ANOVA indicated interaction between feeding habits and seasonality ($F_{15,1989}$= 7.3; $p < 0.001$). Two major groups were formed in respect to seasonal patterns: (1) herbivorous, detritivorous, carnivorous and

piscivorous guilds; and (2) omnivorous and planktivorous guilds. Distance between y-axis values were log-transformed to optimize data visualization.

A positive trend was observed only in the planktivorous fish guild, but the *p*-value was also non-significant. THg concentrations in plankton from Puruzinho Lake were significantly higher during the rainy season, due to the leaching of Hg from soil to the water column and subsequent absorption by phytoplankton, which can explain the positive trend observed in planktivorous fish (Nascimento et al., 2007).

To test the interaction between early-late periods of the seasons and feeding habits, two-way ANOVA was conducted with the six guilds, but in addition considering the early and late periods of each season (Fig. S3). The results also indicated an interaction between feeding habits and seasonality (feeding habits - seasonality, $F_{29,1972}= 7.1$; $p < 0.001$) (Fig. S3), similar to the results presented in Fig. 2. Despite differences in THg concentrations between late RW and early RW in herbivorous, detritivorous, omnivorous, planktivorous and carnivorous guilds, these differences were not significant ($p > 0.05$). The same pattern was observed between early FW and late FW and early LW and late LW (Fig. S3). In general, the comparison between “early” and “late” periods of RW, FW and LW indicated no significant differences in THg concentrations between the two periods ($p > 0.05$).

There was a clear tendency for higher concentrations of THg in carnivorous and piscivorous fish and lower in detritivorous and herbivorous guilds (Fig. 2; Fig. S3). Omnivorous and planktivorous had intermediate concentrations (Fig. 2; Fig. S3). This pattern is well documented in the literature (Wang et al., 2012; Azevedo-Silva et al., 2016; Signa et al., 2017; Arcagni et al., 2018; Rumbold et al., 2018) and is a consequence of the high biomagnification rate of Hg.

4. Discussion

4.1 Seasonal Variation in Fish Guilds

Although Hg accumulation is a relatively slow process, several authors have reported seasonal variation in THg levels in fish from the Amazon (Kehrig and Malm, 1999; Dórea et al., 2006; Sampaio da Silva et al., 2006; Bastos et al., 2007; Bastos et al., 2008; Silva et al., 2019) and from other regions (Fowlie et al., 2008; Burger and Gochfeld, 2011; Greenfield et al., 2013; Braaten et al., 2014; Mills et al., 2018). These studies suggest that ecological and geochemical factors can increase or decrease Hg accumulation in fish efficiently enough to produce a seasonal variation. For instance, Hg levels in perch (*Perca fluviatilis*), from two boreal lakes, was significantly higher during spring and lower in the summer (Braaten et al., 2014). The lower Hg concentration in summer was attributed to growth dilution (i.e., reduction in Hg levels in fish due to higher growth rates with warmer temperatures) (Braaten et al., 2014). In San Francisco Bay, the arrow goby (*Clevelandia ios*) showed consistent seasonal variation with highest concentration in late summer or early fall and lowest in late spring (Greenfield et al., 2013). This consistent pattern is related to the seasonal shifts in net MeHg production (Greenfield et al., 2013). In the Amazon, other authors have also observed seasonal shifts in net MeHg production (Coelho-Souza et al., 2011; Kasper et al., 2014; Brito et al., 2017; Lazaro et al., 2018), which could explain the two consistent patterns of seasonal variation in THg levels: (1) higher levels of THg in RW and LW for detritivorous, herbivorous, carnivorous and piscivorous guilds; (2) lower THg levels during FW and LW for omnivorous and planktivorous guilds.

Higher methylation rates in RW is expected, since increases in water level leads to exactification, thus to anoxia in bottom sediments, creating a suitable environment for sulfate reducing bacteria to convert Hg into MeHg (Coelho-Sousa et al., 2011; Brito et al., 2017; Lazaro et al., 2018). Although during the LW high methylation rates are not expected, Lazaro et al. (2018) reported that in this season MeHg production can reach 15.3-26.2%, against 21.3-37.2% in the HW. Even though the methylation rate is expected to be highest in HW (Coelho-Sousa et al., 2011; Lazaro et al., 2018), in our study THg levels were significantly lower during HW in herbivorous, detritivorous, carnivorous and piscivorous fish guilds. This pattern is related not to the MeHg production, but to the mixing of Puruzinho Lake black water and Madeira River white water.

During the HW season, Madeira River water levels and flow reach peaks, causing mixture with water of Puruzinho lake. The two ecosystems differ in water chemistry and Hg availability (Silva et al., 2009; Vieira et al., 2018). In white water habitats (e.g., Madeira River), with pH close to neutral, photogenerated H_2O_2 is able to reduce Hg^{2+} to Hg^0 (Silva et al., 2009). Hg^0 tends to be deposited in the bottom sediment due to its high density, or escape the system when volatilized, which in turn reduces its availability to biota. Additionally, other authors have observed lower Hg concentration in the dissolved phase when black and white waters are mixed

(Maurice-Bourgoin et al., 2003). Therefore, the lower concentrations of THg observed in HW in this study can be related to the high influx of white water during this season. It is important to mention that in the HW season, the rainfall declines and the high volume of water is derived from the high rainfall in the previous season, RW (Azevedo et al., 2019), so the input of Hg from the soil and the catchment is minimized.

The RW season is marked by a gradual increase in water level due to high rainfall. Processes like resuspension of Hg accumulated in sediments, Hg wet deposition, dissolved Hg accumulation and Hg lixiviation from soil are intensified during high rainfall periods (Suzuki et al., 1991; Roulet et al., 1999; Veiga and Hinton, 2002; Moreno-Brush et al., 2016; Brito et al., 2017). The LW season is considered the peak of the dry season, since the water level reaches the lowest values. Other studies have suggested higher availability of Hg during the dry season (Bisinoti et al., 2007; Jardim et al., 2011) and higher concentration of the contaminant in fish (Azevedo et al., 2018; Martinez et al., 2018). These processes may explain the tendency for increased THg levels in herbivorous, detritivorous, carnivorous and piscivorous guilds during RW and LW. Although geochemical aspects of the floodplain system control Hg availability and speciation, aspects of fish ecology, like feeding, can play an important role in the observed results.

It is well established that the major pathway of fish exposure to Hg is diet. The periodic flooding of floodplain systems changes the proportion of allochthonous and autochthonous inputs (Junk and Wantzen, 2004; Fernandes et al., 2009), which influences fish diet. For instance, the maximum abundance of resources for herbivorous, omnivorous and detritivorous fish guilds occurs during the rising water period (Gomes and Agostinho, 1997; Almeida et al., 1993; Cunico et al., 2002; Winemiller, 2004), when newly flooded areas provide more food, while for piscivorous fish the availability of food is maximum during the LW season when prey are confined in small and shallow systems, facilitating hunting (Vazzoler, 1996; Winemiller, 2004; Luz-Agostinho et al., 2009). Based on these data, THg concentration in herbivorous and omnivorous fish guilds should be higher during RW and in piscivorous fish during LW. Our results are partially in agreement with that assumption, since higher levels of THg were observed in herbivorous and piscivorous fish guilds during RW and LW, respectively. Flood pulse influences fish guilds in trophic habitats differently (Vazzoler, 1996; Cunico et al., 2002; Winemiller, 2004; Luz-Agostinho et al., 2009), so the interaction effect observed in our results is related to how each guild's trophic ecology responds to the periodic flooding. However, to the best of our knowledge there are no studies in the Amazon floodplain system that have related variations in fish guilds' trophic ecology and Hg accumulation.

Although Bisinoti et al. (2007) and Jardim et al. (2011) suggested that Hg availability is higher during the dry season, THg concentrations in RW were similar to those in LW in herbivorous, detritivorous, carnivorous and piscivorous fish. The statistically similar concentrations between the two seasons may be related to the sampling campaign design. The RW season is composed of two distinct periods, the early RW (December 2016 and 2018), when the water level starts to rise, but does not reach its peak, and the late RW (February 2017 and 2018), when the water reaches its highest level. The LW season achieved its peak in the early LW (October 2017) and late LW (December 2017), when there was a slight increase in the water level, marking the beginning of the RW period. The similar conditions between early RW and late LW may account for the similarity in THg concentrations in detritivorous, herbivorous, carnivorous and piscivorous fish.

Our results suggest that pooling the early and late periods to represent the season (e.g., RW season = early RW + late RW) is an adequate approach to evaluate seasonal variation in THg concentration in herbivorous, detritivorous, omnivorous, planktivorous, carnivorous and piscivorous guilds. This information is critical for experimental designs in the study area (Amazon floodplain system). For instance, researchers can choose to sample fish during the early or late stage of a given season without the risk of not representing the season, thus reducing the sampling effort. Remote areas in the Amazon, like the Puruzinho Floodplain System, are difficult to access and a reduced sampling effort means that financial resources can be applied to other needs (e.g., chemical analysis, equipment).

4.2 Considerations about THg Seasonal Variation

In this study, we confirmed the occurrence of two major seasonal patterns of THg variation among six fish guilds. Although we believe the influence of ecological and geochemical mechanisms could explain the seasonal variation patterns, we did not test which factors can account for this variation.

High primary productivity is linked to low THg accumulation in biota (i.e., algal biodilution) in aquatic ecosystem in Africa (Poste et al., 2015), Asia (Razavi et al., 2014; Razavi et al., 2015) and the Amazon (Brito et al., 2017). In Amazon floodplain lakes primary productivity changes drastically along the hydrological seasons with mean values of 0.29 µg L^{-1} of chlorophyll-*a* (a proxy for primary productivity) during HW and 113.03 µg L^{-1} during LW (Affonso et al., 2011). Nitrogen concentration also increases by one order of magnitude in LW (HW= 0.18 mg L^{-1}; LW = 1.62 mg L^{-1}) (Affonso et al., 2011). It is possible that the drastic variation in primary productivity along the hydrological periods accounted for the seasonal variation in THg levels in fish from Puruzinho Lake.

There are several other factors that can influence THg accumulation, like DOC concentration, soil coverage, lake area (Eagle-Smith et al., 2016) and forest harvest (Garcia et al., 2006; Wu et al., 2018). Although the mentioned factors are relevance to understand Hg biogeochemistry, the influence of forest harvest on Hg accumulation in Amazon fish is a hot topic for three reasons. First, after three years of forest harvest, a significant increase of 26% of Hg concentration in large fish was observed (Wu et al., 2018). Second, deforestation in the Amazon is increasing due to expansion of infrastructure and agriculture (Soares-Filho et al., 2006). Third, in the Amazon the ingestion of fish in the diet reaches 369 g per capita day^{-1} in the Lower Amazon River Basin (Cerdeira et al., 1997), 550 g per capita day^{-1} in the Lower Solimões and Upper Amazon basin (Batista et al., 1998) and 243 g per capita day^{-1} in the Madeira River basin (Boischio and Henshel, 2000). Therefore, increased Hg concentration in large fish due to forest harvest and deforestation can impact the health of many people in the Amazon region.

4.3 Considerations about human health risk

Fish consumption in the Amazon is the highest in Brazil (Cerdeira et al., 1997; Batista et al., 1998; Boischio and Henshel, 2000), which increases the risk of exposure to THg and MeHg to the Colombian Amazon, THg concentration in hair of 94% of the sampled population (N= 200) was higher than the WHO (2003) threshold (5 µg g^{-1}) and 79% showed levels higher than 10 µg g^{-1} (Oliveiro-Verbel et al., 2016). In 36 communities distributed along the banks of the Negro, Tapajós and Madeira rivers in the Brazilian Amazon, mean THg levels in hair were above 6.0 µg g^{-1} (Castro and Lima, 2018). High levels of THg in hair is linked to high intake of fish. The Puruzinho Lake community has a fish intake of 406 g day^{-1} (Oliveira et al., 2010) and the mean THg level in hair is 12.1 ± 6.3 µg g^{-1} (Cardoso et al., 2014), which is twice the WHO (2003) threshold.

In Puruzinho Lake during RW, 60% of the sampled fish species showed THg concentrations higher than the threshold of 0.5 mg kg^{-1} wet wt from FAO/WHO (2003) (Table S3). In HW and FW, 50% of the species showed higher concentrations than the threshold (Table S3). Finally, in LW, 40% of the species showed higher levels of THg than the permissible limit of 0.5 mg kg^{-1} wet wt (FAO/WHO, 2003) (Table S3).

Although almost half of the sampled species pose a risk of Hg exposure of human consumers, *Cichla pleiozona*, *Prochilodus nigricans* and *Mylossoma duriventre* are the most economically important and most consumed in communities near Puruzinho Lake and in the Amazon at large (Oliveira et al., 2010; Begossi et al., 2018). From 517 interviews in the basins of the Amazon, Tocantins and Negro rivers, *Prochilodus nigricans* was reported as the most consumed (Begossi et al., 2018). In the Puruzinho Lake community, *Prochilodus nigricans* was the most consumed followed by *Mylossoma duriventre* and *Cichla pleiozona* (Oliveira et al., 2010). Both *Prochilodus nigricans* and *Mylossoma duriventre* mean THg concentrations in the four seasons were below 0.5 mg kg^{-1} wet wt (Table S3), which reduces the risk of exposure to the population. Among the three most consumed species, *Cichla pleiozona* was the only one where mean THg concentration was higher than the permissible limit fo the FAO/WHO (2003) (Table S3).

5. Conclusions

The data supported the study hypothesis that fish guilds will show different patterns of THg concentration due to seasonal variation. Two major seasonal patterns, different from what we expected in predictions 2 and 3, were established: (1) in herbivorous, detritivorous, carnivorous and piscivorous fish guilds, there was tendency for slightly higher THg concentrations during RW and LW; and (2) in planktivorous and omnivorous fish guilds, THg levels were lower during LW. We also confirmed there were no differences in THg levels between early and late periods of each season.

Floodplain systems like Puruzinho are widespread across the Amazon, so the results presented in this paper can be used to understand seasonal variation in THg levels in many Amazonian regions.

6. Acknowledgments

This work was supported by the CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) through the 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). We extend special thanks to the fisherman Raimundo Nonato dos Santos. This study was also financed in part by Coordenação de Aperfeiçoamento de Pessoa de Nível Superior – Brazil (CAPES) – Finance Code 001

7. References

Affonso, A.G., Queiroz, H.L., Novo, E.M.L.M., 2011. Limnological characterization of floodplain lakes in Mamirauá sustainable development reserve, Central Amazon (Amazonas State, Brazil). Acta Limnologica Brasiliensia 23 (1), 95-108.

Affonso, A.G., Queiroz, H.L., Novo, E.M.L.M., 2015. Abiotic variability among different aquatic systems of the central Amazon floodplain during drought and flood events. Braz. J. Biol. 75 (4), 60-69.

Agência Nacional de Águas (ANA) 2019. Rede Hidrometeorológica Nacional, Sistema HIDRO - Telemetria, Estação Humaitá, Código 15630000. Accessed in: http://www.snirh.gov.br/gestorpcd/gerarGrafico.aspx.

Agostinho, A.A., Gomes, L.C., Thomaz, S.M., Hahn, N.S., 2004. The Upper Paraná River and its floodplain: main characteristics and perspectives for management and conservation. In Thomaz, S.M., Agostinho, A.A., Hahn, NS. (Eds.). The upper Paraná River and its floodplain: physical aspects, ecology and conservation. Leiden: Backhuys Publishers. p. 381-393.

Almeida, V.L.L., Resende, E.K., Lima, M.S., Ferreira, C.J.A., 1993. Dieta e atividade alimentar de *Prochilodus lineatus* no pantanal do Miranda-Aquidauana, Mato Grosso do Sul, Brasil. Revista Unimar 15 (3), 125-141.

Almeida, F.F., Melo, S., 2009. Limnological considerations about an Amazonian floodplain lake (Catalão lake- Amazonas State, Brazil). Acta Scientiarum. Biological Sciences 31 (4), 387-395.

Almeida, R., Bernardi, J.V.E., Oliveira, R.C., Carvalho, D.P., Manzatto, A.G., Lacerda, L.D., Bastos, W.R., 2014. Flood pulse and spatial dynamics of Mercury in sediments in Puruzinho Lake, Brazilian Amazon. Acta Amazonica 44, 99-106.

Azevedo, L.S., Pestana, I.A., Rocha, A.R.M., Meneguelli-Souza, A.C., Lima, C.A.I., Almeida, M.G., Bastos, W.R., Souza, C.M.M., 2018. Drought promotes increases in total mercury and methylmercury concentrations in fish from the lower Paraiba do Sul River, southeastern Brazil. Chemosphere 202, 483–490.

Azevedo, L.S., Pestana, I.A., Nery, A.F.C., Bastos, W.R., Souza, C.M.M., 2019. Influence of the flood pulse on mercury accumulation in detritivorous, herbivorous, and omnivorous fish in Brazilian Amazonia. Ecotoxicology 28, 478-485.

Barbosa, C.C.F., Novo, E.M.L.M., Melack, J.M., Gastil-Buhl, M., Pereira Filho, W.P., 2010. Geospatial analysis of spatiotemporal patterns of pH, total suspended sediment and chlorophyll-a on the Amazon floodplain. Limnology 11 (2), 155-166.

Bastos, W.R., Malm, O., Pfeifer, W.C., Cleary, D., 1998. Establishment and analytical quality control of laboratories for Hg determination in biological and geological samples in the Amazon, Brazil. Rev. Ciên. Cult. 50, 255-260.

Bastos, W.R., Almeida, R., Dórea, J.G., Barbosa, A.C., 2007. Annual flooding and fish-mercury bioaccumulation in the environmentally impacted Rio Madeira (Amazon). Ecotoxicology 16, 341-346.

Bastos, W.R., Rebelo, M.F., Fonseca, M.F., Almeida, R., Malm, O., 2008. A description of mercury in fishes from the Madeira River basin, Amazon, Brazil. Acta Amazonica 38 (3), 431-438.

Batista, V.S., Inhamuns, A.J., Freitas, C.D.C., Freire- Brasil, D., 1998. Characterization of the fishery in river communities in the low- solimões/high-amazon region. Fisheries Management and Ecology 5 (5), 419-435.

Begossi, A., Salivonchyk, S.V., Hallwass, G., Hanazaki, N., Lopes, P.F.M., Silvano, R.A.M., Dumaresq, D., Pittock, J., 2018. Fish consumption on the Amazon: a review of biodiversity, hydropower and food security issues. Brazilian Journal of Biology 79, 345-357.

Benoit, J.M., Gilmour, C.C., Heyes, A., Mason, R.P., Miller, C.L., 2002. Geochemical and biological controls over mercury production and degradation in aquatic systems. Biogeochemistry of Environmentally Important Trace Elements. ACS (Am. Chem. Soc.) Symp. Ser. 835, 262e297.

Bisinoti, M.C., Júnior, E.S., Jardim, W.F., 2007. Seasonal Behavior of Mercury Species in Waters and Sediments from the Negro river basin, Amazon Brazil. J. Braz. Chem. Soc. 18 (3), 544-553.

Boischio, A.A.P., Henshel, P., 2000. Fish consumption, fish lore and mercury pollution: risk communication for the Madeira River People. Environmental Research Section A 84 (2), 108-126.

Braaten, H.F.V., Fjeld, E., Rognerud, S., Lund, E., Larssen, T., 2014. Seasonal and year-to-year variation of mercury concentration in perch (*Perca fluviatilis*) in boreal lakes. Environmental Toxicology and Chemistry 33, 2661-2670.

Brito, J.G., Alves, L.F., Espirito Santo, H.M.V., 2014. Seasonal and spatial variations in limnological conditions of a floodplain lake (lake Catalão) connected to both the Solimões and Negro Rivers, Central Amazonia. *Acta Amazonica* 44 (1), 121-133.

Brito, B.C., Forsberg, B.R., Kasper, D., Amaral, J.H.F., Vasconcelos, M.R.R., Sousa, O.P., Cunha, F.A.G., Bastos, W.R., 2017. The influence of inundation and lake morphometry on the dynamics of Mercury in the water and plankton in an Amazon floodplain lake. Hydrobiologia 790, 35-48.

Burger, J., Gochfeld, M., 2011. Mercury and selenium levels in 19 species of saltwater fish from New Jersey as function of specie, size and season. Science of the Total Environment 409, 1418-1429.

Cardoso, N.A., Hoshino, A.C.H., Perez, M.A., Bastos, W.R., Carvalho, D.P., Câmara, V.M., 2014. Tinnitus in a Riverside population exposed to methyl-mercury. Audiol. Commun. Res. 19, 40-44.

Castro, N.S.S., Lima, M.O., 2018. Hair as a Biomarker of Long Term Mercury Exposure in Brazilian Amazon: A Systematic Review. International Journal of Environmental Research and Public Health 15, 500-516.

Cerdeira, R.G.P., Ruffino, M.L., Isaac, V.J., 1997. Consumo de pescado e outros alimentos pela população ribeirinha do Lago Grande de Monte Alegre, PA - Brasil. Acta Amazonica 27 (3), 213-227

Chen, C.Y., Folt, C.L., 2005. High plankton densities reduce mercury biomagnification. Environmental Science & Technology 39, 115–121.

Cunico, A. M., Graça, W. J., Veríssimo, S., Bini, L. M., 2002. Influência do nível hidrológico sobre a assembleia de peixes em lagoa sazonalmente isolada da planície de inundação do alto rio Paraná. Acta Scientarium 24 (2), 383-389.

Dórea, J.G., Barbosa, A.C., Silva, G.S., 2006. Fish mercury bioaccumulation as a function of feeding behavior and hydrological cycles of the Rio Negro, Amazon. Comparative Biochemistry and Physiology, Part C 142, 275-283.

Eagle-Smith, C.A., Ackerman, J.T., Willacker, J.J., Tate, M.T., Lutz, M.A., Fleck, J.A., Stewart, A.R., Wiener, J.G., Evers, D.C., Lepak, J.M., Davis, J.A., Pritz, C.F., 2016. Spatial and temporal patterns of mercury concentrations in freshwater fish across the Western United States and Canada. Science of the Total Environment 568, 1171-1184.

Feitosa, L.B., Huszar, V.L.M., Domingues, C.D., Appel, E., Paranhos, R., Almeida, R.M., Branco, C.W.C., Bastos, W.R., Sarmento, H., 2019. Plankton community interactions in an Amazonian floodplain lake, from bacteria to zooplankton. Hydrobiologia 831, 55-70.

Fernandes, R., Agostinho, A. A., Ferreira, E. A., Pavanelli, C. S., Suzuki, H. I., Lima, D. P., Gomes, L.C., 2009. Effects of the hydrological regime on the ichthyofauna of riverine environments of the Upper Paraná River floodplain. Brazilian Journal of Biology 69 (2), 669-680.

Forsberg, B.R., Devol, A.H., Richey, J.E., Martinelli, L.A, Santos, H., 1988. Factors controlling nutrient concentrations in Amazon floodplain lakes. Limnology and Oceanography 33 (1), 41-56.

Fowlie, A.R., Hodson, P.V., Hickey, M.B., 2008. Spatial and Seasonal Patterns of Mercury Concentrations in Fish from the St. Lawrence River at Cornwall, Ontario: Implication for Monitoring. J. Great Lakes Res. 34, 72-85.

Garcia, E., Carignan, R., Lean, D.R.S., 2007. Seasonal and Inter-Annual Variations in Methyl Mercury Concentrations in Zooplankton from Boreal Lakes Impacted by Deforestation or Natural Forest Fires. Environmental Monitoring Assessment 131, 1-11.

Gomes, LC., Agostinho, AA., 1997. Influence of the flooding regime on the nutritional state and juvenile recruitment of the curimba, *Prochilodus scrofa*, Steindachner, in upper Paraná River, Brazil. Fisheries Management and Ecology 4 (4), 263-274.

Greenfield, B.K., Melwani, A.R., Allen, R.M., Slotton, D.G., Ayers, S.M., Harrold, K.H., Ridolfi, K., Jahn, A., Grenier, J.L., Sandheinrich, M.B., 2013. Seasonal and annual trends in forage fish mercury concentrations, San Francisco Bay. Science of the Total Environment 444, 591-601.

Jardim, W.F., Bisinoti, M.C., Fadini, P.S., Silva, G.S., 2010. Mercury Redox Chemistry in the Negro River Basin, Amazon: The Role of Organic Matter and Solar Light. Aquatic Geochemistry 16, 267-278.

Junk, W.J., 1997. General aspects of floodplain ecology with special reference to Amazonian floodplains. In: W.J. JUNK, ed. *The Central Amazon floodplain, ecology of a pulsing system* Berlin: Springer-Verlag, pp. 3-17.

Junk, W. J., Wantzen, K. M., 2004. The flood pulse concept: new aspects, approaches and applications – an update. In Sustaining Livelihoods and Biodiversity in the New Millennium. Proceedings of the Second International Symposium on the Management of Large Rivers for Fisheries (Welcomme, R. L. & Petr, T., eds), pp. 117-140. Udon Thani: Red Plough International Co., Ltd. Available at www.fao.org/docrep/007/ad525e/ad525e00.htm.

Kasper, D., Forsberg, B.R., Amaral, J.H.F., Leitão, R.P., Py-Daniel, S.S., Bastos, W.R., Malm, O., 2014. Reservoir stratification affects methylmercury levels in river water, plankton, and fish downstream from Balbina Hydroeletric Dam, Amazonas, Brazil. Environmental Science & Technology 48, 1032-1040.

Kehrig, H.A., Malm, O., 1999. Methylmercury in fish as a tool for understanding the Amazon mercury contamination. Applied Organometallic Chemistry 13, 689-696.

Lázaro, W.L., Díez, S., Silva, C.J., Ignácio, A.R.A., Guimarães, J.R.D., 2018. Seasonal changes in peryphytic microbial metabolism determining mercury methylation in a tropical wetland. Science of the Total Environment 627, 1345-1352.

Luz-Agostinho, K.D.G., Agostinho, A.A., Gomes, L.C., Júlio Jr, H.F., Fugi, R., 2009. Effects of flooding regime on the feeding activity and body condition of piscivorous fish in the Upper Paraná River floodplain. Brazilian Journal of Biology 69, 481-490.

Martinez, G., McCord, S.A., Driscoll, C.T., Todorova, S., Wu, S., Araújo, J.F., Vega, C.M., Fernandez, L.E., 2018. Mercury contamination in riverine sediments and fish associated with artisanal and small-scale gold mining in Madre de Dios, Peru. International Journal of Environmental Research and Public Health 15, 1584.

Maurice-Bourgoin, L., Quemerais, B., Moreira-Turcq, P., Seyler, P., 2003. Transport, distribution and speciation of mercury in the Amazon River at the confluence of black and white waters of the Negro and Solimões rivers. Hydrological Processes 17, 1405-1417.

Melack, J.M., Forsberg, B., 2001. Biogeochemistry of Amazon floodplain lakes and associated wetlands. In: M.E. Mcclain, R.L. Victoria and J.E. Richey, eds. The biogeochemistry of the Amazon basin and its role in a changing world. Oxford: Oxford University Press, pp. 235-276.

Mills, N., Cashatt, D., Weber, M.J., Pierce, C.L., 2018. A case study and a meta-analysis of seasonal variation in fish mercury concentrations. Ecotoxicology 6, 641-649.

Moreno-Brush, M., Rydberg, J., Gamboa, N., Storch, I., Biester, H., 2016. Is mercury from small-scale gold mining prevalent in the southeastern Peruvian Amazon? Environ. Pollut. 218, 150-159.

Nascimento, E.L., Gomes, J.P.O., Almeida, R., Bastos, W.R., Bernardi, J.V.E., Miyai, R.K., 2006. Mercurio no plâncton de um lago natural amazônico, Lago Puruzinho (Brasil). J. Braz. Soc. Ecotoxicol. 1,1-6.

Oliveira, R.C., Dórea, J.G., Bernardi, J.V.E., Bastos, W.R., Almeida, R., Manzatto, A.G., 2010. Fish consumption by traditional subsistence villagers of the Rio Madeira (Amazon): Impact on hair mercury. Annals of Human Biology 37, 629-642.

Olivero-Verbel, J., Carranza-Lopez, L., Caballero-Gallardo, K., Ripoll-Arboleda, A., Muñoz-Sosa, D., 2016. Human exposure and risk assessment associated with mercury pollution in the Caqueta River, Colombian Amazon. Environmental Science and Pollution Research 23, 20761-20771.

Pedro, J.P.B., Gomes, M.C.R.L., Trindade, M.E.J., Cavalcante, D.P., Oliveira, J.A., Hercos, A.P., Zucchi, N., Lima, C.B.L., Pereira, S.A., Queiroz, H.L., 2013. Influence of the hydrological cycle on physical and chemical variables of bodies of water in the várzea areas of the Middle Solimões region (Amazonas, Brazil). *Uakari* 9 (2), 75-90.

Pestana, I.A., Almeida, M.G., Bastos, W.R., Souza, C.M.M., 2019. Total Hg and methylmercury dynamics in a river-floodplain system in the Western Amazon:

Influence of seasonality, organic matter and physical and chemical parameters. Science of the Total Environment 656 (15), 388-399.

Pestana, I.A., Azevedo, L.S., Bastos, W.R., Souza, C.M.M., 2019. The impact of hydroeletric dams on mercury dynamics in South America: a review. Chemosphere 219, 546-556.

Pickhardt, P. C., Folt, C.L., Chen, C.Y., Klaue, B., Blum, J.D., 2005. Impacts of zooplankton composition and algal enrichment on the accumulation of mercury in an experimental freshwater food web. Science of the Total Environment 339, 89–101.

Poste, A.E., Muir, D.C.G., Guildford, S.J., Hecky, R.E., 2015. Bioaccumulation and biomagnification of mercury in African lakes: The importance of trophic status. Science of the total Environment 506-507, 126-136.

Razavi, N.R., Qu, M., Jin, B., Ren, W., Wang, Y., Campbell, L.M., 2014. Mercury biomagnification in subtropical reservoir fishes of eastern China. Ecotoxicology 23:133-146.

Razavi, N.R., Qu, M., Chen, D., Zhong, Y., Ren, W., Wang, Y., Campbell, L.M., 2015. Effect on eutrophication on mercury (Hg) dynamics in subtropical reservoirs from a high Hg deposition ecoregion. Limnology and Oceanography 60, 386-401.

Sampaio da Silva, D., Lucotte, M., Roulet, M., Poirier, H., Mergler, D., Crossa, M., 2006. Mercury in fish of the Tapajós river the Brazilian amazon. Journal on Integrated Management of Occupational Health and Environment 1, 1–33.

Si, Y., Zou, Y., Liu, X., Si, X., Mao, J., 2015. Mercury methylation coupled to iron reduction by dissimilatory iron-reducing bacteria. Chemosphere 122, 206 –212.

Silva, G.S., Bisinoti, M.C., Fadini, P.G., Magarelli, G., Jardim, W.F., Fostier, A.H., 2009. Major Aspects of the Mercury Cycle in the Negro River Basin, Amazon. J.Braz.Chem.Soc. 20, 1127-1134.

Silva, S.F., Oliveira, D.C., Pereira, J.P.G., Castro, S.P., Costa, B.N.S., Lima, M.O., 2019. Seasonal variation of mercury in commercial fishes of the Amazon Triple Frontier, Western Amazon Basin. Ecological Indicators 106, 105549.

Sioli H. Studies in Amazonian Waters (1967). In: Atas do simpósio sobre a biota amazônica 3, 9-50. Conselho Nacional de Pesquisas, Rio de Janeiro.

Soares-Filho, B.S., Nepstad, D.C., Curran, L.M., Cerqueira, G.C., Garcia, R.A., Ramos, C.A., Voll, E., McDonald, A., Lefebvre, P., Schlesinger, P., 2006. Modelling conservation in the Amazon basin. Nature 440, 520-523.

Suzuki, T., Imura, N., Clarkson, T.W., 1991. Advances in mercury toxicology. New York: Springer Science & Business Media.

Vazzoler, A.E.A., 1996. Biologia da reprodução de peixes Teleóteos: Teoria e Pratica. Maringá, PR: Editora Universidade Estadual de Maringá.

Veiga, M.M., Hinton, J.J., 2002. Abandoned artisanal gold mines in the Brazilian Amazon: a legacy of mercury pollution. Nat. Resour. Forum 26, 15-26

Vieira, M., Bernardi, J.V.E., Dórea, J.G., Rocha, B.C.P., Ribeiro, R., Zara, L.F., 2018. Distribution and avaliability of mercury and methylmercury in different Waters from the Rio Madeira Basin, Amazon. Environmental pollution 235, 771-779.

Watras, C.J., Morrison, K.A., Host, J., Bloom, N.S., 1995. Concentration of mercury species in relation to other site-specific factors in the surface waters of northern Wisconsin lakes. Limnol. Oceanogr. 40, 556-565.

WHO (2003) Elemental mercury and inorganic mercury compounds: human health aspects. In: Concise International Chemical Assessment Document (CICAD), vol 50. World Health Organization, Geneva.

Winemiller, K.O., 2004. Floodplain river food webs: generalizations and implications for fisheries management. In: Proceedings of the Second International Symposium on the Management of Large Rivers for Fisheries Volume II (Welcomme, R. & Petr, T., eds), pp. 285-309. Udon Thani: Red Plough International Co., Ltd. Available at http://www.fao.org/docrep/007/ad525e/ad525e00.htm

Wissmar, R.C., Richey, J.E., Stallard, R.F., Edmond, J.M., 1981. Plankton metabolism and carbon processes in the Amazon River, its tributaries, and floodplain waters, Peru- Brazil, May-June 1977. Ecology 62, 1622-1633.

Wu, P., Bishop, K., Brömssen, C., Eklöf, K., Futter, M., Hultberg, H., Martin, J., Akerblom, S., 2018. Does forest harvest increase the mercury concentrations in fish? Evidence from Swedish lake. Science of the Total Environment 622-623, 1353-1362.

SUPPLEMENTARY MATERIAL

Mercury concentration in six fish guilds from a floodplain lake in western Amazonia: Interaction between seasonality and feeding habits

Lucas Silva Azevedo[a,*], Inácio Abreu Pestana[a], Adriely Ferreira da Costa Nery[b], Wanderley Rodrigues Bastos[b], Cristina Maria Magalhães Souza[a]

[a]Laboratório de Ciências Ambientais, Centro de Biociências e Biotecnologia, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, Rio de Janeiro, RJ, CEP: 28013-602, Brazil.
[b]Laboratório de Biogeoquímica Ambiental, Universidade Federal de Rondônia, Porto Velho, Rondônia, RO, CEP: 76815-800, Brazil.

This document includes supplementary information about: (1) the water level and flow of Madeira river and the sampling months (Table S1); (2) total length of fish guilds and number of samples (Table S2); (3) total Hg concentration in fish species of Puruzinho lake (Table S3); Map of the study area (Figure S1); Linear regression between THg concentrations (mean values) and water level of Madeira River at Humaitá station (Figure S2); THg concentrations (mg kg^{-1} wet wt) in six guilds (herbivorous, detritivorous, omnivorous, planktivorous, carnivorous and piscivorous) from Puruzinho Lake during the early and late seasonal periods (Figure S3).

Number of tables: 3

Number of figures: 3

Table S1. Water level (m) and Flow (m^3 s^{-1}) of Madeira river in Humaitá Station

Season	Month	Water Level (m)	Flow (m^3 s^{-1})
E-RW	**dec/16**	13.62 ± 0.89	12002.2 ± 2261.2
E-RW	jan/17	17.69 ± 0.95	24053.5 ± 3218.9
L-RW	**feb/17**	19.15 ± 0.75	29370.0 ± 2920.7
E-HW	mar/17	21.68 ± 0.32	39796.6 ± 1449.1
L-HW	**apr/17**	21.72 ± 0.13	39987.4 ± 615.4
L-HW	may/17	20.39 ± 0.61	34272.8 ± 2575.2
E-FW	**jun/17**	18.10 ± 0.82	25425.54 ± 2933.1
E-FW	jul/17	14.34 ± 1.17	13902.35 ± 3108.2
L-FW	aug/17	11.24 ± 0.52	6815.4 ± 995.4
L-FW	sep/17	10.66 ± 0.41	5767.1 ± 705.8
E-LW	**oct/17**	10.22 ± 0.16	5015.8 ± 267.6
E-LW	nov/17	12.58 ± 0.11	9653.05 ± 2486.32
L-LW	**dec/17**	16.14 ± 0.11	18993.22 ± 3435.86
E-RW	jan/18	20.34 ± 0.78	33914.71 ± 3497.23
L-RW	**feb/18**	22.09 ± 0.61	41673.96 ± 2796.72
E-HW	mar/18	22.96 ± 0.13	45702.26 ± 646.10
L-HW	**apr/18**	23.06 ± 0.39	46193.58 ± 1854.35
L-HW	may/18	21.10 ± 0.68	37313.18 ± 2956.87
E-FW	**jun/18**	18.02 ± 1.05	25266.78 ± 3690.01
E-FW	jul/18	13.94 ± 1.31	12920.61 ± 3277.99
L-FW	**aug/18**	11.61 ± 0.28	7488.68 ± 554.16
L-FW	sep/18	10.82 ± 0.43	6048.02 ± 759.10
L-FW	**oct/18**	11.01 ± 0.65	6405.42 ± 1193.81
L-LW	nov/18	14.54 ± 1.24	14450.24 ± 3484.35
E-RW	**dec/18**	19.41 ± 0.70	30374.71 ± 2742.38

E: early
L: late
RW: rising water
HW: high water
FW: falling water
LW: low water
* Bold: sampling months

Table S2: Total length (cm) of fish guilds along the four seasons

	TL (mean ± SD)			
Specie	**Rising Water**	**High Water**	**Falling Water**	**Low Water**
Carnivorous	25.86 ± 10.00 (57)	26.59 ± 11.55 (73)	26.45 ± 10.60 (191)	28.82 ± 15.11 (54)
Piscivorous	25.91 ± 10.10 (109)	26.78 ± 11.58 (65)	26.40 ± 10.40 (212)	30.36 ± 16.00 (93)
Detritivorous	25.70 ± 10.10 (181)	26.51 ± 11.86 (76)	26.35 ± 10.60 (170)	29.66 ± 16.65 (66)
Herbivorous	25.62 ± 10.01 (128)	26.50 ± 12.00 (54)	26.00 ± 11.03 (121)	30.80 ± 10.70 (10)
Planktivorous	25.70 ± 10.00 (68)	26.55 ± 11.57 (8)	25.00 ± 6.90 (71)	28.90 ± 15.37 (65)
Omnivorous	26.20 ± 10.20 (22)	19.00 (1)	26.50 ± 10.55 (86)	27.65 ± 22.10 (29)

*Number of samples (N) between parentheses

Table S3. THg concentration (mg kg^{-1} wet wt) in muscle of fish species from Puruzinho lake

Season	Specie	N	Mean	SD
High water	*Anodus elongatus*	**2**	**0.56**	**0.18**
	Cichla pleiozona	**33**	**0.87**	**0.29**
	Hoplias malabaricus	**6**	**0.82**	**0.17**
	Hypophthalmus marginatus	**6**	**0.77**	**0.14**
	Mylossoma duriventri	50	0.06	0.03
	Pellona castelnaeana	**16**	**1.26**	**0.61**
	Pellona flavipinnis	**4**	**1.17**	**0.46**
	Plagioscion squamosissimus	30	0.8	0.27
	Potamorhina altamazonica	38	0.15	0.06
	Potamorhina latior	3	0.25	0.08
	Prochilodus nigricans	32	0.19	0.06
	Rhaphiodon vulpinus	**39**	**0.74**	**0.37**
	Schizodon fasciatus	4	0.25	0.18
	Semaprochilodus insignis	3	0.2	0.06
	Serrasalmus rhombeus	**10**	**1.65**	**0.69**
	Triportheus albus	1	0.11	
Low water	*Acestrorhynchus falcirostris*	**12**	**1.37**	**0.82**
	Ageneiosus inermis	1	0.32	
	Anodus elongatus	17	0.38	0.17
	Calophysus macropterus	**12**	**1.42**	**0.97**
	Cichla pleiozona	**32**	**1.18**	**0.46**
	Hemiodus unimaculatus	8	0.18	0.08
	Hoplias malabaricus	**7**	**1.05**	**0.72**
	Hypophthalmus marginatus	48	0.4	0.23
	Leporinus friderici	7	0.29	0.11
	Mylossoma duriventri	6	0.05	0.02
	Pellona castelnaeana	**33**	**1.25**	**0.62**
	Pimelodus blochii	2	0.29	0.06
	Plagioscion squamosissimus	**2**	**0.89**	**0.01**
	Potamorhina latior	7	0.25	0.09
	Prochilodus nigricans	42	0.2	0.08
	Psectrogaster rutiloides	9	0.16	0.04
	Rhaphiodon vulpinus	**25**	**1.3**	**0.52**
	Schizodon fasciatus	4	0.14	0.12
	Serrasalmus rhombeus	**23**	**1.49**	**0.41**
	Triportheus albus	20	0.18	0.14
Rising water	*Acestrorhynchus falcirostris*	**5**	**1.48**	**0.27**
	Ageneiosus inermis	**2**	**0.97**	**0.01**
	Anodus elongatus	**15**	**0.72**	**0.36**
	Calophysus macropterus	**7**	**1.39**	**0.83**
	Cichla pleiozona	**40**	**1.05**	**0.4**

	Hemiodus unimaculatus	4	0.31	0.05
	Hoplias malabaricus	**2**	**1.06**	**0.18**
	Hypophthalmus edentatus	**8**	**0.94**	**0.13**
	Hypophthalmus marginatus	**45**	**0.52**	**0.19**
	Mylossoma duriventri	80	0.07	0.03
	Pellona castelnaeana	**47**	**1.14**	**0.6**
	Pimelodus blochii	4	0.42	0.04
	Pinirampus pirinampu	**6**	**1.23**	**0.4**
	Plagioscion squamosissimus	**6**	**0.92**	**0.58**
	Potamorhina altamazonica	52	0.22	0.07
	Potamorhina latior	78	0.26	0.11
	Prochilodus nigricans	21	0.25	0.12
	Psectrogaster amazonica	26	0.1	0.07
	Rhaphiodon vulpinus	**24**	**1.06**	**0.61**
	Schizodon fasciatus	48	0.33	0.14
	Serrasalmus rhombeus	**27**	**0.81**	**0.52**
	Triportheus albus	18	0.27	0.12
Falling water	*Acestrorhynchus falcirostris*	**55**	**1.35**	**0.44**
	Ageneiosus inermis	**1**	**0.55**	
	Anodus elongatus	17	0.42	0.18
	Brycon sp.	9	0.11	0.04
	Calophysus macropterus	**2**	**0.92**	**0.12**
	Cichla pleiozona	**125**	**0.93**	**0.43**
	Hemiodus unimaculatus	27	0.36	0.19
	Hoplias malabaricus	**43**	**0.98**	**0.24**
	Hypophthalmus marginatus	**54**	**0.58**	**0.19**
	Mylossoma duriventri	100	0.07	0.04
	Pellona castelnaeana	**32**	**1.17**	**0.52**
	Pesectrogaster amazonica	2	0.14	0,00
	Phractocephalus hemioliopterus	**17**	**0.68**	**0.3**
	Pimelodus blochii	7	0.25	0.11
	Pinirampus pirinampu	**6**	**0.91**	**0.62**
	Plagioscion squamosissimus	**20**	**0.79**	**0.45**
	Potamorhina altamazonica	20	0.14	0.04
	Prochilodus nigricans	98	0.21	0.1
	Psectrogaster amazonica	9	0.1	0.04
	Psectrogaster rutiloides	29	0.13	0.07
	Rhaphiodon vulpinus	**40**	**1.05**	**0.34**
	Schizodon fasciatus	21	0.22	0.09
	Serrasalmus rhombeus	**85**	**0.59**	**0.28**
	Triportheus albus	38	0.45	0.34

Bold: Concentrations higher than FAO/WHO (2003) threshold of 0.5 mg kg^{-1} wet wt.

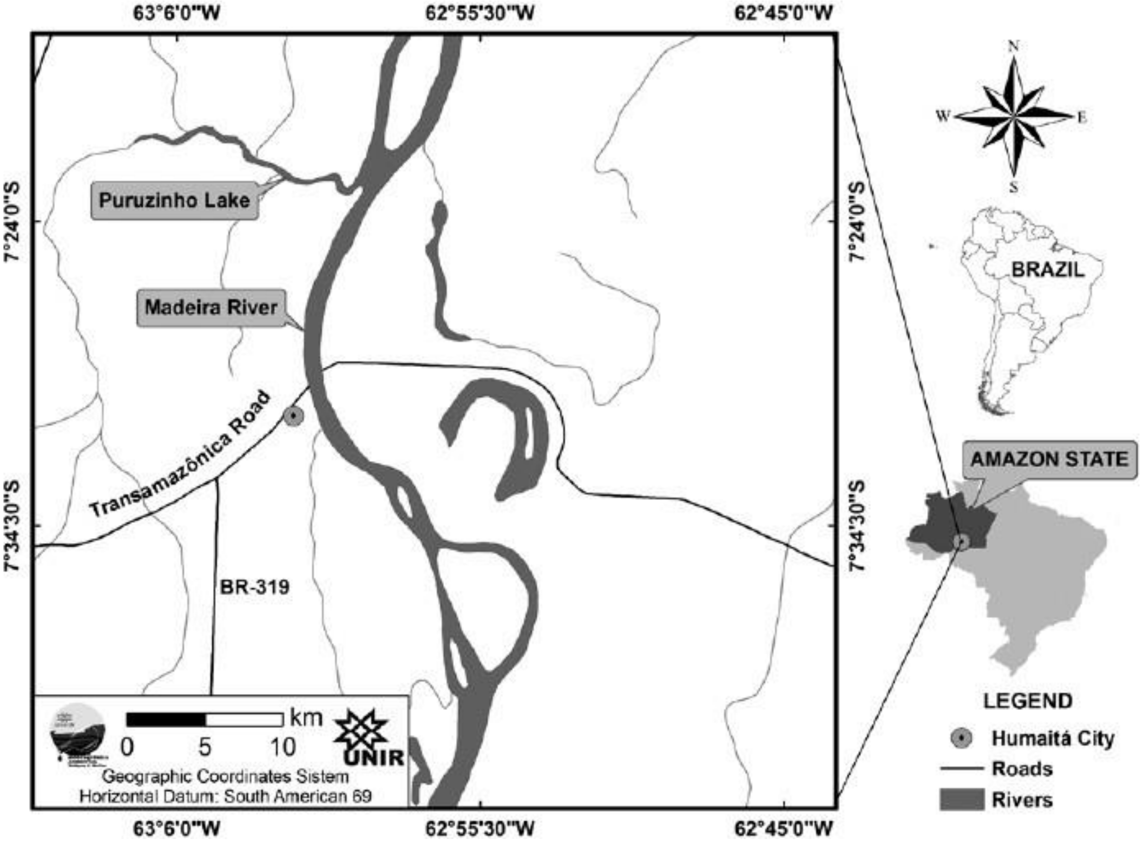


Figure S1: Map of the study area (Extracted from Almeida et al., 2014).

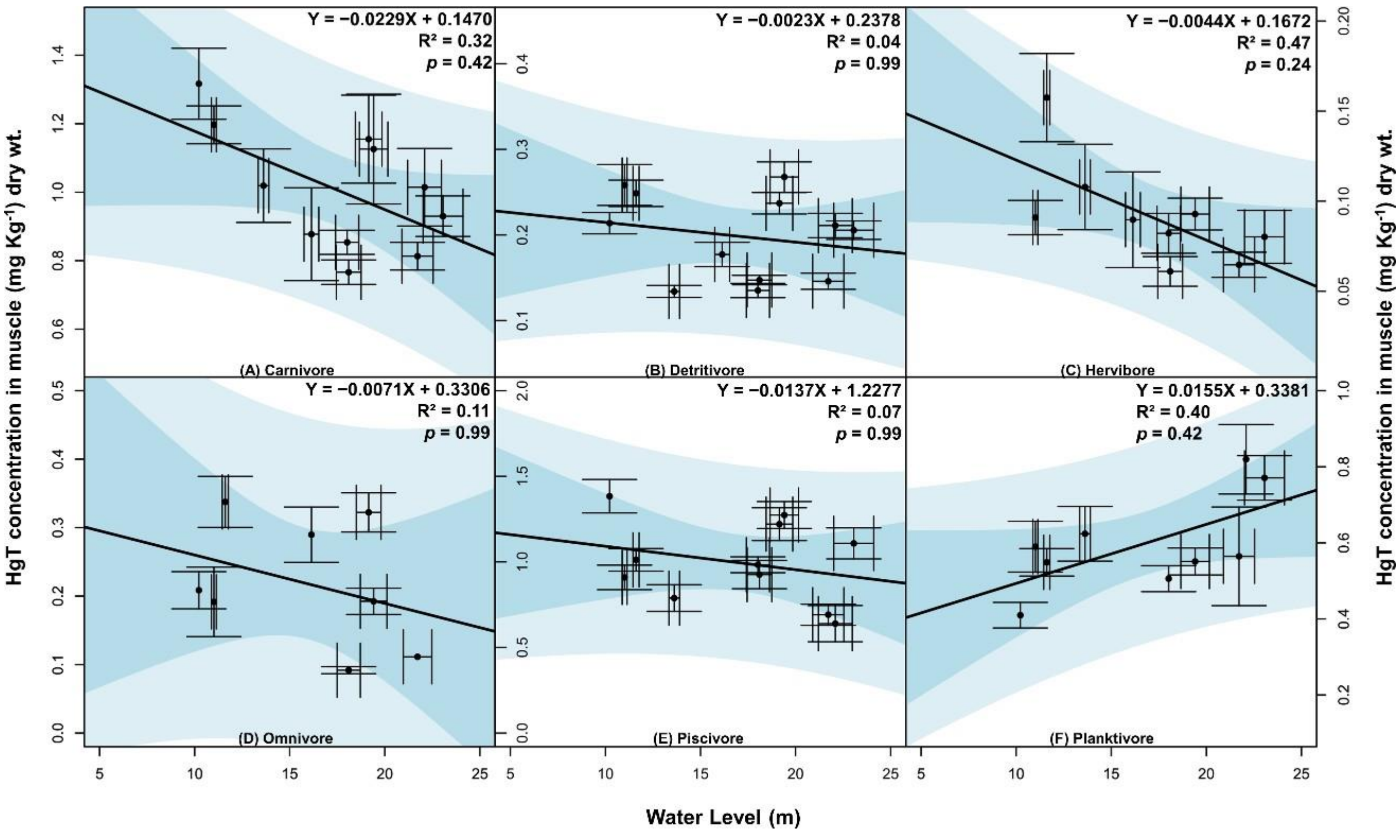


Figure S2. Linear regression between THg concentrations (mean values) and water level of Madeira River at Humaitá station (Puruzinho Lake is located 20 km from the urban area of Humaitá). Vertical and horizontal error bars represent mean standard error of respective variables. Dark blue and light blue areas represent the confidence and prediction intervals of regression models, both calculated with 95% certainty.

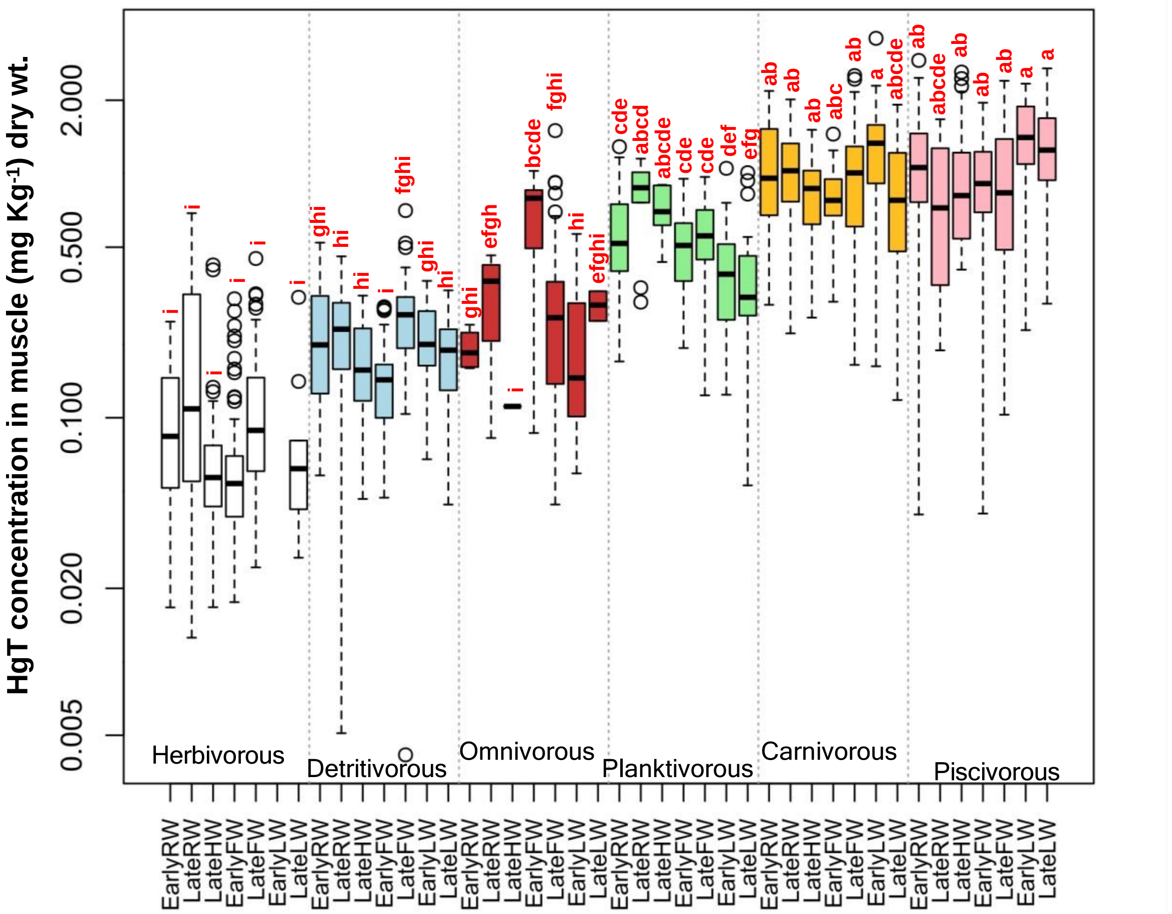


Figure S3. THg concentrations (mg kg^{-1} wet wt) in six guilds (herbivorous, detritivorous, omnivorous, planktivorous, carnivorous and piscivorous) from Puruzinho Lake during the early and late seasonal periods. Different letters indicate statistical differences of mean (α = 0.05). Two-way ANOVA indicates interaction between feeding habits and seasonality ($F_{29,1972} = 7.1$; $p < 0.001$). Distance between y-axis values were log-transformed to optimize data visualization.

CAPÍTULO 2: **Mercury biomagnification in an ichthyic food chain of an Amazon floodplain lake (Puruzinho Lake): influence of seasonality and food chain modeling**

Publicado na revista *Ecotoxicology and Environmental Safety* (Qualis A2 – Biodiversidade), Fator de Impacto (4,8). DOI:10.1016/j.ecoenv.2020.111249

Mercury biomagnification in an ichthyic food chain of an Amazon floodplain lake (Puruzinho Lake): influence of seasonality and food chain modeling

Lucas Silva Azevedo[a,*], Inácio Abreu Pestana[a], Marcelo Gomes Almeida[a], Adriely Ferreira da Costa Nery[b], Wanderley Rodrigues Bastos[b], Cristina Maria Magalhães Souza[a]

[a]Laboratório de Ciências Ambientais, Centro de Biociências e Biotecnologia, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Parque Califórnia, CEP: 28013-602, Campos dos Goytacazes, Rio de Janeiro, RJ, Brazil.
[b]Laboratório de Biogeoquímica Ambiental, Universidade Federal de Rondônia, Olaria, CEP: 76815-800, Porto Velho, Rondônia, RO, Brazil.

ABSTRACT

Mercury (Hg) biomagnification in fish food chains is a relevant subject due to the high fish consumption of the Amazonian population and the high toxicity of this metal. In the Amazon, floodplain lake hydrodynamics change considerably along the four seasons of the hydrological cycle (rising water, high water, falling water and low water), which can influence Hg bioaccumulation in fish. The main aim of this study was to evaluate if Hg biomagnification is influenced by seasonality in a floodplain lake (Puruzinho Lake) in the Brazilian Amazon. Additionally, the influence of food chain modeling on measurement of Hg biomagnification was tested. Hg concentrations and stable isotope signatures (carbon and nitrogen) were estimated in four species, *Mylossoma duriventre* (herbivorous), *Prochilodus nigricans* (detritivorous), *Cichla pleiozona* (piscivorous) and *Serrasalmus rhombeus* (piscivorous). The "trophic magnification slope" (TMS) of the food chain composed by the four species was calculated and compared among the four seasons. There was no significant seasonal variation in TMS among rising water, high water, falling water and low water seasons ($p = 0.08$), suggesting that Hg biomagnification does not change seasonally. However, there was significant variation in TMS among different food chain models. Lower TMS was observed in a food chain composed of detritivorous and piscivorous fish (0.20) in comparison with a food chain composed of the four species (0.26). The results indicate food chain modeling influences TMS results.

Keywords: Mercury; Food chain modeling; Amazon; Biomagnification; Seasons; Floodplain

1. Introduction

The Amazon is a relevant area for studies about mercury (Hg) due to its naturally high concentrations in the region's old soils (Roulet et al., 1998; Roulet et al., 2001; Johnson, 2003) and intense gold mining activities in past years that has resulted in a legacy of 2,000 metric tons of Hg released in the environment (Malm, 1998). There are many published studies about Hg geochemistry in Amazonian aquatic environments (Lechler et al., 2000; Silva et al., 2006; Maia et al., 2009; Nevado et al., 2010; Béliveau et al., 2017; Bonotto et al., 2018; Vieira et al., 2018; Lino et al., 2019; Pestana et al., 2019) and Hg bioaccumulation in fish (Kehrig and Malm, 1999; Bastos et al., 2007; Bastos et al., 2008; Bastos et al., 2015; Lino et al., 2019; Silva et al., 2019; Azevedo et al., 2020), indicating the relevance of understanding Hg dynamics in the region. Hg bioaccumulation in fish is of special interest since some Brazilian Amazon populations rely mostly on fish for protein consumption (Oliveira et al., 2010). For example, the intake of fish can reach 406 g day^{-1} and the annual consumption is 148.2 kg per capita in the Puruzinho Lake community (Oliveira et al., 2010). Hg intake through fish consumption in the Amazon ranges from 0.4 to 3.0 µg kg day^{-1} (Bidone et al., 1997; Passos et al., 2008). Adverse neurological effects related to Hg exposure, like attention deficit, poor leg coordination, sensory and balance disturbances, tremors, hyperreflexia and dysarthria (Grandejan et al., 1999; Harada et al., 2001; Pacheco-Ferreira, 2001; Cordier et al., 2002) have been observed Amazonian communities were fish consumption is high.

The Minamata incident in Japan, from 1940 to 1960, was an environmental disaster that exposed many people to high levels of Hg though consumption of fish and shellfish. The first well-documented case of severe mercury poisoning (called "Minamata disease") was reported in 1956 in a five-year-old girl suffering unusual neurological symptoms like convulsions and difficulty of walking and speaking (Hachiya, 2006). After that, 2,955 other people showed symptoms of Minamata disease until 2005 (Hachiya, 2006). Since this incident, the major concerns regarding Hg in fish have been its high toxicity and high potential for biomagnification. This process can be measured by different approaches, involving field or controlled experiments. Under controlled conditions, the biomagnification factor (BMF), which is the ratio between Hg concentration in a predator and in one prey, is a reliable tool. However, in field experiments the BMF is not a reliable approach because fish feed on more than one prey. Conversely, the trophic magnification slope (TMS) is the slope of the regression line between $\log_{10}$-transformed Hg concentration in a given organism and its $\delta^{15}N$ signature. This metric considers biomagnification not as single prey-predator relationship but through a community. It has been extensively used in field studies to estimate biomagnification (Borga et al., 2012; Kwon et al., 2012; Lavoie et al., 2013; Pouilly et al., 2013; Azevedo-Silva et al., 2016; Mussy, 2018). The main advantage of using TMS is the assessment of the biomagnification as it happens in the field, which provides a more realistic and conclusive measure than laboratory-derived metrics (Borga et al., 2012).

Hg biomagnification in ichthyic food chains is influenced by several factors, including water chemistry (Verburg et al., 2014), hydrology (Lavoie et al., 2013) and trophic chain modeling (Ouédraogo et al., 2015). In the meta-analysis carried out by Lavoie et al. (2013), the authors also concluded that much of the variability of TMS among different ecosystems was unexplained. In the Amazon, there is significant variation in water chemistry among aquatic ecosystems (Sioli, 1967): “black water” ecosystems have high concentration of dissolved organic carbon and acid pH (e.g., Negro River); “white water” ecosystems have high concentration of particulate matter and neutral pH (e.g., Madeira and Solimões rivers); and “clear water” ecosystems have low concentrations of suspended particulate matter (e.g., Tapajós River). In black water ecosystems, there are suitable conditions (i.e., acid pH and high concentration of dissolved organic matter) for higher Hg methylation and availability. In acid environments, methylmercury production can increase from two to three-fold in the sediment-water interface (Xun et al., 1987), and the release of this contaminant from sediment particles is also enhanced (Miller and Akagi, 1979). These two factors combined (i.e., increased methylation and Hg availability) result in higher Hg concentration in the whole aquatic food chain. In addition, Hg availability varies with the hydrodynamics of aquatic ecosystems, which specifically for Amazonian floodplain lakes can change drastically along the local hydrological cycle.

Floodplain lakes (locally known as “Várzea” lakes) are composed of a lentic section (i.e., the lake), which is connected to a lotic section (i.e., the river) through a narrow channel. During the rising water season (RW), the system’s water level gradually increases and reaches the peak in the high water season (HW), when often the river overflows and mixes with the water of floodplain lakes. In contrast, during the falling water season (FW), the water level gradually decreases and reaches the lowest values in the low water season (LW), when there is no mixing of lentic and lotic waters.

Food resource availability and trophic interactions change along the seasons in floodplain systems, due to the natural cycles of flooding and drying (Pool et al., 2017). For instance, during the dry season piscivores can encounter their prey more easily (Wantzen et al., 2002) because fish are confined in a smaller habitat. In this circumstance, the $\delta^{15}N$ signature of piscivorous fish should be higher. Also, Hg concentration in piscivorous fish should be higher as well since the diet is the main pathway of exposure to this contaminant. Basal resource origin (i.e., allochthonous or autochthonous), indicated by the $\delta^{13}C$ signature, can also change along the seasons due to the higher influx of terrestrial resources in the flood season. The seasonal changes in allochthonous resource influx can influence Hg concentration in biota because there is evidence of higher Hg concentration in water and biota in lakes with higher influx of terrestrial organic matter (Poste et al., 2019).

Significant variation in fish Hg concentrations among the seasons of the hydrological cycle have been observed (Silva et al., 2019; Azevedo et al., 2020). Silva et al. (2019) found higher Hg concentration in fish during the RW season in comparison with the FW season. In agreement with this, Azevedo et al. (2020) reported higher Hg concentrations in herbivorous, detritivorous, carnivorous and piscivorous fish guilds in RW and LW. Although Hg seasonal variation have been studied, the variation in TMS among seasons have not been evaluated.

The biomagnification rate of total mercury (i.e., all Hg chemical species; THg) can be influenced by food chain modeling (Ouédraogo et al., 2015). The two major concerns regarding food chain modeling are: (1) the inconsistency of how each research group composes it; and (2) whether they include other taxa, like invertebrates or mammals, along with fish to be more accurate in representing a natural food chain. For example, Kwon et al. (2012) selected 24 fish species with seven diets to compose the food chain while Pouilly et al. (2013) considered only three species. In this case, Kwon et al. (2012) observed a TMS of 0.10 and Pouilly et al. (2013) found a TMS of 0.43, both studying a clear water river. If the number of species and diets can influence the TMS results, modeling the food chain is a decisive step to make comparisons of TMS among food chains more reliable.

The aim of this study was to evaluate THg biomagnification in an ichthyic food chain of an Amazon floodplain lake during the region's four hydrological cycles. Due to the mixing of Puruzinho Lake's black water with Madeira River's white water, which can reduce Hg availability (Pestana et al., 2019) to species, we expected a lower TMS during the RW and HW seasons in comparison with FW and LW. Also, we tested the influence of different food chain models on the TMS results.

2. Material and Methods

2.1 Study area

The sampling occurred in Puruzinho Lake (see Supplementary Material 3 for a map of the study area), located in the municipality of Humaitá, Amazonas state, Brazil (63°6'0''W; 7°24'0''S). The lake is a black water ecosystem connected through an 8 km channel with the Madeira River, a white water ecosystem. The river overflows and mixes with the lake during the late stage of the RW and in the HW seasons, while during the FW and LW there is little or no mixing of waters. Due to the connection between lentic and lotic sections, Puruzinho Lake's water depth is strongly related to the variation in the Madeira River's water level (Feitosa et al., 2019; Azevedo et al., 2020). Variation in water chemistry along the seasons was described by Nascimento et al. (2006): temperatures of 27.5 ºC (RW), 28.5ºC (HW) and 31.9ºC (LW); dissolved oxygen contents of 5.9 mg L^{-1} (RW), 3.0 mg L^{-1} (HW) and 5.7 mg L^{-1} (LW); pH values of 5.4 (RW), 5.2 (HW) and 5.6 (LW); and conductivity values of 8.3 µS cm^{-1} (RW), 9.2 µS cm^{-1} (HW) and 15.2 µS cm^{-1} (LW).

2.2 Sampling

The sampling occurred during the four hydrological cycles during 2017-2018: RW (December 2017−February 2018), HW (April 2018), FW (September 2018) and LW (October 2018). There is a five-month gap between the end of the rainy season (April) and the beginning of the dry season (September). This is enough time to accommodate the turnover rates of stable isotopes in fish muscle, which have a half-life of 42-66 days for nitrogen and 39-61 days for carbon (Thomas et al., 2015). These half-life values are based on animals weighing from 100 g to 1 kg at 30 ºC. Four species with different diets were sampled: *Mylossoma duriventre* (common name pacu; herbivorous; n = 64), *Prochilodus nigricans* (common name curimatã; detritivorous; n = 74), *Cichla pleiozona* (common name tucunaré; piscivorous; n = 73) and *Serrasalmus rhombeus* (common name black piranha; piscivorous; n = 54). In a study of fish from the Madeira River Basin, Cella-Ribeiro et al. (2017) described the diet of the four sampled species by stomach content analysis: *Mylossoma* *duriventre* (fruit = 58.54%, plants = 36.79%, seeds = 3.94%, arthropods = 0.63%); *Prochilodus nigricans* (detritus = 97.0%, algae = 3.0%); *Cichla pleiozona* (fish = 100%); *Serrasalmus rhombeus* (fish = 100%). These species were selected based on their: I- occurrence in the study area during the four hydrological periods; II- relevance as food items to the fishing community and Amazon population in general (Oliveira et al., 2010); and III- high prevalence of fish in the diet of *Cichla pleiozona* and *Serrasalmus rhombeus*, assuring the two species are top predators. Samples were obtained using nets with mesh sizes of 30, 45 and 60 mm. The sampling was done only in the lentic section of the floodplain system. Only adult fish were sampled to avoid the influence of ontogenetic shift in diet. Individuals were stored in ice bags and taken to the Environmental Biogeochemistry Laboratory of Rondônia Federal University (UNIR), where the biometric (total length and weight) and chemical analyses were performed.

2.3 Stable isotope analysis

A portion of the dorsal white muscle was extracted, freeze-dried (FreezeDry System, Labconco, Model 7522900, Kansas City, USA) and macerated. Stable isotope analysis was performed with a Thermo Finnigan Delta V Advantage mass spectrometer (isotope ratio mass spectrometer, IRMS, Thermo Scientific, Milan, Italy) coupled with a Flash 2000 organic elemental analyzer (Thermo Scientific, Milan, Italy). Pee Dee Belemnite (PDB) and atmospheric nitrogen were used as standards for $\delta^{13}C$ and $\delta^{15}N$, respectively. Triplicates of each 20 samples were used to test analytical precision ($\delta^{13}C$ was ±0.2‰ and $\delta^{15}N$ was ±0.3‰). For the elemental and isotopic composition, analytical precision was determined by certified standards

(Protein OAS/Isotope Cert 114859; Elemental Microanalysis, United Kingdom). The C:N atomic ratio (C:Na) of some individuals was higher than 3.5, so correction was necessary to remove the influence of lipids on the $\delta^{13}C$ signature. The equations from McConnaughey and McRoy (1979) were used to normalize the $\delta^{13}C$ signature:

$$L = 93/[1 + (0.246\ C{:}Na - 0.775)^{-1}]$$

$$\delta_{lipid\text{-}free} = \delta_{raw} + D\ [-0.207 + 3.90/(1+287/L)]$$

where: L is the estimated lipid content; $\delta_{lipid\text{-}free}$ is the corrected carbon isotopic signature and D is the difference of the $\delta^{13}C$ signature between protein and lipid (6‰ based on literature values) (McConnaughey and McRoy, 1979).

2.4 Total Hg data

Data of THg in muscle of the four sampled species during the hydrological cycles were obtained from Azevedo et al. (2020). The method of THg determination is described in detail in Supplementary Material 4 and in Azevedo et al. (2020). Samples for THg determination were not freeze-dried. THg data were expressed as ng g^{-1} wet wt.

2.5 Data analysis

All statistical analysis and graphing were carried out with the R software (R Core Team, 2019). Two-way ANOVA (aov function, base package; R Core Team, 2019) was performed to evaluate differences in stable isotopic signatures ($\delta^{13}C_{lipid\text{-}free}$, $\delta^{15}N$; reported in Table 1) and THg (reported in Table 2) among species and the four hydrological cycles. Since the interaction between species and the four hydrological cycles was significant in all cases ($p < 0.05$), we analyzed it using

Tukey's multiple comparison test (TukeyHSD, base package; R Core Team, 2019) and reported the results accordingly.

To assess the influence of length on the THg concentrations (reported in Supplementary Material 5) and isotopic signatures of carbon and nitrogen ($\delta^{13}C_{lipid-free}$, $\delta^{15}N$; reported in Figure 1), linear regressions between $\log_{10}$-transformed THg concentrations and these variables were carried out (lm function, base package, R Core Team, 2019). The influence of weight on THg concentration was also assessed by linear regressions (Supplementary Material 6). Data from the four hydrological cycles were pooled for these regression analyses because ANCOVA did not show significant differences among them (see Figure 1 and Supplementary Material 5 and 6 captions for *p*-values).

Due to the prevalence of non-significant relationships between THg concentration and length and/or weight and the low R^2 of the significant ones, we did not consider the influence of size in the statistical analysis (See Supplementary Material 5 and 6 for details).

To obtain the trophic magnification slope (TMS), linear regressions between $\log_{10}$-transformed THg concentrations and $\delta^{15}N$ signature were carried out (lm function, base package, R Core Team, 2019) for each hydrological cycle. Similarly, linear regressions were also used to evaluated the relationship between $\log_{10}$-transformed THg concentrations and $\delta^{13}C$ signature. For these analyses, we used the same four species in different hydrological cycles with their respective degrees of freedom (reported in Figure 2). The interaction between seasonality and isotope signatures ($\delta^{15}N$ and $\delta^{13}C$) was not significant ($p = 0.08$ and $p = 0.55$, respectively), and size was not considered as a covariable due to its small variation, since only adult fish were sampled (Supplementary Material 5).

To evaluate the effect of the composition of the food chain on TMS, both in relation to the number of species and their diets, food chain models were constructed considering all possible combinations between the number of species and their feeding habits, totaling 11 models (reported in Figure 3 and Supplementary Material 1). Since interaction between THg and $\delta^{15}N$ was not significant ($p = 0.08$), data from the four hydrological cycles were pooled for this analysis. The slopes, their standard errors and the degrees of freedom for each regression model were used to generate 95% confidence intervals. Then all models were compared to the reference food chain (the only model with all species and all diets) and the lack of overlap between them was interpreted as statistical support for a slope difference.

In all cases, *a priori* type I error of 5% ($\alpha = 0.05$) was assumed.

3. Results

3.1 Biometric variables and stable isotope signatures

In general, total length (TL) and total weight (TW) of the studied species followed the pattern: *Mylossoma duriventre* < *Serrasalmus rhombeus* < *Prochilodus nigricans* < *Cichla pleiozona* in all seasons.

he most enriched mean $\delta^{13}C$ signature was observed in the herbivorous *Mylossoma duriventre*, with values ranging from -26.81 ± 0.47‰ to -26.35 ± 0.50‰ ($p < 0.05$; Table 1). For *Prochilodus nigricans,* $\delta^{13}C$ ranged from -34.42 ± 1.38‰ to -33.50 ± 1.44‰; for *Cichla pleiozona* it varied from -33.60 ± 0.82‰ to -32.75 ± 1.32

Table 1. Total length (TL, cm), total weight (TW, g), stable isotopic signatures ($\delta^{13}C_{\text{lipid-free}}$, ‰) and ($\delta^{15}N$, ‰), C:Na, organic carbon (%) and nitrogen (%) of the sampled species during the four hydrological cycles.

Rising Water

Species	TL	TW	$\delta^{13}C_{\text{lipid-free}}$	$\delta^{15}N$	C:Na	C Org	N Org
Mylossoma duriventre	14.84 ± 1.28	177.70 ± 42.45	-26.62 ± 0.95^{Ab}	6.00 ± 1.18^{Ac}	4.47 ± 0.61	48.69 ± 2.60	12.82 ± 1.11
Prochilodus nigricans	24.33 ± 2.42	286.66 ± 29.02	-34.27 ± 0.76^{Aa}	7.81 ± 0.50^{Ab}	3.85 ± 0.08	44.74 ± 1.80	13.54 ± 0.51
Cichla Pleiozona	27.16 ± 1.16	311.66 ± 14.71	-33.27 ± 0.91^{Aa}	9.60 ± 0.34^{Aa}	3.76 ± 0.06	45.07 ± 2.58	14.00 ± 0.74
Serrasalmus rhombeus	21.00 ± 1.55	233.33 ± 20.00	-34.14 ± 0.97^{Aa}	10.30 ± 0.23^{Aa}	4.01 ± 0.25	46.67 ± 1.01	13.60 ± 0.74

High Water

Species	TL	TW	$\delta^{13}C_{\text{lipid-free}}$	$\delta^{15}N$	C:Na	C Org	N Org
Mylossoma duriventre	16.00 ± 0.80	184.00 ± 39.12	-26.62 ± 0.63^{Ab}	5.90 ± 0.87^{Ac}	5.28 ± 0.89	50.90 ± 3.93	11.44 ± 1.36
Prochilodus nigricans	26.35 ± 1.87	395.00 ± 75.91	-34.42 ± 1.38^{Aa}	7.45 ± 0.65^{Ab}	4.22 ± 0.37	47.00 ± 3.30	13.04 ± 1.06
Cichla Pleiozona	26.90 ± 2.57	442.10 ± 120.50	-33.60 ± 0.82^{Aa}	9.80 ± 0.20^{Aa}	3.77 ± 0.08	44.42 ± 4.51	13.73 ± 1.37
Serrasalmus rhombeus	22.70 ± 2.60	405.00 ± 106.60	-33.25 ± 0.40^{Aa}	10.74 ± 0.22^{Aa}	3.80 ± 0.09	45.52 ± 1.00	13.95 ± 0.38

Falling Water

Species	TL	TW	$\delta^{13}C_{\text{lipid-free}}$	$\delta^{15}N$	C:Na	C Org	N Org
Mylossoma duriventre	16.10 ± 1.40	182.50 ± 38.40	-26.35 ± 0.50^{Ab}	6.40 ± 0.90^{Ac}	4.83 ± 0.86	50.16 ± 2.22	12.35 ± 1.51
Prochilodus nigricans	26.10 ± 2.00	297.72 ± 33.51	-33.50 ± 1.44^{Aa}	7.40 ± 1.10^{Ab}	4.05 ± 0.26	46.57 ± 1.50	13.42 ± 0.57
Cichla Pleiozona	29.25 ± 4.17	352.85 ± 74.89	-33.31 ± 0.74^{Aa}	10.05 ± 0.37^{Aa}	3.83 ± 0.08	45.36 ± 1.40	13.80 ± 0.55
Serrasalmus rhombeus	24.00 ± 2.30	277.00 ± 34.20	-33.85 ± 1.12^{Aa}	10.76 ± 0.33^{Aa}	4.01 ± 0.19	46.26 ± 2.48	13.45 ± 0.54

Low Water

Species	TL	TW	$\delta^{13}C_{\text{lipid-free}}$	$\delta^{15}N$	C:Na	C Org	N Org
Mylossoma duriventre	15.16 ± 0.98	138.33 ± 31.88	-26.81 ± 0.47^{Ab}	6.47 ± 0.98^{Ac}	3.93 ± 0.05	45.16 ± 1.54	13.38 ± 0.33
Prochilodus nigricans	23.10 ± 1.74	287.00 ± 66.34	-33.82 ± 0.84^{Aa}	7.80 ± 0.66^{Ab}	3.79 ± 0.11	45.13 ± 2.00	13.91 ± 0.60
Cichla Pleiozona	28.10 ± 4.14	462.50 ± 187.23	-32.75 ± 1.32^{Aa}	9.93 ± 0.37^{Aa}	3.74 ± 0.09	45.18 ± 0.80	14.07 ± 0.47
Serrasalmus rhombeus	22.85 ± 2.38	400.00 ± 124.03	-34.94 ± 1.02^{Aa}	10.81 ± 0.37^{Aa}	3.77 ± 0.09	46.38 ± 0.85	14.33 ± 0.35

Lowercase letter: indicates Tukey test comparisons ($\alpha = 0.05$) in $\delta^{13}C_{\text{lipid-free}}$ and in $\delta^{15}N$ signature among species in the same hydrological cycle.

Upper case letters: indicates Tukey test comparisons ($\alpha = 0.05$) in $\delta^{15}N$ and in $\delta^{13}C_{\text{lipid-free}}$ signature for a species among the four hydrological cycles

‰; and for *Serrasalmus rhombeus* it ranged from -33.25 ± 0.40‰ to -33.85 ± 1.12‰ (Table 1). $\delta^{13}C$ signatures were statistically significant among these three species ($p < 0.05$). The variation in $\delta^{13}C$ signature along the seasons was not significant ($p > 0.05$) for any of the sampled species.

Mean $\delta^{15}N$ signatures followed the pattern: *Mylossoma duriventre* < *Prochilodus nigricans* < *Cichla pleiozona* = *Serrasalmus rhombeus* (Table 1) and were significant among all species except for *Cichla pleiozona* and *Serrasalmus rhombeus*. This pattern reflects the position of each species in the trophic chain: the herbivorous *Mylossoma duriventre*, lower link of the trophic chain, showed depleted $\delta^{15}N$ values while the piscivorous *Serrasalmus rhombeus*, a top predator in the trophic chain, had the highest $\delta^{15}N$ signature (Table 1). For the four species, there were no significant variations in $\delta^{15}N$ signature along the four hydrological cycles ($p > 0.05$) (Table 1). Organic carbon (C Org) and nitrogen (N Org) levels were similar among the four species, ranging from 44.74 ± 1.80% to 50.90 ± 3.93% and from 11.44 ± 1.36% to 14.33 ± 0.35%, respectively.

Linear regression between total length and stable isotope signatures ($\delta^{13}C_{lipid\text{-}free}$, $\delta^{15}N$) were non-significant ($p > 0.05$) for *Mylossoma duriventre* (Fig. 1A-B), *Prochilodus lineatus* (Fig. 1C-D), *Serrasalmus rhombeus* (Fig. 1E-F) and *Cichla pleiozona* (Fig. 1G-H).

3.2 THg concentration

Mean THg concentration in the herbivorous *Mylossoma duriventre* was the lowest, in all hydrological cycles ($p < 0.05$; Table 2). The mean THg concentration

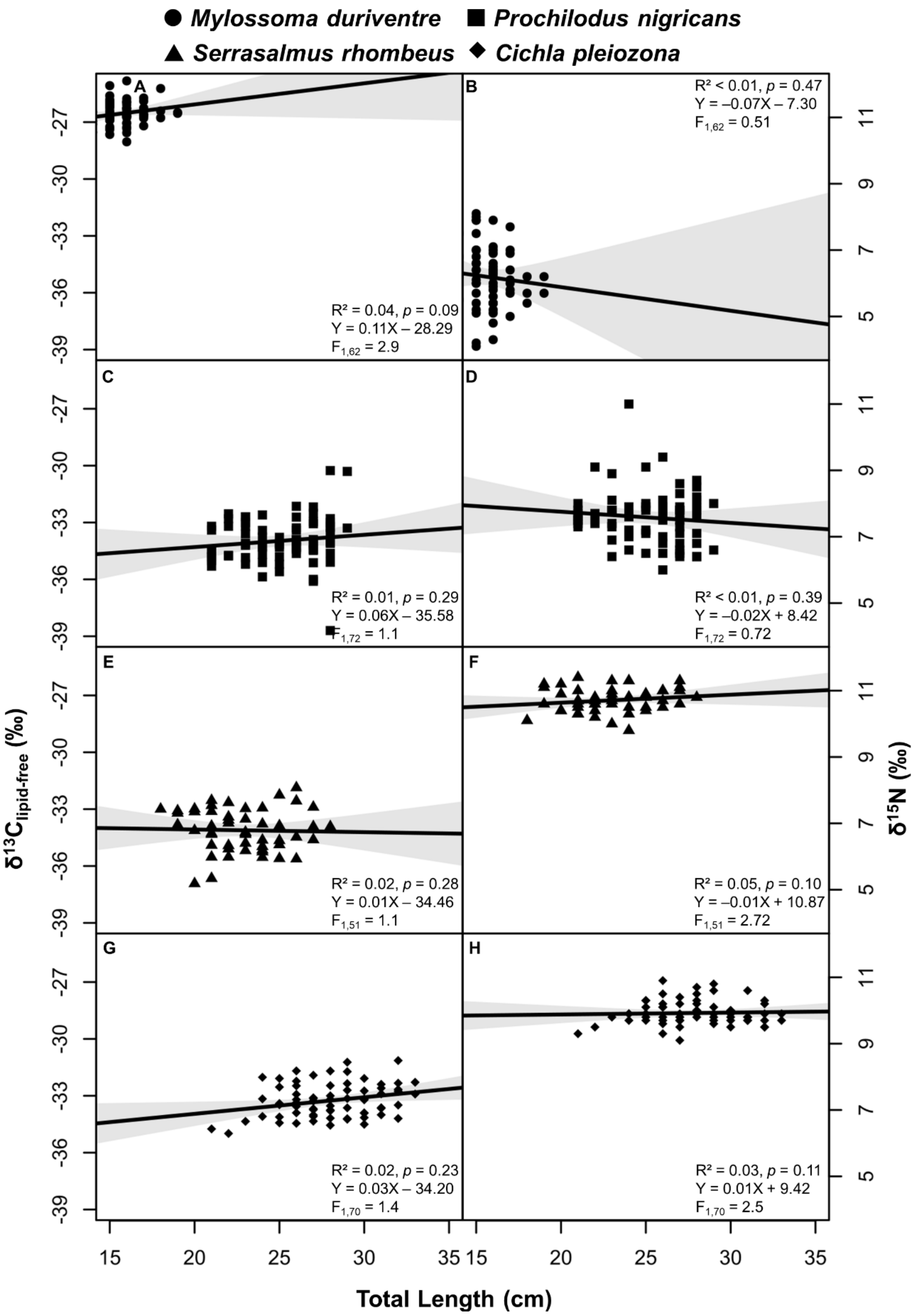


Figure 1. Linear regression between stable isotope signature ($\delta^{13}C_{lipid\text{-}free}$ and $\delta^{15}N$) and total length. **A, B:** *Mylossoma duriventre*; **C,D:** *Prochilodus nigricans*; **E,F:** *Serrasalmus rhombeus*; **G,H:** *Cichla pleiozona*. The shaded area around regressions represents the 95% confidence intervals. Model statistics (Equation, R², *p* and F) are within each graph. Data from the four hydrological cycles were pooled for regression analyses because ANCOVA did not show significant differences among them (*p* = 0.38, 0.44, 0.22, 0.08, 0.29, 0.48, 0.11, 0.44 for graphs A, B, C, D, E, F, G and H, respectively).

of *Mylossoma duriventre* was one order of magnitude lower than *Prochilodus nigricans* ($p < 0.05$; Table 2) and *Cichla pleiozona* ($p < 0.05$; Table 2) in all hydrological cycles and two orders of magnitude lower than the piscivorous *Serrasalmus rhombeus* in RW ($p < 0.05$; Table 2), HW ($p < 0.05$; Table 2) and LW ($p < 0.05$; Table 2). *Mylossoma duriventre* mean THg concentration was similar along the four hydrological cycles, with no significant variation ($p > 0.05$) (Table 2).

The detritivorous *Prochilodus nigricans* showed intermediate THg concentration, with higher values than the herbivorous species ($p < 0.05$; Table 2) and lower than the two piscivorous ones ($p < 0.05$; Table 2). In the RW hydrological cycle, THg concentration was significantly higher (0.33 ± 0.06 mg kg^{-1} wet wt.) ($p < 0.05$; Table 2) than in HW (0.20 ± 0.06 mg kg^{-1} wet wt.), FW (0.17 ± 0.06 mg kg^{-1} wet wt.) and LW (0.18 ± 0.06 mg kg^{-1} wet wt.), while the THg concentration among these three latter hydrological cycles was similar ($p > 0.05$) (Table 2).

Although *Serrasalmus rhombeus* and *Cichla pleiozona* have overlapping niches (Cella-Ribeiro et al., 2017), mean THg concentration in first was one order of magnitude higher than in the second in HW and LW (Table 2). Mean THg concentration in *Cichla pleiozona* was similar in RW, HW, FW and LW ($p > 0.05$; Table 2). *Serrasalmus rhombeus* was the only species to show a substantial increase in THg concentration among the hydrological cycles, with higher mean values in HW (1.64 ± 0.69 mg kg^{-1}) and lower in FW (0.85 ± 0.30 mg kg^{-1}) ($p < 0.05$; Table 2).

Table 2. THg concentration (mg kg^{-1} wet wt.) in fish species (Mean±SD) and their diets, for each hydrological cycle. The number of samples is within parentheses.

Species	Diets	Rising Water	High Water	Falling Water	Low Water
Mylossoma duriventre	Herbivorous	0.04 ± 0.02 (13)^{Ca}	0.05 ± 0.02 (20)^{Ca}	0.05 ± 0.02 (24)^{Ca}	0.05 ± 0.02 (6)^{Ca}
Prochilodus nigricans	Detritivorous	0.33 ± 0.06 (12)^{Ba}	0.20 ± 0.06 (20)^{Bb}	0.17 ± 0.06 (22)^{Bb}	0.18 ± 0.06 (20)^{Bb}
Cichla Pleiozona	Piscivorous	0.95 ± 0.52 (5)^{Aa}	0.96 ± 0.21 (20)^{Aa}	0.85 ± 0.27 (35)^{Aa}	0.94 ± 0.38 (12)^{Aa}
Serrasalmus rhombeus	Piscivorous	1.03 ± 0.39 (9)^{Aa}	1.64 ± 0.69 (11)^{Aa}	0.85 ± 0.30 (20)^{Ab}	1.44 ± 0.42 (14)^{Aa}

*Lowercase letters indicate significant differences (Tukey's multiple comparison test; α = 0.05) of THg concentration of a species among hydrological cycles.

*Uppercase letters indicate significant differences (Tukey's multiple comparison test; α = 0.05) of THg concentration among species in the same hydrological cycle.

THg concentration was negatively associated with $\delta^{13}C$ signature in RW (R^2= 0.81; $p < 0.0001$; Fig. 2A), HW (R^2= 0.48; $p < 0.0001$; Fig. 2C), FW (R^2= 0.60; $p < 0.0001$; Fig. 2E) and LW (R^2= 0.31; $p < 0.0001$; Fig. 2G).

3.3 THg biomagnification

TMS was similar among all hydrological cycles, as indicated by the ANCOVA interaction term ($p = 0.08$).

In this study, we tested the influence of food chain modeling on TMS results by constructing models based on all possible combinations between the number of species and their feeding habits (Figure 3 and Supplementary Material 1). A food chain considering the four species and their respective diets (*Mylossoma duriventre* - herbivorous + *Prochilodus lineatus* - detritivorous + *Cichla pleiozona* - piscivorous + *Serrasalmus rhombeus* - piscivorous) is the "reference model", against which all other models were compared. The reference model showed a mean TMS of 0.26 and 95% confidence interval of 0.24-0.27. Significantly lower TMS values were found in food chains composed of *Prochilodus lineatus* and *Serrasalmus rhombeus* (TMS = 0.20; 95% confidence interval: 0.18-0.23); *Cichla pleiozona* and *Serrasalmus rhombeus* (TMS = 0.03; 95% confidence interval: -0.02 – 0.10); and *Prochilodus lineatus*, *Cichla pleiozona* and *Serrasalmus rhombeus* (TMS = 0.21; 95% confidence interval: 0.19-0.23) (Figure 3 and Supplementary Material 1). These three models' 95% confidence intervals showed no overlap with the 95% confidence interval of the model with four diets. The results showed that food chain modeling can influence the TMS results in an Amazonian lake.

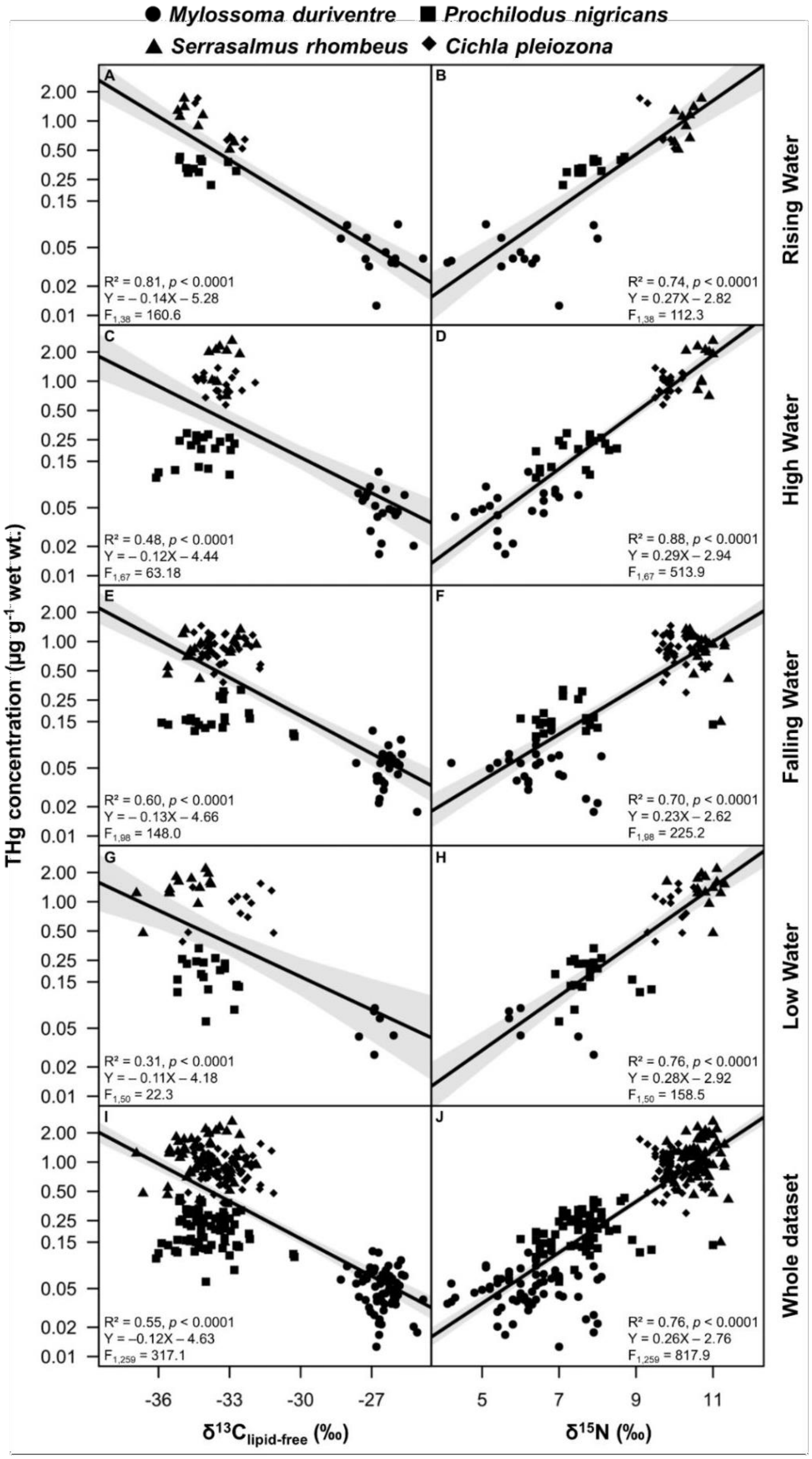


Figure 2. Linear regressions between THg concentrations (mg kg^{-1} wet wt.) and stable isotope signature ($\delta^{13}C_{\text{lipid-free}}$ and $\delta^{15}N$, on x-axis) for each hydrological cycle (on secondary y-axis). The shaded area around regressions represents the 95% confidence intervals. Model statistics (Equation, R², *p* and F) for regressions between THg concentrations and isotope signatures are within each graph. The relationship between THg and $\delta^{13}C_{\text{lipid-free}}$ and TMS was similar among all hydrological cycles, as reported by the ANCOVA interaction term (*p* = 0.55 and 0.08, respectively).

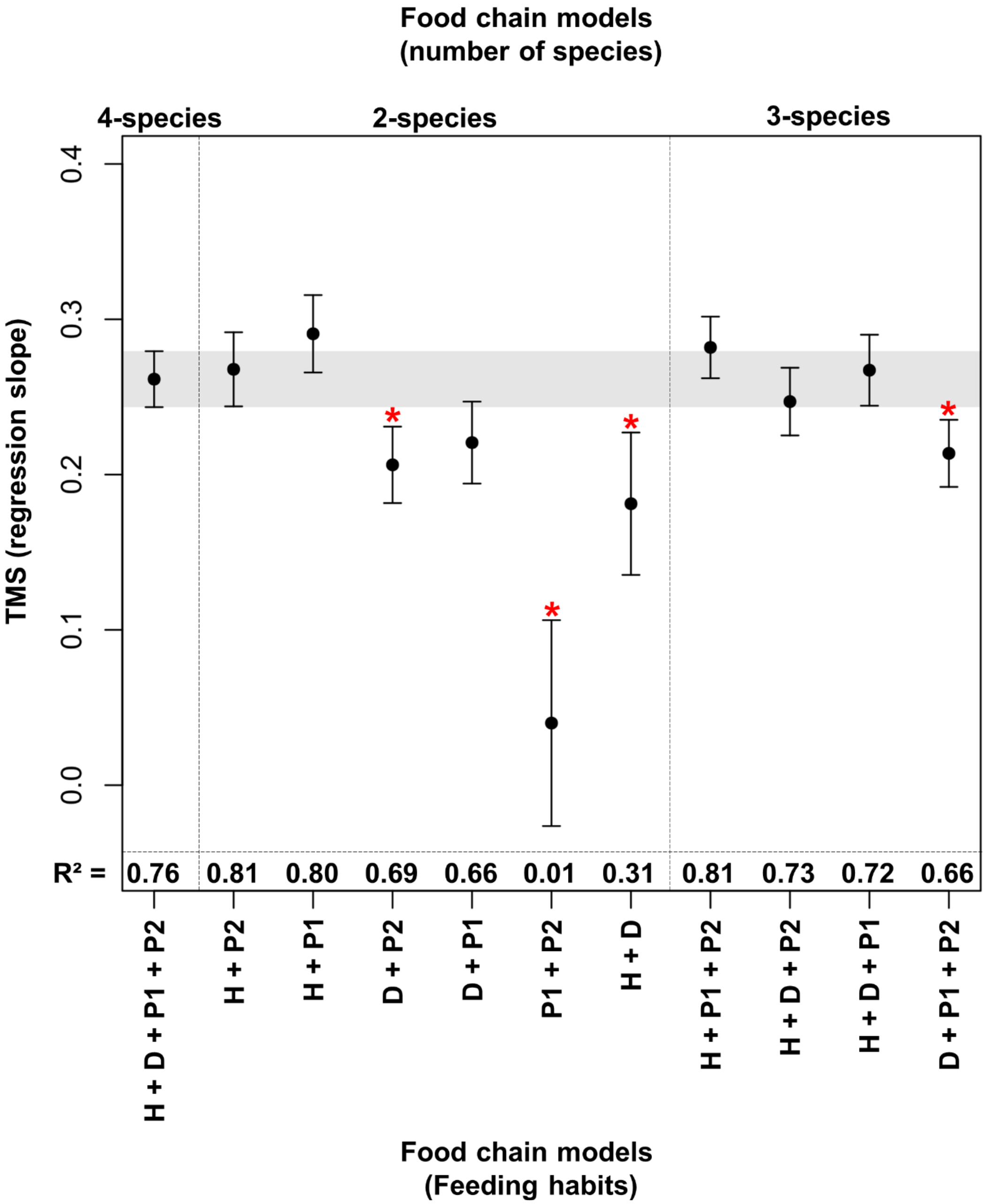


Figure 3. TMS (slope of the regression of THg with $\delta^{15}N$) in different food chain models. Bars represent the TMS 95% confidence intervals. Grey shading indicates the 95% confidence interval of TMS from the 4-species food chain model. Red asterisks indicate which food chain models differed from the 4-species food chain model (non-overlapping confidence intervals). H: herbivorous (*Mylossoma duriventre*); D: detritivorous (*Prochilodus nigricans*); P1: piscivorous (*Cichla pleiozona*); P2: piscivorous (*Serrasalmus rhombeus*). The coefficient of determination (R²) from the regressions of each food chain model is present right above them. All regressions were significant, except for the P1+P2 model (p=0.23). Additional details are in the **Supplementary Material 1**.

4. Discussion

4.1 Seasonal variation: isotopic signature

The variation of $\delta^{13}C$ signature was not significant among the hydrological cycles. In agreement with our results, Silva et al. (2005) observed little variation in $\delta^{13}C$ signature in non-piscivorous and piscivorous fish from Bom Intento and Cupu lakes (both in the Amazon region) between HW (sampled in April-May 2000) and RW (sampling in January 2001). Oliveira et al. (2006) observed a significant variation in $\delta^{13}C$ signature in *Mylossoma duriventre* among RW (-20.5 ± 3.8‰), HW (-23.7 ± 3.7‰) and FW (-23.5 ± 4.3‰) in Camaleão Lake, and the authors reported a high degree of omnivory for this species. The same authors also observed that species with no omnivorous behavior like *Schizodon fasciatus* (herbivorous), *Prochilodus nigricans* (detritivorous) and *Cichla monocolus* (piscivorous) showed low variation in $\delta^{13}C$ signature along the four hydrological cycles. In the Madeira River Basin, *Mylossoma duriventre* is classified as exclusively herbivorous (Cella-Ribeiro et al., 2017), so the non-significant variation in $\delta^{13}C$ signature was expected. Carvalho et al. (2015) observed significant variation in $\delta^{13}C$ signature in *Astyanax altiparanae*, *Hisonotus paracanjuba* and *Knodus moenkhausii* among different types of land use (i.e., natural cover, sugarcane and pasture), which suggests that watershed characteristics can influence carbon isotope signature. The sampling was done only in the lake section of the floodplain system, so the land use was mostly natural vegetation and the influence of land use on the observed results was small, partially explaining the similarity of $\delta^{13}C$ values among species.

The non-significant variation in $\delta^{15}N$ signature suggests no dietary shift among the hydrological cycles. This result agrees with that reported by Silva et al. (2005), who observed similar results in piscivorous and non-piscivorous fish from Bom Intento, Pereira and Cupu lakes (Amazon region). Changes in $\delta^{15}N$ signature are related to ontogenetic dietary shift (Cornelissen et al., 2018; Park et al., 2018), different land use (Carvalho et al., 2015) and spatial gradients (Marsh et al., 2017). In this study, only adult fish were considered, which rules out the influence of ontogenetic dietary shift. Carvalho et al. (2015) observed enriched $\delta^{15}N$ signature in fish from streams flowing through sugarcane plantations and pastures due to the influence of chemical/organic fertilizers and dung, with significant change in trophic level of *Characidium zebra* and *Hisonotus piracanjuba*. Puruzinho Lake is a relatively pristine area with a small population and no sugarcane plantations or grazing land that could increase the $\delta^{15}N$ signature.

4.2 Seasonal variation: THg concentration

THg levels among species followed the pattern often reported in the literature (Bastos et al., 2008; Bastos et al., 2015; Økelsrud et al., 2016): piscivorous > detritivorous > herbivorous. THg concentration in fish is influenced by several factors, including seasonality (Azevedo et al., 2020). Two major patterns of THg during the hydrological cycles were observed by Azevedo et al. (2020): (1) higher concentrations during RW and LW for herbivorous ($n_{species}$ = 2), detritivorous ($n_{species}$ = 7), carnivorous ($n_{species}$ = 6) and piscivorous ($n_{species}$ = 5) guilds; and (2) lower concentration during LW season for omnivorous ($n_{species}$ = 5) and planktivorous ($n_{species}$ = 3) guilds. THg concentrations for the studied species did not follow any of the patterns reported by Azevedo et al. (2020). THg concentrations in *Mylossoma duriventre* and *Cichla pleiozona* did not show any significant variation among hydrological cycles. This result may be related to similar diet during the hydrological cycles, as indicated by the absence of significant variation in stable isotope signatures. Although $\delta^{13}C$ signature of *Prochilodus nigricans* did not change over the hydrological cycles, THg concentrations were significantly higher in RW. Phytoplankton is a major component of the diet of *Prochilodus nigricans* (Araujo-Lima et al., 1986; Forsberg et al., 1993) and THg concentration in these primary producers increases significantly during RW in Puruzinho Lake possibly due to Hg leaching from soil to the lake (Nascimento et al., 2006). Thus, the higher concentrations observed in *Prochilodus nigricans* can be attributed to higher concentrations of Hg in food. *Serrasalmus rhombeus* showed higher THg concentration in HW in comparison with RW and FW. This result is not in agreement that of Azevedo et al. (2020), who observed higher Hg concentrations in fish guilds in RW and lower in HW.

THg concentration was not influenced by $\delta^{13}C$ signature in individual species (Supplementary Material 2). The studies that have reported significant relationship between these variables (Eagles-Smith et al., 2008; Arcagni et al., 2018; Croizier et al., 2019) investigated more than one species. For example, Arcagni et al. (2018) considered pelagic and demersal species. In this study, the analysis of the four species (Fig. 2A; 2C; 2E; 2G; 2I) resulted in significant negative relationships due to lower THg concentration in the most enriched $\delta^{13}C$ signature species (i.e., *Mylossoma duriventre*) and higher concentrations in the most depleted $\delta^{13}C$

signature species (i.e., *Serrasalmus rhombeus* and *Cichla pleiozona*). However, when just one species was considered in the analysis (Supplementary Material 2), the relationship was weak and non-significant.

There was no seasonal variation in the relationship between $\delta^{13}C$ signature and THg concentration, as evidenced by the similar slopes among RW (-0.14; Fig. 2A), HW (-0.12; Fig. 2C), FW (-0.13; Fig. 2E) and LW (-0.11; Fig. 2G) and the ANCOVA interaction term ($p = 0.55$).

4.3 Seasonal variation: THg biomagnification

There was no significant variation in TMS among RW, HW, FW and LW in Puruzinho Lake ($p = 0.08$). To the best of our knowledge, there are no studies that have evaluated variation in TMS among hydrological cycles in the Amazon region, making it difficult to compare results. However, other studies have tested seasonal variation in other parts of the world (Zhang et al., 2012; Razavi et al., 2014; Marrugo-Negrete et al., 2017; Verhaert et al., 2019). In Ayapel and LaRaya marshes, northern Colombia, Marrugo-Negrete et al. (2017) reported no significant variation in TMS between the rainy season (September to November 2010) (Ayapel: 0.13; LaRaya: 0.17) and dry season (January to March 2010) (Ayapel: 0.15; LaRaya: 0.20), which agrees with our results. Also, in Xin'anjiang Reservoir in Qiandao Hu, China, the TMS between summer (May 2009 and June 2011) (0.13) and fall (October 2008) (0.10) was similar (Razavi et al., 2014). Conversely, along the shore of Lake Ontario, Canada, TMS values were 0.18, 0.17 and 0.27 during spring (May-June 2009, summer (July-mid September 2009) and fall (mid September-November 2009), respectively (Zhang et al., 2012). In the Olifants River Basin, South Africa, Verhaert et al. (2019) reported differences in the trophic magnification factor (TMF) during the summer high flow in April 2012 (1.9 and 2.5) and winter low flow in September 2012 (3.7 and 4.2).

In the most recent meta-analysis about Hg biomagnification, Lavoie et al. (2013) reported TMS values significantly higher in colder environments. At lower temperatures, fish metabolism is relatively slower, which in turn results in lower Hg excretion and size growth rates (Trudel and Rasmussen, 1997; Greenfield et al., 2001). Under these conditions, biomagnification rates are higher (Lavoie et al., 2013). Verhaert et al. (2019) reported a higher biomagnification rate during the colder season, corroborating Lavoie et al. (2013). In tropical regions, temperatures remain relatively high throughout the year, which may explain the lack of variation in TMS in this study. However, temperature alone cannot explain the results. Other factors, like food chain modeling, can influence TMS (Yan et al., 2010; Cheng et al., 2011; Cheng & Hu, 2012; Poste et al., 2012; Ouédraogo et al., 2015; Azevedo-Silva et al., 2016).

4.4 Food chain modeling influence on TMS and considerations for standardization

In studies of Hg biomagnification, researchers consider species with different diets to represent the food chain. Some authors have considered more than five diets (Kidd et al., 2003; Poste et al., 2012) and others with less than four (Silva et al., 2005; Li et al., 2015). However, there are no criteria on how many diets should be used. In this study, a food chain with three diets (herbivorous, detritivorous and piscivorous) and four species resulted in a TMS of 0.24 and 0.26, respectively, which is similar to other results in the Amazon (Azevedo-Silva et al., 2016). The TMS is significantly higher in a food chain with only herbivorous and piscivorous species (TMS= 0.29) and significantly lower with detritivorous and piscivorous (TMS = 0.20), which indicates that food chain modeling influences TMS results. As its name implies, TMS is the slope of linear regression between $\log_{10}$-THg and $\delta^{15}N$. Therefore, the slope will be steeper as bigger differences in THg concentration are observed among the diets. For example, the TMS with herbivorous-piscivorous food chain is significantly higher than the detritivorous-piscivorous pair due to bigger differences in THg concentration between the herbivorous and piscivorous species compared to detritivorous and piscivorous fishes. Kwon et al. (2012) observed a very low TMS (0.10) in the Amazon aquatic food web due to the lack of significant differences in THg concentration among the diets (piscivorous, carnivorous, invertivorous, omnivorous, algivorous, herbivorous and detritivorous), with the only exception being piscivorous THg concentrations, which were significantly higher than for herbivorous species. If these authors considered the food chain with only piscivorous and herbivorous species, the TMS would be higher. Borga et al. (2012) suggested that the TMS is influenced by the enrichment of $\delta^{15}N$ at the base of the food web and by the organism selected to represent the lower trophic level. The authors recommended using organisms with lower seasonal variation of $\delta^{15}N$ to avoid overestimation or underestimation of TMS. Considering other animals than fish can influence TMS as well. Clayden et al. (2015) demonstrated that in a small artic polynya ecosystem, a food chain composed of invertebrates and fish had TMS of 0.15 while one of invertebrates and seabirds had TMS of 0.26.

Based on our results, the critical step to modeling a food chain for biomagnification studies is to decide which species is going to represent the base and which one is going to represent the top of the food chain. Greater difference in THg levels and $\delta^{15}N$ signatures between the base and top species will result in a higher TMS values. Therefore, it is advisable to include an herbivorous species to denote the base and a piscivorous species to represent the top of the food chain to not introduce bias in TMS estimation. Including a “intermediate” species, like the detritivorous *Prochilodus nigricans*, in an herbivorous-piscivorous food chain did not change the TMS significantly, indicating it is optional for the researcher to considerer or not this diet when modeling a food chain. However, it is not advisable to use an “intermediate” species, like a detritivore, to represent the base of the food chain due to underestimation of TMS.

The results presented in this paper bring useful information for researchers who study Hg bioaccumulation/biomagnification. First, the results indicate that to assess Hg biomagnification with TMS, it is not necessary to sample the species along the four hydrological seasons. Second, sampling a high number of species of different diets is not necessary. The results showed clearly that TMS did not change when an “intermediate” diet, like detritivorous, was included in a herbivorous-piscivorous foot chain. Researchers can sample one species to represent the lower trophic level and other one to represent the higher trophic levels to obtain a reliable TMS evaluation. These methodological implications are fundamental because the Amazon is a relatively remote area and sampling logistics is not always easy, especially for researchers from other regions of Brazil or other countries.

No major seasonal variations in Hg accumulation patterns emerged in this study. However, Azevedo et al. (2020) observed that herbivorous, detritivorous, carnivorous and piscivorous fish guilds showed increased Hg concentration during RW and LW and lower concentrations during HW and FW. Based on this pattern, the consumption guidelines should advise consumers to reduce their fish intake during the seasons of higher Hg concentrations (i.e., RW and LW). Other studies in the Amazon aiming to establish consumption guidelines should consider seasonality and pooling of species in guilds.

5. Conclusions

In this study, we report for the first time the TMS in all four Amazonian hydrological cycles. There was no significant difference in TMS among RW, HW, FW and LW, which suggests that the variation in the hydrological cycle did not influence TMS. The food chain modeling influenced TMS, with the biomagnification rate being significantly higher in herbivorous-piscivorous food chains (TMS= 0.29) and lower with detritivorous-piscivorous diets (TMS= 0.20). In summary, we recommend that in new studies about Hg biomagnification in fish food chains that researchers carefully the species to represent the top and base of the food chain, where preferentially the base species should feed exclusively on plants and the top one exclusively on fish, so as not to introduce bias the TMS value. Including one or more species with “intermediate” diets does not change the TMS significantly in an herbivorous-piscivorous food chain.

Since only adult fish were sampled, stable isotope signatures and size showed non-significant relationship for the majority of the species, suggesting a lack of diet shift. When the four species were pooled together, the relationship was negative for $\delta^{13}C$ and total length and positive for $\delta^{15}N$ and total length.

Total Hg concentration was not significantly associated with $\delta^{13}C$ signature of individual species, possibly due to low variation in the latter variable. However, when the species were pooled, the relationship was significant and negative. THg concentration among hydrological cycles in fish species did not follow the pattern for fish guilds reported by Azevedo et al. (2020)

6. Acknowledgments

This work was supported by the CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) through the CNPq/CTUniversal project (Grant no. 458977 2014-4) and by the Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ; grant number: E-26/010.001984/2014 and E-26/210.071/2018). We are grateful to IBAMA (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis) for the fish collection license (DIFAP/IBAMA no. 091). We extend special thanks to the fisherman Raimundo Nonato dos Santos. This study was also financed in part by Coordenação de Aperfeiçoamento de Pessoa de Nível Superior – Brazil (CAPES) – Finance Code 001.

7. References

Araújo-Lima, C.A.R.M., Forsberg, B.R., Victoria, R., Martinelli, L.A., 1986. Energy sources for detritivorous fishes in the Amazon. Science 234, 1256-1258.

Arcagni, M., Juncos, R., Rizzo, A., Pavlin, M., Fajon, V., Arribére, M.A., Horvat, M., Guevara, S.R., 2018. Species- and habitat-specific bioaccumulation of total mercury and methylmercury in the food web of a deep oligotrophic lake. Science of the Total Environment 612, 1311-1319.

Azevedo-Silva, C.E., Almeida, R., Carvalho, D.P., Ometto, J.P.H.B., Camargo, P.B., Dorneles, P.R., Azeredo, A., Bastos, W.R., Malm, O., Torres, J.P.M., 2016. Environmental Research 151, 286-296.

Azevedo, L.S.; Pestana, I.A.; Nery, A.F.C.; Bastos, W.R.; Souza, C.M.M. 2020. Mercury concentration in six fish guilds from a floodplain lake in western Amazonia: Interaction between seasonality and feeding habits. Ecological Indicators, 111:106056.

Bastos, W.R., Almeida, R., Dórea, J.G., Barbosa, A.C., 2007. Annual flooding and fish-mercury bioaccumulation in the environmentally impacted Rio Madeira (Amazon). Ecotoxicology 16, 341-346.

Bastos, W.R., Rebelo, M.F., Fonseca, M.F., Almeida, R., Malm, O., 2008. A description of mercury in fishes from the Madeira River basin, Amazon, Brazil. Acta Amazonica 38 (3), 431-438.

Bastos, W.R., Dórea, J.G., Bernardi, J.V.E., Lauthartte, L.C., Mussy, M.H., Lacerda, L.D., Malm, O., 2015. Mercury in fish of the Madeira River (temporal and spatial assessment), Brazilian Amazon. Environmental Research 140, 191-197.

Béliveu, A., Lucotte, M., Davidson, R., Paquet, S., Mertens, F., Passos, C.J., Romana, C.A., 2017. Reduction of soil erosion and mercury losses in agroforestry systems compared to forests and cultivated fields in the Brazilian Amazon. Journal of Environmental Management 203, 522-532.

Bidone, E.D., Castilhos, Z.C., Santos, T.J.S., Souza, T.M.C., Lacerda, L.D., 1997. Fish contamination and human exposure to mercury in Tartarugalzinho River, Amapa State, Northern Amazon, Brazil. A screening approach. Water, air and soil pollution 97, 9-15.

Bonotto, D.M., Wijesiri, B., Vergotti, M., Silveira, E.G., Goonetilleke, A., 2018. Assessing mercury pollution in Amazon River tributaries using a Bayesian Network approach. Ecotoxicology and Environmental Safety 166, 354-358.

Borga, K., Kidd, K.A., Muir, D.C.G., Berglund, O., Conder, J.M., Gobas, F.A.P.C., Kucklick, J., Malm, O., Powel, D.E., 2012. Trophic magnification factors: consideration ecology, ecosystem, and study design. Integrated Environmental Assessment and Management 8, 64-84.

Carvalho, D.R., Castro, D., Callisto, M., Moreira, M.Z., Pompeu, P.S., 2015. Isotopic variation in five species of stream fishes under the influence of different land uses. Journal of Fish Biology 87, 559-578.

Cella-Ribeiro, A., Torrente-Vilara, G., Lima-Filho, J.A., Doria, C.R.C., 2017. Ecologia e Biologia de peixes do Rio Madeira. EDUFRO, Porto Velho.

Cheng, Z., Liang, P., Shao, D.D., Wu, S.C., Nie, X.P., Chen, K.C., Li, K.B., Wong, M.H., 2011. Mercury biomagnification in the aquaculture pond Ecosystem in the Pearl River Delta. Archives of Environmental Contamination and Toxicology 61, 491-499.

Cheng, H., Hu, Y., 2012. Understanding the paradox of mercury pollution in china: High concentrations in environmental matrix yet low levels in fish on market. Environmental Science & Technology 46, 4695-4696.

Clayden, M.G., Arsenault, L.M., Kidd, K.A., O'Driscoll, N.J., Mallory, M.L., (2015). Mercury bioaccumulation and biomagnification in a small Arctic polynya ecosystem. Science of the Total Environment 509-510, 206-215.

Cordier, S., Garel, M., Mandereau, L., Morcel, H., Doineau, P., Gosme-Seguret, S., Josse, D., White, R., Amiel-Tison, C., 2002. Neurodevelopmental investigations among methyl mercury exposed children in French Guiana. Environmental Research, 89:1-11.

Cornelissen, I.J.M., Vijverberg, J., van den Beld, A.M., Helmsing, N.R., Verreth, J.A.J., Nagelkerke, L.A.J., 2018. Stomach contents and stable isotopes confirm ontogenetic diet shifts of Nile perch, Lates niloticus, in southern Lake Victoria. Journal of Great Lakes Research 44, 1264-1272.

Croizier, G., Schaal, G., Point, D., Loc'h, F., Machu, E., Fall, M., Munaron, J.M., Boyé, A., Walter, P., Laë, R., Morais, L.T., 2019. Stable isotope analyses revealed the influence of foraging habitat on mercury accumulation in tropical coastal marine fish. Science of the Total Environment 650, 2129-2140.

Eagles-Smith, C.A., Suchanek, T.H., Colwell, A.E., Anderson, N.L., 2008. Mercury trophic transfer in a eutrophic lake: the importance of habitat-specific foraging. Ecological Aplications 18, 196-212.

Feitosa, I.B., Huszar, V.L.M., Domingues, C.D., Appel, E., Paranhos, R., Almeida, R.M., Branco, C.W.C., Bastos, W.R., Sarmento, H., 2019. Plankton Community interactions in an Amazonian floodplain lake, from bacteria to zooplankton. Hydrobiologia 831, 55-70.

Forsberg, B.R., Araujo-Lima, C.A.R.M., Martinelli, L.A., Victoria, R.L., Bonassi, J.A., 1993. Autotrophic carbon sources for fish of the central amazon. Ecology 74:643-652.

Grandjean, P., White, R.F., Nielsen, A., Cleary, D., Santos, E.C.O., 1999. Methyl mercury neurotoxicity in Amazonian children downstream from gold mining. Environmental Health Perspectives 107, 587-591.

Greenfield, B.K., Hrabik, T.R., Harvey, C.J., Carpenter, S.R., 2001. Predicting mercury levels in yellow perch: use of water chemistry, trophic ecology, and spatial traits. Canadian Journal of Fisheries and Aquatic Sciences 58, 1419-1429.

Hachiya, N., 2006. The history and the present of Minamata disease – entering the second half a century. Japan Medical Association Journal 49, 112-118.

Harada, M., Nakanishi, J., Yasoda, E., Pinheiro, M.C.N., Oikawa, T., Guimarães, G.A., Cardoso, B.S., Kizaki, T., Ohno, H., 2001. Mercury pollution in the Tapajós River Basin, Amazon: mercury level of head hair and health effects. Environmental International 27, 285-90.

Johnson, M., 2003. Case Study: Mercury Contamination in the Amazon. Reducing soil erosion may provide a lasting solution. International Development Research Centre. https://idl-bnc-idrc.dspacedirect.org/handle/10625/52893.

Kehrig, H.A., Malm, O., 1999. Methylmercury in fish as a tool for understanding the Amazon mercury contamination. Applied Organometallic Chemistry 13, 689-696.

Kidd, K.A., Bootsma, H.A., Hesslein, R.H., Lockhart, W.L., Hecky, R.E., 2003. Mercury concentrations in the food webs of lake Malawi, East Africa. Journal of Great Lakes Research 29, 258-266.

Kwon, S.Y., McIntyre, P.B., Flecker, A.S., Campbell, L.M., 2012. Mercury biomagnification in the food web of a neotropical stream. Science of the Total Environment 417-418, 92-97.

Lavoie, R.A., Jardine, T.D., Chumchal, M.M., Kidd, K.A., Campbell, L.M., 2013. Biomagnification of Mercury in Aquatic Food Webs: A Worldwide Meta-Analysis. Environmental Science & Technology 47, 13385-13394.

Lechler, P.J., Miller, J.R., Lacerda, L.D., Vinson, D., Bonzongo, J.C., Lyons, W.B., Warwick, J.J., 2000. Elevated mercury concentrations in soils, sediments, water, and fish of the Madeira River basin, Brazilian Amazon: a function of natural enrichments? The Science of the Total Environment 260, 87-96.

Li, J., Zhou, Q., Yuan, G., He, X., Xie, P., 2015. Mercury bioaccumulation in the food web of Three Gorges Reservoir (China): Tempo-spatial patterns and effect of reservoir management. Science of the Total Environment 527-528, 203-210.

Lino, A.S., Kasper, D., Guida, Y.S., Thomaz, J.R., Malm, O., 2019. Total and methyl mercury distribution in water, sediment, plankton and fish along the Tapajós River basin in the Brazilian Amazon. Chemosphere 235, 690-700.

Maia, P.D., Maurice, L., Tessier, E., Amouroux, D., Cossa, D., Pérez, M., Moreira-Turcq, P., Rhéault, I., 2009. Mercury distribution and Exchange between the Amazon River and connected floodplain lake. Science of the Total Environment 407, 6073-6084.

Malm, O., 1998. Gold mining as a source of mercury exposure in the Brazilian Amazon. Environmental Research, Section A 77, 73-78.

Marsh, J.M., Mueter, F.J., Iken, K., Danielson, S., 2017. Ontogenetic, spatial and temporal variation in trophic level and diet of Chukchi Sea fishes. Deep-Sea Research II 135, 78-94.

Marrugo-Negrete, J.L., Ruiz-Guzmán, J.A., Ruiz-Fernández, A.C., 2017. Biomagnification of mercury in fish from two gold mining-impacted tropical marshes in northern California. Archives of Environmental Contamination and Toxicology 74, 121-130.

McConnaughey, T., McRoy, C.P., 1979. Food-Web Structure and the Fractionation of Carbon Isotopes in the Bering Sea. Marine Biology 53, 257-262.

Miller, D.R., Akagi, H., 1979. pH affects mercury distribution, not methylation. Ecotoxicology and Environmental Safety 3, 36-38.

Mussy, M.H., 2018. Mercúrio em peixes na área de influência da hidrelétrica – Santo Antônio no Rio Madeira no Pré e Pós Enchimento (unpublished PhD thesis), Universidade Federal do Rio de Janeiro.

Nascimento, E.L., Gomes, J.P.O., Almeida, R., Bastos, W.R., Bernardi, J.V.E., Miyai, R.K., 2006. Mercurio no plâncton de um lago natural amazônico, Lago Puruzinho (Brasil). J. Braz. Soc. Ecotoxicol. 1:1-6.

Nevado, J.J.B., Martin-Doimeadios, R.C.R., Bernardo, F.J.G., Moreno, M.J., Herculano, A. M., do Nascimento, J.L.M., Crespo-Lopez, M.E., 2010. Mercury in the Tapajos River basin, Brazilian Amazon: A review. Environmental International 36, 593-608.

Økelsrud, A., Lydersen, E., Fjed, E., 2016. Biomagnification of mercury and selenium in two lakes in southern Norway. Science of the Total Environment 566-567, 596-607.

Oliveira, A.C.B., Soares, M.G.M., Martinelli, L.A., Moreira, M.Z., 2006. Carbon sources of fish in an Amazonian floodplain lake. Aqua. Sci. 68, 229-238.

Oliveira, R.C., Dórea, J.G., Bernardi, J.V.E., Bastos, W.R., Almeida, R., Manzatto, A.G., 2010. Fish consumption by traditional subsistence villagers of the Rio Madeira (Amazon): Impact on hair mercury. Annals of Human Biology 37, 629-642.

Ouédraogo, O., Chételat, J., Amyot, M., 2015. Bioaccumulation and Trophic Transfer of Mercury and Selenium in African Sub-Tropical Fluvial Reservoirs Food Webs (Burkina Faso). PLoS One 10, e0123048.

Pacheco-Ferreira H. Mercúrio na Amazônia: efeitos sobre a saúde das populações ribeirinhas [Doctoral Dissertation]. Belém: Núcleo de Altos Estudos Amazônicos, Universidade Federal do Pará; 2001.

Park, H.J., Park, T.H., Lee, C.I., Kang, C.K., 2018. Ontogenetic shifts in diet and trophic position of walleye pollock, Theragra chalcogramma, in the western East Sea (Japan Sea) revealed by stable isotope and stomach content analyses. Fisheries Research 204, 297-304.

Passos, C.J.S., Silva, D.S., Lemire, M., Fillion, M., Guimarães, J.R.D., Lucotte, M., Mergler, D., 2008. Daily mercury intake in fish-eating populations in the Brazilian Amazon. Journal of Exposure Science and Environmental Epidemiology 18, 76-87.

Pestana, I.A.; Almeida, M.G.; Bastos, W.R.; Souza, C.M.M. 2019. Total Hg and methylmercury dynamics in a river-floodplain system in the Western Amazon: Influence of seasonality, organic matter and physical and chemical parameters. Science of The Total Environment, 656 (15):388-399.

Poste, A.E., Muir, D.C.G., Mbabazi, D., Hecky, R.E., 2012. Food web structure and mercury trophodynamics in two contrasting embayments in northern Lake Victoria. Journal of Great Lakes Research 38, 699-707.

Pouilly, M., Rejas, D., Pérez, T., Duprey, J.L., Molina, C.I., Hubas, C., Guimarães, J.R.D., 2013. Trophic structure and mercury biomagnification in Tropical Fish Assemblages, Iténez River, Bolivia. PLoS One 8, e65054.

Razavi, N.R., Qu, M., Jin, B., Ren, W., Wang, Y., Campbell, L.M., 2014. Mercury biomagnification in subtropical reservoir fishes of eastern China. Ecotoxicology 23, 133-146.

Rosende-Pereiro, A., Flores-Ortega, J.R., González-Sansón, G., Corgos, A., 2020. Stomach content and stable isotopes reveal an ontogenetic dietary shift of young-of-the-year scalloped hammerhead sharks (Sphyrna lewini) inhabiting costal nursery areas. Environ. Biol. Fish 103, 49-65.

Roulet, M., Lucotte, M., Saint-Aubin, A., Tran, S., Rheault, I., Farella, N., Silva, E.J., Dezencourt, J., Passos, C.J.S., Soares, G.S., Guimarães, J.R.D., Mergler, D., Amorim, M., 1998. The geochemistry of Hg in Central Amazonian soils developed on the Alter-do-Chao formation of the lower Tapajos river valley, Para State, Brazil. Science of Total Environment 223, 1-24.

Roulet, M., Lucotte, M., Canuel, R., Farella, N., Goch, Y.G.F., Peleja, J.R.P., Guimarães, J.R.D, Mergler, D., Amorim, M., 2001. Spatio-Temporal geochemistry of mercury in waters of the Tapajós and Amazon rivers, Brazil. Limnology and Oceanography 46, 1141-1157.

Silva, S., Lucotte, M., Roulet, M., Poirier, H., Mergler, D., Santos, E.O., Crossa, M., 2005. Trophic Structure and bioaccumulation of mercury in fish of three natural lakes of the Brazilian Amazon. Water, Air, and Soil Pollution 165, 77-94.

Silva, G.S., Jardim, W.F., Fadini, P.S., 2006. Elemental gaseous mercury flux at the water/air interface over the Negro River basin, Amazon, Brazil. Science of the Total Environment 368, 189-198.

Silva, S.F., Oliveira, D.C., Pereira, J.P.G., Castro, S.P., Costa, B.N.S., Lima, M.O., 2019. Seasonal variation of mercury in commercial fishes of the Amazon Triple Frontier, Western Amazon Basin. Ecological Indicators 106, 105549.

Sioli, H., 1967. Studies in Amazonian Waters. In: Atas do simpósio sobre a biota amazônica 3, 9-50. Conselho Nacional de Pesquisas, Rio de Janeiro.

Thomas, S.M., Crowther, T.W., 2015. Predicting rates of isotopic turnover across the animal kingdom: a synthesis of existing data. Journal of Animal Ecology 84, 861-870.

Trudel, M., Rasmussen, J.B., 1997. Modeling the elimination of mercury by fish. Environmental Science & Technology 31, 1716-1722.

Verburg, P., Hickey, C.W., Phillips, N., 2014. Mercury biomagnification in three geothermally-influenced lakes differing in chemistry and algal biomass. Science of the Total Environment 493, 342-354.

Verhaert, V., Teuchies, J., Vlok, W., Wepener, V., Addo-Bediako, A., Jooste, A., Blust, R., Bervoets, L., 2019. Bioaccumulation and trophic transfer of total mercury in the subtropical Olifants River Basin, South Africa. Chemosphere 216, 832-843.

Vieira, M., Bernardi, J.V.E., Dórea, J.G., Rocha, B.C.P., Ribeiro, R., Zara, L.F., 2018. Distribution and avaliability of mercury and methylmercury in different Waters from the Rio Madeira Basin, Amazon. Environmental pollution 235, 771-779.

Wantzen, K.M., Machado, F.A., Voss, M., Boriss, H., Junk, W.J., 2002. Seasonal isotopic shifts in fish of the Pantanal wetland, Brazil. Aquatic Sciences 64, 01-13.

Xun, L., Campbell, N.E.R., Rudd, J.W.M., 1987. Measurements of specific rates of net methyl mercury production in the water column and surface sediments of acidified and circumneutral lakes. Canadian Journal of Fisheries and Aquatic Sciences 44, 750-757.

Yan, H., Rustadbakken, A., Yao, H., Larssen, T., Feng, X., Liu, T., Shang, L., Haugen, T.O., 2010. Total mercury in wild fish in Guizhou reservoirs, China. Journal of Environmental Sciences 22, 1129-1136.

Zhang, L., Campbell, L.M., Johnson, T.B., 2012. Seasonal variation in mercury and food web biomagnification in Lake Ontario, Canada. Environmental Pollution 161, 178-184.

SUPPLEMENTARY MATERIAL

Mercury biomagnification in an ichthyic food chain of an Amazon floodplain lake (Puruzinho Lake): influence of seasonality and food chain modeling

Lucas Silva Azevedo[a,*], Inácio Abreu Pestana[a], Marcelo Gomes Almeida[a], Adriely Ferreira da Costa Nery[b], Wanderley Rodrigues Bastos[b], Cristina Maria Magalhães Souza[a]

[a]Laboratório de Ciências Ambientais, Centro de Biociências e Biotecnologia, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Parque Califórnia, CEP: 28013-602, Campos dos Goytacazes, Rio de Janeiro, RJ, Brazil.
[b]Laboratório de Biogeoquímica Ambiental, Universidade Federal de Rondônia, Olaria, CEP: 76815-800, Porto Velho, Rondônia, RO, Brazil.

This document includes supplementary information about: TMS in different food chain structures (Supplementary Material 1); Linear regressions between THg concentrations (mg kg^{-1} wet wt.) and $\delta^{13}C_{lipid\text{-}free}$ for each species (Supplementary Material 2); Map of study area (Supplementary Material 3); Total Hg determination method (Supplementary Material 4); Linear regression between total Hg concentration and total length (Supplementary Material 5); Linear regression between total Hg concentration and total weight (Supplementary Material 6).

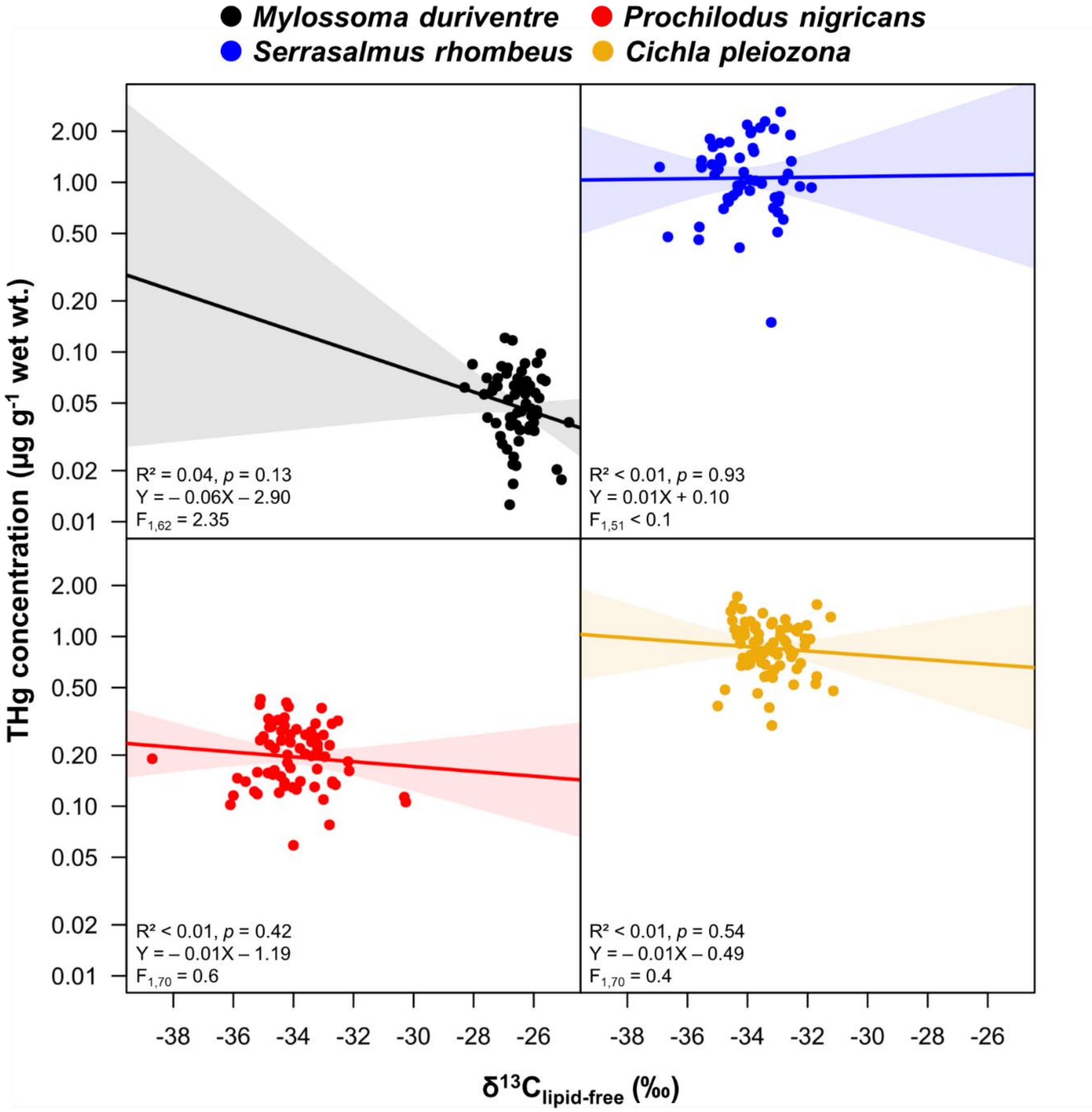
Number of Tables: 1

Number of Figures: 4

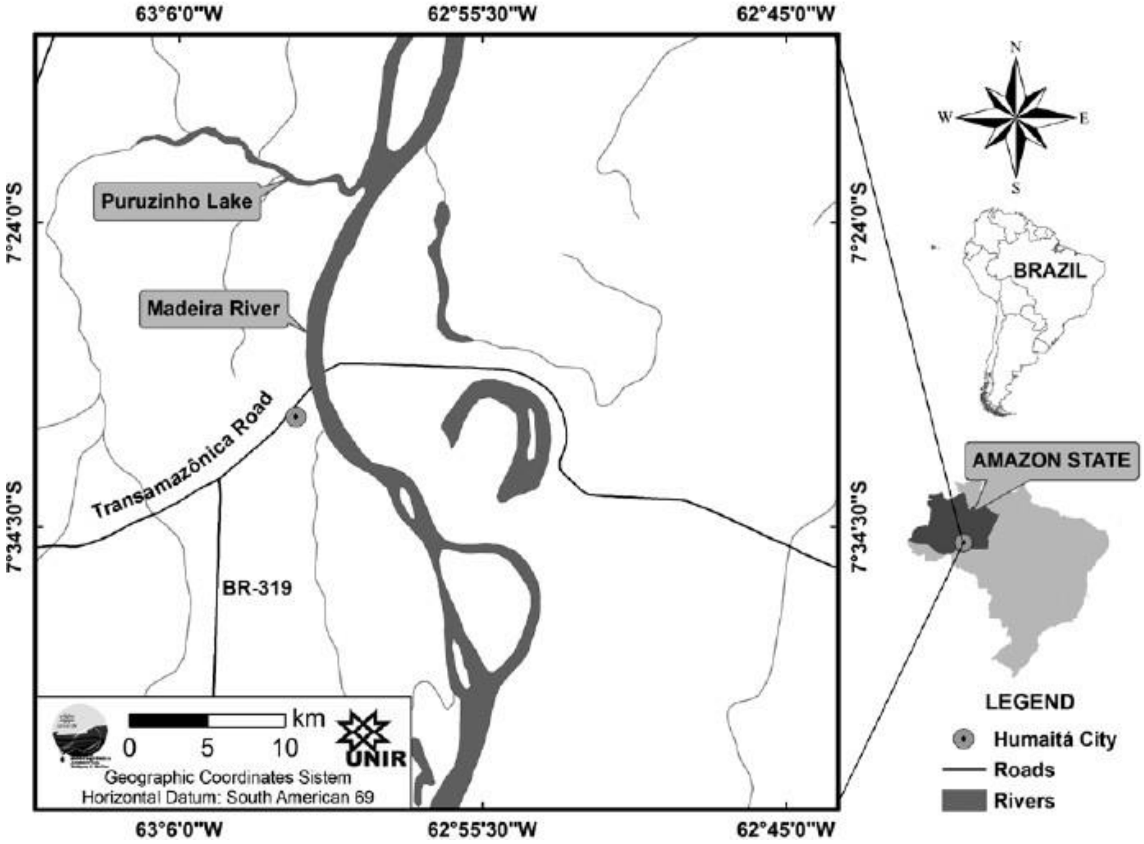
Supplementary Material - 1. TMS in different food chain models. Lowercase letters indicate significant differences of TMS among food chain models.

Food chain models	**F**	**R^2**	**TMS**	**TMS-CI**	**Intercept**	***p*-value**
H + D + P1+ P2	$F_{1,259}$ = 817.9	0.76	0.26	0.24-0.27^{a}	-2.76	0.0001
H + P2	$F_{1,115}$ = 494.0	0.81	0.26	0.24-0.29^{ab}	-2.91	0.0001
H + P1	$F_{1,134}$ = 532.7	0.67	0.29	0.26-0.3^{a}	-3.03	0.0001
D + P2	$F_{1,123}$ = 275.2	0.69	0.20	0.18-0.23^{bcd}	-2.23	0.0001
D + P1	$F_{1,142}$ = 273	0.65	0.22	0.19-0.24^{abc}	-2.32	0.0001
P1 + P2	$F_{1,123}$ = 1.4	0.01	0.03	-0.02-0.10^{e}	-0.43	0.23
H + D	$F_{1,134}$ = 61.15	0.31	0.18	0.13-0.22^{d}	-2.25	0.0001
H + P1 + P2	$F_{1,187}$ = 786.7	0.80	0.28	0.26-0.30^{ab}	-2.96	0.0001
H + D + P2	$F_{1,187}$ = 499.7	0.72	0.24	0.22-0.26^{ab}	-2.68	0.0001
H + D + P1	$F_{1,206}$ = 530.8	0.72	0.26	0.24-0.29^{a}	-2.80	0.0001
D + P1 + P2	$F_{1,195}$ = 380.2	0.66	0.21	0.19-0.23^{cd}	-2.25	0.0001

H: herbivorous (*Mylossoma duriventre*)
D: detritivorous (*Prochilodus nigricans*)
P1: piscivorous (*Cichla pleiozona*)
P2: piscivorous (*Serrasalmus rhombeus*)
TMS-CI: Confidence Interval (2.5% - 97.5%)



Supplementary Material 2 – Linear regressions between THg concentrations (mg kg^{-1} wet wt.) and $\delta^{13}C_{lipid\text{-}free}$ for each species. Model statistics (Equation, R², *p* and F) for regressions are within each graph. The shaded area around regressions represents the 95% confidence intervals.



Supplementary Material 3: Map of the study area (Extracted from Almeida et al., 2014).

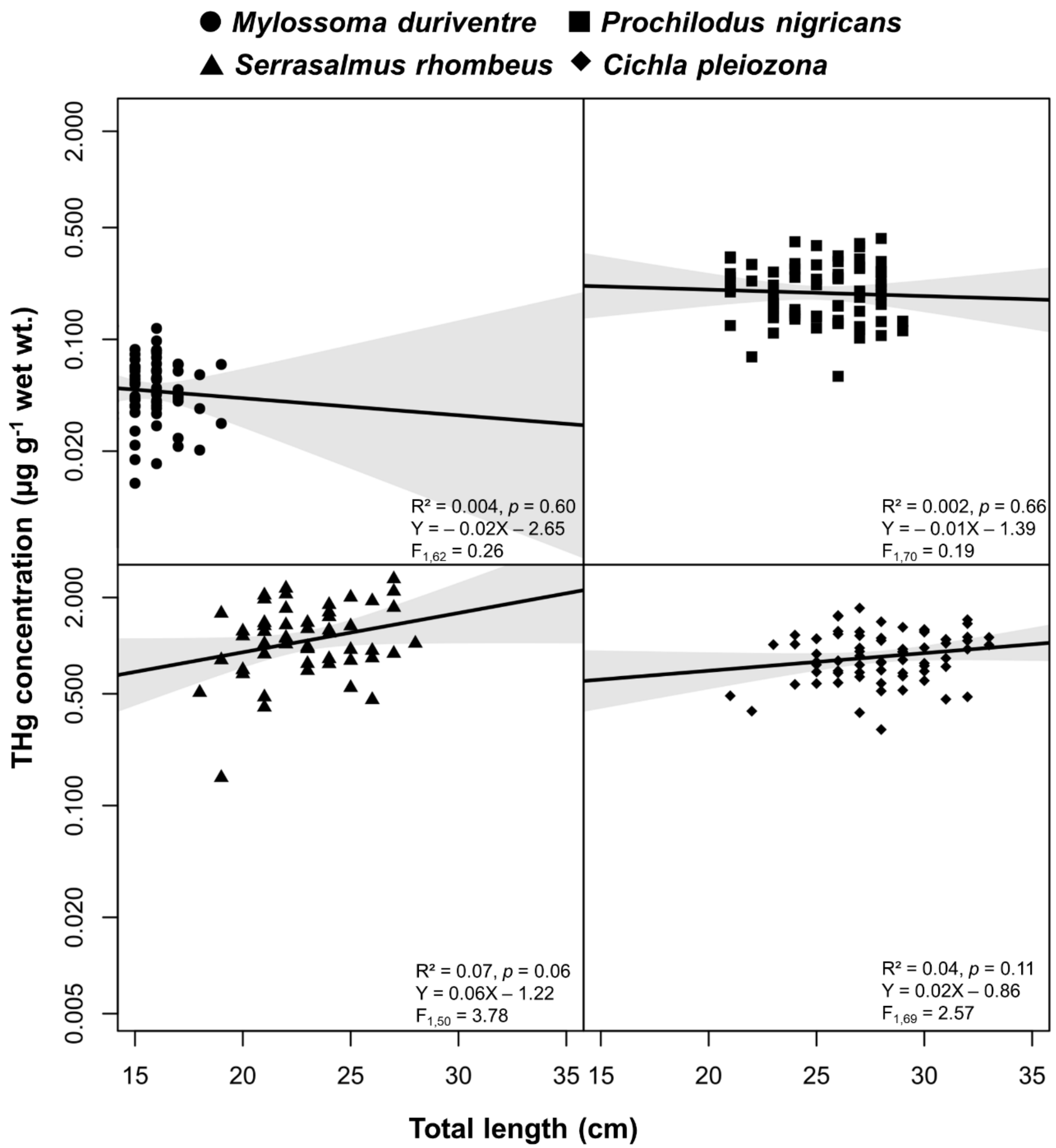
Supplementary material 4

THg determination methods:

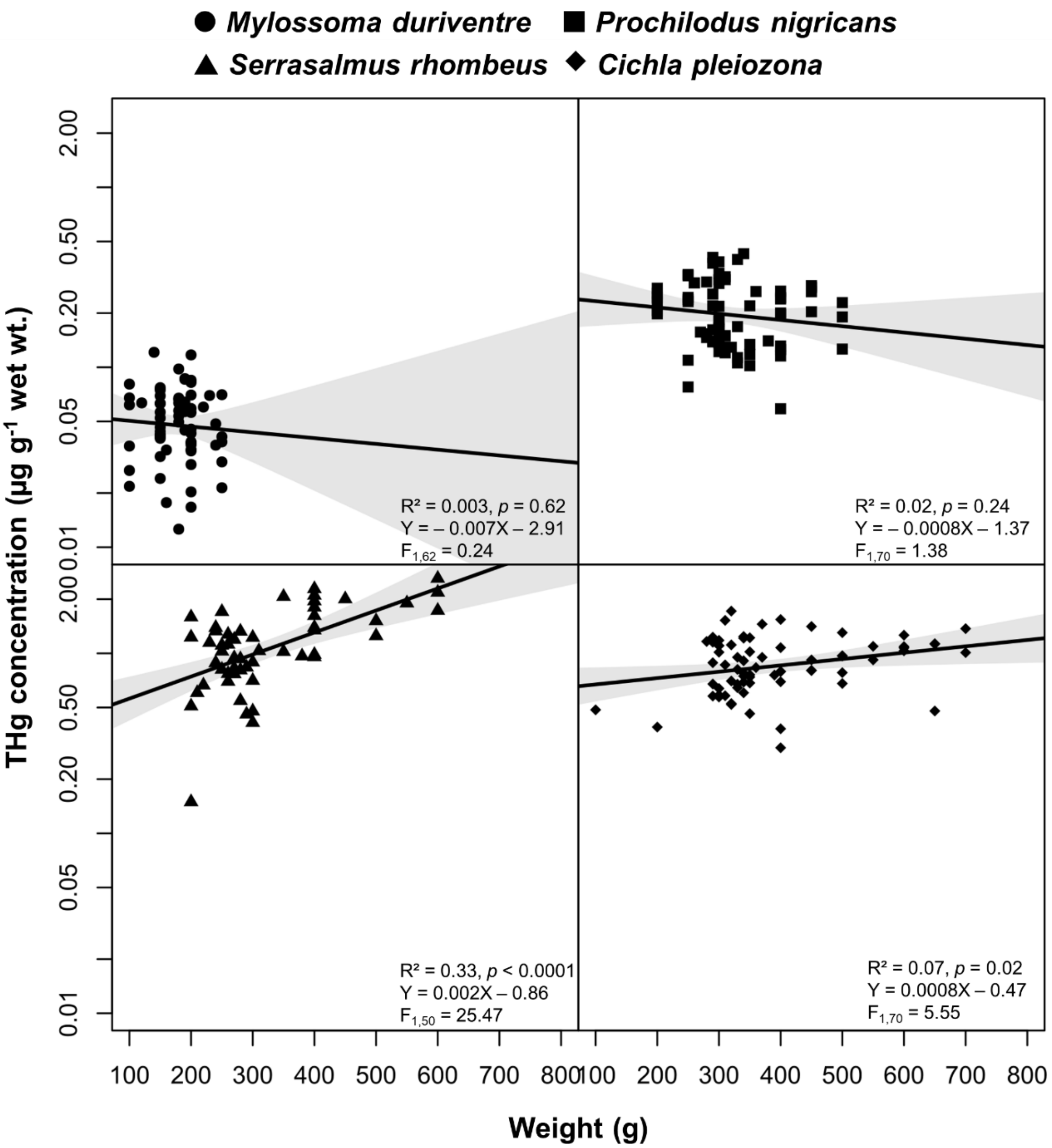
Digestion of fish muscle (0.2 g wet wt) followed the method described by Bastos et al. (1998): (i) 1 mL of 30% H_2O_2 and 4 mL of 65% HNO_3: 98% H_2SO_4 (1:1); (ii) heating in a Tecnal digestion block (model: TE04/25) for 30 min at 70 ºC; (iii) cooling at room temperature; (iv) 5% $KMnO_4$; heating for 15 min; (v) cooling at room temperature overnight; (vi) 12% $NH_2OH.HCl$; and (vii) addition of ultrapure water to a final volume of 12 mL. Determination was performed by cold vapor atomic absorption spectrometry (CVAAS), with a flow injection mercury system (FIMS-400) from PerkinElmer (Germany). Blanks were used to control the quality of the reaction medium. The method's detection limit was 0.007 mg kg^{-1}. Certified material (DORM-2) was analyzed in triplicate for each 30 samples (recuperation: 99±2.5%).

Reference:

Bastos, W.R., Malm, O., Pfeifer, W.C., Cleary, D., 1998. Establishment and analytical quality control of laboratories for Hg determination in biological and geological samples in the Amazon, Brazil. Rev. Ciên. Cult. 50, 255-260.



Supplementary Material 5 – Linear regressions between log-transformed THg concentrations (mg kg^{-1} wet wt.) and total length (cm) for each species. Model statistics (Equation, R², *p* and F) for regressions are within each graph. The shaded area around regressions represents the 95% confidence intervals. Data from the four hydrological cycles were pooled for this analysis because ANCOVA did not show significant interaction between length and the four hydrological cycles ($p = 0.58$).



Supplementary Material 6 – Linear regressions between log-transformed THg concentrations (mg kg^{-1} wet wt.) and weight (g) for each species. Model statistics (Equation, R², *p* and F) for regressions are within each graph. The shaded area around regressions represents the 95% confidence intervals. Data from the four hydrological cycles were pooled for this analysis because ANCOVA did not show significant interaction between length and the four hydrological cycles (*p* = 0.83).

CAPÍTULO 3: **Do fish isotopic niches change in an Amazon floodplain lake over the hydrological regime?**

Artigo aceito para publicação a “Ecology of Freshwater Fish” (Qualis A2 – Biodiversidade), Fator de Impacto (1,95).

Do fish isotopic niches change in an Amazon floodplain lake over the hydrological regime?

Lucas Silva Azevedo[a,*], Inácio Abreu Pestana[a], Marcelo Gomes Almeida[a], Wanderley Rodrigues Bastos[b], Cristina Maria Magalhães Souza[a]

[a]Laboratório de Ciências Ambientais, Centro de Biociências e Biotecnologia, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, Rio de Janeiro, RJ, CEP: 28013-602, Brazil.
[b]Laboratório de Biogeoquímica Ambiental, Universidade Federal de Rondônia, Porto Velho, Rondônia, RO, CEP: 76815-800, Brazil.

ABSTRACT

Floodplain lakes are ecosystems characterized by annual flood and dry cycles. Fish ecology is influenced by the flood pulse due to the large influx of allochthonous food resources and diversification of habitats during the flood cycle, while during the dry cycle, fishes tend to be confined in reduced habitat. The aim of this study was to evaluate the seasonal variation in trophic niche width and overlap of four species – *Mylossoma duriventre*, *Prochilodus nigricans*, *Cichla pleiozona* and *Serrasalmus rhombeus* – in an Amazonian floodplain lake. Stable isotope analyses were used to estimate trophic niche width and overlap during the flood and dry seasons. We hypothesized broader niche width for all species during the flood cycle and a higher degree of overlap between the two piscivorous fishes during the dry cycle. Isotopic niche width was 72% broader for *Prochilodus nigricans*, 61% for *Serrasalmus rhombeus* and 54% for *Cichla pleiozona* during the dry cycle, which did not support our hypothesis. Core niche width overlaps were not observed between piscivorous species in either flood or dry cycle. The results indicate that seasonal variation in isotopic niche width is specific to feeding habit. Understanding how fish trophic ecology responds to changes in the hydrological regime during the seasons is crucial

for sustainable fishery management in a region where many people rely heavily on fish for nutritional and economic purposes.

Keywords: Seasonal Variation; Stable Isotopes; Trophic Ecology; Overlap; Floodplain Lake

1. Introduction

Floodplains are widespread geological formations in the Amazon region. Approximately 300,000 km^2 of land is flooded annually by large rivers (Junk et al. 1989). Lakes' limnological and ecological features are subject to strong seasonal variation due to regular flooding and receding of river water (Junk et al. 1989). For example, during the peak flood season, maximum depth of a lake can reach 12 m while at the extreme low water season, maximum depth may drop to less than 1 m (Lesack and Melack, 1995; Almeida et al. 2014). Primary productivity is another factor that varies seasonally, with higher mean values during low water season (chlorophyll-*a* concentration: 19.1 ± 5.3 µg L^{-1}) in comparison with the flood season (8.3 ± 2.1 µg L^{-1}) (Brito et al. 2014).

The seasonal cycle of rising and falling water changes the availability of food for fishes (Meroná and Rankin-de-Meroná, 2004; Oliveira et al. 2006) affecting a fishes' trophic ecology and resulting in wider or narrower trophic niches (Pool et al. 2017). During the flood season, fish have access to terrestrial food resources like fruits, seeds, leaves and insects, while during the dry season the main food resources are phytoplankton, zooplankton and the resident macrofauna (Junk et al. 1997). For instance, Quirino et al. (2017) reported a wider trophic niche of *Moenkhausia forestii*, a small characid from the Upper Paraná River floodplain, in the flood season due to higher availability of allochthonous resources such as Hymenoptera, Coleoptera and Orthoptera, while in the dry season, niche contraction was observed due to lower availability of those resources. Zaret and Rand (1971) also observed low food abundance in the dry season for nine sympatric fishes in the Panama lowland stream. Although the link between higher influx of allochthonous resources during the flood season and increases in niche width seems intuitive, some studies have shown narrower niche breadth in this season (Walker et al. 2013) and others have shown no consistent pattern of niche breadth during the flood pulse (Correa and Winemiller, 2014). Even though trophic ecology of Amazonian fishes has been extensively studied (Araujo-Lima et al. 1986; Leite et al. 2002; Mortillaro et al. 2015; Rejas 2018; Cardoso et al. 2019), there is no defined pattern of seasonal variation of fish trophic niche width in floodplain lakes (Meroná and Rankin-de-Meroná, 2004). Understanding the natural seasonal variation in trophic niche dynamics is an important step towards Amazonian fish conservation.

Trophic niche width and trophic niche overlap are two useful concepts for understanding the trophic relationships among consumers. Species that have a broad trophic niche exploit a wide variety of resources (generalists), while species that have a narrow niche have a more restricted diet (specialists) (Cody 1974). Consumers with similar trophic ecology exploit similar resources, so the niche space of a consumer can be shared by other consumers (overlap) (Colwell and Futuyama, 1971). Although niche overlap does not directly imply interspecific competition, its measurement provides information about resource sharing among consumers in a community. Regarding the influence of the flood and dry cycles on niche overlap, Pool et al. (2017) observed higher overlap during the flood, when fish species exploit similar resources. Other studies like Jaeger et al. (2010) and Pokharel et al. (2015) also observed seasonal variations in trophic niche, but in seabirds and mammals, respectively.

The plausible mechanism underlying the seasonal changes in trophic niche overlap is the variation in food resource diversity/quantity caused by the periodical flood of terrestrial environments followed by a dry period when water recedes. To evaluate niche width and overlap, stomach content analysis and stable isotope analysis (SIA) of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) are two suitable approaches. Stomach content analysis indicates the food consumed recently but not yet assimilated by the consumer, while SIA provides information about trophic level ($\delta^{15}N$) and carbon flow ($\delta^{13}C$) (Post 2002; Park et al. 2015). SIA provides reliable information on fish trophic ecology and has been successfully used to understand trophic dynamics in fish communities to evaluate the influence of invasive species on native fish, and to assess biomagnification of pollutants (Herlevi et al. 2018; Rumbold et al. 2018; Wang et al. 2018).

The aim of this study was to evaluate seasonal variation in trophic niche width and overlap of four fish species (*Mylossoma duriventre*, common name Pacu – herbivorous; *Prochilodus nigricans*, common name Curimatã – detritivorous; *Serrasalmus rhombeus*, common name Piranha-preta – piscivorous; and *Cichla pleiozona*, common name Tucunaré – piscivorous) from a floodplain lake (Puruzinho

Lake) in western Amazonia. The hydrological regime in the region is represented by: (1) the flood cycle, which consists of rising water (river and lake water levels increase) and high water seasons (river and lake water levels reach their peak); and (2) the dry season, which is composed of the falling water (river and lake water levels decrease) and low water seasons (river and lake water levels reach their lowest values). The hypotheses of this study were: (I) the isotopic niche width for all species would be broader during the flood cycle than the dry cycle due to higher influx of allochthonous resources; and (II) the trophic niche overlap among piscivorous species would be higher during the dry cycle (falling and low water seasons) than in the flood cycle (rising and high water seasons) due to higher interspecific competition in shallow lakes.

2. Material and Methods

2.1 Study Area

Puruzinho Lake is a floodplain lake located in the municipality of Humaitá, State of Amazonas, Brazil, in the Madeira River Basin (63°6'0''W; 7°24'0''S) (Supplementary Material 1). The study lake is a black water system with high concentration of dissolved organic matter and acid pH (4.9 – 5.4), in contrast the Madeira River is a white water system with pH close to neutral and high concentrations of suspended particulate matter (Wissmar et al. 1981; Almeida et al. 2014).

The annual flood pulse influences the variation in limnological variables, lake morphometry and the mixture between lentic (i.e., lake) and lotic (i.e., river) sections of the floodplain (Thomaz et al. 2007; Fantin-Cruz et al. 2008). During the flood cycle, represented by the rising water and high water seasons, riverine influx to the lake section is maximized, leading to homogenization and subsequent similarity in limnological parameters between the two sections (Thomaz et al. 2007). Conversely, in the dry cycle – falling water and low water seasons – the two sections are isolated, which increases dissimilarity in limnological parameters (Thomaz et al. 2007).

There are significant differences in lake depth between the flood and dry cycles. In the flood cycle, the lentic section's depth reaches 11.00 ± 0.90 m (mean ±SD) while during the dry cycle the value drops to 0.90 ± 0.60 m (Almeida et al. 2014). The variations in lake's depth follow the variation in the Madeira River's depth (Feitosa et al. 2019). Nutrient concentrations are higher during the flood cycle, with nitrate concentrations reaching 905.0 ± 90.9 µg L^{-1} (mean ±SD), while in the dry cycle the mean value is lower (796.1 ± 94.6 µg L^{-1}) (Feitosa et al. 2019). Average dissolved organic carbon concentration is also higher during the flood cycle (5.0 ± 0.6 mg L^{-1}; mean ±SD) in comparison with the dry cycle (2.7 ± 0.3 mg L^{-1}) (Feitosa et al. 2019). The increase in nutrients and organic matter in the lake in the flood cycle reflects the influx of allochthonous materials. Details about the Madeira River's depth and flow during the sampling period can be found in Azevedo et al. (2020).

2.2 Sampling

Sampling was done in the four hydrological seasons of 2017-2018 at the beginning of each month: rising water (December 2017−February 2018), high water (April 2018), falling water (September 2018) and low water (October 2018). The half-lives of stable isotopes are 42-66 days for nitrogen and 39-61 days for carbon (Thomas and Crowther, 2015), which is enough time to accommodate the turnover rates since there is a five-month gap between the end of the rainy season (April) and the beginning of the dry season (September). These half-life values are based on animals weighing from 100 g to 1 kg at 30 ºC (Thomas and Crowther, 2015). Adult individuals were obtained using gillnets with bar mesh sizes of 30, 45 and 60 mm in the lentic section of the floodplain system. Four species were sampled: detritivorous *Prochilodus nigricans*, herbivorous *Mylossoma duriventre*, piscivorous *Cichla pleiozona* and piscivorous *Serrasalmus rhombeus* (Table 2). Sample size is summarized in Table 2. Species feeding habits were identified based on Cella-Ribeiro et al. (2017), who described the diet of the four sampled species in the Madeira River Basin by stomach content analysis: *Mylossoma duriventre* (fruit = 58 %, plants = 37%, seeds = 4.0%, arthropods = 1.0%); *Prochilodus nigricans* (detritus = 97.0%, algae = 3.0%); *Cichla pleiozona* (fish = 100%); and *Serrasalmus rhombeus* (fish = 100%). Only adult fish were used in the analyzes to avoid the influence of ontogenetic shift in diet. The mean total length of fish species were: *Prochilodus nigricans* (rising water: 243.3 ± 24.2 mm; high water: 263.5 ± 18.7 mm; falling water: 261.0 ± 20.0 mm; low water: 231.0 ± 17.4 mm); *Mylossoma duriventre* (rising water: 148.4 ± 12.8 mm; high water: 160.0 ± 8.0 mm; falling water: 161.0 ± 14.0 mm; low water: 151.6 ± 9.8 mm); *Serrasalmus rhombeus* (rising water: 210.0 ± 15.5 mm; high water: 227.0 ± 26.0 mm; falling water: 240.0 ± 23.0 mm; low water: 228.5 ± 23.8 mm); *Cichla pleiozona* (rising water: 271.6 ± 11.6 mm; high water: 269.0 ± 25.7 mm; falling water: 292.5 ± 41.7 mm; low water: 281.0 ± 41.4 mm) (Table S1). The white muscle tissues of the sampled fish were extracted using scalps and scissors. After extraction, the muscle tissues were stored in ice and transported to the laboratory. Samples were freeze-dried and pulverized at the laboratory.

2.3 Stable Isotope Analysis

Samples of freeze-dried white muscle were pulverized and analyzed. Stable isotope analysis (SIA) was performed with a Thermo Finnigan Delta V Advantage mass spectrometer (isotope ratio mass spectrometer, IRMS, Thermo Scientific, Milan, Italy) coupled to a Flash 2000 organic elemental analyzer (Thermo Scientific, Milan, Italy). The standards for $\delta^{13}C$ and $\delta^{15}N$ were Pee Dee Belemnite (PDB) and atmospheric nitrogen, respectively. The isotopic ratio was calculated based on the following equation:

$$\delta X (‰) = (R_{Sample}/R_{Standard} - 1) \times 1000$$

where: R_{Sample} and $R_{Standard}$ are the ratios $^{13}C/^{12}C$ or $^{15}N/^{14}N$ of the sample and the standard, respectively.

Triplicates of 20 samples were used to test analytical precision ($\delta^{13}C$ was ±0.2‰ and $\delta^{15}N$ was ±0.3‰). For the elemental and isotopic composition, analytical precision was determined by certified standards (Protein OAS/Isotope Cert 114859; Elemental Microanalysis, United Kingdom).

The C:N ratio of some individuals was higher than 3.5, so correction was necessary to remove the influence of lipids on the $\delta^{13}C$ signature. The equations from McConnaughey and McRoy (1979) were used to normalize the $\delta^{13}C$ signature (Table S1):

$$L = 93/[1 + (0.246\ C{:}Na - 0.775)^{-1}]$$

$$\delta_{lipid\text{-}free} = \delta_{raw} + D\ [-0.207 + 3.90/(1+287/L)]$$

Where: L is the estimated lipid content; $\delta_{lipid-free}$ is the corrected carbon isotopic signature and D is the difference of the $\delta^{13}C$ signature between protein and lipid (6‰ based on literature values) (McConnaughey and McRoy 1979).

2.4 Data Analysis

All statistical analyses were performed with the R program (R Core Team 2020) and *a priori* error of 5% was assumed ($\alpha = 0.05$) in all hypothesis tests. The differences in species' stable isotopes signatures ($\delta^{13}C_{lipid-free}$ and $\delta^{15}N$) among seasons was evaluated through ANOVA (aov function, base package, R Core Team, 2020) followed by Tukey's multiple comparison test (TukeyHSD function, base package, R Core Team 2020) (Table S1). ANOVA's assumptions were validated through the use of diagnostic plots (Altman & Krzywinski 2016).

To evaluate trophic niche dynamics of the studied community, the six Layman metrics (Layman et al. 2007) and the standard ellipse area corrected by sample size (SEAc) (Jackson et al. 2011) were calculated using the SIBER package in R (Jackson et al. 2011). The six Layman metrics calculated were: (1) carbon range (CR): distance between the highest and lowest values of $\delta^{13}C$ within a community of the four species, which indicates the variety of basal food resources exploited (higher values of CR imply greater diversification of basal resource use); (2) nitrogen range (NR): distance between the highest and the lowest $\delta^{15}N$ signature, which indicates the vertical structure of the food web (higher NR values suggest greater degree of trophic diversity among consumers); (3) total area (TA): total amount of niche space occupied by a community, thus serving as a proxy for trophic diversity within a food web (TA is influenced by extreme values of $\delta^{13}C$ and $\delta^{15}N$); (4) mean distance from the centroid (CD): a measure of average trophic diversity (higher values of CD indicate greater trophic diversity); (5) mean nearest neighbor distance (MNND): an estimate of the average trophic redundancy (higher values of MNND imply lower trophic redundancy and lower values of MNND imply higher trophic redundancy); and (6) standard deviation of the mean nearest neighbor distance (SDNND): a measure of evenness of species packing.

SEAc represents the core niche width and was calculated considering 40% of all the data for a species (Jackson et al. 2011). Ellipse size heavily affects trophic niche overlap estimates (Jackson et al. 2011; Syväranta et al. 2013). To compared SEAc values among species and seasons (hypothesis I), we fitted a Bayesian multivariate normal distribution to our data (siberMVN function, SIBER package, Jackson et al. 2011) using Gibbs sampling (rjags package, Plummer, 2019) and assuming the prior distributions defined by Jackson et al. (2011). Then we extracted the posterior means and their respective credible intervals (i.e., an interval within which an unobserved parameter value falls with a particular probability) to present the results. Lack of overlap in the 95% credible intervals was interpreted as statistical support for differences in the means of the calculated metrics. Layman metrics were also compared with this method.

To compare the core niche width among species in the same season, we calculated the percentage overlap of SEAc (maxLikOverlap function; SIBER package, Jackson et al., 2011) (hypothesis II).

3.0 Results

The highest NR and CR values within a community of the four species (Fig. 1; Table 1) were observed during high water (NR= 4.83) and low water (CR= 8.14) periods, respectively. However, the 95% credible intervals for both metrics showed high overlap among all seasons. The TA value in the high-water season (13.90) was higher than during rising water (10.50), falling water (12.00) and low water (11.30) (Fig.1; Table 1), suggesting a higher degree of trophic diversity during the high-water season, although this difference was not significant. The CD, another metric that measures trophic diversity, was slightly higher during high water (3.33) in comparison with rising water (2.21), falling water (3.27) and low water (3.17) seasons (Fig.1; Table 1). The indicators of trophic redundancy, MNND and SDNND showed inconsistent patterns, with a higher value of MNND during low water (3.47), indicating lower trophic redundancy. The low SDNND value in the low water season (2.27) suggests low evenness of species packing.

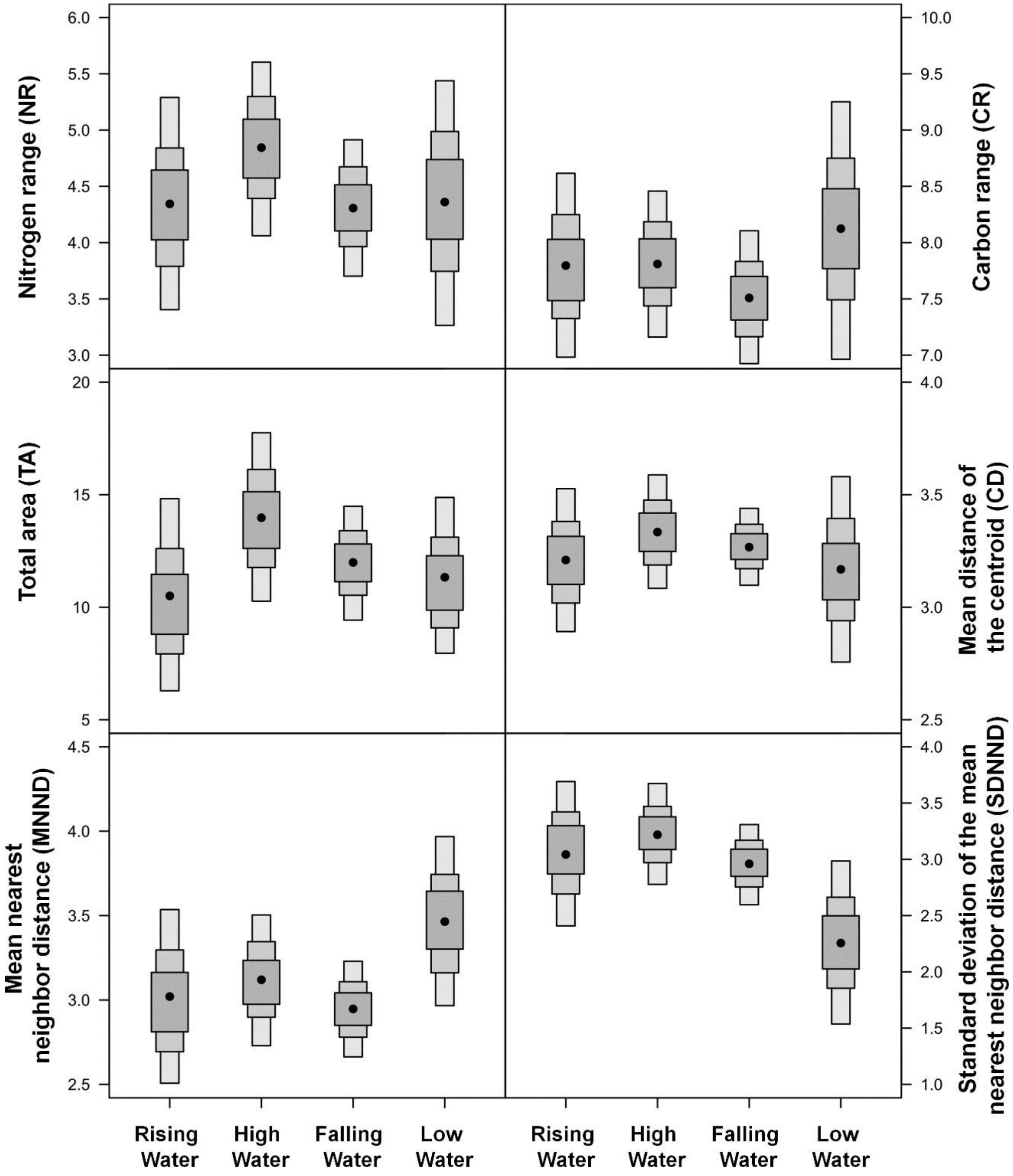


Figure 1. Layman metrics of the studied community during the four hydrological seasons: nitrogen range (NR), carbon range (CR), total area (TA), mean distance from the centroid (CD), mean nearest neighbor distance (MNND), standard deviation of the mean nearest neighbor distance (SDNND) calculated for each season. The bars represent 95%, 75% and 50% credible intervals while the central point represents the mean value.

Table 1. Means and 95% credible intervals of SEAc (Standard ellipse area corrected by sample size) calculated by Seasons and Cycles. The flood and dry cycles represent rising water + high water seasons and falling water + low water seasons, respectively.

Species	Sample Size	Seasons	SEAc (95% credible intervals)	Cycle	SEAc (95% credible intervals)
P.nigricans	12	**Rising Water**	1.28 (0.63 – 2.06)	**Flood cycle**	2.02 (1.35 – 2.74)
	20	**High Water**	2.21 (1.31 – 3.21)		
	22	**Falling Water**	5.14 (3.09 – 7.43)	**Dry Cycle**	3.49 (2.49 – 4.61)
	20	**Low Water**	1.74 (1.02 – 2.53)		
M.duriventre	13	**Rising Water**	3.25 (1.65 – 5.15)	**Flood cycle**	2.26 (1.54 – 3.09)
	20	**High Water**	1.79 (1.06 – 2.63)		
	25	**Falling Water**	1.39 (0.87 – 1.97)	**Dry Cycle**	1.50 (1.00 – 2.03)
	6	**Low Water**	1.55 (0.51 – 2.95)		
C.pleiozona	6	**Rising Water**	0.71 (0.22 – 1.37)	**Flood cycle**	0.68 (0.43 – 0.95)
	20	**High Water**	0.51 (0.30 – 0.75)		
	35	**Falling Water**	0.80 (0.55 – 1.07)	**Dry Cycle**	1.05 (0.77 – 1.35)
	12	**Low Water**	1.54 (0.73 – 2.52)		
S.rhombeus	9	**Rising Water**	0.79 (0.34 – 1.34)	**Flood cycle**	0.83 (0.49 – 1.23)
	11	**High Water**	0.30 (0.14 – 0.50)		
	20	**Falling Water**	1.25 (0.74 – 1.84)	**Dry Cycle**	1.34 (0.92 – 1.81)
	14	**Low Water**	1.28 (0.66 – 2.01)		

The observed trophic niche width variation between dry and flood cycles did not support the hypothesis of broader niche width during rising water and high water for all species (hypothesis I). Isotopic niche width was 72% broader for *Prochilodus nigricans*, 61% for *Serrasalmus rhombeus* and 54% for *Cichla pleiozona* during the dry cycle, which did not support our first hypothesis. Only *Mylossoma duriventre* showed a broader niche during rising water (SEAc= 3.25) (Table 2; Fig. 2). *Prochilodus nigricans* had the broadest isotopic niche among all species (falling water: SEAc = 5.14) (Table 2; Fig.2). For *Cichla pleiozona* and *Serrasalmus* *rhombeus*, the isotopic niche width was higher during low water (SEAc = 1.54 and 1.28, respectively) (Table 2; Fig. 2).

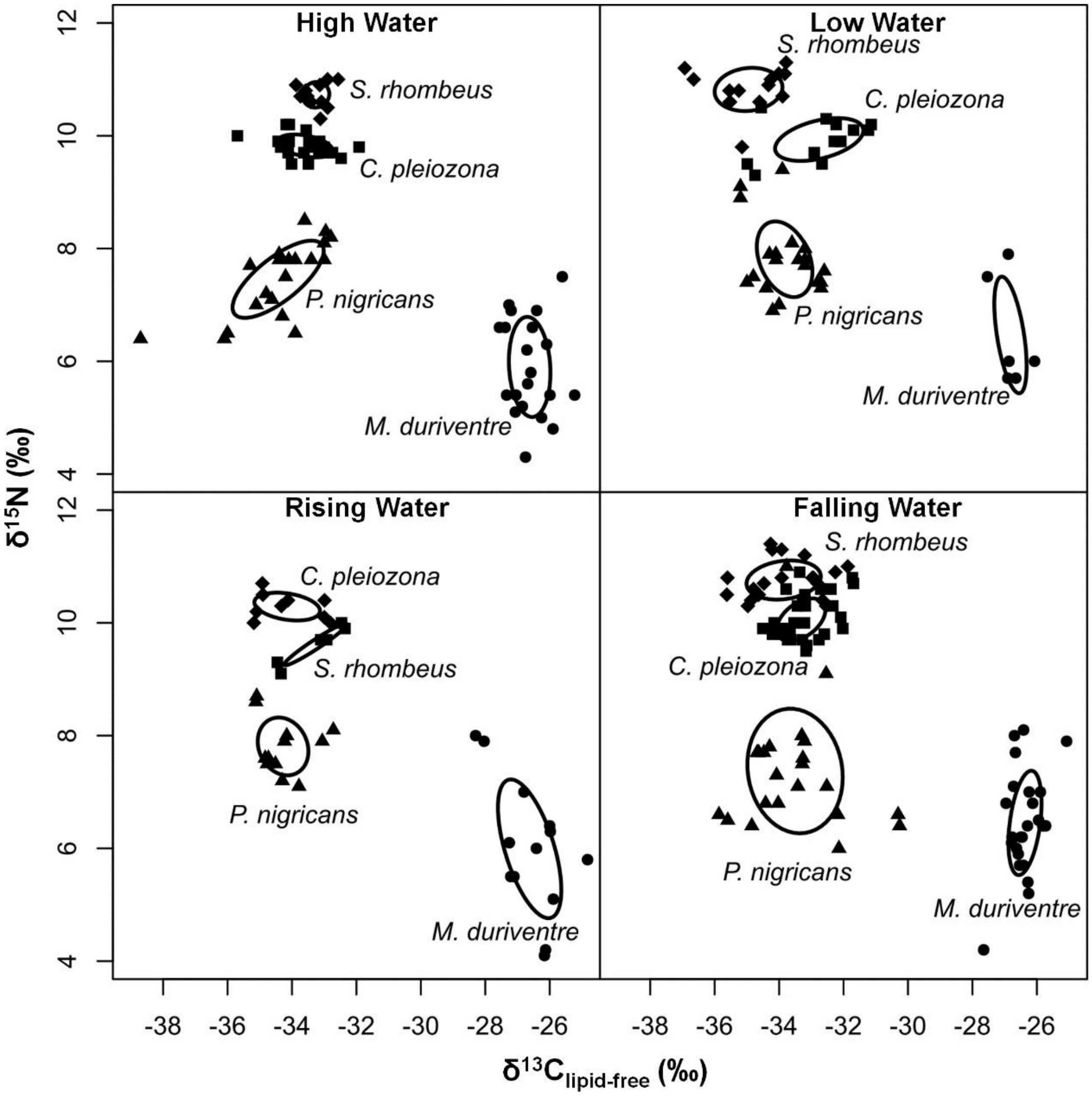


Figure 2. Standard ellipse area corrected for sample size (SEAc) of the four species during the four hydrological seasons. Red: *Prochilodus nigricans* (detritivorous); Black: *Mylossoma duriventre* (herbivorous); Blue: *Serrasalmus rhombeus* (piscivorous); Yellow: *Cichla pleiozona* (piscivorous). Ellipses represent the core niche width (40% confidence interval).

Table 2. Mean and 95% credible intervals (in parentheses) of Layman Metrics

Season	Nitrogen range (NR)	Carbon range (CR)	Total area (TA)	Mean distance of the centroid (CD)	Mean nearest neighbor distance (MNND)	Standard deviation of the mean nearest neighbor distance (SDNND)
High Water	4.83 (4.03 - 5.61)	7.81 (7.17 - 8.48)	13.90 (10.30 - 17.60)	3.33 (3.07 - 5.58)	3.12 (2.73 - 3.50)	3.21 (2.79 - 3.65)
Low Water	4.37 (3.25 - 5.48)	8.14 (7.02 - 9.25)	11.30 (7.89 - 15.10)	3.17 (2.76 - 3.58)	3.47 (2.96 - 3.99)	2.27 (1.55 - 2.99)
Rising water	4.35 (3.37 - 5.29)	7.80 (6.97 - 8.61)	10.50 (6.07 - 15.00)	2.21 (2.88 - 3.52)	3.03 (2.50 - 3.58)	3.03 (2.38 - 3.65)
Falling Water	4.30 (3.70 - 4.93)	7.52 (6.93 - 8.13)	12.00 (9.51 - 14.50)	3.27 (3.10 - 3.44)	2.95 (2.66 - 3.23)	2.96 (2.61 - 3.32)

Even though *Cichla pleiozona* and *Serrasalmus rhombeus* shared the same feeding habit (piscivorous), these species' isotopic niches did not follow the predicted pattern of higher overlap during the dry cycle in comparison with the flood cycle (Fig. 2). A very small overlap in isotopic niche of these two species was observed during the falling and rising water seasons (Fig. 2; both < 1%), which did not support our second hypothesis. The results suggest that the piscivorous species *Cichla pleiozona* and *Serrasalmus rhombeus* exploit different food resources in Puruzinho floodplain lake. The isotopic niche of the detritivorous *Prochilodus nigricans* and the herbivorous *Mylossoma duriventre* did not overlap during the annual dry and flood cycles.

4. Discussion

The similar results of the Layman metrics NR, TA and CD (indicators of trophic diversity) and CR (indicator of resource variability use) suggest that, when pooling all four species together, trophic diversity did not change along the seasons. The trophic redundancy, indicated by MNND, and species evenness, indicated by SDNND, also followed the same trend of no significant changes among the seasons. These results are consistent with Abrantes et al. (2014) who reported no significant seasonal variation in the Layman metrics in the Zambezi and Rianila estuaries, east Africa. However, Abrantes et al. (2014) observed seasonal changes in other estuaries of east Africa. This inconsistency of seasonal variation in Layman metrics among different areas was also observed by Masese et al. (2017) in an African savanna river. Since the sampling of this study was done only in the lentic section of the Puruzinho floodplain system, it’s not possible to known if Layman metrics would show significant seasonal variation in the channel or in the main river.

This study showed that trophic niche width is influenced by the annual flood and dry cycle. However, the seasonal variation in isotopic niche width was not in agreement with our hypothesis of broader niches during the flood cycle for all species. The only species that followed the predicted pattern was *Mylossoma duriventre*, which had an isotopic niche width, estimated by the SEAc, approximately two times greater at the beginning of the flood cycle (rising water season) than in the falling water and low water seasons. *Prochilodus nigricans* showed a niche width more than four times higher in the falling water in comparison with the rising water season, while *Cichla pleiozona* and *Serrasalmus rhombeus* showed slightly broader niches in both seasons of the dry cycle (falling and low water). The results suggest interspecific differences in foraging habits in response to seasonal changes in water level and hydrodynamics. Variations in niche width reflect the influence of several environmental drivers, such as ecosystem fragmentation (Layman et al. 2007), changes in resource availability and diversity (Lehmann et al. 2015; Pool et al. 2017) and particular responses to seasonal environments (Martínez del Rio et al. 2009).

The greater trophic niche width of *Mylossoma duriventre* in the rising water season is related to the greater diversity of available food resources. The results of Pool et al. (2017) corroborate this inference. Studying a flood-pulse river in Cambodia, they observed that during the flood cycle, fish of the same species tended to have broader isotopic niches, reflecting higher trophic diversity. Also, Sepulveda-Lozada et al. (2017) reported broader isotopic niche during the flood cycle of a floodplain system in Mexico due to diversification of basal resources. The increase in trophic diversity of *Mylossoma* diet during the flood cycle can be related to the use of allochthonous resources like fruits, seeds and leaves, which are more plentiful during that season (Gonzalez and Vispo 2003, Knab-Vispo et al. 2003). Species of the genus *Mylossoma* are known for eating fruits from palm trees during the flood cycle (Parolin et al. 2013). In flooded forests, it is common for frugivorous fish to wait beneath trees and consume fruits that fall into the water (Parolin et al. 2013). Although allochthonous resources are abundant during the flood cycle, the isotopic niche of *Mylossoma duriventre* declined by 57% from the rising water (3.25) to the falling water (1.39) seasons, which can reflect the fact that some species expand their diet for a brief period by including a wider variety of food resources and then return to specializing in a more dominant food, thus reducing diet range and keeping it relatively constant on average during the seasons (Correa and Winemiller, 2014).

Prochilodus nigricans was the species with broadest isotopic niche during the falling water season, possibly due to diet diversification. The study of Benedito et al. (2018) in three areas of the Paraná river floodplain (Baía, Paraná and Ivinheima rivers) during the falling water season indicates that the detritivorous species of the genus *Prochilous* have a diet composed of a mixture of autochthonous phytoplankton, periphyton detritus and allochthonous organic matter. These authors reported higher relative importance, more than 88%, of autochthonous resources for *Prochilodus lineatus* in the Baía and Parana rivers, possibly due to higher primary productivity. Other studies of detritivorous fish with morphological adaptations to exploit phytoplankton and periphyton detritus, such as *Prochilodus lineatus* and *Prochilodus nigricans*, also have reported higher relative importance of autochthonous resources (Babler et al. 2011). However, in the Ivinheima River, the contribution of autochthonous resources was lower (72%), suggesting more diversification in the diet of *Prochilodus lineatus*. The results of Benedito et al. (2018) indicate that even during the falling water season, fish of the *Prochilodus* genus can exploit a wide spectrum of resources, thus having a wider isotopic niche. Correa and Winemiller (2014) also observed increased niche width of *Brycon falcatus* and *Myloplus asterias* in an Amazon floodplain forest area during the falling water season due to diversification in food resource use. This mixed use of allochthonous and autochthonous resources may explain the progressive increase of niche width of *Prochilodus nigricans* through rising water (SEAc= 1.28), high water (SEAc= 2.21) and falling water (SEAc=5.14).

Despite sharing the same feeding habit (piscivorous), *Cichla pleiozona* and *Serrasalmus rhombeus* had no overlap of their isotopic niche in any of the seasons. The higher niche separation was observed in the Low water season, which is in agreement with Zaret and Rand (1971) that have reported a higher degree of niche separation in the dry season in Pedro Miguel River, Panama. However, Zaret and Rand (1971) observed higher niche overlap during the wet season, which differ from the pattern reported in this study. In contrast, Lowe-McConnel (1964) found a higher niche overlap during the dry season when a large number of fish are confined in small drying ponds and pools. It is plausible that many aspects of the studied environment and fish morphology influences the high, low or lack of niche overlaps. Regarding this study, the lack of niche overlaps between the two piscivorous can be explained by their differences in morphology. *Serralsamus rhombeus* have a smaller mouth with sharp teeth that rip apart the flesh of their prey, which make it possible for this specie to feed on larger preys (Shelis and Berkovitz, 1976). In contrast, *Cichla pleiozona* has a much bigger mouth that swallows the whole prey alive, not only parts of it (Santos et al., 2011). Therefore, the striking differences in mouth/teeth morphologies between these two piscivorous species can explain the lack of trophic niche overlap observed.

The isotopic niche width of *Cichla pleiozona* and *Serrasalmus rhombeus* showed similar seasonal variation, with broader niches during the seasons of the dry cycle. Prey densities increase during the low water season due to habitat compression, which in turn facilitates encounters between piscivorous fish and their prey (Arrington et al. 2005). In line with the findings of Arrington et al. (2005), Sá-Oliveira et al. (2017) observed that for *Serrasalmus rhombeus*, the relative frequencies of full stomachs were approximately 50% during the dry cycle and 30% in the flood cycle in an Amazonian reservoir. Although *Serrasalmus rhombeus* and *Cichla pleiozona* are classified as piscivorous, these fish also feed, with lower frequency, on other organisms like insects, crustaceans and microcrustaceans (Sá-Oliveira et al. 2017), which can make their isotopic niche broader. It is possible that the effect of habitat compression during the dry cycle makes it easier for both fish to feed on their regular (fish) and alternative preys (insects, crustaceans and microcrustaceans), thus increasing niche width.

Although we observed significant variation in isotopic niche width of the four studied species and confirmed the influence of the flood pulse on this aspect, we also observed an apparent stability in niche width during the seasons of the same cycle. *Mylossoma duriventre,* for example, showed similar niche widths during falling and low water seasons while the niches of *Cichla pleiozona* and *Serrasalmus* *rhombeus* were similar during rising and high water seasons. Similar niche widths between seasons of the same cycle suggests that resource uses do not change significantly from rising to high water or from falling to low water. However, this information should be considered with caution, because *Prochilodus lineatus, Mylossoma duriventre* and *Cichla pleiozona* also showed significant variation in niche width between seasons of the same cycle. In this case, *Mylossoma duriventre* showed a decrease in niche width from rising to high water, *Cichla pleiozona* an increase from falling to low water, and *Prochilodus nigricans* had variations in both dry and wet cycles. Therefore, the results suggest that the influence of the flood pulse on fish trophic ecology is species specific.

5. Conclusions

The first hypothesis of the study was not supported by the observed results because seasonal variations in isotopic niche width were different for species with each feeding habit. For the herbivorous *Mylossoma duriventre*, the isotopic niche was broader during the beginning of the flood cycle (rising water season). The detritivorous *Prochilodus nigricans* showed a progressive increase in isotopic niche width from rising water to falling water. The piscivorous *Cichla pleiozona* and *Serrasalmus rhombeus* both showed broader niche in low water season.

Our results also do not support the second hypothesis, due to no overlap of isotopic niche between piscivorous fish in the dry cycle. This result indicates that interspecific competition did not increase during the dry cycle. The two species occupied different niches, allowing coexistence.

This study highlights the influence of the flood pulse on species with different feeding habits in a relatively pristine floodplain lake. For three out of the four studied species, the isotopic niche expanded during the dry cycle, suggesting a higher trophic diversity during this season. Although we addressed in the discussion the possible causes of this result, the phenomenon is not fully understood due to lack of data on fish trophic ecology in the evaluated area. New studies should analyze the influx of allochthonous resources to the lake section during the flood cycle and whether species continue to exploit these resources during the dry cycle.

6. Acknowledgments

This work was supported by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) through the CNPq/CTUniversal project (Grant no. 458977 2014-4), and by Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ; grant number: E-26/010.001984/2014 and E-26/210.071/2018). We are grateful to IBAMA (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis) for the fish collection license (DIFAP/IBAMA no. 091). We extend special thanks to the fisherman Raimundo Nonato dos Santos. This study was also financed in part by Coordenação de Aperfeiçoamento de Pessoa de Nível Superior – Brazil (CAPES) – Finance Code 001. This paper is dedicated to the memory of Cristina Maria Magalhães de Souza.

7. Conflicts of interest

The authors disclose no conflicts of interest.

8. Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

9. References:

Abrantes, K.G., Barnett, A., & Bouillon, S., (2014). Stable isotopes-based community metrics as a tool to identify patterns in food web structure in east African estuaries. *Functional Ecology*, 28, 270-282.

Almeida, R., Bernardi, J.V.E., Oliveira, R.C., Carvalho, D.P., Manzatto, A.G., Lacerda, L.D., & Bastos, W.R. (2014). Flood pulse and spatial dynamics of mercury in sediments in Puruzinho Lake, Brazilian Amazon. *Acta Amazonica*, 44, 99-106.

Altman, N., & Krzywinski, M. (2016). Regression diagnostics. *Nature Methods*, 13, 385–386.

Arauajo-Lima, C.A., Forsberg, B.R., & Martinelli, L. (1986). Energy sources for detritivorous fishes in the Amazon. *Science*, 234, 1256-8.

Arrington, D.A., Winemiller, K.O., & Layman, C.A. (2005). Community assembly at the patch scale in a species rich tropical river. *Oecologia*, 144, 157-167.

Azevedo, L.S., Pestana, I.A., Nery, A.F.C., Bastos, W.R., & Souza, C.M.M. (2020). Mercury concentration in six fish guilds from a floodplain lake in western Amazonia: Interaction between seasonality and feeding habits. *Ecological Indicators*, 111, 106056.

Babler, A.L., Pilati, A., & Vanni, M.J. (2011). Terrestrial support of detritivorous fish populations decreases with watershed size. *Ecosphere*, 2, art76.

Benedito, E., Santana, A.R.A., & Werth, M. (2018). Divergence in energy sources for *Prochilodus lineatus* (Characiformes: Prochilodontidae) in Neotropical floodplains. *Neotropical Ichthyology*, 16, e160130.

Brito, J.G., Alves, L.F., & Espirito Santo, H.M.V. (2014). Seasonal and spatial variation in limnological conditions of a floodplain lake (Lake Catalão) connected to both the Solimões and Negro Rivers, Central Amazonia. *Acta Amazonica*, 44, 121-134.

Cardoso, D.C., deHart, P., Freitas, C.E.C., & Siqueira-Souza, F.K. (2019). Diet and ecomorphology of predator fish species of the Amazonian floodplain lakes. *Biota Neotropica*, 19(3), e20180678.

Cella-Ribeiro, A., Torrente-Vilara, G., Lima-Filho, J.A., & Doria, C.R.C. (2017). Ecologia e Biologia de peixes do Rio Madeira. EDUFRO, Porto Velho.

Cody, M.L. (1974). Competition and the structure of bird communities. Princeton University Press, Princeton, New Jersey, 318 pp.

Colwell, R.K., & Futuyma, D.J. (1971). On the measurement of niche breadth and overlap. *Ecology*, 52, 567-576.

Correa, S.B., & Winemiller, K.O. (2014). Niche partitioning among frugivorous fishes in response to fluctuating resources in the Amazonian floodplain forest. *Ecology*, 95, 210–224.

Fantin-Cruz, I., Loverde-Oliveira, S.M., & Girard, P. (2008). Caracterização morfométrica e suas implicações na limnologia de lagos do Pantanal Norte. *Acta Scientiarum. Biological Sciences*, 30, 133-140.

Feitosa, I.B., Huszar, V.L.M., Domingues, C.D., Appel, E., Paranhos, R., Almeida, R.M., Branco, C.W.C., Bastos, W.R., & Sarmento, H. (2019). Plankton community interactions in an Amazonian floodplain lake, from bacteria to zooplankton. *Hydrobiologia*, 831, 55-70.

Gonzalez, N., & Vispo, C. (2003). Aspects of the diets and feeding ecologies of fish from nine floodplain lakes of the lower Caura, Venezuelan Guayana. Pp. 329-366 *in* Vispo, C. & C.

Knab-Vispo (eds.). Plantas y vertebrados acuáticos en el corredor ribereño del bajo Rio Caura. Scient. Guaian. 12.

Herlevi, H., Aarnio, K., Puntila-Dodd, R., & Bonsdorff, R. (2018). The food web positioning and trophic niche of the non-indigenous round goby: a comparison between two Baltic Sea populations. *Hydrobiologia*, 822, 111-128.

Jackson, A. L., Inger, R., Parnell, A.C., & Bearhop, S. (2011). Comparing isotopic niche widths among and within communities: SIBER-Stable Isotope Bayesian Ellipses in R. *Journal of Animal Ecology*, 80, 595-602.

Jaeger, A., Connan, M., & Richard., P. (2010). Use of stable isotopes to quantify seasonal changes of trophic niche and levels of population and individual specialisation in seabirds. *Marine Ecology Progress Series*, 401, 269-277.

Junk, W.J., Barley, P.B., & Sparks, R.E. (1989). The flood pulse concept in river floodplain systems. *Canadian Special Publication of Fisheries and Aquatic Sciences*, 106, 110-127.

Junk, W.J. (1997). Structure and function of the large Central Amazonian river floodplains: Synthesis and Discussion, p. 455- 468. In: Junk W.J. (Ed.). The Central Amazon Floodplain. v. 126. Springer-Verlag Berlin Heidelberg, Germany.

Knab-Vispo, C., Daza, F., Vispo, C.R., & Gonzalez, N. (2003). The diet of morocoto (*Piaractus brachypomus*) in the lower Rio Caura in relation to its ecological role and its conservation. Pp. 367-391 in Vispo C. & C. Knab-Vispo (eds.). Plantas y vertebrados acuáticos en el corredor ribereño del bajo Rio Caura. Scientia Guaianae 12.

Layman, C.A., Quattrochi, J.P., Peyer, C.M., & Allgeier, J.E. (2007). Niche width collapse in a resilient top predator following ecosystem fragmentation. *Ecological Letters*, 10, 937–944.

Lehmann, D., Mfune, J.K.E., Gewers, E., Brain, C., & Voigt, C.C. (2015). Individual variation of isotopic niches in grazing and browsing desert ungulates. *Oecologia*, 179,75–88.

Leite, R.G., Araújo-Lima, C.A.R.M., Victoria, R.L., & Martinelli, L.A. (2002). Stable isotope analysis of energy source for larvae of eight fish species from the Amazon floodplain. *Ecology of Freshwater Fish*, 11, 56–63.

Lesack, L.F., & Melack, J.M. (1995). Flooding hydrology and mixture dynamics of lake water derived from multiple sources in an Amazon floodplain lake. *Water Resources Research*, 31, 329-345.

Lowe-McConnell, R.H. (1964). The fishes of the Rupununi savanna district of British Guiana, South America: Part 1. Ecological grouping of fish species and effects of the seasonal cycle on the fish. *Zoological Journal of the Linnean Society*, 45, 103-144.

Martínez del Rio, C., Sabat, P., Anderson-Sprecher, R., & Gonzalez, S.P. (2009). Dietary and isotopic specialization: the isotopic niche of three Cinclodes ovenbirds. *Oecologia*, 161,149-159.

McConnaughey, T., & McRoy, C.P. (1979). Food-Web Structure and the Fractionation of Carbon Isotopes in the Bering Sea. *Marine Biology*, 53, 257-262.

Mérona, B., & Rankin-de-Mérona, J. (2004). Food resource partitioning in a fish community of central Amazon floodplain. *Neotropical Ichthyology*, 2(2), 75-84.

Masese, F.O., Abrantes, K.G., Gettel, G.M., Irvine, K., Bouillon, S., & McClain, M.E. (2017). Trophic structure of an African savanna river and organic matter inputs by large terrestrial herbivores: a stable isotopes approach. *Freshwater Biology*, 63, 1365-1380.

Mortillaro, J.M., Pouilly, M., Wach, M., Freitas, C.E.C., Abril, G., & Meziane, T. (2015). Trophic opportunism of central Amazon floodplain fish. *Freshwater Biology*, 60 (8), 1659-1670.

Oliveria, A.C.B., Soares, M.G.M., Matinelli, L.A., & Moreira, M.Z. (2006). Carbon sources of fish in an Amazonian floodplain lake. *Aquatic Science*, 68, 229-238.

Park, H.J., Yeon, I., Han, E., Lee, Y.J., Hanchet, S.M., Baeck, G.W., Kwon, Y., Choi, S.G., Lee, D.W., & Kang, C.K. (2015). Diet study of Antarctic toothfish caught in the east Antarctic based on stomach content, fatty acid and stable isotope analyses. *Commission for the Conservation of Antarctic Marine Living Resources*, 22, 29–44.

Parolin, P., Wittmann, F., &Ferreira, L. (2013). Fruit and seed dispersal in Amazonian floodplain trees - a review. *Ecotropica*, 19, 19–36.

Plummer, M. (2019). rjags: Bayesian Graphical Models using MCMC. R package version 4-10. https://CRAN.R-project.org/package=rjags

Pokharel, K.P., Yohannes, E., & Salvarina, I., (2015). Isotopic evidence for dietary niche overlap between barking deer and four-horned antelope in Nepal. *Journal of Biological Research-Thessaloniki*, 22, 6.

Pool, T., Holtgrieve, G., Elliott, V., McCann, K., McMeans, B., Rooney, N., Smits, A., Phanara, T., Cooperman M., Clark, S., Phen, C., & Chhuoy, S. (2017). Seasonal increase in fish trophic niche plasticity within a flood-pulse river ecosystem (Tonle Sap Lake, Cambodia). *Ecosphere*, 8(7), e01881.

Post, D.M. (2002). Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology*, 83, 703–718.

Quirino, B.A., Carniatto, N., Guglielmetti, R., & Fugi, R. (2017). Changes in diet and niche breadth of a small fish species in response to the flood pulse in a Neotropical floodplain lake. *Limnologica*, 62, 126-131.

R Core Team, (2020). R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Austria, Vienna. http://www.R-project.org/.

Rejas, D. (2018). Trophic structure of a floodplain fish assemblage in the upper Amazon basin, Bolivia. *Revista de Biologia Tropical*, 66(3), 1258-1271.

Rumbold, D.G., Lange, T.R., Richard, D., DelPizzo, G., & Hass, N. (2018). Mercury biomagnification through food webs along a salinity gradient down-estuary from a biological hotspot. *Estuarine*, *Coastal and Shelf Science*, 200, 116-125.

Sá-Oliveira, J., C., Ferrari, S. F., Vasconselos, H. C.G., Araujo, A. S., Campos, C. E. C., Mattos-Dias, C. A., Fecury, A. A., Oliveira, E., Mendes-Junior, R.N.G., & Issac, V.J. (2017). Resource Partitioning between Two Piranhas (Serrasalmus gibbus and Serrasalmus rhombeus) in an Amazonian Reservoir. *The Scientific World Journal*, 17, 8064126.

Santos, A.F.G.N., Santos, L.N., & Araújo, F.G. (2011). Feeding morphology of the Neotropical piscivorous fish *Cichla kelberi* (Perciformes: Cichlidae) introduced into an oligotrophic Brazilian reservoir. *Revista de Biologia Tropical*, 59, 000-000.

Sepúlveda-Lozada, A., Saint-Paul, U., Mendoza-Carranza, M., Wolff, M., & Yáñez-Arancibia, A. (2017). Flood pulse induced changes in isotopic niche and resource utilization of consumers in a Mexican floodplain system. *Aquatic Sciences*, 79, 597-616.

Shellis, R.P., & Berkovitz, B.K.B. (1976). Observations on the dental anatomy of piranhas (Characidae) with special reference to tooth structure. *Journal of Zoology*, 180, 69-84.

Syväranta, J., Lensu, A., Marjomaki, T.J., Oksanen, S., & Jones, R.I. (2013). An empirical evaluation of the utility of convex hull and standard ellipse areas for assessing population niche widths from stable isotope data. *PloS ONE*, 8, e56094.

Thomas, S.M., & Crowther, T.W. (2015). Predicting rates of isotopic turnover across the animal kingdom: a synthesis of existing data. *Journal of Animal Ecology*, 84, 861-870.

Thomaz, S.M., Bini, L.M., & Bozelli, R.L. (2007). Floods increase similarity among aquatic habitats in river-floodplain systems. *Hydrobiologia*, 579, 1-13.

Walker, R.H., Kluender, E.R., Inebnit, T.E., & Adams, S.R. (2013). Differences in diet andfeeding ecology of similar-sized spotted (*Lepisosteus oculatus*) and shortnose (*Lepisosteus platostomus*) gars during flooding of a south-eastern US river. *Ecology of Frewshwater Fish*, 22, 617-625.

Wang, J., Chapman, D., Xu, J., Wang, Y., & Gu, B. (2018). Isotope niche dimension and trophic niche overlap between bighead carps and native filter-feeding fish in the lower Missouri River, USA. *PLoS ONE*, 13(5), e0197584.

Wissmar, R.C., Richey, J.E., Stallard, R.F., & Edmond, J.M. (1981). Plankton metabolism and carbon processes in the Amazon River, its tributaries, and floodplain waters, Peru- Brazil, May-June 1977. *Ecology*, 62, 1622-1633.

Zaret, T.M., & Rand, A.S. (1971). Competition in Tropical Stream Fishes: Support for the Competitive Exclusion Principle. *Ecological Society of America*, 52, 336-442.

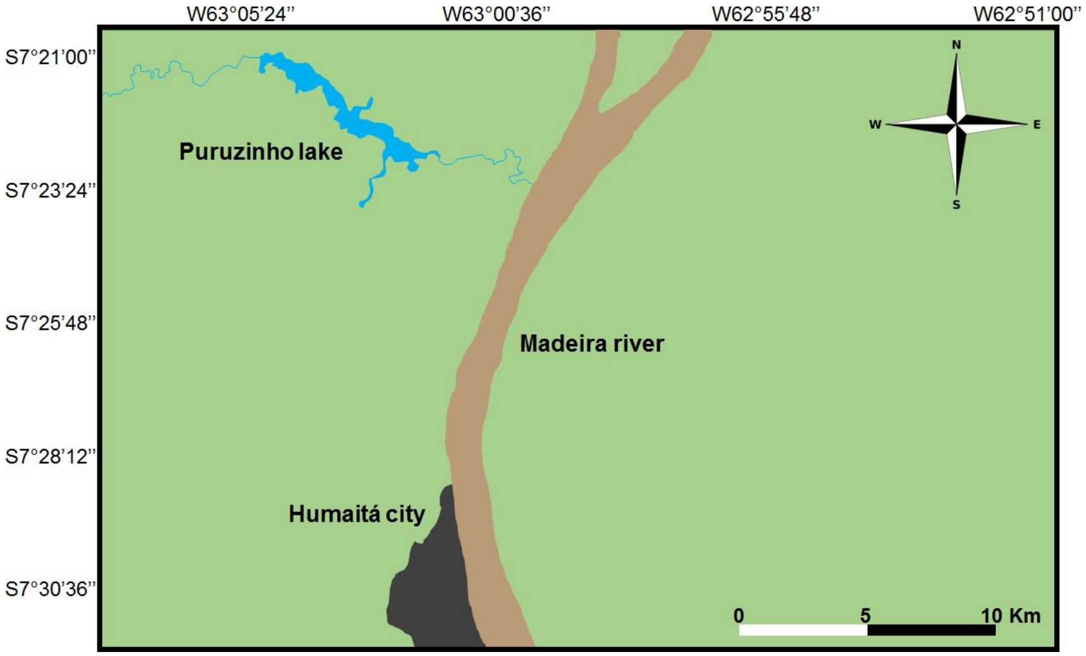
SUPPLEMENTARY MATERIAL

Do fish isotopic niches change in an Amazon floodplain lake over the hydrological regime?

Lucas Silva Azevedo[a,*], Inácio Abreu Pestana[a], Marcelo Gomes Almeida[a],
Wanderley Rodrigues Bastos[b], Cristina Maria Magalhães Souza[a]

[a]Laboratório de Ciências Ambientais, Centro de Biociências e Biotecnologia, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, Rio de Janeiro, RJ, CEP: 28013-602, Brazil.
[b]Laboratório de Biogeoquímica Ambiental, Universidade Federal de Rondônia, Porto Velho, Rondônia, RO, CEP: 76815-800, Brazil.

This document have: one map of the study area (Supplementary Material 1); a table with total length, total weight, stable isotopic signatures ($\delta^{13}C_{lipid\text{-}free}$, $\delta^{15}N$), C:Na, organic carbon and nitrogen of the sampled species during the four hydrological seasons (Data extracted from Azevedo et al. 2021) (Table S1).



Supplementary Material 1: Map of the study area

Table S1. Total length, total weight, stable isotopic signatures ($\delta^{13}C_{\text{lipid-free}}$, $\delta^{15}N$), C:Na, organic carbon and nitrogen of the sampled species during the four hydrological seasons (Data extracted from Azevedo et al. 2021). ANOVA reported significant interaction between seasons and species for $\delta^{13}C_{\text{lipid-free}}$ ($F_{9,249} = 3.76$; $p = 0.00018$) while no significant interaction was detected for $\delta^{15}N$ ($F_{9,249} = 1.58$; $p = 0.12$). Lowercase letters indicate Tukey test comparisons ($\alpha = 0.05$) for a species among the four hydrological cycles.

Rising Water

Species	TL (mm)	TW (g)	$\delta^{13}C_{\text{lipid-free}}$ (‰)	$\delta^{15}N$ (‰)	C:Na	C Org (%)	N Org (%)
Mylossoma duriventre	148.4 ± 12.8	177.70 ± 42.45	-26.62 ± 0.95^{a}	6.00 ± 1.18^{a}	4.47 ± 0.61	48.69 ± 2.60	12.82 ± 1.11
Prochilodus nigricans	243.3 ± 24.2	286.66 ± 29.02	-34.27 ± 0.76^{a}	7.81 ± 0.50^{a}	3.85 ± 0.08	44.74 ± 1.80	13.54 ± 0.51
Cichla pleiozona	271.6 ± 11.6	311.66 ± 14.71	-33.27 ± 0.91^{a}	9.60 ± 0.34^{a}	3.76 ± 0.06	45.07 ± 2.58	14.00 ± 0.74
Serrasalmus rhombeus	210.0 ± 15.5	233.33 ± 20.00	-34.14 ± 0.97^{a}	10.30 ± 0.23^{a}	4.01 ± 0.25	46.67 ± 1.01	13.60 ± 0.74

High Water

Species	TL (mm)	TW (g)	$\delta^{13}C_{\text{lipid-free}}$ (‰)	$\delta^{15}N$ (‰)	C:Na	C Org (%)	N Org (%)
Mylossoma duriventre	160.0 ± 8.0	184.00 ± 39.12	-26.62 ± 0.63^{a}	5.90 ± 0.87^{a}	5.28 ± 0.89	50.90 ± 3.93	11.44 ± 1.36
Prochilodus nigricans	263.5 ± 18.7	395.00 ± 75.91	-34.42 ± 1.38^{a}	7.45 ± 0.65^{a}	4.22 ± 0.37	47.00 ± 3.30	13.04 ± 1.06
Cichla pleiozona	269.0 ± 25.7	442.10 ± 120.50	-33.60 ± 0.82^{a}	9.80 ± 0.20^{a}	3.77 ± 0.08	44.42 ± 4.51	13.73 ± 1.37
Serrasalmus rhombeus	227.0 ± 26.0	405.00 ± 106.60	-33.25 ± 0.40^{a}	10.74 ± 0.22^{a}	3.80 ± 0.09	45.52 ± 1.00	13.95 ± 0.38

Falling Water

Species	**TL (mm)**	**TW (g)**	**$\delta^{13}C_{lipid\text{-}free}$ (‰)**	**$\delta^{15}N$ (‰)**	**C:Na**	**C Org (%)**	**N Org (%)**
Mylossoma duriventre	161.0 ± 14.0	182.50 ± 38.40	-26.35 ± 0.50[a]	6.40 ± 0.90[a]	4.83 ± 0.86	50.16 ± 2.22	12.35 ± 1.51
Prochilodus nigricans	261.0 ± 20.0	297.72 ± 33.51	-33.50 ± 1.44[a]	7.40 ± 1.10[a]	4.05 ± 0.26	46.57 ± 1.50	13.42 ± 0.57
Cichla pleiozona	292.5 ± 41.7	352.85 ± 74.89	-33.31 ± 0.74[a]	10.05 ± 0.37[a]	3.83 ± 0.08	45.36 ± 1.40	13.80 ± 0.55
Serrasalmus rhombeus	240.0 ± 23.0	277.00 ± 34.20	-33.85 ± 1.12[a]	10.76 ± 0.33[a]	4.01 ± 0.19	46.26 ± 2.48	13.45 ± 0.54

Low Water

Species	**TL (mm)**	**TW (g)**	**$\delta^{13}C_{lipid\text{-}free}$ (‰)**	**$\delta^{15}N$ (‰)**	**C:Na**	**C Org (%)**	**N Org (%)**
Mylossoma duriventre	151.6 ± 9.8	138.33 ± 31.88	-26.81 ± 0.47[a]	6.47 ± 0.98[a]	3.93 ± 0.05	45.16 ± 1.54	13.38 ± 0.33
Prochilodus nigricans	231.0 ± 17.4	287.00 ± 66.34	-33.82 ± 0.84[a]	7.80 ± 0.66[a]	3.79 ± 0.11	45.13 ± 2.00	13.91 ± 0.60
Cichla pleiozona	281.0 ± 41.4	462.50 ± 187.23	-32.75 ± 1.32[a]	9.93 ± 0.37[a]	3.74 ± 0.09	45.18 ± 0.80	14.07 ± 0.47
Serrasalmus rhombeus	228.5 ± 23.8	400.00 ± 124.03	-34.94 ± 1.02[a]	10.81 ± 0.37[a]	3.77 ± 0.09	46.38 ± 0.85	14.33 ± 0.35

Reference: Azevedo, L.S., Pestana, I.A., Almeida, M.G., Nery, A.F.C., Bastos, W.R., & Souza, C.M.M. (2021). Mercury biomagnification in an ichthyic food chain of an Amazon floodplain lake (Puruzinho Lake): influence of seasonality and food chain structure**.** *Ecotoxicology and Environmental Safety*,207, 111249, 2021. http://dx.doi.org/10.1016/j.ecoenv.2020.111249

2. Discussão Geral

Os resultados apresentados neste estudo compõem um corpo teórico para fundamentar as explicações sobre como as concentrações de Hg em peixes de lagos de várzea da Amazônia mudam ao longo do regime hidrológico característico da região (o pulso de inundação). Verificou-se que os padrões sazonais na acumulação de Hg são mais consistentes quando as espécies foram agrupadas em guildas tróficas (carnívoros, piscívoros, onívoros, detritívoros, herbívoros e planctívoros; Capítulo 1). A reprodutibilidade desse resultado ainda precisa ser testada em outros lagos de várzea e em outros ecossistemas aquáticos de água doce (rios de águas brancas e claras, igarapés) para confirmar se os padrões observados neste estudo são, de fato, gerais e podem ser aplicados para os outros ecossistemas.

O estudo sobre a biomagnificação de Hg (Capítulo 2) mostrou que a composição da cadeia trófica, ou seja, as espécies que o autor decide amostrar para compor a sua cadeia trófica, exerce influência sobre o resultado do “Trophic Magnification Factor”. Ficou claro, portanto, que o pesquisador que decide estudar biomagnificação de Hg usando apenas duas espécies pode obter um resultado diferente daquele que opta por conduzir seu estudo com dez espécies diferentes. Uma padronização na construção de cadeias tróficas para esse tipo de análise é devida para que a comparabilidade entre estudos, necessária para a compreensão dos fatores que influenciam o fenômeno da biomagnificação, possa ser feita de forma adequada.

O uso dos isótopos estáveis para caracterizar a ecologia trófica das espécies do lago Puruzinho produziu informações inéditas pois, até o momento da submissão do Capítulo 3, a variação do nicho isotópico dos peixes desta região ao longo das quatro estações do regime hidrológico ainda não tinha sido caracterizada. Os resultados não seguiram o padrão esperado de variação na amplitude de nicho, mostrando que a ecologia trófica dos peixes dos lagos de várzea é complexa e sujeita a fatores que não foram considerados nesta pesquisa. Importante destacar também que os próximos estudos no lago Puruzinho sobre a ecologia trófica das espécies devem dar ênfase à caracterização do conteúdo estomacal dos peixes mais representativos (tucunaré, pacu, curimbatá e etc) e à caracterização isotópica das fontes de recursos alimentares (insetos e plantas terrestres, sementes, frutas e etc), de forma que se possa detalhar qual das fontes exerce maior influência na assinatura isotópica dos peixes.

Por fim, os objetivos que conduziram essa pesquisa foram atingidos e os estudos publicados certamente acrescentarão na discussão sobre os fatores que influenciam a acumulação de Hg em peixes de lagos de várzea da Amazônia.

3. Considerações Finais

As pesquisas para elucidar a influência dos fatores ambientais sobre a acumulação de Hg em peixes Amazônicos ainda têm um longo caminho para percorrer. Os artigos apresentados nesta tese trouxeram informações novas e preencheram algumas lacunas, mas ainda há muitas perguntas que não foram respondidas tanto sobre a relação entre o pulso de inundação e acumulação de Hg quanto sobre a relação do pulso de inundação sobre a ecologia alimentar das espécies. É importante que os próximos estudos na região Amazônica, que busquem avaliar a influência da sazonalidade sobre a acumulação de Hg em peixes em outros ecossistemas, considerem as guildas tróficas ao invés das espécies individiduais, pois, como foi demonstrado, os padrões sazonais são mais consistentes nas guildas. Seria de grande valia para o corpo teórico da biogeoquímica de Hg em ambientes amazônicos se outros autores avaliassem a variação sazonal nas guildas tróficas em diferentes ecossistemas considerando aspectos como, físico-quimica (águas pretas, brancas e claras), área (lagos pequenos, médios e grandes), area de inundação e uso do solo ao redor do ecossistema aquático. Um outro aspecto importante que foi abordado nesta tese é a ecologia alimentar dos peixes do lago Puruzinho, que ainda não tinha sido feita. As lacunas que precisam ser preenchidas nesta área são maiores do que aquelas presentes nos estudos sobre acumulação de Hg e muito ainda precisa ser feito. Como foi mencionado anteriormente, estudos preliminares sobre o conteúdo estomacal das espécies representativas ao longo das estações do regime hidrológico firmaria uma base importante para o estudo da ecologia trófica das espécies do lago Puruzinho e outros lagos de várzea onde elas também são representativas. Paralelamente, a caracterização isotópica das fontes também acrescentaria informações uteis. Em suma, a tese em questão apresenta novas informações sobre a dinâmica do Hg e sobre a ecologia alimentar de peixes da Amazônia e aponta um caminho para novos estudos ecológicos.

4. Referências Bibliográficas

Amaro, C.S.O., Junior, D.R., Silva, M.C.F., Lima, A.A.S., Santos, G.F.S., Pinheiro, M.C.N. (2014). Concentração de Mercúrio Total (Hg-T) em peixes comercializados em diferentes períodos sazonais no Mercado do Ver-o-Peso, Belém, Estado do Pará, Brasil. Revista Pan-Amazônia de Saúde, 5:53-60.

Azevedo, L.S., Pestana, I.A., Nery, A.F.C., Bastos, W.R., Souza, C.M.M. (2020). Mercury concentration in six fish guilds from a floodplain lake in western Amazonia: Interaction between seasonality and feeding habits. Ecological Indicators, 111:106056.

Bisinoti, M.C., Júnior, E.S., Jardim, W.F., 2007. Seasonal Behavior of Mercury Species in Waters and Sediments from the Negro river basin, Amazon Brazil. J. Braz. Chem. Soc. 18 (3), 544-553.

Cordier, S., Garel, M., Mandereau, L., Morcel, H., Doineau, P., Gosme-Seguret, S., Josse, D., White, R., Amiel-Tison, C. (2002). Neurodevelopmental investigations among methyl mercury exposed children in French Guiana. Environmental Research, 89:1-11.

Herlevi, H., Aarnio, K., Puntila-Dodd, R., & Bonsdorff, R. (2018). The food web positioning and trophic niche of the non-indigenous round goby: a comparison between two Baltic Sea population. *Hydrobiologia*, 822, 111-128.

Junk, W.J. (1997). General aspects of floodplain ecology with special reference to Amazonian floodplains. In: W.J. JUNK, ed. The Central Amazon floodplain, ecology of a pulsing system Berlin: Springer-Verlag, pp. 3-17.

Lino, A.S., Kasper, D., Guida, Y.S., Thomaz, J.R., Malm, O., (2019). Total and methyl mercury distribution in water, sediment, plankton and fish along the Tapajós River basin in the Brazilian Amazon. Chemosphere, 235, 690-700.

Lopes, I.G., Oliveira, R.G., Ramos, F.M. (2016). Perfil do consumo de peixes da população brasileira. Biota Amazônia, 6, 62-65.

Mérona, B., Rankin-de-Mérona, J. (2004). Food resource partitioning in a fish community of central Amazon floodplain. *Neotropical Ichthyology*, 2(2), 75-84.

Oliveria, A.C.B., Soares, M.G.M., Matinelli, L.A., & Moreira, M.Z. (2006). Carbon source of fish in an Amazonian floodplain lake. *Aquatic Science*, 68, 229-238.

Oliveira, R.C., Dórea, J.G., Bernardi, J.V.E., Bastos, W.R., Almeida, R., Manzatto, A.G. (2010). Fish consumption by traditional subsistence villagers of the Rio Madeira (Amazon): Impact on hair mercury. Annals of Human Biology, 37, 629-642.

Pacheco-Ferreira H. Mercúrio na Amazônia: efeitos sobre a saúde das populações ribeirinhas [Tese de doutorado]. Belém: Núcleo de Altos Estudos Amazônicos, Universidade Federal do Pará; 2001.

Pool, T., Holtgrieve, G., Elliott, V., McCann, K., McMeans, B., Rooney, N., Smits, A., Phanara, T., Cooperman M., Clark, S., Phen, C., & Chhuoy, S. (2017). Seasonal increase in fish trophic niche plasticity within a flood-pulse river ecosystem (Tonle Sap Lake, Cambodia). *Ecosphere*, 8(7), e01881.

Renzoni, A., Zino, F., Franchi, E. (1998). Mercury levels along the food chain and riskvfor exposed populations. Environ. Res., 77, 68–72.

Silva, S.F., Oliveira, D.C., Pereira, J.P.G., Castro, S.P., Costa, B.N.S., Lima, M.O., 2019. Seasonal variation of mercury in commercial fishes of the Amazon Triple Frontier, Western Amazon Basin. Ecological Indicators 106, 105549.

Sioli H. Studies in Amazonian Waters (1967). In: Atas do simpósio sobre a biota amazônica 3, 9-50. Conselho Nacional de Pesquisas, Rio de Janeiro.

Vieira, M., Bernardi, J.V.E., Dórea, J.G., Rocha, B.C.P., Ribeiro, R., Zara, L.F., 2018. Distribution and avaliability of mercury and methylmercury in different Waters from the Rio Madeira Basin, Amazon. Environmental pollution 235, 771-779.