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Trophic dynamics of methylmercury and trace elements in a remote Amazonian Lake

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A B S T R A C T

Information on pollutant trophodynamics can be crucial for public health, as contaminated food consumption may lead to deleterious effects. This study was performed in Puruzinho Lake, a remote body of water in the Brazilian Amazon from which a riparian human population obtains an important part of its animal protein intake. Samples from 92 individuals, comprising 13 species and four trophic guilds (iliophagous, planktivorous, omnivorous, and piscivorous fish) were analysed for the determination of trace elements (Fe, Cr, Mn, Ni, Zn, Ca, Sr, Cd, Sn, Tl and Pb) and methylmercury concentrations. Samples from the same individuals had already been analysed for stable isotope (SI) measurements ($\delta^{13}C$ and $\delta^{15}N$) in a previous investigation and the SI data have been statistically treated with those generated in this study for the evaluation of trophic dynamics of contaminants. Methylmercury was the only analyte that biomagnified, presenting TMF values of 4.65 and 4.55 for total and resident ichthyofauna, respectively. Trace elements presented either trophic dilution or independence from the trophic position, constituting a behaviour that was coherent with that found in the scientific literature. The similarity between Ni behaviour through the trophic web to that of essential elements contributes to the discussion on the essentiality of this metal to fish. Considering the Non-cancer Risk Assessment, the calculated Target Hazard Quotient (THQ) values were higher than 1.0 for all analysed individuals for methylmercury, as well as for only one individual for nickel. No other analyte rendered THQ values higher than 1.0.

1. Introduction

Information on the trophodynamics of trace elements is crucial for public health, as contaminated food consumption may lead to deleterious effects on the population. These data are important to the decision-making organizations, *i.e.*, to the organs that play a role in monitoring programs, in the evaluation of impacted areas, as well as in the regulation of fish consumption, for example: the United States Environmental Protection Agency (USEPA, 2000a; b). Knowing the behaviour of trace elements in the biota allows us to infer in which species, according to their trophic position, the highest concentrations are more likely to be found. For instance: the highest concentrations of elements that are well-known to biomagnify (such as Hg) are found in animals that occupy the top positions of the aquatic food webs (Campbell et al., 2008; Bisi et al., 2012; Lavoie et al. 2013; Azevedo-Silva et al. 2016). Mercury biomagnification is related to its organic form (Lavoie et al. 2013), subsequently to the methylation process and methylmercury (MeHg) synthesis (Coelho-Souza et al. 2011). On the other hand, elements such

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as nickel (Ni) tend to undergo trophic dilution (concentrations decrease upwards the trophic web, *i.e.*, the top predator species present the lowest ones) in aquatic ecosystems (Nfon et al. 2009; Pereira et al. 2010; Guo et al. 2016; Monferrán et al. 2016; Bungala et al. 2017).

Knowledge on the behaviour of trace elements in the biota constitutes also an important tool for ecological studies, as we can use occurrence and concentrations of multiple elements in certain tissues and structures of fish for the understanding of migration process, as the concentrations of essential elements in fish are related, among other factors, to the ecosystem inhabited by the animals (Lall, 2002; Albuquerque et al. 2010; Collins et al. 2013). The occupation of habitats can also be inferred by trace element concentrations. For example, Carvalho et al. (2005) found differences in Fe and Sr concentrations between marine fish from different habitats, on the Portuguese coast. Significant differences in Fe levels were found between pelagic and benthic species, with higher concentrations in the latter group. For Sr, on the other hand, differences were found between fish from sandy/muddy bottom or rocky environments, with higher concentrations in individuals from the former habitat.

Therefore, knowledge on the trophodynamics of trace elements is important for the comprehension of their biogeochemical cycles, filing an important data gap for essential (*e.g.*, iron - Fe, calcium - Ca, manganese - Mn and zinc - Zn) and non-essential (*e.g.*, cadmium - Cd, mercury - Hg and lead - Pb) elements, as well as for elements whose essentiality is still a matter of debate, such as Ni (Payle and Couture, 2011).

Barwick and Maher (2003) highlight that there still are many gaps on the comprehension of trace element trophodynamics in aquatic food webs. Although Hg biomagnification has been consistently demonstrated in different ecosystems, for other elements (*e.g.*, Cd and silver - Ag) the occurrence of trophic magnification depends on the ecosystem investigated, as well on the toxicokinetic of the chemical species of the studied elements, among other factors (Lavoie et al. 2013; Bungala et al. 2017; Liu et al. 2019). Zinc constitutes a good example of contrasting scenarios for the same element. For instance, Ikemoto et al. (2008) observed that Zn concentrations were not related to the trophic position occupied by organisms of the Mekong River Delta food web; Zeng et al. (2013) found Zn trophic dilution in the food web of the Pearl River estuary; and Espejo et al. (2020) observed Zn biomagnification in the food web of invertebrates from Paradise Bay (Antarctica). Although less discrepant than the data on Zn, other elements can also present divergences in their trophodynamics in aquatic ecosystems, as chrome (Cr) and Mn presented either trophic dilution or independence from the trophic position in different ecosystems (Pereira et al., 2010; Ikemoto et al., 2008; Nfon et al., 2009). It is important to highlight that Zn, Cr and Mn are essential elements; consequently, their toxicokinetics is mediated by physiological processes (NRC, 1993; Prabhu et al., 2016).

Therefore, peculiarities of the environment are determinants for the trophodynamics of trace elements and the study of complex ecosystems, such as the Amazon ones, enriches the comprehension of trace element cycling (Vilhena et al. 2021). The Amazon basin presents a wide diversity of freshwater ecosystems, with different geomorphological, physicochemical, and biological features, including river-floodplain systems (Furch and Junk, 1997; Junk et al. 1989). The floodplain constitutes a mosaic of open waters (lakes and rivers), flooded forests and floating meadows that have their areas and proportions modified according to the water level seasonal variation (Junk et al. 1989; Junk, 1997a). In addition, the fish assemblage of the Amazon region presents a high diversity (Junk, 1997b) with migratory species playing a significant role in energy flow and matter cycling (Hoeinghaus et al. 2006) and hence being important for trace element cycling as well. Moreover, the generation of more knowledge on trace element trophodynamics in Amazonian environments is important from the human health point of view as well, given the high importance of fish for the nutrition of Amazonian riparian communities (Dorea, 2003), for whom fish constitute the main protein source (Boischio and Hensel, 2000; Bastos et al. 2006, 2015).

As it will be detailed in Material and Method section, the samples analysed in the present study were collected in the first decade of this century (2006, 2007). Therefore, the data set generated by our investigation owns an additional scientific value, as it presents a picture of an Amazon Ecosystem in a certain past time. Amazon environments are suffering alterations, such as deforestation and construction of hydroelectric power plants (Qin et al. 2023; Nickerson et al. 2022), which can interfere in the biochemical processes of methylmercury and the evaluated trace elements. Therefore, the present study ends up constituting a reference for the trophodynamics of these contaminants in an Amazon ecosystem.

Due to all the exposed above, the present study had the following goals. The first goal was to evaluate the trophodynamics of trace elements (Fe, Cr, Mn, Ni, Zn, Ca, Sr, Cd, Sn, Tl and Pb) and methylmercury (MeHg) in the ichthyofauna from Puruzinho Lake - Brazilian Amazon, using $\delta^{13}C$ and $\delta^{15}N$, through Trophic Magnification Factor (TMF) and Trophic Magnification Slope (TMS). This evaluation was performed considering (A) all species and (B) only the resident ones. The second goal was to address the potential use of multi-element assessment as a tool for discriminating migratory and resident fish species from Puruzinho Lake. The third goal was to evaluate human exposure to MeHg, Cr, Mn, Ni, Zn, and Pb through the consumption of fish from Puruzinho Lake.

2. Study area

This study was performed in Puruzinho Lake, which is part of the Puruzinho River drainage basin (Fig. 1). The Puruzinho River is located at the Lower Plateau of the Western Amazon, covering an area of "cerrado" vegetation downstream to the middle section of its course. Subsequently, the rivers cover an area of dense ombrophylous forest and rainforest within its area of seasonal flooding (IBGE, 2003; Almeida, 2006). The study area is part of a region characterized by Junk et al. (1989) as a river-floodplain system and presents monomodal flooding. Puruzinho Lake has an area of 4.84 Km^2 during the dry season and is located between the latitudes 07° 20′ 53″S and 07° 22′ 38″S and between the longitudes 63° 05′ 05″ W and 63° 00′ 57″W. The lake has been classified as a blackwater system, with anoxic and hypoxic periods in the water column (Almeida, 2006; Nascimento et al. 2006; Azevedo-Silva, 2011).

3. Material and Methods

This section addresses, among other aspects, the sampling of seston, bottom litter and superficial sediment, which were used as energy sources (baseline) for the calculation of the trophic position (TP), and fishes. In addition, information on stable isotope measurements and treatment of $\delta^{13}C$ and $\delta^{15}N$ data are available in Azevedo-Silva et al. (2016). Sampling was performed in two phases of the Puruzinho Lake hydrological cycle. The first sampling procedure was carried out at the end of the dry season, in September 2006. The second sampling period was in February 2007, during the high-water period.

Concerning the expression of trace element and methylmercury concentrations, we have opted for expressing them in dry weight for two reasons: 1) the samples had been dried before having been sent to the Laboratory of Oceanology, University of Liege (Belgium), where they were analysed for the determination of trace element concentrations; 2) expressing element concentrations in dry weight minimize the variation in water content among samples and species, generating data more appropriate for (a) comparison among fish species, (b) comparison between resident and migratory species, and (c) evaluation of the trophodynamics of contaminants. Although human consumption is normally evaluated in wet weight, the present study presents more aspects for which dry weight constitutes the most adequate form of data presentation. In addition, the human consumption aspect was evaluated only for

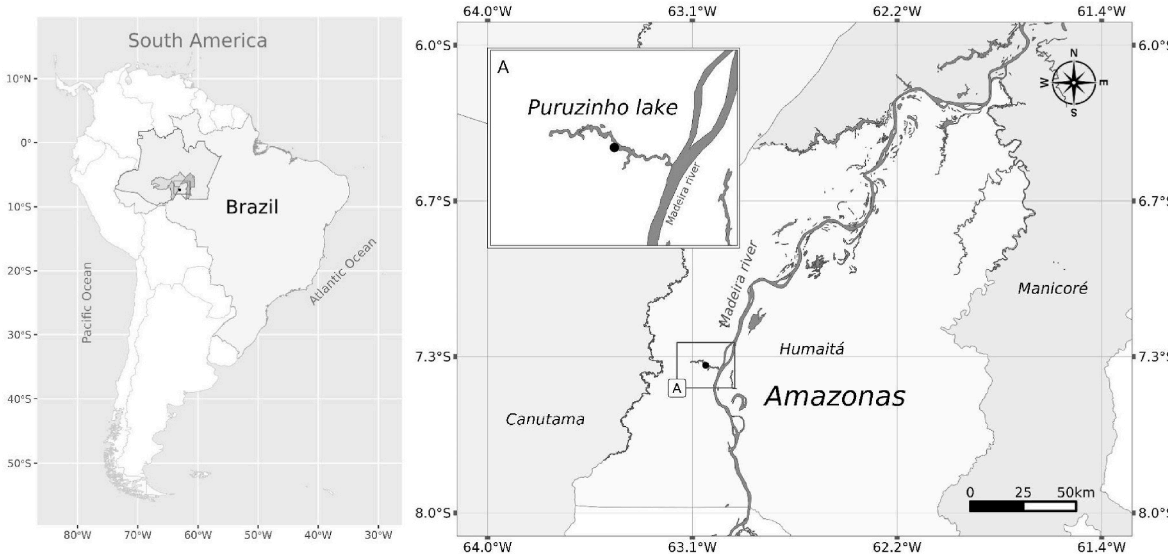

Fig. 1. South American map, highlighting Brazil and Amazonas state and amplifying the Madeira River basin, stressing Puruzinho Lake.

methylmercury and five elements.

3.1. Fish sampling

Complete information about the species can be found in Azevedo-Silva et al. (2016). In the present study, 92 fish were sampled, comprising 13 species, which were sorted out into four trophic guilds: iliophagous, planktivorous, omnivorous and piscivorous fish. This classification was based on feeding habit data from published studies that dealt with stomach content analyses and stable isotope measurement. The iliophagous species analysed were *Potamorhina altamazonica*; *Potamorhina latior* and *Potamorhina pristigaster*. Two out of the three species of the genus *Potamorhina*, *i.e.*, *P. latior* and *P. altamazonica*, present a migratory behaviour. The omnivorous fish were *Mesonauta festivus*, *Satanoperca jurupari* and *Triportheus elongatus*. The species *T. elongatus* constituted the only migrating omnivorous fish in the present study. The two planktivorous fish analysed, *Anodus elongatus* and *Hypophthalmus edentatus*, were migratory species. The piscivorous fish studied were *Acaronia nassa*, *Acestrorhynchus falcirostris*, *Cichla monoculus*, *Hoplias malabaricus* and *Plagioscion squamosissimus*. All piscivorous fish are resident species.

The sampled fish species are consumed by the human riverine population from the Puruzinho Lake, as well as from the Amazon region in general (Barthem and Fabré, 2004, Cardoso and Freitas, 2008; Oliveira et al., 2010; Doria et al., 2012; Lima et al., 2016). In fact, fish consumption was high in Puruzinho Lake region during the 2000s (the decade of the sampling), as it can be seen in Oliveira et al. (2010). It is worth mentioning that the human riverine population in the Amazon region in general had a high dependence on fish as a source of animal protein (Boischio and Hensel, 2000; Dorea, 2003, Bastos et al., 2006, 2015).

3.2. Determination of trace element concentrations

The present study determined the following elements in fish muscle: Fe, Cr, Mn, Ni, Zn, Ca, Sr, Cd, Sn, Tl and Pb. Prior to element quantification, aliquots (200 mg dry weight – d.w.) of muscle tissues were mineralized by adding 2 mL of HNO_3 65% (Merck®), 1 mL of H_2O_2 30% (Merck®) and 5 mL of ultrapure water (18.2 M Ωcm – Milli-Q, Merck Millipore®) in a 100 mL polytetrafluorethylene (PTFE) tube. Microwave-assisted (Ethos D, Milestone®) digestion was performed with an initial temperature set at 120 °C with a 5-min hold time, ramped to 160 °C and held for 5 min, to finally raise the temperature to 190 °C for 15 min. After finishing the digestion program, samples were carried to a cold chamber while cooling (30 min). The mineralized samples were transferred to graduated 50-mL screw-capped PTFE tubes, and the extracts were made up to 50 mL with ultrapure deionised water. From these samples, 1 mL was taken to the analytical plastic vial, and added 9 mL of an internal standard solution of Rhodium (^{103}Rh – Sigma-Aldrich®) and Rhenium (^{185}Re – Sigma-Aldrich®) in concentrations of 1.2 ng g^{-1} and 4 ng g^{-1}, respectively. Finally, the element concentrations were determined by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Elan DRC II - PerkinElmer®) (Richir et al. 2013). The trace element measurements were performed at the Freshwater and Oceanic Sciences Unit of Research (FOCUS), Laboratory of Oceanology, University of Liege, Belgium.

3.3. Determination of methylmercury concentrations

The samples mineralization was achieved by an alkaline treatment (3 ml of KOH: $CH_3.OH$) (Sigma®, purity levels 85 and 99%, respectively), tanking aliquots of 30 mg d.w. of fish muscle tissues in screw-caped PTFE flasks (14 mL) and taken to 65 °C for 6h, shaking in each hour by using a vortex mixer. After 12h, samples were centrifuged (2136 g force for 10 min). From these mineralized samples, an aliquot of 30 μl was taken to a 40 ml amber vial half-filled with ultrapure water (Direct 8 -Millipore®) and acetate buffer (300 μl) (Aldrich®, purity level 97%). Finally, ethylation reagent was added (50 μl NaBET4, Brooks Rand Instruments®) and vials were fully filled (inverted meniscus), immediately, with ultrapure water. Measurements were performed in an Automated Methylmercury System (MERX – Brooks Rand Instruments®) and detected by Atomic Fluorescence Spectrophotometer. Nitrogen (99.998% purity) acted as the purging and dryer gas, while argon (99.999% purity) was the carrier gas (Taylor et al. 2011; USEPA, 2001). The methylmercury measurements were performed at the Radioisotope Laboratory (LREPF), Biophysics Institute (IBCCF), Federal University of Rio of Janeiro (UFRJ), Brazil.

3.4. Quality control

The method accuracy was assayed by analysing certified reference material aliquots (CRM) in parallel to the environmental samples. The recovery percentage to MeHg (DORM-3, fish protein) ranged from 70% to 92%. Three CRMs were used to assess the method precision of metals: DORM-2 (Dogfish muscle), NIST 1566b (Oyster), and NIST 2976 (Mussel). The recoveries of all analytes were in accordance with the acceptable range suggested by US Environmental Protection Agency (EPA), which is between 85% and 115% for metals, and from 70% to 130% for MeHg (USEPA, 2000b). Quality control was also assessed by analytical blanks, and repeatability in sample duplicates by the coefficient of variation (CV = standard deviation x 100/mean value) below 15%.

3.5. Stable isotope measurements

Information on sample treatment, $\delta^{13}C$ and $\delta^{15}N$ measurements, arithmetic lipid normalization and trophic position assessment are available in Azevedo-Silva et al. (2016).

3.6. Biomagnification estimation

Biomagnification of methylmercury and trace elements was assessed through the Trophic Magnification Factor (TMF) and slope (b) of a log-linear regression between pollutant concentration and $\delta^{15}N$. TMF is the anti-log of the slope of a log-linear regression between pollutant concentration and TP. A TMF value higher than 1.0 would indicate biomagnification, whereas a TMF<1.0 would point out to trophic dilution of the contaminant (Fisk et al. 2001; Kelly et al. 2008; Jæger et al. 2009; Borgå et al., 2012). Therefore, the adopted formula is $TMF = 10^b$, which comes from the equation $Log_{10}[Hg] = a+b(TP)$, where *a* is the dimension at the point of intersection of the axis of ordinates and *b* is the slope of the regression line.

A significant and positive slope ($b > 0$) of a log-linear regression between pollutant concentration and $\delta^{15}N$ ($Log_{10}[Pollutant] = a+b(\delta^{15}N)$) indicates pollutant biomagnification in a food web (Lavoie et al. 2013). The slope (b) of regression, which is called Trophic Magnification Slope (TMS), has been used to estimate contaminant biomagnification in different food webs (Kidd et al. 2003; Campbell et al. 2005; Lavoie et al. 2013; Poste et al. 2015; Dorneles et al. 2020), facilitating the comparison between ecosystems.

Trace element and methylmercury concentrations have been log-transformed (Log_{10}) because they did not present normal distributions. It is important to bear in mind that TMF is calculated from a linear regression; consequently, it is based on the premise that the data present normal distributions. In addition, Borgå et al. (2012), who standardized the TMF calculation, suggest the log-transformation (Log_{10}) of contaminant concentrations. As it has been standardized this way, several researchers log-transform (Log_{10}) their data. As an example, all authors that are quoted in the present study for comparison purposes on TMF values have log-transformed (Log_{10}) their data. This standardization allows for a more robust comparison among studies.

3.7. Non-cancer risk assessment - Target Hazard Quotient (THQ) estimation

The THQ was calculated according to Keshavarzi et al. (2018) using the following equation.

where: EF is exposure frequency = 365 days/year; ED is exposure duration for non-cancer risk = 72.6 years for the Amazon population (IBGE, 2020); FIR is fish ingestion rate = 406g/person/day (Oliveira et al. 2010); CF is the conversion factor to convert fresh weight to dry weight calculated for each specie; CM is the calculated fish trace element concentration (mg/kg; dry weight basis); WAB is the average body weight = 70 kg (Yipel and Yarsan, 2014); ATn is the average exposure time for non-carcinogens, defined as EF × ED, as used in characterizing non-cancer risk (Keshavarzi et al. 2018), and RfD is the element reference dose. The RfD values adopted in the present study were: 1.5 mg/kg/day for trivalent Cr (IRIS EPA, 1987; Jiang et al., 2016); 0.0001 for MetilHg (IRIS EPA, 2001); 0.14 for Mn (IRIS EPA, 1988); 0.02 for Ni (Saleh and Marie, 2015); 0.004 for Pb (Saha et al. 2016); and 0.3 for Zn (IRIS EPA, 1991a).

3.8. Statistical treatment

The statistical software used comprised the Primer 6.0 and the Bioestat 5.0. The significance level was defined at $p < 0.05$. The Shapiro-Wilk's test was applied for verifying the distribution of the data, while the Levene's test was used for investigating the homogeneity of the variance. Two tests, *i.e.*, the Kruskal-Wallis test and the Dunn's multiple-comparison test (which followed the KW test) were applied for verifying the significance of the difference among *k* series of data using the Bioestat 5.0.

We applied a non-metric multidimensional scaling (nMDS) to map dissimilarities by Euclidean distances between fish species considering their trophic guild and migratory behaviour in function of all measured data (trace elements, MeHg, and isotopes), plotting correlations greater than 0.5 using the Primer software (version 6).

4. Results and discussion

4.1. Distribution in the food web

Methylmercury and five of the trace elements were found in all analysed fish. The metals that were not found in all specimens were Ni, Cr, Pb, Cd and Sn, which occurred in 97.8%, 95.6%, 88.9%, 18.9% and 13.3% of the fish samples, respectively. Due to the low representativity (low percentage of individuals with concentrations above the quantification limit), Cd and Sn concentrations were expressed exclusively on Table 2 of the Supplementary Material. Despite having been found in low percentages of the samples, it is important to highlight the species in which these metals were detected. For Cd, the highest frequencies of occurrence were found in the omnivorous *M. festivus* (100%), as well as in the piscivorous *A. nassa* (80%) and *C. monoculus* (33.3%). The two first species are associated to the littoral vegetation, with the omnivorous feeding either on the macrophyte bank species (mostly filamentous algae) or on the associated invertebrates (Lowe-McConnell, 1969; Machado, 1983; Winemiller et al. 1995; Ferreira, 1998; Mérona et al. 2001; Mérona and Mérona, 2004; Santos et al. 2006; Santos et al., 2009; Prado et al. 2010). The highest values found in this omnivorous may be related to Cd solubility (ATSDR, 2008; Mason, 2013), which propitiates an efficient absorption/adsorption by algae and macrophytes. Cadmium bioaccumulation by the invertebrates associated to macrophytes and algae (Ng et al. 2005; Currie et al. 1998; Rubio-Franchini et al. 2016; Javed et al., 2019), which are the main items of the diet of this fish, may also explain this finding. Mason et al. (2000) found higher Cd concentrations in herbivore invertebrates associated to periphyton and macrophytes than in predator insects in two rivers from Virginia, USA. Therefore, it seems plausible that Cd has been assimilated by the ichthyofauna through submerse vegetation and associated biota. Another metal for which there was low representativity (low percentage of individuals with concentrations above the quantification limit) in Puruzinho Lake ichthyofauna was Sn. The highest frequencies of occurrence of tin were found in the iliophagous species *P. latior* (50%) and *P. pristigaster* (40%), which also presented the highest mean values. These results suggest tin assimilation by the biota through sediment detritus and associated microorganisms (Bowen, 1983; Carvalho, 1984; Pouilly et al. 2004, 2013; Azevedo-Silva et al. 2016). In a previous study, performed with the same sampled individuals, the $\delta^{13}C$ values of the iliophagous species was associated to the phytoplanktonic and detritivore food webs, including methanotrophic bacteria (Azevedo-Silva et al. 2016). The $\delta^{13}C$, $\delta^{15}N$ and trophic position values appear on Table 3 of the supplementary material.

The iliophagous species *P. pristigaster* presented the highest median values for Cr and Fe, being significantly higher than those found in five and six species from other trophic guilds, respectively (Dunn, $p < 0.05$; Table 4 – Supplementary Material). The other two iliophagous species also presented higher Cr and Fe values than piscivorous and omnivorous fish, except for the migrating omnivorous *T. elongatus*, which presented

Table 1
Trophic Magnification Factor (TMF) values for all (resident and migratory) and resident species, intercept (a), slope (b), determination coefficient (R^2) and significance (p) for elements and methylmercury (MeHg). The number of samples below the quantification limit are between parentheses.

Elements (<L.Q.)	TMF	Intercept (a)	Slope (b)	R^2	p*[1]	TMF	Intercept (a)	Slope (b)	R^2	p*[1]
	All species (migratory + residents)					Resident species				
MeHg*[2]	4.65	0.9860	0.6670	0.49	$p < 0.0001$	4.55	0.9664	0.6578	0.51	$p < 0.0001$
Fe	0.40	5.9665	- 0.4049	0.11	$p = 0.0016$	0.35	5.9411	- 0.4605	0.23	$p = 0.0005$
Cr (4)	0.26	5.2009	- 0.5778	0.11	$p = 0.0017$	0.23	5.1584	- 0.6423	0.19	$p = 0.0018$
Mn	0.36	4.5598	- 0.4462	0.14	$p < 0.0001$	0.47	4.0595	- 0.3258	0.18	$p = 0.0024$
Sr	0.27	5.1800	- 0.5618	0.14	$p < 0.0001$	0.45	4.7405	- 0.4542	0.22	$p = 0.0009$
Ni (2)	0.40	3.7866	- 0.3988	0.08	$p = 0.0088$	0.23	4.4693	- 0.6348	0.25	$p < 0.0001$
Ca	0.50	6.9919	- 0.3022	0.06	$p = 0.0216$	0.70	6.4292	- 0.1573	0.05	$p = 0.1224$
Zn	0.87	4.4401	- 0.0629	0.02	$p = 0.2018$	0.80	4.5305	- 0.0983	0.13	$p = 0.0104$
Pb (10)	0.55	2.8066	- 0.2570	0.08	$p = 0.0069$	0.69	2.4921	- 0.1643	0.05	$p = 0.1116$
Tl	0.92	1.5979	- 0.0347	0.01	$p = 0.4510$	0.95	1.3844	0.0242	0.01	$p = 0.6497$

*[1] - Values $p < 0.05$ were regarded as significant.
*[2]- The TMF assessment for MeHg was performed with 82 samples.

Table 2
Trophic Magnification Slope (TMS) values for all (resident and migratory) and resident species, intercept (a), slope (b), determination coefficient (R^2) and significance (p) for elements and methylmercury (MeHg). The number of samples below the quantification limit are between parentheses.

Elements (<L.Q.)	Intercept (a)	Slope (b)	R^2	p*[1]	Intercept (a)	Slope (b)	R^2	p*[1]
	All species (migratory + residents)				Resident species			
MeHg*[2]	1.1966	0.1959	0.49	$p < 0.0001$	1.1746	0.1932	0.51	$p < 0.0001$
Fe	5.8397	- 0.1191	0.11	$p = 0.0016$	5.7976	- 0.1355	0.23	$p = 0.0005$
Cr (4)	5.0199	- 0.1699	0.11	$p = 0.0017$	4.9541	- 0.1885	0.19	$p = 0.0018$
Mn	4.4200	- 0.1312	0.14	$p < 0.0001$	3.9570	- 0.0959	0.18	$p = 0.0024$
Sr	5.0041	- 0.1652	0.14	$p < 0.0001$	4.5963	- 0.1334	0.22	$p = 0.0009$
Ni (2)	3.6617	- 0.1173	0.08	$p = 0.0088$	4.2691	- 0.1865	0.25	$p < 0.0001$
Ca	6.8973	- 0.0889	0.06	$p = 0.0216$	6.3787	- 0.0461	0.05	$p = 0.1235$
Zn	4.4204	- 0.0185	0.02	$p = 0.2018$	4.4980	- 0.0287	0.13	$p = 0.0108$
Pb (10)	2.7261	- 0.0756	0.08	$p = 0.0069$	2.4395	- 0.0482	0.05	$p = 0.1127$
Tl	1.5870	- 0.0102	0.01	$p = 0.4510$	1.3906	0.0073	0.01	$p = 0.6386$

*[1] - Values $p < 0.05$ were regarded as significant.
*[2] - The TMS assessment for MeHg was performed with 82 samples.

the second highest median value for both elements. However, there was no significant difference, except for *P. latior*, which had Cr concentrations significantly higher than those found in three species (*A. nassa*, *P. squamosissimus* and *S. jurupari*). The planktivorous fish presented a similar pattern for Fe to that observed for iliophagous fish in relation to other trophic guilds (Fig. 2). Nevertheless, the species that represent the planktivorous guild present different patterns for Cr. *A. elongatus* presents a pattern similar to that of iliophagous species (with values close to those from *P. altamazonica* and *P. latior*), whereas *H. edentatus* presented lower values, close to those found for piscivorous (*e.g.*, *H. malabaricus*) and omnivorous (*e.g.*, *M. festivus*) species, except for *T. elongatus* (Fig. 2).

Manganese, Ca, and Sr presented similarities in the distribution of the elements among guilds and species. As it had been observed for Cr, an important discrepancy was verified between the two planktivorous species for all elements, with *A. elongatus* and *H. edentatus* presenting the highest and the lowest values, respectively (Fig. 2). The planktivorous fish *H. edentatus* presented the lowest values for the three elements. The pattern related to the higher concentrations found in iliophagous species than in piscivorous and omnivorous fish was also observed for these elements, as it had been found for Fe and Cr. The three highest median values were basically found in the same species, *P. altamazonica*, *A. elongatus* and *T. elongatus*; varying only in the order of values. Strontium constituted an exception, as *P. pristigaster* presented the third highest value, taking the place of *T. elongatus* (Fig. 2). Considering the distribution pattern of these elements in the species of the Puruzinho Lake ichthyofauna, it is important to highlight that they are essential for fish and their toxicokinetic is mediated by physiological processes (NRC, 1993; Prabhu et al., 2016). The exception is strontium, which presents no known physiological function for fish, *i.e.*, it would not be an essential element (NRC, 2011). However, strontium is an alkaline-earth element like calcium, an element for which Sr has a strong chemical affinity, being considered an analogous element. Sr replaces Ca in certain fish tissues, such as the otoliths (Suzuki et al. 1972; Farrell and Campana, 1996; Carvalho et al. 2005; Albuquerque et al. 2010). It is believed Sr is mainly absorbed through water; an absorption that has been associated to same transport mechanism that Ca undergoes (Farrell and Campana, 1996; Walther et al. 2017), which would explain the similarity in the distribution of both elements in biota (Fig. 2). Lall (2002) reports that essential elements take part in vital processes for live beings. Among these processes are the regulation of colloidal systems and the acid-base equilibrium, the formation of the skeleton structure. In addition, essential elements end up being constituents of hormones, enzymes, enzyme activators and other important compounds. Moreover, they have their concentrations actively regulated by organisms (Muyssen et al. 2004), which may have contributed to the similarity observed for these bivalent cations.

Although it is not an essential element, Pb presented a distribution that was similar to that of those elements, with iliophagous fish presenting the highest values, along with *A. elongatus* and *T. elongatus*. The planktivorous fish also presented great discrepancy between the two species, with *H. edentatus* presenting lower values (Fig. 2). However, the observed values were less divergent among species and guilds than the registered for other elements, with a low variation in concentrations and a few species presenting significant differences (Table 4 – Supplementary Material). Lead mimics bivalent cations (*e.g.* Ca, Mg, Fe, Zn and Mn) in biological systems, which explains the observed similarity. Pb is distributed through organisms while substituting essential elements in membrane transport systems. Its capacity of dislocating and replacing those cations disrupts the homeostasis of those elements and affects the functioning of the metabolism dependent on them (ions themselves,

Table 3
Highest, mean, and median values of calculated THQ.

Species	THQMethylHg	THQCr	THQMn	THQNi	THQZn	THQPb
A. falcirostris						
highest value	40.6486	0.0017	0.0114	0.0558	0.0713	0.0465
mean	24.7570	0.0009	0.0074	0.0343	0.0596	0.0307
median	23.2276	0.0008	0.0070	0.0326	0.0587	0.0316
A. elongatus						
highest value	24.1923	0.0335	0.0933	0.7232	0.1907	0.0855
mean	17.6291	0.0144	0.0584	0,.880	0.1168	0.0534
median	17.2969	0.0092	0.0591	0.0478	0.0988	0.0493
C. monoculus						
highest value	44.9086	0.0069	0.0206	0.2881	0.1362	0.0598
mean	28.1984	0.0032	0.0110	0.0741	0.0992	0.0310
median	26.3270	0.0019	0.0095	0.0203	0.0920	0.0279
H. malabaricus						
highest value	28.7488	0.0088	0.0298	0.1687	0.1130	0.1883
mean	13.8533	0.0037	0.0173	0.0468	0.0858	0.0612
median	13.1194	0.0027	0.0170	0.0189	0.0881	0.0453
H. edentatus						
highest value	41.5277	0.0201	0.0384	0.1508	0.0730	0.0766
mean	20.8692	0.0062	0.0120	0.0500	0.0493	0.0319
median	19.4368	0.0030	0.0067	0.0082	0.0464	0.0077
P. squamosissimus						
highest value	27.9734	0.0014	0.0286	0.0471	0.0726	0.0538
mean	14.8571	0.0009	0.0120	0.0220	0.0647	0.0336
median	11.4955	0.0010	0.0083	0.0176	0.0686	0.0302
P. altamazonica						
highest value	9.8388	0.0153	0.0787	0.0301	0.1033	0.0981
mean	6.5570	0.0084	0.0634	0.0202	0.0929	0.0729
median	7.5231	0.0083	0.0705	0.0204	0.0939	0.0736
P. latior						
highest value	6.8442	0.0205	0.1066	0.1475	0.0768	0,1470
mean	5.2031	0.0093	0.0428	0.0408	0.0698	0.0720
median	5.4263	0.0068	0.0300	0.0289	0.0694	0,0514
P. pristigaster						
highest value	5.9449	0.0541	0.0935	1.4212	0.0027	0.2005
mean	4.2162	0.0323	0.0383	0.4217	0.0770	0.0849
median	3.6836	0.0348	0.0234	0.0855	0.0810	0.0472
S. jurupari						
highest value	2.7823	0.0013	0.0126	0.0257	0.0855	0.0501
mean	2.3604	0.0008	0.0201	0.0168	0.0692	0.0325
median	2.6322	0.0008	0.0237	0.0172	0.0711	0.0344
T. elongatus						
highest value	23.6690	0.0221	0.0904	0.1477	0.2299	0.2201
mean	16.1326	0.0135	0.0429	0.0538	0.1397	0.0674
median	15.8896	0.0104	0.0434	0.0417	0.1463	0.0474

enzymes, and proteins). Among these elements, Ca is one of the most influenced, with Pb acting in several metabolic processes. In muscles, Pb causes a decrease in the activity of complexes I to IV of the respiratory chain and interferes in the activity of troponin C, which plays a role in muscle contraction (Campbell et al. 2005; ATSDR, 2020).

The iliophagous species presented the highest mean Fe, Cr, Ca, Mn, Sr and Pb concentrations. These results suggest an assimilation of these elements through detrital food chains, including methanotrophic bacteria (Azevedo-Silva et al. 2016). The δ^{13}C values of the same *A. elongatus* (planktivorous species) individuals suggest an association to the phytoplanktonic and detrital food chains, including methanotrophic bacteria. On the other hand, the δ^{13}C values found for *T. elongatus* (omnivorous species) did not allow us to infer with clarity which chain the species would be inserted in. This may be related to the fact that the species presents a migratory behaviour, which generates a distinct signature that does not represent the environment investigated (Azevedo-Silva et al. 2016). The studies that evaluated the feeding of *T. elongatus* and *A. elongatus* suggest assimilation of these elements through the food web of allochthonous material from the flooded forest (Almeida, 1984; Braga, 1990; Ferreira, 1998; Mérona et al. 2001; Claro-Jr et al., 2004; Mérona and Mérona, 2004) and plankton (Braga, 1990; Mérona and Mérona, 2004), respectively.

Zinc is a cofactor of enzyme systems and an integral part of metalloenzymes, including carbonic anhydrase, glutamic dehydrogenase, alkaline phosphatase, and DNA polymerase (NRC, 1993; 2011). Despite this importance to organisms, Zn did not present the same distribution pattern found for the other abovementioned essential elements (Fig. 2), with similarity appearing only for *H. edentatus*. This fish species presented the lowest Zn concentrations, with significant differences for some species (Dunn, $p < 0.05$; Table 4 – Supplementary Material). Zn exhibited a higher representativity for omnivorous species, with the highest central value found in *T. elongatus*, followed by *M. festivus*. Concerning these two fish, the former species presents a feeding spectrum highly diversified, which includes terrestrial and aquatic invertebrates, fruits, and seeds, being associated to the food web of allochthonous material from the flooded forest as highlighted above. The same *M. festivus* specimens presented δ^{13}C values that associate them to seston and therefore to the phytoplanktonic food web (Azevedo-Silva et al. 2016). However, δ^{13}C values of phytoplankton and periphyton may overlap in river-floodplain systems (Hamilton et al. 1992; Hamilton and Lewis, 1992; Wantzen et al. 2002; Jepsen and Winemiller, 2007). Considering that the species feeds on macrophyte bank species, including filamentous algae and associated invertebrates (Lowe-McConnell, 1969; Machado, 1983; Winemiller et al. 1995; Mérona et al. 2001; Santos et al. 2006; Santos et al., 2009), we may associate the Zn concentrations found in *M. festivus* to the food web related to the macrophyte bank. The species *A. elongatus* presented values that were close to those from these two omnivorous fish (*T. elongatus* and *M. festivus*), whereas iliophagous and piscivorous had similar values (Fig. 2).

A low variation in concentrations was found for thallium among species from Puruzinho Lake. However, it is important to highlight the planktivorous fish *A. elongatus* as the species that presented the highest median value, as well as the omnivorous *S. jurupari* and *M. festivus* as representatives of the lowest concentrations (Fig. 2).

Nickel was the only element for which there was no significant difference among species. The pattern observed in this study – similar concentrations in 13 species from different trophic guilds, including migratory fish – may contribute to investigations that evaluate Ni essentiality (Fig. 2). Authors who defend nickel as an essential element for fish have used the fact that Ni concentrations in fish tissues remain relatively constant, suggesting the regulation of this metal by fish, as this behaviour is observed for essential elements. However, evidence is still not stinging (Payle and Couture, 2011). Muyssen et al. (2004) argue that the active regulation of nickel by different aquatic organisms would suggest its essentiality, but more studies are needed for confirming this. Ni essentiality to aquatic organisms has only been proved for cyanobacteria, phytoplankton, and a few higher plants (Muyssen et al. 2004).

For MeHg, as expected, there were significant differences among species (Dunn, $p < 0.05$; Table 4 of Supplementary Material). Fish that occupy the highest trophic positions (such as the piscivorous species *C. monoculus* and *A. falcirostris*) presented higher MeHg concentrations than those that are on the proximity of the bottom of the food web, such as iliophagous (*P. latior*) and even omnivorous (*M. festivus*) fish (Fig. 2). This pattern is consistent with the well-known biomagnification capacity of MeHg in aquatic food webs (Gray, 2002; Lavoie et al. 2013). The δ^{13}C values from our previous investigations on the diet of these piscivorous fish suggest that these species are associated to the trophic webs of phytoplankton, bottom litter and detritus (Azevedo-Silva et al. 2016).

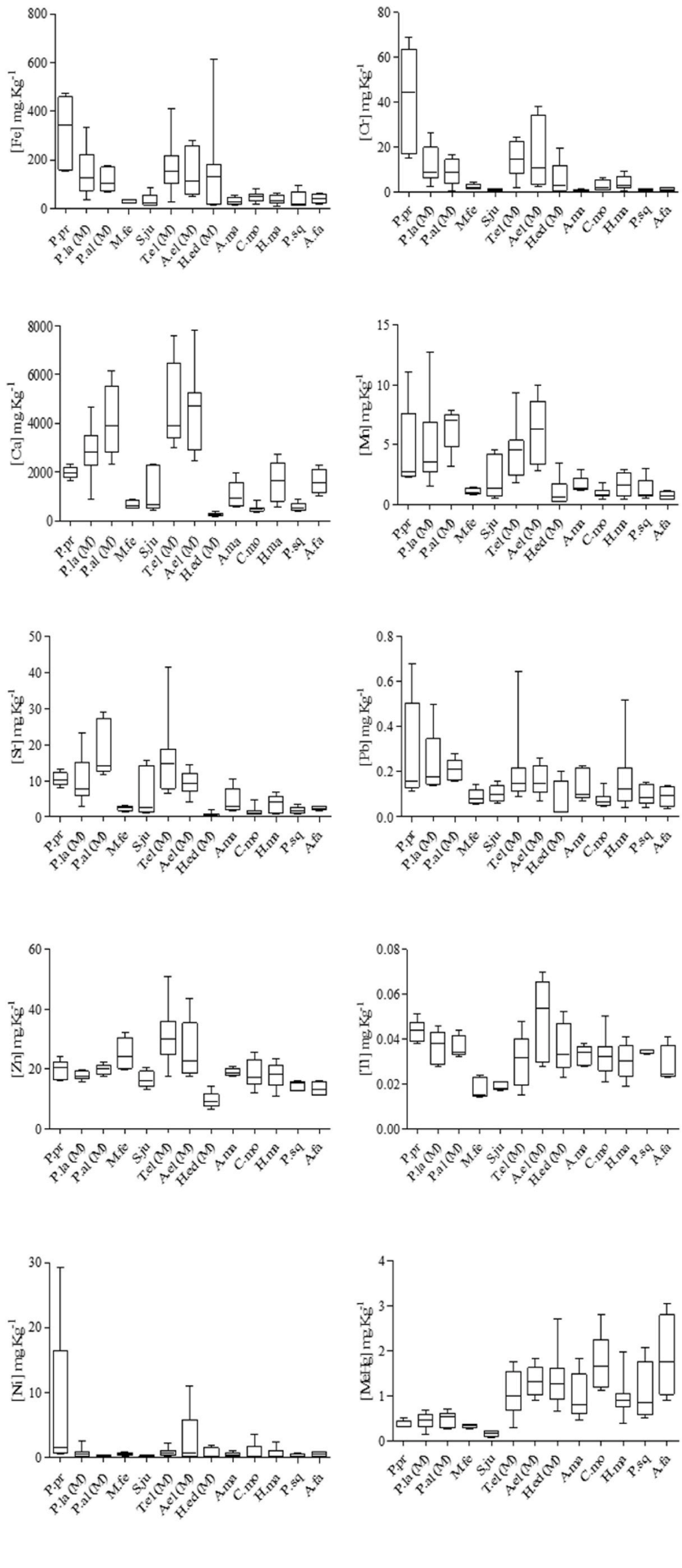

Fig. 2. Boxes represent the first and third quartiles, median, minimum, and maximum concentrations (mg.kg^{-1} in dry weight) of elements and methylmercury (MeHg). Species (abbreviation): *A. massa* (A.ma), *A. falcirostris* (A.fa), *P. squamosissimus* (P.sq), *C. monoculus* (C. mo), *H. malabaricus* (H.ma), *A. elongatus* (A.el), *H. edentatus* (H.ed), *M. festivus* (M.fe), *S. jurupari* (S.ju), *T. elongatus* (T.el), *P. pristigaster* (P. pr), *P. altamazonica* (P.al) and *P. latior* (P.la). Those classified as Migratory species, are labelled with "M" in parentheses (M).

4.2. Resident versus migratory species

There was no relation between the measured variables and the trophic guilds of the sampled species. A high variability of the functional groups was observed, except for piscivorous fish (Fig. 3A). However, it was possible to observe that some migratory fish (especially *A. elongatus* and *T. elongatus* and, in a lesser extent *P. altamazonica* and *P. latior*) differed from the other species for Ca, Sr, Mn and Zn, and, in a lesser

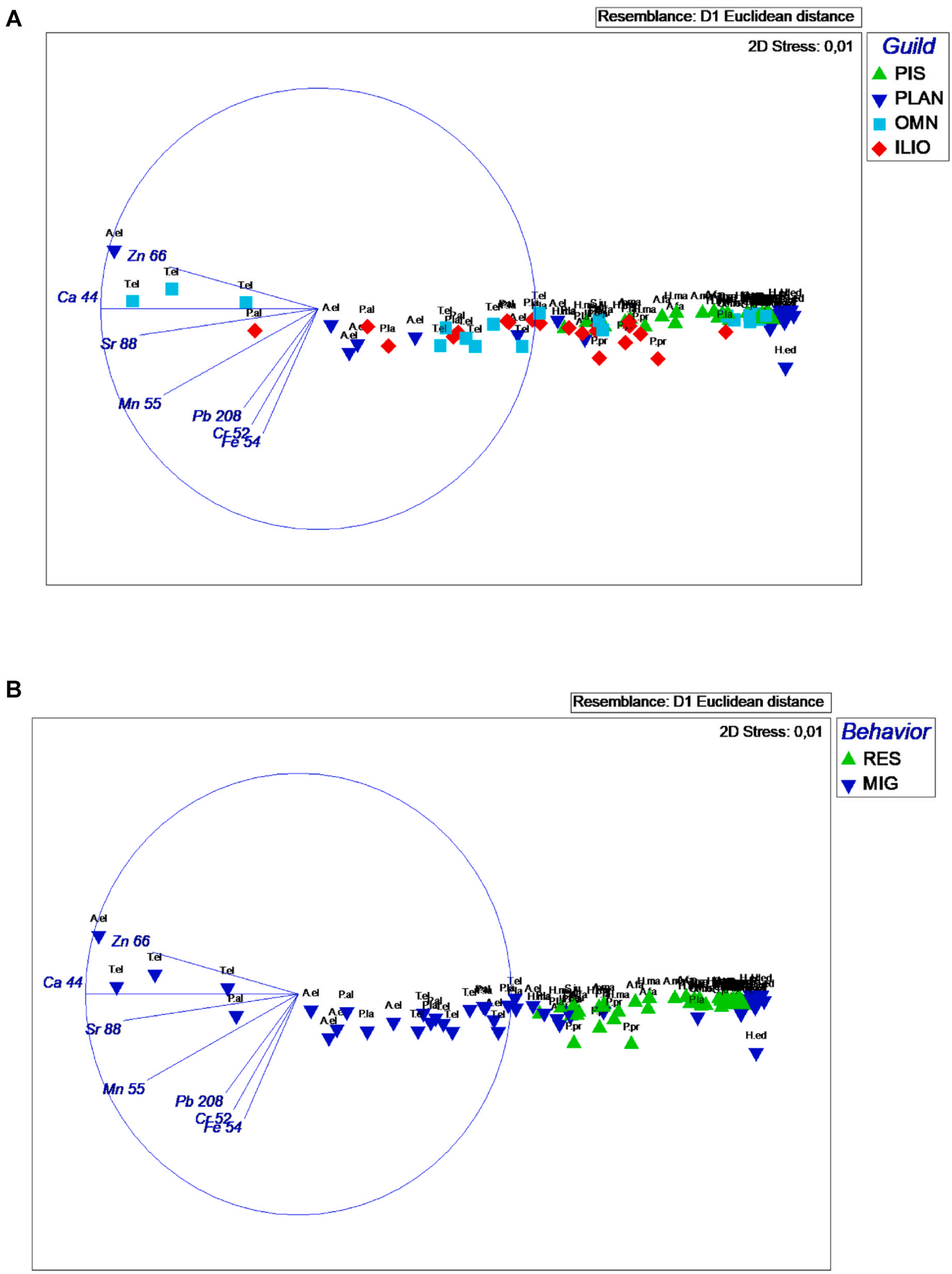

Fig. 3. nMDS analysis showing distribution of fishes (letters represent species) in function of their (A) guild (piscivorous, planktivorous, omnivorous, and iliophagous) and (B) migratory behaviour (resident and migratory), considering all variables measured. Zinc, Ca, Sr, Mn, Pb, Cr and Fe were the main elements separating groups in a correlation greater than 0.5.

extent, for Pb, Cr and Fe (Fig. 3B). Although not always significant, Fe, Cr, Ca, Mn, Sr and Pb values tend to be higher in migratory species (Fig. 2) (Dunn, $p < 0.05$; Table 4 of Supplementary Material). The exception was the migratory fish *H. edentatus*, which presented the lowest Mn, Sr and Pb concentrations (Fig. 2; Fig. 3B), with values that differed from those of the resident species. The sole discrepancy in the pattern observed between resident and migratory fish was provided by the high values found for the resident species *P. pristigaster* for all elements (Fig. 2).

A study performed in lakes of the former Soviet Union observed that Fe, Mn, Cu and Zn concentrations in fish muscle and liver reflected geochemical features of the drainage basin around the lake (Love, 1980 *apud* Lall, 2002). This helps explaining the discrepancy in the concentrations of these elements between resident and migratory fish, since these species are originated from sub-basins that present distinct geochemical features. Lall (2002) reports that trace-element concentrations in fish are related: i) to the ecosystem inhabited by the animal; ii) to its feeding; iii) to the species; iv) as well as to the age and physiological state of the individual. Therefore, the muscle concentrations of these elements in fish may be used for discriminating species of distinct habitats, important information when the migratory species from the present study are considered. Strontium and its relationship with calcium and barium in otoliths have been used for inferring on origin and movements of fish among freshwater, estuarine, and marine ecosystems (Lucas and Baras, 2000; Campana and Thorrold, 2001; Albuquerque et al. 2010; Collins et al. 2013; Maciel et al. 2020). Other elements in otoliths are also used with the same goal. Maciel et al. (2020) used Ba:Ca, Cu:Ca, Mg:Ca, Mn:Ca, Ni:Ca and Zn:Ca ratios of juvenile and adult Guri sea catfish (*Genidens genidens*) from four tropical and subtropical estuaries for distinguishing different reproduction areas and fish stocks. The authors considered that the used ratios were capable of differentiating populations of the investigated species from the four evaluated estuaries.

However, the pattern found in the present study should be seen with caution, as there was no equilibrium between resident and migratory species within the trophic guilds. Variations in the toxicokinetic of the elements in the different investigated species should also be considered. Another important aspect is related to the fact that the iliophagous species presented the highest values for the abovementioned elements, being important to keep in mind that two of them (out of the three) are migratory fish. Additional studies with a more specialized sampling design should be performed for evaluating the use of muscle trace-element concentrations for discriminating resident and migratory fish.

4.3. Trophic dynamic of methyl mercury and trace elements

Borgå et al. (2012) comment that migrators may influence the results for not representing the study ecosystem, neither in δ^{15}N values nor in trace element concentrations. An important feature of the present study sampling is the presence of migrator species, and, for this reason, we have opted for evaluating biomagnification considering both scenarios, *i.e.*, (1) all samples and (2) only resident species. Although the total number of individuals has decreased to less than 50 specimens (48 for trace elements and 46 for methylmercury) in the second scenario, the sampling number continues higher than the minimum suggested by Borgå et al. (2012), *i.e.*, between 30 and 40 individuals. Considering all fish, the sampling numbers were 90 and 82 for trace elements and methylmercury, respectively.

Methylmercury was the only analyte that has undergone biomagnification considering TMF and TMS values in both scenarios, *i.e.*, considering (1) the resident and (2) the total ichthyofauna. On the other hand, trophic dilution was found for Cr, Ni, Fe, Sr, and Mn considering all variations of the performed assessments. Although it is not common to include thallium in studies comprising the trophic flow of trace elements in aquatic environments, we have decided to perform measurements of this element due to the following reasons. Thallium is a non-essential toxic element for which data on trophodynamics are scarce, with apparent contrasting information on its behaviour in the trophic webs. For example, Ikemoto et al. (2008) did not observe biomagnification or trophic dilution for thallium, whereas Jardine et al. (2019) found trophic dilution and Asante et al. (2008) verified biomagnification of this metal. In the present study, thallium did not present significant variation through the food web, neither biomagnifying nor biodiluting in all scenarios ($p \geq 0.05$; Tables 1 and 2; Figs. 4 and 5). Therefore, the data suggest that the migratory species did not influence TMF and TMS calculations for the abovementioned elements and methylmercury and, consequently, the evaluation of magnification.

For Zn, trophic dilution was found among resident species and independence from the trophic position was verified when the entire ichthyofauna was evaluated, whereas the opposite scenario was found for Ca and Pb, *i.e.*, trophic independence among the resident species and biodilution for all sampled specimens (Tables 1 and 2; Figs. 4 and 5). The similarity in behaviour between Ca and Pb presents coherency, as Pb is an analogous of Ca (Moreira and Moreira, 2004). Therefore, it can be considered that migratory species have interfered in the calculation of biomagnification for these three elements. The low determination coefficients (R_2), *i.e.*, the weak correlation coefficients ($R_2 \leq 0.10$; Nfon et al., 2009) found for Ca ($R_2 = 0.06$) and Pb ($R_2 = 0.08$), considering all the sampled specimens (total ichthyofauna), suggest that the presence of migratory species may have influenced the calculation, as well as that the trophic dilution found may not have been robust (Tables 1 and 2; Figs. 4 and 5).

Methylmercury biomagnification is consistent (Gray, 2002) having been observed in several investigations. In this context, the study of Lavoie et al. (2013) should be highlighted as the authors compiled many investigations, generating a database with 124 TMS values for methylmercury distributed in different types of aquatic ecosystems. Taking the numbers found by Lavoie et al. (2013) as references, the values observed in the present study (Table 2) were lower than the mean TMS value (0.24) for freshwater ecosystems ($n = 110$), but higher than the mean TMS value (0.16) for tropical freshwater ecosystems ($n = 8$). Nevertheless, the values found in the present study were close to those verified in the meta-analysis performed by Lavoie et al. (2013).

The present results have evidenced that the essential elements measured have either undergone trophic dilution or kept stable through the food web. The same holds for the non-essential elements Ni and Tl, as well as for those that present a calcium-like toxicokinetic (Sr and Pb). Some factors may have contributed to this scenario. Firstly, essential elements are physiologically regulated, which would contribute to the maintenance of stable levels through the food web. According to Nfon et al. (2009), concentrations of the essential elements Fe, Zn and Mn, as well as of the non-essential metal Pb, are regulated by metallothioneins and metallothionein-like proteins, which turns their biomagnification into unlikely events. In addition, muscle is not regarded as a storage tissue for those elements as, for example, bones and scales are for Ca, Sr and Pb, or liver, spleen and bone marrow are for Fe (NRC, 1993; Lall, 2002; ATSDR, 2020).

In the trophic web evaluated in the present study, the representatives of the primary consumers were species associated to sediments, the iliophagous fish. This is an aspect that may have contributed to the trophic dilution found for Cr, Ni, Fe, Sr and Mn. However, these five elements have also undergone trophic dilution in two situations reported in the scientific literature. It was the case for Fe, Mn, Cr and Ni in Lagoa Mãe Bá, Espírito Santo State, Brazil (Pereira et al. 2010), as well as for Cr, Ni, Fe, Mn and Sr in San Roque Reservoir, Córdoba, Argentina (Monferrán et al. 2016). In addition, Ikemoto et al. (2008) also found trophic dilution for Mn, but Cr and Sr presented independence from the trophic level in the delta of the Mekong River, Vietnam. The data generated by the present study on Ni and Cr ratified the behaviour found for these two metals by Guo et al. (2016) in the food web of the Daliao River estuary, China. Nfon et al. (2009) observed trophic dilution of Fe and Ni in the pelagic food web of the Baltic Sea, but Mn and Cr did not

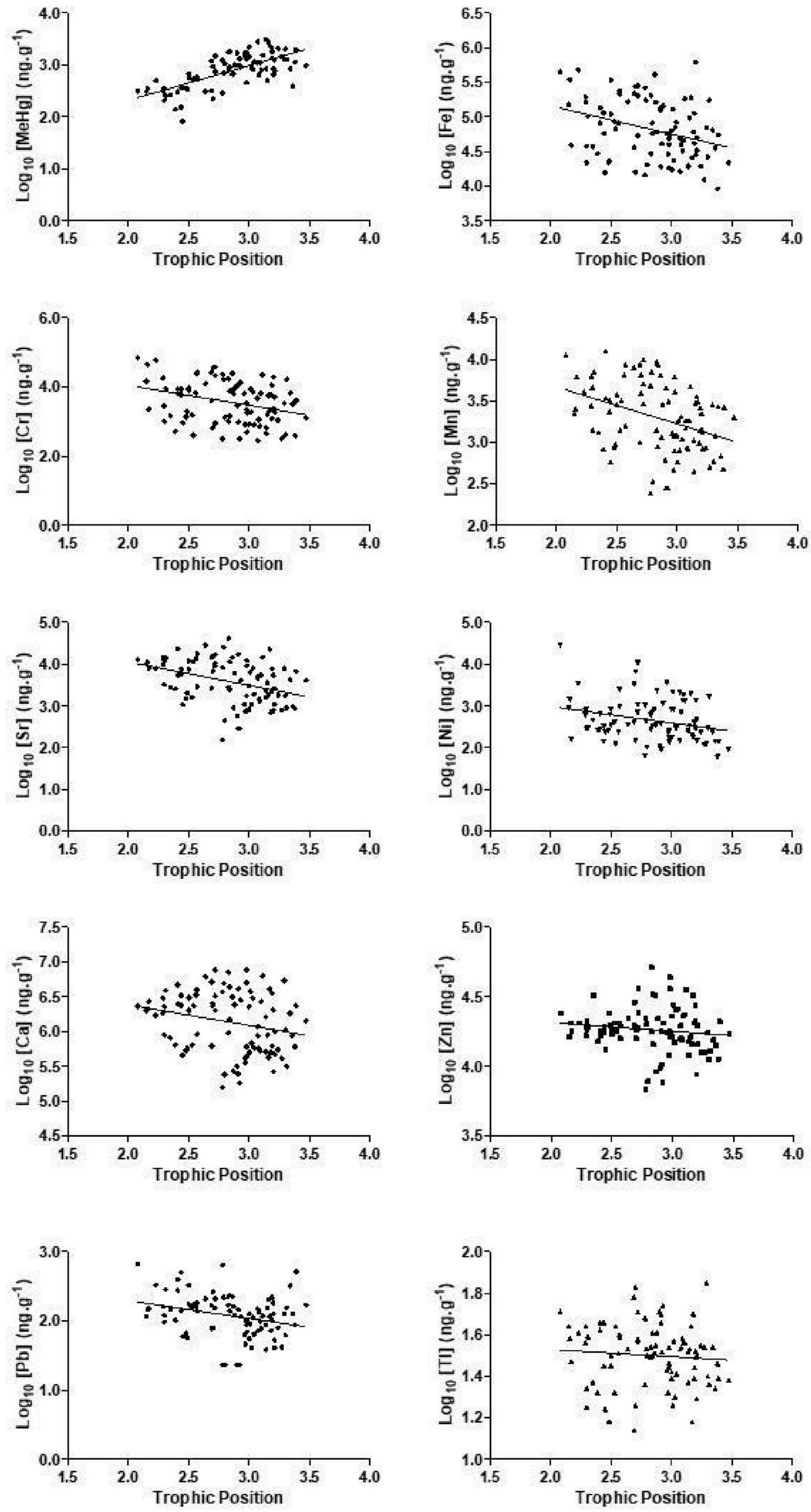

Fig. 4. Log-linear regressions between element and methylmercury (MeHg) concentrations and trophic positions of ichthyofauna (resident and migratory species) components, from Puruzinho Lake.

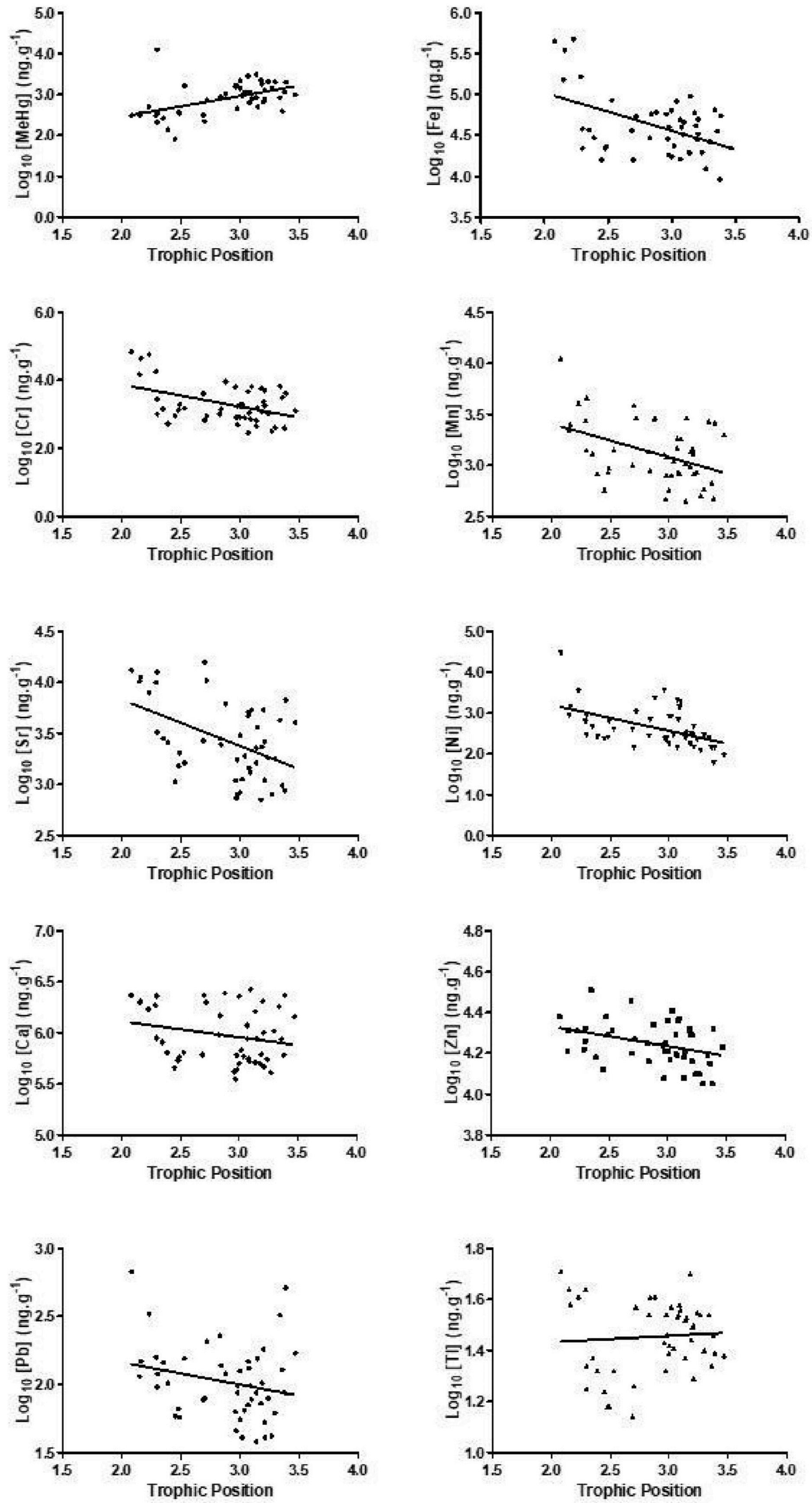

Fig. 5. Log-linear regressions between element and methylmercury (MeHg) concentrations and trophic positions of resident species from Puruzinho Lake ichthyofauna.

present correlation with the trophic position of organisms.

Although the trophic dilutions of Ca, Zn and Pb were not found in an overwhelming manner, the environmental behaviour of the two latter elements corroborated the scenario observed in previous studies. The independence between element concentration and trophic position was found: (1) by Ikemoto et al. (2008) for Tl, Zn and Pb in the food web of the delta of the Mekong River; (2) by Guo et al. (2016), for Pb, in the food web of the Daliao River estuary; (3) by Zeng et al. (2013), for Pb, in the food web of the Pearl River estuary; as well as (4) by Nfon et al. (2009), for Zn and Pb, in a pelagic food web from the Baltic Sea. On the other hand, trophic dilution was observed for Zn and Pb in Lagoa Mãe Bá (Pereira et al. 2010), for Pb in the San Roque Reservoir (Monferrán et al. 2016), for Zn in Pearl River estuary (Zeng et al. 2013). Therefore, the behaviour of the studied elements in the Lake Puruzinho food web was coherent with those found in the scientific literature.

The divergence in the environmental behaviour of trace elements may be related to different factors, such as: physicochemical features of ecosystems; predominant chemical species of the element; interactions with other elements in the ecosystem; season and sampling areas within the ecosystem (habitat; benthic versus pelagic); and investigated species (physiological differences among taxonomic groups; seasonal changes in feeding habits; analysed tissues) (Mason et al. 2000; Pereira et al. 2010; Revenga et al. 2012; Zeng et al. 2013; Lavoie et al. 2013; Monferrán et al. 2016).

5. Non-cancer risk assessment

The calculated THQ (Target Hazard Quotient) for Cr, Mn, Zn, and Pb rendered results lower than 1.0, and do not represent risk through fish consumption. The scenario for Ni constitutes an exception among trace elements and should be highlighted; however, only one individual (of the species *P. pristigaster*) presented calculated THQ value higher than 1.0 (1.4212). The USEPA (IRIS EPA, 1991b) suggests that the associated risk related to nickel refers to decreased body and organ weights as the critical effect. According to Goyer (2001), these elements are considered the major toxic metals with multiple toxic effects. Regarding methylmercury, the calculated THQMethylHg values were higher than 1.0 for all analysed specimens, ranging from 1.0252 for *S. jurupari* to 44.9086 for *C. monoculus*. The latter species also presented the highest median value of THQMethylHg (26.3270) (Table 3).

Mansilla-Rivera and Rodríguez-Sierra (2011) studied marine fishes (*Scomberomorus cavalla*, *Lutjanus synagris*, *Micropogon undulates*, and *Lutjanus analis*) from sites influenced by industries, agricultural activities, and residential impacts. The authors consider fish consumption in 1, 2, 3, 4, 5, and 7 days in a week, being the fishermen the most exposed population due to its high fish consumption (227 $g.day^{-1}$). The mean mercury concentrations varied from 0.09 mg kg^{-1} in *L. analis* to 0.26 mg kg^{-1} in *S. cavalla*, and yielded Hazard Quotients values that varied from 2.9 to 8.4 for the same species, respectively. Mean THQ values obtained in the present study were higher than those found by Mansilla-Rivera and Rodríguez-Sierra (2011).

Keshavarzi et al. (2018) analysed *Anodontostoma chacunda* (pelagic), *Belangerii* sp (mesopelagic), and *Cynoglossurs arel* (benthic) samples from Musa Estuary and Mahshahr Harbour, Persian Gulf, where there are impacts of urban, agricultural, shipping port activities, oil and metal industries, and petrochemical plants. The pelagic and benthic species presented the lower (2.04 mg kg^{-1}) and highest (5.82 mg kg^{-1}) Hg concentrations respectively. The THQ levels obtained by the authors were estimated using total Hg values and applying the same RfD adopted in the present study. Lower (1.43) and upper (18.87) average THQ values were obtained analysing *Belangerii* sp and *C. arel* samples, respectively. The THQ maximum value (54.55) was observed in *C. arel* and it corresponded to the petrochemical centre sampling site. Samples from Puruzinho Lake, a remote area of the Brazilian Amazon, presented THQ values in the same magnitude as those obtained by Keshavarzi et al. (2018) and Mansilla-Rivera and Rodríguez-Sierra (2011), both from potentially contaminated areas.

Łuczyńska et al. (2017) analysed bream (*Abramis brama*), perch (*Perca fluviatilis*), ide (*Leuciscus idus*), carp (*Cyprinus carpio*), rainbow trout (*Oncorhynchus mykiss*), flounder (*Platichthys flesus*), and herring (*Clupea harengus*) samples purchased from the Polish market. Hg concentrations revealed THQ values ranging from 0.011 to 0.254, which were lower than those observed in the present study. Łuczyńska et al. (2018) determined Hg values in gills, liver, gonads, and muscle samples of *Rutilus rutilus* and *Perca fluviatilis* from Pluszne Lake, Olsztyn Lake District (Poland). The obtained THQ values for mercury in muscle tissue were lower than 1 for *R. rutilus* (THQ = 0.135) and *P. fluviatilis* (THQ = 0.303).

Bonsignore et al. (2013) determined mercury THQ in fish species caught inside and outside Augusta Bay, Southern Italy. Considering the species caught inside Augusta Bay, THQ values for mercury varied from 1.53 to 15.8 for *Pagellus acarne* and *Murena helena* respectively and were in the same order of magnitude as our results. The authors described that *Engraulis encrasicolus* and *Mullus barbatus* samples caught outside Augusta Bay, presented THQ of 0.31 and 4.20, respectively. Felix et al. (2022) calculated mercury THQ in muscle samples of dourada (*Brachyplatystoma rousseauxii*), filhote (*Brachyplatystoma filamentosum*), pescada branca (*Cynoscion leiarchus*), and piramutaba (*Brachyplatystoma vaillanti*) from Belém City (Para State), North Brazilian region. All THQ values were also below 1, showing no risk to humans through fish consumption. Exposure to methylmercury represents health risks to nervous system development and other nervous impairments (IRIS EPA, 2001).

Chromium was also a representative metal once considered the calculated individual and mean values. The calculated THQCr for all samples was below 1 when considering the Reference Dose of trivalent chromium (1.5 mg of chromium/kg/day) (Table 3). In another scenario, considering the Reference Dose of hexavalent form (0.003 mg of chromium/kg/day) to the CrTHQ calculation, we estimated THQ maximal value around 27. Zhang and Li (1987) consider that chromium exposure induces the development of signals and symptoms such as mouth sores, stomach-ache, indigestion, vomiting, and diarrhoea. It was also observed by the same authors that chromium increases the blood white cells counting in the exposed group when compared with the control one.

6. Conclusion

The essential elements Fe, Cr, Ca, and Mn presented a similar pattern in their distribution through Puruzinho Lake ichthyofauna. Although not always significant, Zn, Pb, Fe, Cr, Ca, Mn, and Sr concentrations suggest distinct patterns between migratory and resident species, except for *P. pristigaster*. Therefore, these elements could be used as markers in studies that evaluate migration processes between aquatic environments that present distinct physicochemical features, as well as between freshwater and marine environments.

On the evaluation of trophic magnification, the expected pattern was expressed, *i.e.*, methylmercury biomagnification and the elements presenting either trophic dilution or independence from the trophic position, *i.e.*, a pattern that corroborates other studies that evaluated the trophodynamics of methylmercury and trace elements in freshwater, estuarine and marine ecosystems. Although the insertion of migratory species may influence the biomagnification assessment through TMS and TMF, such interference was only found for Zn, Ca and Pb. In addition to the fact that the mentioned interference has been found for three elements only, it had low representativity due to the weak correlations between element concentration and trophic position found for Zn, Ca and Pb.

The absence of significant difference in Ni concentrations among the species and the similarity in trophodymamics between Ni and essential metals (Cr, Fe and Mn) in the trophic web of Puruzinho Lake contribute to the evaluation of the essentiality of this element to fish. The essentiality of Ni was confirmed to cyanobacteria, phytoplankton, and a few higher plants (Muyssen et al., 2004).

Therefore, considering the risk magnitude revealed by our findings linked to the THQMethylHg, authorities in Surveillance in Environmental Health should be contacted and communicated about our results, aiming the construction of actions and public politics to mitigate the exposure of individuals through fish consumption. Still in the context of the non-cancer risk assessment, future studies on Amazon ichthyofauna should include nickel as well.

Future works dedicated to investigations (i) on chromium speciation considering the hexavalent form, (ii) on chromium content in drinking water and other important dietary items (mainly those related to Amazonian traditional alimentary habits), as well as (iii) on additional environmental exposure sources of Cr (such as soil and air), and carcinogenic risk assessment (by deterministic and probabilistic estimations) are strongly recommended.

The present study performed the evaluation of the trophodymamics and human exposure through fish consumption of methylmercury and elements in the Puruzinho Lake area. This evaluation was carried out through the analyses of fish sampled in 2006 and 2007, which allow the access to information from more than 15 years ago, as well as comparison to the current and future scenarios. This way, the present study may work as a temporal mark of an Amazon ecosystem.

When it is considered that the study was performed in the Amazon region, the presented scenario owns great relevance due to the deforestation and goldmining in recent years. The deforestation accumulated during the 2016–2020 period was of 254,465 km^2, which promotes, among other effects, soil degradation and alterations in the hydrological regime (da Silva et al. 2023). The loss of the original vegetal covering leads to soil leaching, which may cause environmental contamination by certain elements [(*e.g.*, Hg (Roulet et al. 2000)], influencing their (1) trophodymamics, (2) concentration in fish, and (3) human exposure through fish consumption. In addition, environmental Hg concentrations are also influenced by goldmining activities in Amazon Region, as this element is used as an amalgamator. In 2020 only, the assessed gold production in Brazil reached 92 tons, 90% of it coming from the non-industrial mining. The artisanal (non-industrial) mining in Brazil occurs predominantly (94%) in Amazon. From these data, it is assessed that the goldmining activity used 300 tons of Hg in Amazon in 2020 only (Crespo-Lopez et al., 2023).

Hence, we should highlight the importance of performing new studies on trophodynamics of the contaminants evaluated herewith, as well as on human exposure to them through fish consumption in Amazon. This would be particularly important regarding Cr, Ni and Hg, or its organic form, methylmercury. These investigations will allow an evaluation over time on the mentioned topics, contributing with valuable information to legislators and public authorities on the environmental and health fields.

Credit author statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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