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Original Article

Selenium in *Cichla melaniae* in Areas Influenced by the Belo Monte Hydroelectric Power Plant, Xingu River, Amazon

Luziane Barbosa Macedo[1*], Thais Pereira Nascimento[2], Hildegard Silva Holanda[1], Marcelo de Oliveira Lima[3], Tatiana da Silva Pereira[1]

[1] Programa de Pós Graduação em Biodiversidade e Conservação, Universidade Federal do Pará (UFPA), Altamira, PA, Brazil
[2] Laboratorio de Ecotoxicología, Instituto de Investigaciones Marinas y Costeras (IIMYC, CONICET), Universidad Nacional de Mar del Plata, Mar del Plata, Argentina
[3] Instituto Evandro Chagas, Ananindeua, PA, Brazil

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Abstract

Selenium (Se) is an essential micronutrient, but can be toxic to fish at elevated concentrations. Studies have shown that the construction of hydroelectric dams increases the presence of toxic elements in reservoir areas. This study was conducted in March 2019 (Amazon flood season) and October 2020 (Amazon dry season) at four sites influenced by the Belo Monte Hydroelectric Plant. Conductivity showed a correlation with Se concentrations in the tissues of *Cichla melaniae,* as well as standard length (SL), particularly in the liver and muscle. During the flood season, significant differences in Se concentrations were observed between sites L1 and L3, L1 and L4, L2 and L3, and L2 and L4 ($p<0.01$). Differences were also found between gills and liver, and between liver and muscle ($p<0.01$). During the dry season, concentrations varied significantly across tissues ($p<0.01$). A comparative analysis between the seasons indicated higher Se concentrations during the flood season. The liver exhibited the highest concentrations in both seasons, especially at the reservoir site (L2) and the reference site (L1). These findings underscore the influence of seasonal factors on Se concentrations and highlight the need for continuous monitoring in areas impacted by hydroelectric plants.

Keywords: Amazon, bioaccumulation, bioconcentration, sediment, tissues.

INTRODUCTION

Trace elements like selenium (Se) are vital for fish growth, metabolism, reproduction, and overall health when maintained within physiologically safe concentrations. (Lall & Kaushik, 2021), being an indispensable micronutrient for the proper functioning of the organism (Aramli *et al*., 2023). However, Se has a narrow range between concentrations considered essential and those that are toxic to the organism (Hamilton, 2004). The chemical forms of Se that are constantly present in fish diets are the organic forms, selenomethionine (Se-Met) and selenocysteine (Se-Cyst), which are considered the most toxic compared to the toxicity of the inorganic forms, selenide Se(-II), selenite Se(IV), and selenate Se(VI) (Janz *et al*., 2010a). Se can be absorbed by aquatic organisms from both water and the diet. However, the dietary route, especially from organoselenium, tends to be the main pathway responsible for Se accumulation in higher trophic levels (Hamilton, 2004; Stewart *et al*., 2004).

Fish are widely used in environmental impact assessment studies and are recognized as effective bioindicators of pollution in aquatic environments (Azevedo *et al*., 2009). Many studies have used fish as bioindicators of pollution by Potentially Toxic Elements (PTEs), including Se, as the concentrations of these elements in their tissues reflect environmental conditions and the risks to human consumption (Birungi *et al*., 2007; Luczynska *et al*., 2018; Findik and Cicek, 2011).

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*Corresponding author: annemacedo563@gmail.com

Cichla melaniae (tucunaré) is one of the fish species endemic to the Xingu River region (Kullander *et al*., 2006). It is a carnivorous/piscivorous predator with diurnal hunting behavior (Japsen *et al*., 1997), and is among the most commonly consumed species by the local population. It is also a popular target for sport fishing, which highlights its regional socioeconomic importance (Camargo *et al*., 2011). In this context, fish like *C. melaniae* can serve as excellent indicators of environmental contamination, due to the accumulation of pollutants in their tissues, which allows for the assessment of contamination levels and the potential risks to human health through the consumption of contaminated fish (Ural *et al*., 2012; Ali & Khan, 2018). As also reported by Albuquerque *et al*. (2021), tissues such as muscle and liver of a tucunaré species (*Cichla temensis*) are excellent for biomonitoring concentrations of toxic elements in the aquatic environment.

The increasing anthropogenic activities have intensified the release of selenium from its natural sources (rocks and soils), making it available to the aquatic environment and, consequently, to humans, considering that the primary route of exposure is through dietary intake (Seixas & Kehrig, 2007). One example of these anthropogenic activities is the construction of hydroelectric dams, which has caused significant impacts, such as declines in water quality, silting of water bodies, and alterations in the natural hydrological flow of rivers (Carvalho *et al*., 2014; Fearnside, 2019).

These changes transform the lotic system into a lentic one and lead to vegetation suppression in areas affected by construction due to subsequent reservoir filling (Berman, 2007; Araújo *et al*., 2019). Thus, the changes caused by a hydroelectric dam can mobilize and redistribute PTEs (Campos *et al*., 2018).

The Belo Monte Hydroelectric Plant (BMH), located on the Xingu River, had its original design modified to operate under a run-of-river regime, reducing the flooded area from 1,225 km² to 478 km². Construction of BMH began in 2011, with reservoir filling starting in 2015, and the plant officially began operations in 2016 (NESA, 2018). However, there is a lack of studies addressing the occurrence of Se in this area, while studies such as Lemly (2002a) have shown that Se contamination in aquatic ecosystems poses a high risk of fish population mortality. Thus, monitoring the impact of anthropogenic activities in this region is of great importance,particularly given the lack of studies on Se in this location.

The concentration of selenium in organic detritus within aquatic sediments plays a more significant role in contaminating the food chain than its dissolved concentration in the water column (Seixas *et al*., 2005). Rainfall rate and hydroelectric projects are important factors contributing to the deposition rate of elements in the aquatic environment, particularly in sediments (Zanin *et al*., 2017; Paz *et al*., 2022). Lemly (2002b) identified that elevated Se concentrations in fish tissues were associated with skeletal deformities, gill malformations, and spinal anomalies, as well as negative effects on species reproduction.

Thus, the present study aimed to analyze selenium concentrations in the tissues of *Cichla melaniae* in the Xingu River region, in areas under the direct influence of BMH, during two hydrological seasons in the Amazon: dry and wet.

MATERIALS AND METHODS

Study Area

This study was conducted in the Xingu River, in areas influenced by the Belo Monte Hydroelectric Plant (BMH), which spans the municipalities of Altamira and Vitória do Xingu, in the southwestern region of the state of Pará, Brazil (Figure 1). In the Xingu River, the months of April and May correspond to the peak of the rainy season, while the months from September to October are the driest (Neto *et al*., 2021; Freire *et al*., 2019).

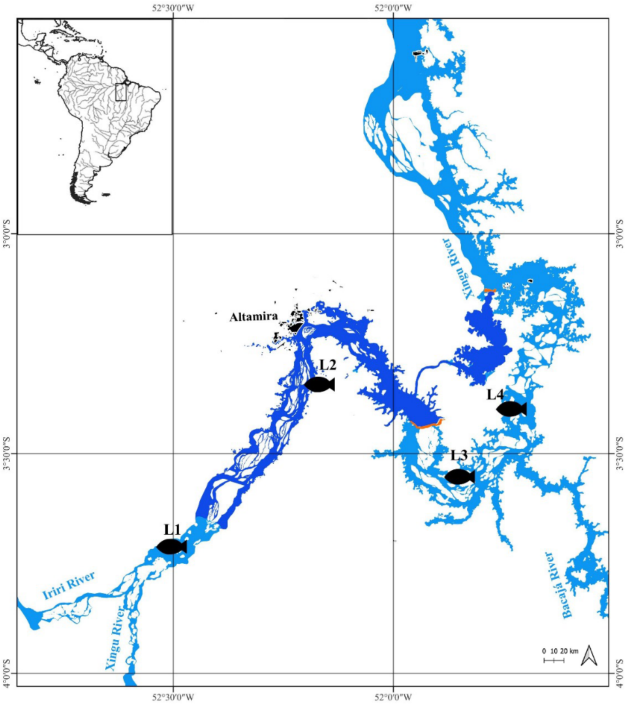

Figure 1. Map of the sampling sites in the Xingu River (dark blue – hydroelectric reservoirs; light blue – area without BMH influence; red – BMH powerhouses).

Sampling

Sampling was conducted in May 2019 (flood season) and October 2020 (dry season) at four collection sites under the influence of the Belo Monte Hydroelectric Plant (BMH) (Table 1).

Table 1. Sampling locations in the Xingu River.

Sampling Sites	Location
L1	Upstream of the hydroelectric power plant's main reservoir. Area used as a reference for being outside the influence of the BMH
L2	Main reservoir of the BMH, upstream of the city of Altamira
L3	Volta Grande, a section of the river diverted for the construction of the power plant, upstream of the Bacajá River.
L4	Volta Grande, downstream of the Bacajá River.

Limnological parameters and tissue

Samples of Cichla melaniae

At the four collection sites, limnological parameters were measured in situ, including pH, conductivity, and temperature, using a HANNA$^{®}$ multiparameter probe. Samples were collected using small aluminum motorboats, locally known as *voadeiras*, in backwater areas along the riverbank, at a depth of approximately 50 centimeters.

Tissue samples were collected under SISBIO authorization No. 71763-1 and approval from the Animal Research Ethics Committee (CEUA - UFPA) No. 8166251119, for species handling and euthanasia. Specimens were captured by a local fisherman using a rod-and-reel method with artificial lures.

The species *Cichla melaniae* (Cichlidae), commonly known as tucunaré, is found throughout the Middle Xingu River region. *Cichla melaniae* is a carnivorous species (Kullander & Ferreira, 2006) and is widely consumed by local populations.

For our study, after being collected from the river, the specimens were euthanized using the anesthetic eugenol diluted in alcohol. Then, their weight, total length (TL) — measured from the anterior portion of the head to the end of the caudal fin — and SL — which corresponds to the body length up to the base of the tail — were recorded. Subsequently, in the field, tissues (muscle, liver, and gills) were extracted and stored at refrigeration temperatures.

The tissues were analyzed at the Evandro Chagas Institute (Pará, Brazil). Tissue samples (muscle, liver, and gills) were homogenized and weighed in Teflon vials. For each analysis, 0.1 g of tissue was used per sample. They were then subjected to acid digestion using 1 mL of nitric acid (HNO_3) and 0.5 mL of hydrogen peroxide (H_2O_2). The samples were placed in a microwave digestion system (MarsXpress, CEM Corporation) for 20 minutes to complete digestion. After cooling, they were transferred to Falcon tubes and diluted with 1% HNO_3. The total Se concentrations were determined using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS) 820-MS (Bruker) (Sousa-Araujo *et al.*, 2022). For the analysis, the certified reference material DORM-3 from the National Research Council of Canada (NRC-CNRC) was used, with a recovery rate of 109%. The reference material was analyzed in duplicate.

Statistical analysis

To perform the analyses, both the response and covariate matrices were first standardized using the Z-score method to minimize the scale effect.

Then, a Euclidean distance matrix was constructed. A permutational multivariate analysis of variance (PERMANOVA) with 999 permutations was applied to evaluate the effects of Se concentrations in tissues, limnological parameters, and seasonality on the variability of biological data.

In parallel, a Principal Coordinates Analysis (PCoA) was conducted based on the distance matrix, with the principal axis scores (PCoA1 and PCoA2) incorporated into the original dataset. Additionally, correlations between response variables and covariates were calculated, with results adjusted using a scaling factor to facilitate graphical visualization.

After verifying assumptions, it was found that the data did not follow a normal distribution. Therefore, to test differences among tissues, between tissues and sampling sites, as well as between tissues and seasons, the non-parametric Kruskal-Wallis test was applied, followed by Dunn's *post hoc* test. All analyses were performed in the R Studio environment (version 3.4.2), adopting a significance level of $p < 0.05$.

RESULTS

Limnological parameters

The physicochemical data presented in Table 2 show that during the wet season, water temperature varied little among sampling sites, whereas in the dry season, greater variability was observed along with higher temperatures. Regarding the limnological parameter, the values showed distinct patterns between seasons, with pH being slightly acidic during the flood season and close to neutrality in the dry season.

Table 2. Limnological variables (depths of approximately 50 cm) in the two rainfall seasons, dry and wet, at the sampling sites in the Xingu River.

Sampling Sites	Limnological Parameters					
	Flood 2019			Dry 2020		
	Temp (º C)	Cond (µS/cm)	pH	Temp (º C)	Cond (µS/cm)	pH
L1	27,5	23	6,8	31,9	16	7,0
L2	27,9	21	6,9	30,6	16	7,1
L3	27,6	23	6,4	31,8	19	7,2
L4	27,7	23	6,7	31,7	19	7,2

To better understand how these variables influenced the overall data structure, a Principal Component Analysis (PCA) was performed. The analysis revealed a significant seasonal effect, accounting for approximately 31.5% of the total variation (Sum of Squares = 88.747; F = 51.861; $p < 0.001$). Among the environmental variables, conductivity and pH contributed significantly to the observed patterns, explaining 3.8% (Sum of Squares = 10.762; F = 6.289; p = 0.002) and 9.8% (Sum of Squares = 27.502; F = 16.071; $p < 0.001$) of the variation, respectively. In contrast, temperature did not have a statistically significant effect (Sum of Squares = 0.978; F = 0.571; p = 0.600). Overall, the model explained 45.4% of the total variation, with residual variance accounting for the remaining 54.6%.

Although temperature did not significantly influence the structure of the environmental dataset, electrical conductivity showed a clear relationship with Se concentrations in tissues (p = 0.002), highlighting its potential role as a key driver in Se accumulation.

Total selenium concentration in *Cichla melaniae* tissues

Selenium concentrations in *C. melaniae* tissues varied according to both tissue type and hydrological season (Table 3). To further explore these seasonal patterns, Principal Coordinates Analysis (PCoA) was applied, revealing clear differences between periods. Samples from the dry season formed a tighter cluster in the ordination space, while those from the rainy season exhibited greater dispersion (Figure 2).

Table 3. Average (± SD) Selenium (µg/g) levels in tissues of *Cichla melaniae* at the four sampling sites in the Xingu River, during the wet and dry seasons.

Rainfall Station	Sites	Muscle (N) (µg/g)	Liver (N) (µg/g)	Gills (N) (µg/g)
Flood	L1	0.499±0.07 (12)	3.316±1.70 (11)	0.532±0.10 (11)
	L2	0.67±0.11 (8)	4.018±1.12 (8)	0.433±0.10 (7)
	L3	0.219±0.03 (15)	2.736±0.60 (14)	0.313±0.05 (15)
	L4	0.213±0.03 (15)	2.352±0.46 (14)	0.409±0.47 (15)
Dry	L1	0.147±0.02 (15)	1.409±0.59 (13)	0.248±0.08 (14)
	L2	0.142±0.04 (17)	1.278±0.66 (16)	0.307±0.14 (17)
	L3	0.128±0.04 (18)	1.410±0.62 (17)	0.315±0.09 (17)
	L4	0.101±0.02 (13)	1.512±0.55 (13)	0.381±0.12 (10)

Note: N = number of specimens analyzed.

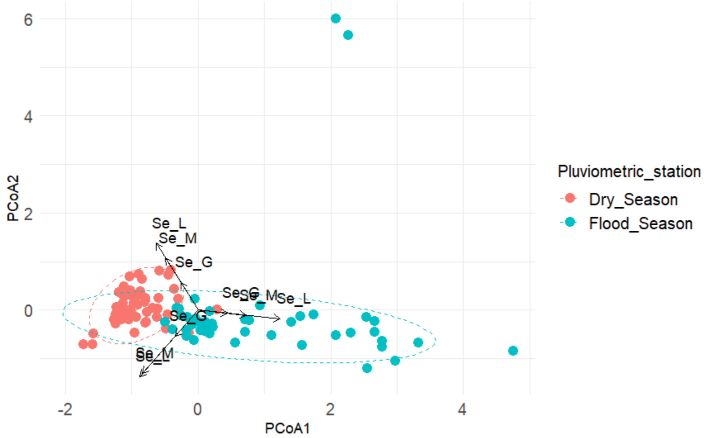

Figure 2. Distribution of *Cichla melaniae* samples based on Se concentrations in tissues and rainfall seasons. Se_L - selenium in the liver; Se_M - selenium in the muscle; Se_G - selenium in the gills.

Additionally, the analyzed environmental and biological variables showed distinct influences on sample distribution. Selenium concentrations in the liver (Se_L) and muscle (Se_M) were strongly associated with the dry season, whereas concentrations in the gills (Se_G) were predominantly associated with the rainy season.

Selenium in *Cichla melaniae* tissues at sampling sites

During the flood season, a significant variation in Se concentrations was observed among the sampling sites ($p < 0.05$). Multiple comparisons using Dunn's *post hoc* test indicated statistically significant differences between sites L1 and L3, L1 and L4, L2 and L3, and L2 and L4 ($p < 0.01$) in all comparisons. Additionally, significant differences were found among tissues, with variations in Se concentrations between gills and liver, and between liver and muscle ($\chi^2 = 225.17$; df = 2; $p < 0.01$). Figure 3 provides a detailed illustration of these differences, comparing total Se concentrations across tissues and the four sampling sites.

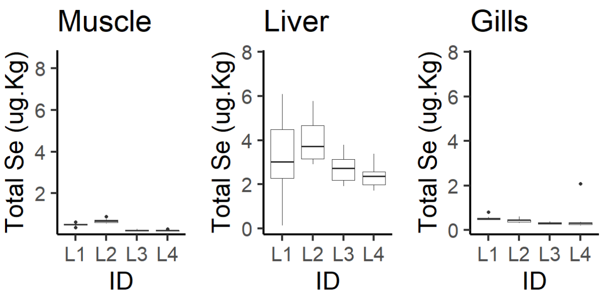

Figure 3. Comparison between the tissues of *C. melaniae* at different sampling locations during the collection period.

During the flood season, the highest Se concentrations in muscle were recorded at L2, whereas the highest values in liver and gills were found at L1, followed by L2. Furthermore, correlation analysis between Se concentration and sampling sites indicated a significant variation ($p < 0.01$). In the dry season, significant differences in Se concentrations were detected among tissues ($p < 0.01$), with variations between gills and liver, gills and muscle, and liver and muscle. However, no statistically significant differences were observed among the sampling sites.

Comparative analysis between tissues and pluviometric seasons

Differences in Se concentrations were observed between the pluviometric seasons ($p<0.01$). Figure 4 presents the comparison of total Se concentrations in *C. melaniae* tissues between the dry and flood seasons.

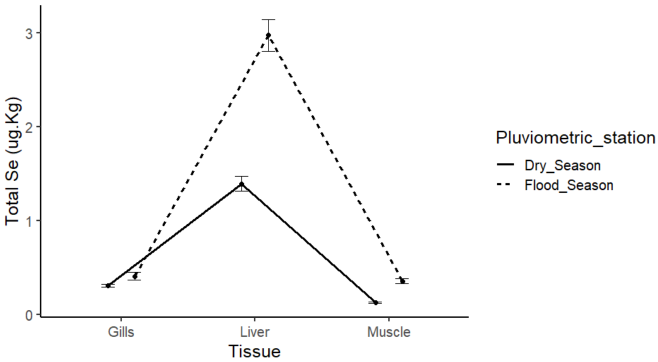

Figure 4. Comparison between tissues (gills, liver, and muscle) during the dry and rainy seasons in the Amazon.

The highest Se concentrations were recorded during the flood season, and in both seasons, the liver exhibited the highest Se concentrations.

DISCUSSION

Environmental changes are known to influence the bioavailability and accumulation of chemical elements in aquatic organisms. This is particularly relevant for selenium, an essential trace element that, in excess and depending on its chemical form, can become toxic —

leading to effects such as egg failure and reproductive anomalies in fish (Janz *et al*., 2010b). In the present study, variations in Se concentrations among tissues and seasons reflect this sensitivity to environmental conditions.

These seasonal variations appear to be influenced, at least in part, by limnological parameters. For instance, although pH varied between the dry and flood seasons, it remained within the reference limits for clear-water Amazonian rivers, as established by Brazilian regulations (CONAMA, 2005). However, other parameters, such as electrical conductivity, may offer deeper insights into the dynamics of Se availability.

Silva *et al*. (2017) highlight electrical conductivity as a key indicator of aquatic environmental quality, given that it reflects the concentration of dissolved ions. In this context, the observed correlation between electrical conductivity and Se concentrations may point to areas affected by anthropogenic contamination, as increased salinity and the presence of certain elements tend to raise conductivity levels (Silva *et al*., 2022; Andrade *et al*., 2012).

Thus, it is suggested that variations in electrical conductivity in response to contamination may influence the availability of potentially toxic elements such as Se, consequently increasing the risk of fish exposure (Pereira *et al*., 2017; Arcos & Cunha, 2021). This hypothesis is supported by our results, which demonstrate variation in Se concentrations among the analyzed fish, between the two Amazonian hydrological seasons, and across different sampling sites in the Xingu River.

Our results indicate that the liver exhibited the highest Se concentrations, particularly at L1 (reference site) and L2 (within the reservoir), while muscle tissue showed higher levels within the reservoir. The elevated bioaccumulation in both muscle and liver at L2 suggests that the reservoir may be influencing Se availability and uptake. The flooding of these areas transforms the environment into a lentic system, promoting the accumulation of organic matter and sediments (Meena *et al*., 2018).

Leaching in this region transports various elements, including selenium, to the dammed river, where they may be dissolved in water, associated with sediments, or present in animal tissues (Sim *et al*., 2014). However, the increase in Se levels at L1 during the rainy season suggests that natural sources, such as local geology influenced by seasonality, may also contribute to the observed concentrations.

The liver is commonly used for environmental contamination assessment due to its potential to concentrate elements (Albuquerque *et al*., 2021). This tissue's predisposition to accumulate selenium may be related to its physiological role in the detoxification and metabolism of trace metals (Hauser-Davis *et al*., 2012), as it serves as a primary site for bioaccumulation due to the presence of metallothioneins and other binding proteins that facilitate the retention of toxic elements (Sinaie *et al*., 2010).

Muscle tissue, on the other hand, tends to exhibit lower Se concentrations since the element accumulates more intensely in metabolic organs and tissues with high biochemical activity, rather than being directly incorporated into muscle (Seixas *et al*., 2005). Conversely, the seasonal variation observed in the gills may be related to direct exposure to the aquatic environment, as this tissue is one of the main sites for ion exchange and absorption of dissolved elements (Mehmood *et al*., 2017). Thus, the differences found between tissues reflect both physiological mechanisms and the environmental dynamics of Se availability.

In the present study, the highest selenium (Se) concentration in muscle (0.67 µg/g) was recorded within the reservoir during the rainy season. Studies conducted in the Tapajós River with carnivorous fish species similar to the tucunaré (*C. melaniae*) found values lower than those quantified in the Xingu River, such as Faial *et al*. (2015), with concentrations of 0.34 µg/g, and Lino *et al*. (2018), with concentrations of 0.28 µg/g.

The selenium (Se) concentrations found in our study in the reservoir area are higher than those reported in previous studies in other regions. This finding is significant, as this study is the first to report Se concentrations in *Cichla melaniae* in areas influenced by the Belo Monte Hydroelectric Power Plant, highlighting the need for further investigations to assess the environmental impacts and potential risks associated with these elevated concentrations.

Selenium requirements for adequate physiological function are not uniform across fish species, as they are influenced by a range of biological and environmental factors. These include the species and size of the fish, its health sensitivity, the chemical speciation of selenium (whether organic or inorganic), and the specific ecological conditions of its habitat (Khan *et al*., 2016, 2017).

In line with these variations, the toxicity of Se also differs among species and is closely related to their physiological characteristics and the dynamics of selenium transfer — both from the diet and from tissues to eggs (Passomai *et al*., 2022). Moreover, the organism's ability to oxidize organic Se plays a crucial role in determining its vulnerability, as excessive selenium can impair reproductive capacity (Muscatello *et al*., 2008). At high concentrations, selenium can cause embryonic mortality and teratogenic malformations during the larval stage, including skeletal, craniofacial, and fin deformities (Janz *et al*., 2010b; Chapman *et al*., 2009).

However, the increased Se concentrations in muscle tissue in the reservoir area make the consumption of fish caught in this area one of the primary routes of human intoxication by these elements (Castro-González; Méndez-Armenta, 2008; Zhong *et al*., 2018).

The decrease in Se observed in our study during the dry season corroborates with Zucco *et al*. (2015), who highlight that the variation in the availability of chemical elements in river waters is consistently associated with changes in hydrological conditions. Rainfall and changes in hydrodynamic mechanisms reflect the increase in organic matter in these areas during the flood period, leading to the mobilization of sediments that influence elevated concentrations of elements (Bastos *et al*., 2020). Considering these factors, it is possible to explain the differentiated bioaccumulation patterns between the sampling sites. During the dry season, with lower water flow and greater limnological stability, Se distribution appears to be more homogeneous among the sampled sites, reducing detectable spatial differences.

In our study, we found that the Se concentrations in *C. melaniae* are influenced by seasonal variations, with higher levels observed during the rainy season. These results are consistent with those of Meche *et al.* (2010), who, when analyzing several metals and essential nutrients, including Se, found an increase in Se concentrations during the rainy season, which could lead to increased fish exposure. Similarly, Albuquerque *et al*. (2020) observed that rising water levels cause changes in diet and the availability of toxic elements, which may explain the observed variation.

For human consumption, the Se concentrations found in the muscle of *C. melaniae*, the tissue used for human food, were below the limits set by Brazilian legislation for food, which is 0.30 mg/g (ANVISA, 1965). However, it is important to understand the factors that contribute to the high values found during the flood season, considering the health of this species and the local population, as well as the health of traditional communities who consume it (Burger & Gochfeld, 2021). Considering its essential role in metabolism, the adequate intake of Se is estimated at 70 mg/day for adult men and 60 mg/day for adult women. However, exposure to levels above 400 mg/day is considered toxic and may lead to adverse effects such as skin disorders, neurological dysfunctions, and, in severe cases, paralysis (Silva Junior *et al*., 2017).

This study identified elevated concentrations of Se in the tissues of *Cichla melaniae* in the area influenced by the Belo Monte Hydroelectric Plant (BMH), with higher levels observed in the reservoir and a clear seasonal pattern, showing an increase during the flooding period. To mitigate risks to human health, it is recommended to consume fish from different trophic levels, especially during this period. The absence of specific reference values for *C. melaniae* prevents a precise assessment of the risks that these concentrations may pose to the species; however, when compared to studies conducted in other river systems, the concentrations observed in this study are considerably higher.

In light of these findings, the implementation of continuous monitoring of water, sediments, and aquatic organisms becomes essential. Furthermore, ecotoxicological studies focusing on *C. melaniae* are urgently needed to assess the sublethal and lethal effects of Se on this species. These actions are crucial not only for the conservation of aquatic biodiversity but also for safeguarding the food security and health of human populations that rely on this species as a dietary resource

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AUTHOR'S CREDIT STATEMENT

LM: Writing – Original Draft, Conceptualization, Contextualization, Investigation, Visualization, Methodology. **TN:** Field collection, Sample Analysis, Writing – Review & Editing, Supervision, Methodology, Data Curation. **HH:** Field collection, Sample Analysis, Writing – Review, Conceptualization, Data Curation, Methodology. **ML:** Supervision and Project Administration. **TP:** Field collection, Writing – Review & Editing, Supervision, Data Curation, Funding Acquisition, Project Administration.

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