Linking trophic ecology and metal bioaccumulation to assess a widespread fish as a bioindicator following a large-scale mining disaster

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Abstract

Fish are key bioindicators for understanding the impacts of human-induced environmental disasters. We assessed trophic ecology and metal accumulation (Fe, Mn, Hg) in the small, abundant characid *Astyanax lacustris* in the Doce River basin, following the 2015 mining tailings dam collapse. Stable isotope and metal analyses were conducted in fish from six affected sites and two reference sites. Aquatic invertebrates dominated the diet, except near the dam rupture, where algae predominated, and metal concentrations were highest. Fe and Mn concentrations decreased with fish length, Hg increased with fish nitrogen isotopic composition ($\delta^{15}N$), and only Fe showed clear associations with dietary sources. This study is novel in integrating trophic ecology and metal contamination assessment in a sentinel fish species after a major mining disaster. The findings provide insights for biomonitoring and metal risk assessment in freshwater ecosystems worldwide, and highlight *Astyanax lacustris* as a

powerful sensitive bioindicator, reflecting both contamination legacy and ecological pathways of metal accumulation.

Keywords: Fish, Stable isotopes, Carbon, Nitrogen, Fundão dam, Mariana disaster, Ore tailings.

1. Introduction

The Doce River basin is notable for its long history of environmental impacts from multiple pollution sources, which were dramatically exacerbated by the collapse of the Fundão iron ore tailings dam in 2015 (Sánchez et al. 2018). A global review of tailings dam failures over the past century (1915–2020) documented 366 incidents, with Brazil's failures being the most severe due to their high cumulative impact (Islam and Murakami 2021). Much of this is attributed to the Fundão collapse, regarded as the largest disaster in the history of the global mining industry in terms of both the volume of tailings released and the scale of environmental damage (Do Carmo et al. 2017). Unlike other major mining dam failures, such as Mount Polley (Canada, 2014), whose impacts were largely localized and quickly addressed through efficient corrective measures (Islam and Murakami 2021), the Fundão dam collapse disrupted dozens of municipalities and livelihoods across an entire river basin. By comparison, some disasters were more lethal (Stava, Italy, 1985, with 268 deaths – Simeoni et al. 2017) or more toxic (Baia Mare, Romania, 2000, with cyanide and heavy metal contamination – WWF Hungary, 2002), but none combined such magnitude, geographic extent, and long-term ecological and legal repercussions.

The iron ore tailings from the Fundão dam, rich in Fe, Al, and Mn, pose environmental risks to soils, water, and biotic communities, as their mineral components can influence the mobility of trace metals under variable redox conditions (Duarte et al. 2023). Among the organisms most affected by the Fundão collapse were freshwater fishes, which experienced severe impacts including accumulation of potentially toxic metals (Ferreira et al. 2020; Freitas et al. 2025), histopathological changes (Macêdo et al. 2020; Vieira et al. 2022), morphological damage (Bonecker et al. 2019), and stress responses (Yamamoto et al. 2023). The dam rupture was also responsible for alterations in assemblage structure and trophic dynamics (Andrades et al. 2020, 2021; de Carvalho et al. 2024; Paiva et al. 2024). These responses to environmental impacts across multiple scales, from the cellular to the organismal level, make fish suitable bioindicators of environmental quality.

Studies evaluating the trophic ecology of fish are necessary as they provide essential information for understanding relationships within a community. They offer insights into energy flow within and between ecosystems, resource partitioning, habitat and prey preferences, predation, evolution, competition, and other ecological processes (Braga et al. 2012). Studies on fish trophic ecology are also crucial to evaluate whether all these factors change along the organisms' development (i.e., ontogenetic stages, Vitule et al. 2008; Dala-Corte et al. 2016). Feeding habits are also closely related to fish health, as these organisms absorb both essential and non-essential metals (Ali and Khan

2018a, b), as well as other chemical substances—such as polychlorinated biphenyls (PCBs) (Masset et al. 2019)—through food intake, retaining them in their tissues. Thus, knowing what fish consume reveals important properties about the functioning of the ecosystem they inhabit (Villéger et al. 2017).

Fish species that are widely distributed and abundant are ideal candidates for bioindicator studies assessing ichthyofauna responses to environmental degradation. Fish of the genus *Astyanax*, commonly known as "lambaris" or "piabas", have a broad geographical distribution and comprise more than 120 neotropical species (Eschmeyer 2025). The species of this genus are relatively small (10-12 cm for adults) and gather in shoals. They are non-migratory and capable of reproducing in both lotic and lentic environments, with a long reproductive period featuring multiple peaks of active reproduction, short generations, and a high number of progeny (Godinho et al. 2010). Moreover, they can be used as a bioindicator of environmental quality regarding aquatic contamination (Alonso et al. 2019; Passos et al. 2020; Merçon et al. 2021, 2022). Among the species of this genus, *Astyanax lacustris* stands out due to its ability to adapt to both lotic and lentic environments, being abundant in those habitats. Additionally, they have an omnivorous diet, feeding on both animal and plant items (Bastian et al. 2021), with a tendency towards herbivory and insectivory (Da Silva et al. 2012; Vidotto-Magnoni et al. 2021). This species' dietary plasticity is also reported in different environments, such as reservoirs, rivers, and streams (Alonso et al. 2019; Vidotto-Magnoni et al. 2021). The environmental disaster in the Doce River basin has caused documented consequences for *A. lacustris*. Previous studies have reported histological damage in gonads (Merçon et al. 2021), liver, and gills (Macêdo et al. 2020) of fish exposed to contaminated water, with more severe effects near the dam rupture (Macêdo et al. 2020). Additionally, seasonal peaks of metals, particularly aluminium (Al) and iron (Fe), have been linked to biochemical modifications in the liver of *A. lacustris* (Merçon et al. 2022). Although these findings reinforce the species' bioindicator potential, little is known about its trophic ecology in the basin after the disaster. The only available data indicate that *A. lacustris* occupies basal to intermediate trophic positions in the food web, with spatial variations across different sites (de Carvalho et al. 2024).

Given that fish are an important pathway of metal exposure to humans (Isangedighi and David 2019), understanding the factors driving metal assimilation in this widespread species remains essential. Therefore, this study aimed to compare concentrations of Fe, Mn, and Hg in the muscle tissue of *Astyanax lacustris* across different regions of the Doce River basin and to evaluate how trophic ecology and ontogenetic stages influence metal accumulation. To access the trophic ecology, we used the stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), since this approach enables advancements in research on trophic ecology and energy flow in ecosystems through the reconstruction of animal diets and food webs (e.g., Layman et al. 2007; Perkins et al. 2014; Post 2002). Recently, this tool has been increasingly used to assess anthropogenic impacts on fish fauna (e.g., Albrecht et al. 2021; Burbank et al. 2022; de Carvalho et al. 2020, 2024). However, there is still significant potential for integrating stable isotopes with other methodologies aiming to assess human-induced changes in aquatic ecosystems, such as investigating the relationship between fish trophic ecology and metal intake. Considering that dietary shifts can modify metal uptake by altering both the type and contamination levels of consumed resources, and that ontogenetic changes (e.g., body size and trophic position) affect assimilation efficiency and tissue distribution, we tested two main hypotheses:

H1) Influence of ontogenetic stages on metal accumulation: We hypothesise that fish enriched in $\delta^{15}N$, reflecting higher trophic position or assimilation of ^{15}N-enriched resources, will exhibit higher metal concentrations. A similar positive relationship is expected with body size, due to the metal's bioaccumulative nature, where concentrations are expected to increase along the food web and throughout ontogenetic stages.

H2) Influence of dietary resources on metal concentrations: We hypothesise that metal concentrations vary according to the type of consumed resources. We expect higher concentrations of metals in fish from sites where aquatic invertebrates are more consumed compared to terrestrial resources. Additionally, periphyton availability and assimilation are expected to be higher in non-affected sites, as this resource is likely more impacted by the dam collapse (e.g., increased turbidity, abrasion). Consequently, fish with higher periphyton assimilation should exhibit lower concentrations of metals strongly associated with mine tailings.

By testing these hypotheses, we expect to gain insights into how resource use and fish traits modulate metal bioaccumulation following large-scale mining tailings dam failures, shedding light on contaminant transport, retention, and ecological pathways in freshwater systems. Additionally, we aim to advance the understanding of environmental conditions in the Doce River basin, providing information to support risk assessment and guide management strategies to mitigate the impacts of mining-related contamination.

2. Material and Methods

2.1 Study area

The present study was conducted in the Doce River basin, which originates at an altitude of 1200 meters in the municipality of Ressaquinha, Minas Gerais, Brazil (Figure 1). The Doce River flows over 853 km, spanning 230 municipalities across Minas Gerais (MG) and Espirito Santo (ES). Land use in the Doce River basin is characterized by agriculture, mining, and livestock farming, and since the 1970s, strong regional economic development in the Doce River basin has led to a high degree of environmental degradation (Cupolillo et al. 2008). The rapid economic growth and infrastructure improvements have resulted in environmental impacts such as high levels of air and water pollution (Marcuzzo et al. 2011). Additionally, poor land use and deforestation have caused siltation throughout the basin's drainage, resulting in significant fish habitat loss and posing a major conservation challenge for the river's ichthyofauna (Vieira 2009). In November 2015, the Fundão tailings dam collapsed in the municipality of Mariana, Minas Gerais, worsening the historical conditions by releasing mining waste that impacted the ecosystems and communities of the Doce River, particularly those along its watercourses. The impacts on aquatic ecosystems were severe, affecting both aquatic organisms and riparian communities directly and indirectly (Dias et al. 2018; Espindola et al. 2019). Approximately 40 million cubic meters of tailings, contaminated with metals such as Fe, As, Al, Hg, and Mn, were discharged into the Doce River basin (Sánchez et al. 2018).

2.2 Sampling design

Eight sampling sites were sampled along the Doce River basin, six of them located along the path of the mining waste mudflow (I1 – I6), and the other two were set on sites unexposed to the Fundão material, thus considered as control sites (C1 – C2) (Figure 1, Table 1). Site C1 is located on the Santo Antônio River, known for its good environmental quality and importance for conserving Doce River biodiversity (Vieira 2009). Site C2 is situated on the Manhuaçu River, in the lower part of the Doce River basin. The sites in the mud's path are located near the Candonga Reservoir (Risoleta Neves Hydroelectric Power Plant) (I1), São José do Goiabal (I2), Naque (I3), Tumiritinga (I4), Aimorés (I5), and Colatina (I6). Thus, the impacted sites were distributed from the reservoir, which retained a significant portion of the released mud (Sánchez et al. 2018), to Colatina, the closest site to the Doce River mouth.

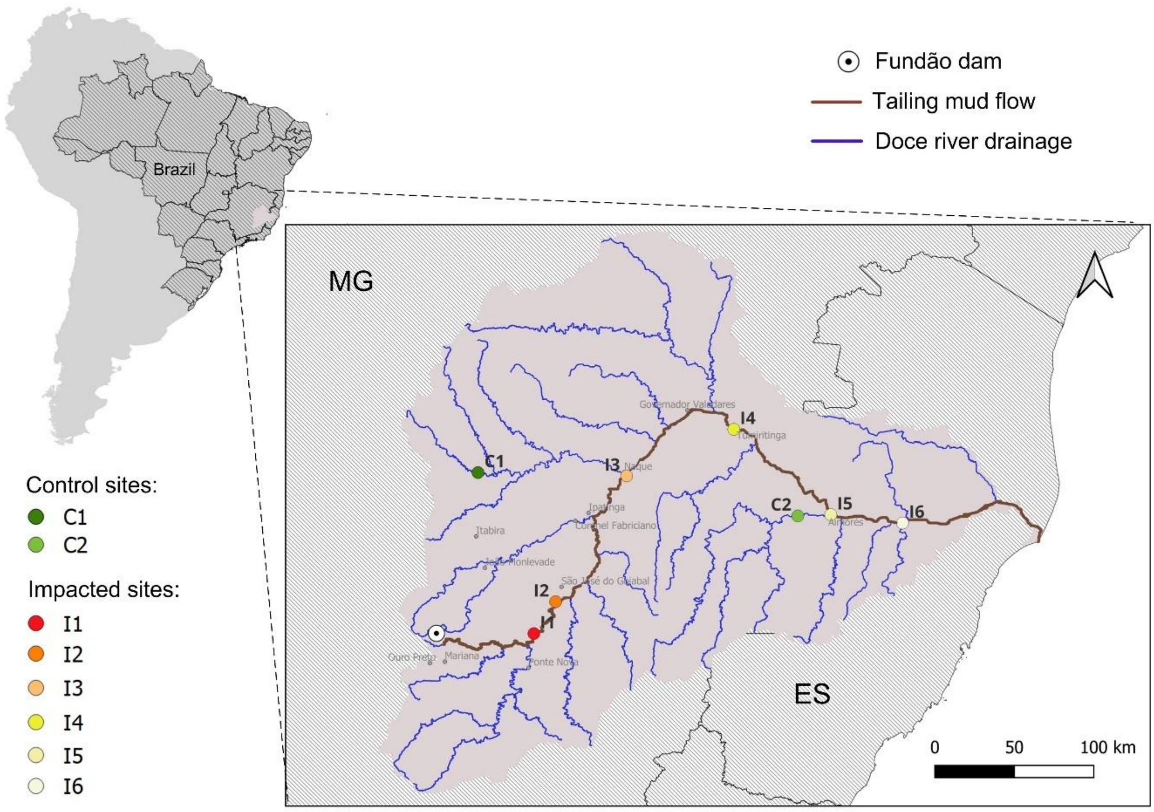

Fig. 1 Control sites (C1 and C2) and sites impacted by the Fundão dam rupture (I1 to I6) sampled in the Doce River basin. C1 = Santo Antônio River; C2 = Manhuaçu River; I1 = Doce River upstream of the Risoleta Neves Hydroelectric Power Plant (UHE); I2 = Doce River (São José do Goiabal); I3 = Doce River (Naque); I4 = Doce River (Tumiritinga); I5 = Doce River (Aimorés); I6 = Doce River (Colatina)

Table 1 Geographic coordinates of sampled sites for stable isotopes along the Doce River basin. I = Sites impacted by the dam rupture (I-1 to I-6); C = Control sites, unaffected by the dam rupture (C-1 and C-2)

Site	Condition	Drainage (City)	State	Longitude	Latitude
C1	Control	Santo Antônio River (S. Ant. Rio abaixo)	MG	-43.21522792	-19.23122506
C2	Control	Manhuaçu River (Aimorés)	MG	-41.27548295	-19.49390858
I1	Impacted	Doce River (HPP Risoleta Neves)	MG	-42.87635589	-20.20798406
I2	Impacted	Doce River (São José do Goiabal)	MG	-42.74447348	-20.01466177
I3	Impacted	Doce River (Naque)	MG	-42.31401131	-19.25154488
I4	Impacted	Doce River (Tumiritinga)	MG	-41.66371293	-18.96876677
I5	Impacted	Doce River (Aimorés)	MG	-41.07506333	-19.48643624
I6	Impacted	Doce River (Colatina)	ES	-40.639851	-19.53728383

Samples of *A. lacustris* and their primary food resources were collected at each site during the dry season, from August to September 2020. All procedures, including fish collection, euthanasia, and transportation, were conducted with approval from the Animal Research Ethics Committee of the Federal University of Viçosa (protocol no. 55430-2). Additionally, authorization was granted by the Biodiversity Authorization and Information System (SISBIO) under protocol no. 80532-1, and by the Chico Mendes Institute for Biodiversity Conservation (ICMBio) and the Ministry of the Environment (MMA).

2.3 Sampling of *Astyanax lacustris* and putative food sources

At each site, *A. lacustris* specimens were collected using gill nets (10 meters long, 1.5 meters high, mesh sizes from 3, 4, 5, and 6 - opposite knot length) and sieves (80 cm in diameter, 1 mm mesh). Each captured specimen was immediately euthanized and measured (mm), and a sample of muscle tissue was extracted using sterilized ceramic knives for isotopic analysis and metal concentration analyses. These samples were stored in labeled plastic containers and kept on ice until stocked in -20°C laboratory freezers. In the laboratory, samples intended for isotopic analysis underwent freeze-drying for a minimum of 24 hours before being ground and homogenized using a mortar and pestle, then stored in plastic tubes for shipment to the laboratory. Samples intended for potentially toxic metal analysis were sent preserved on ice for laboratory analysis.

We standardized the collection of five samples of each of the following food sources at each sampling site: filamentous algae (AL), periphyton/biofilm (PE), coarse particulate organic matter (CPOM), suspended matter (SM), macrophytes (MA), aquatic invertebrates (BE), and terrestrial invertebrates (IT). The sampling and processing of resources occurred as detailed in de Carvalho et al. (2024). Briefly, all resources were randomly collected at the sampling sites, aiming to cover the widest possible distribution area to capture variations in isotopic compositions. All samples were dried and ground into a fine, homogeneous powder before being sent to a specialized laboratory. Approximately 2 to 5 mg of animal tissue was used for isotopic analysis, and around 10 mg was required for basal resource samples.

2.3 Isotopic analysis

The samples were isotopically analyzed for carbon and nitrogen at the Center for Nuclear Energy in Agriculture (CENA) at the University of São Paulo. A total of 340 samples were analyzed, comprising 74 samples of *A. lacustris* individuals, 40 samples of filamentous algae (AL), 40 of periphyton (PE), 40 of coarse particulate organic matter (CPOM), 40 of fine particulate organic matter (FPOM), 40 of aquatic invertebrates (BE), 40 of terrestrial invertebrates (IT), and 26 of macrophytes (MA) (Supplementary material Table S1).

The isotopic ratios were measured using a continuous-flow isotope ratio mass spectrometer (CF-IRMS) coupled with a Carlo Erba elemental analyzer (CHN 1110) and connected to a Delta Plus mass spectrometer (Thermo Scientific). The results were reported as deviations from international reference standards in delta (δ) notation in parts per thousand (‰), calculated using the formula: $\delta X = [(R_{sample}/R_{standard}) - 1] \times 10^3$, where X represents ^{13}C or ^{15}N, and R represents the isotopic ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ (Barrie and Prosser 1996).

2.4 Metal concentrations analysis

A total of 58 specimens were examined to evaluate metal concentrations in the tissues of *A. lacustris* (Supplementary material Table S2). A muscle tissue sample from each fish was sent to the Tommasi Ambiental Environment and Food Analysis laboratory, located in Vitória, ES, to quantify Fe, Hg, and Mn. Processing occurred as detailed in Ferreira et al. (2020). In the laboratory, approximately 1.5 g of each sample was ground and subjected to digestion in a Microwave Multiwave GO system (Anton-Paar, Austria) using a solution composed of 65% nitric acid and 30% hydrogen peroxide. The digested material was then quantitatively transferred to a polypropylene tube, the volume was adjusted to 10 mL with type I water, and the solution was subsequently analysed using ICP-MS for the determination of trace elements by Inductively Coupled Plasma Mass Spectrometry.

Quality control measures for the metal analysis method were rigorously implemented to ensure analytical reliability and accuracy. Calibration standards were prepared at 5% HNO_3 (v/v) concentrations using appropriate dilutions from 1000 $mg.L^{-1}$ stock solutions acquired from Absolute Standards Inc (Hamden, Connecticut, USA) with ISO GUIDE 34 certification, all traceable to NIST standards. Rhodium (Rh) 1 $\mu g.L^{-1}$ was used as internal standard for Fe, Hg, and Mn, while Gold (Au) 10 $\mu g.L^{-1}$ was employed to reduce mercury memory effects. Detection (LOD) and quantification (LOQ) limits were established as follows: Fe (LOD: 0.00179 $mg.kg^{-1}$, LOQ: 0.0482 $mg.kg^{-1}$), Hg (LOD: 0.00008 $mg.kg^{-1}$, LOQ: 0.00006 $mg.kg^{-1}$), and Mn (LOD: 0.00004 $mg.kg^{-1}$, LOQ: 0.00013 $mg.kg^{-1}$). The laboratory employed Dorm-4: Fish Protein Certified Reference Material for Trace Metals as the most suitable standard for the analyzed samples (fish protein). Recovery rates (i.e., (Found value / Certified value) × 100) obtained were satisfactory: Fe (104.4%), Hg (92.7%), and Mn (107.9%), demonstrating analytical precision and accuracy. Results were expressed in $mg.kg^{-1}$ on wet basis according to the conversion formula: $C(mg.kg^{-1}) = c(\mu g.L^{-1}) \times V(mL) / [m(g) \times 1000]$, where C represents the final concentration, c is the solution concentration, V is the final solution volume, m is the sample mass, and 1000 is the conversion factor.

Statistical Analyses

To test whether $\delta^{13}C$ and $\delta^{15}N$ isotopic compositions, fish length (representing different ontogenetic stages), and Fe, Mn, and Hg concentrations in *A. lacustris* tissues varied among sampling sites, analysis of variance (ANOVAs) were performed after testing for normality and/or homogeneity of variance (Shapiro-Wilk and Levene's tests, respectively). The non-parametric Kruskal-Wallis test was used for non-normally distributed data. When significant differences were observed ($p < 0.05$), pairwise comparisons were conducted using Tukey's post-hoc test (normal distribution) and Dunn's test with Bonferroni correction (non-normal distribution) to identify significantly different means.

To evaluate whether the concentrations of each assessed metal in *A. lacustris* varied across the eight sampling sites and were related to individual size/fish length (ontogenetic stages) or $\delta^{15}N$ values, mixed-effects generalized linear models (GLMM) were performed, where the sampling locations were considered random effects. The concentrations of metals (Fe, Mn, and Hg) in fish tissues were log-transformed prior to analysis. To verify that the assumptions of the linear mixed-effects model were met, we conducted a set of diagnostic tests. Conditional independence was assessed by visual inspection of residuals versus fitted values, the autocorrelation function (ACF) of residuals, and the Durbin–Watson test. We also calculated the intraclass correlation coefficient (ICC) to evaluate the proportion of variance explained by the grouping factor. Exogeneity of random effects was examined by comparing fixed- and random-effects specifications through the Hausman test, and by inspecting potential correlations between random and fixed effects. Normality of random effects was evaluated using Q–Q plots of the random intercepts, the Shapiro–Wilk test, and kernel density estimates of their distribution. Independence of random effects was assessed based on the estimated covariance matrices of the random intercepts, likelihood ratio tests comparing models with and without random effects, and scatterplots of the random-effect estimates. Random effects (samplings sites) were initially included in the model; however, when variance estimates reached the boundary (singular fit), indicating that the random component did not contribute to explaining the variability, the random effect was excluded and the model was re-estimated without it (linear regression).

Bi-plot graphs were constructed to assess the trophic structure at each sampling site, using the isotopic compositions of *A. lacustris* individuals and food resources (x-axis: $\delta^{13}C$, y-axis: $\delta^{15}N$). The primary carbon sources assimilated by *A. lacustris* at each site were determined using Bayesian mixing models for stable isotopes (Parnell et al. 2010), using the MixSIAR package in R (Stock and Semmens, 2016). The selection of food resources for partitioning analysis was based on dietary habits documented in the literature and from the evaluation of the bi-plot graph. Thus, the following resources were considered for this analysis: periphyton, filamentous algae, suspended matter, and terrestrial and aquatic invertebrates. Coarse particulate organic matter (CPOM) and macrophytes were excluded from partitioning analysis as their isotopic compositions differed markedly from those of fish (Figure 2, Supplementary Material Table S1). Fractionation values used for consumers were 1.3 ± 0.3 ‰ for carbon and 2.9 ± 0.32 ‰ for nitrogen per trophic level (McCutchan et al. 2003). The relationship between the assimilation percentage of each item and the average metal concentration at each site was evaluated using linear regression, backward selection (Metal ~AL + BE + IT + MS + PE), after verifying its main assumptions. Linearity was assessed through diagnostic plots of fitted values versus residuals, the normal distribution of residuals was evaluated using both graphical methods (Q–Q plots) and Shapiro-Wilk test, homoscedasticity was checked through Breusch-Pagan test, and independence of observations was examined using Moran's I test.

3. Results

Isotopic compositions, fish length, and metal concentrations in *A. lacustris* tissues varied among sampling sites (Figure 2). *Astyanax lacustris* exhibited distinct $\delta^{13}C$ values among sites, with the highest enriched $\delta^{13}C$ values observed at site I1 and the most depleted at I3 (Figure 2a, Supplementary material Table S1). Differences in $\delta^{15}N$ were less pronounced, with the highest enrichment observed in *A. lacustris* from site I5 and the lowest values at C1 (Figure 2b, Supplementary material Table S1). The largest individuals were collected at sites C1 and I6, while the smallest were collected at I1, I2, and I5 (Figure 2c). Among the analyzed metals, mercury (Hg) showed the greatest variation in concentrations in fish tissues, with the highest concentrations observed in sites exposed to the iron ore derived from de Fundão dam, being particularly high at I1 and I2 sites (Figure 2e, Supplementary material Table S2). Both iron (Fe) and manganese (Mn) exhibited higher concentrations in fish from site I1, located in the reservoir of the Risoleta Neves Hydroelectric Plant (Figure 2d and 2f, Supplementary material Table S2).

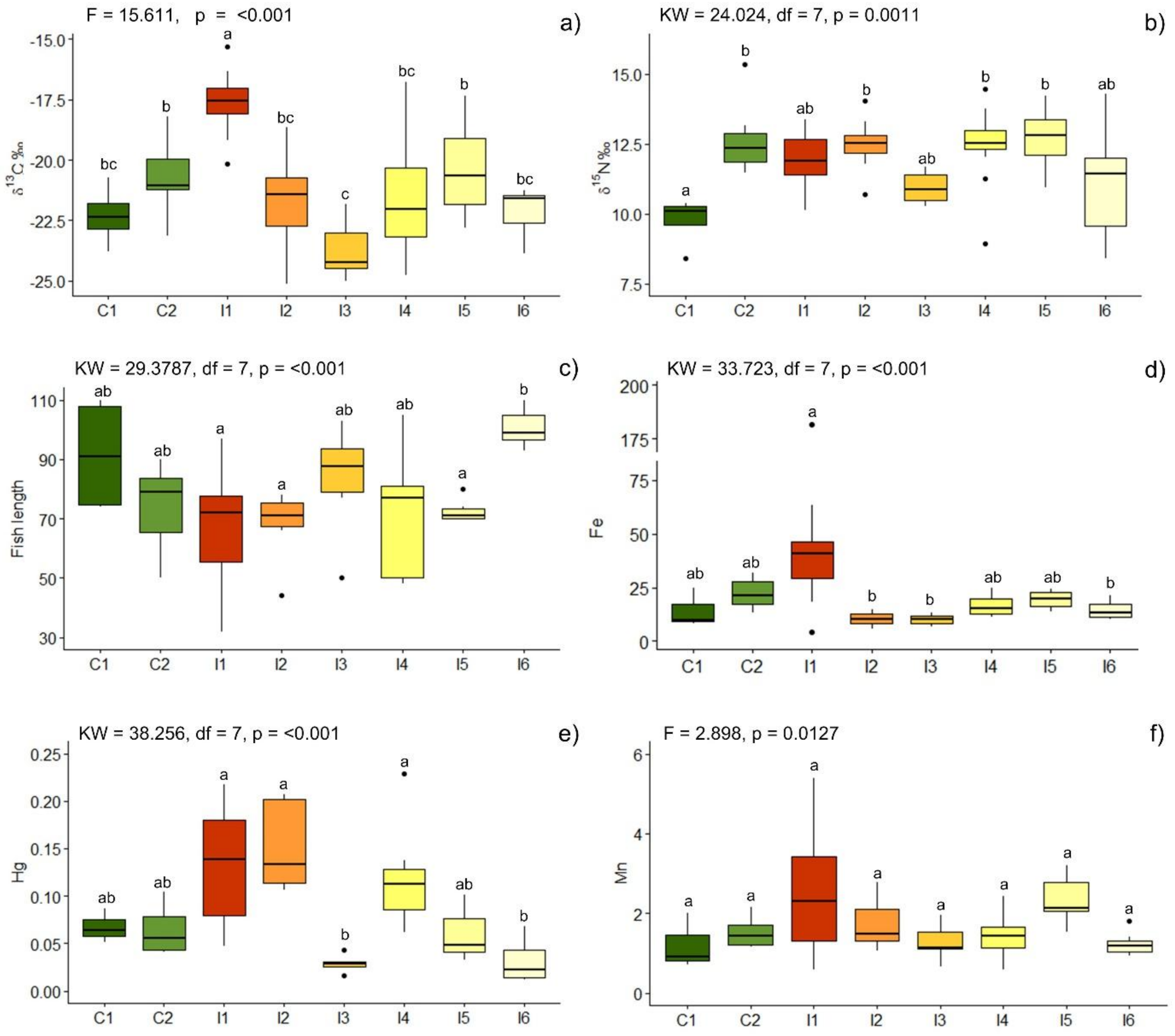

Fig. 2 Variation in carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic composition, fish length (mm), and metal concentrations (Fe, Hg, and Mn mg.kg-1) in tissues of *Astyanax lacustris* across the Doce River basin, at control sites (C1 – C2) and sites impacted (I1 – I6) by the mining tailings dam break

The sampling site factor accounted for 38% and 64% of the variation in Fe and Hg concentrations, respectively, while it did not influence Mn concentrations. A negative relationship was observed between fish length (mm) and Fe and Mn concentrations, explaining 16% and 21% of the concentrations, respectively (Table 2). This relationship was observed for the entire dataset but can also be verified for most individual sampling sites (Figure 3). Mercury was the only metal that showed a significant relationship between its concentration and $\delta^{15}N$ (Table 2), a pattern observed for the entire fish sample and most sampling sites (Figure 3).

Table 2 Relationships between metal concentrations (log-transformed Fe, Mn, Hg) and size or $\delta^{15}N$ of *A. lacustris*. In the tested GLMM models for Fe and Hg, sampling sites were used as a random effect ((log(metals) ~ Size + $\delta^{15}N$ + (1|Site)). For Mn, a linear regression (LM) provided a better fit (logMn ~ Size + $\delta^{15}N$). β ± SE represents the estimated coefficient of the predictor (β) ± standard error (SE), indicating the magnitude and precision of the effect; p represents the p-value of the test, with values <0.05 considered statistically significant; R^2_m = marginal variance explained by fixed effects (Size and $\delta^{15}N$); R^2_c = conditional variance explained by the total model (including both fixed and random effects)

Metal	**Fixed effects**	**β ± SE**	**p**	**R^2m**	**R^2c**
Fe	Size	-0.0093 ± 0.0029	**0.002**	0.16	0.54
	$\delta^{15}N$	-0.0023 ± 0.0226	0.919		
Mn	Size	-0.0081 ± 0.0022	**<0.001**	0.21	--
	$\delta^{15}N$	0.0043 ± 0.0203	0.832		
Hg	Size	0.0023 ± 0.0027	0.413	0.08	0.72
	$\delta^{15}N$	0.0789 ± 0.0207	**<0.001**		

The distribution of *A. lacustris* individuals in the bi-plot graph varied among sampling sites, differing even between the two control sites (C1 and C2) (Figure 4). A relatively wider range of $\delta^{13}C$ values was observed in fish tissues at sites I4 (-24.8 to -16.8‰) and I2 (-25.1 to -18.6‰), respectively, while the smallest $\delta^{13}C$ range was observed at site I6 (-23.9 to -21.2‰). Regarding $\delta^{15}N$, the highest range was observed at sites I6 (8.4 to 14.3‰), and I4 (8.9 to 14.5‰), and the lowest variation in $\delta^{15}N$ values was found at site I3 (10.3 to 11.7‰). The carbon sources assimilated by *A. lacustris* also differed among sites, although aquatic invertebrates (BE) were consistently among the primary assimilated resources (Figure 4, Table 3). Among primary producers, filamentous algae were more assimilated at site I1, while periphyton was more assimilated at sites C1 and I3. Terrestrial invertebrates were more assimilated at site I5 and suspended matter at I6 (Table 3).

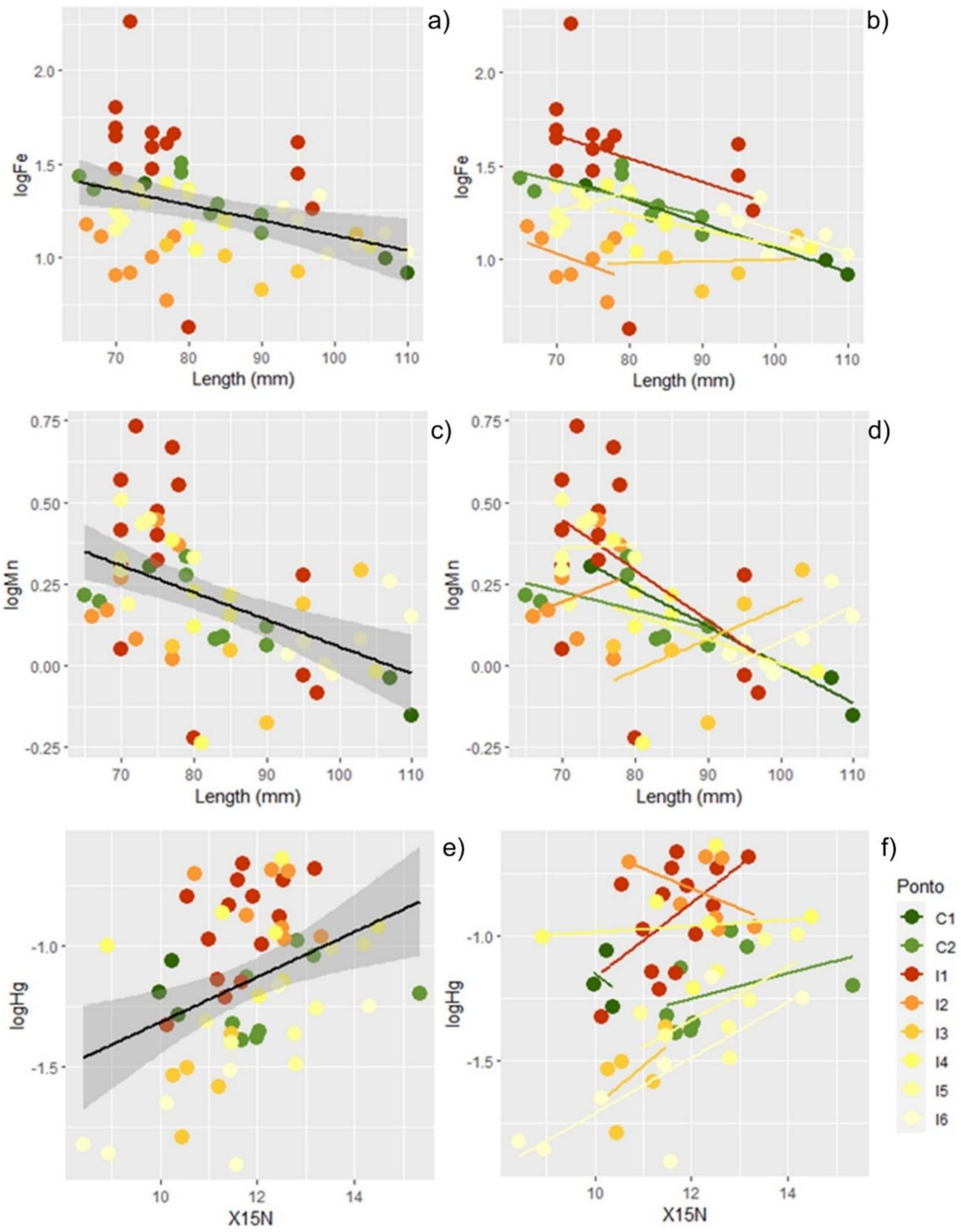

Fig. 3 GLMM models with significant relationships between iron (Fe) concentration and individual size (mm) (a and b); manganese (Mn) concentration and individual size (mm) (c and d); mercury (Hg) concentration and $\delta^{15}N$ enrichment in *Astyanax lacustris* individuals (e and f), evaluated with all sites combined (letters a, c, and e) and each site separately (letters b, d, and f)

Table 3 Proportion of assimilation (%) of each food resource by *Astyanax lacustris* at the eight sampling sites along the Doce River basin. AL = filamentous algae; BE = aquatic macroinvertebrates; IT = terrestrial invertebrates; MS = suspended matter; PE = periphyton. C = control sites; I = sites impacted by the mining tailings dam break

Site	AL	BE	IT	MS	PE
C1	0.094	0.325	0.122	0.223	0.235
C2	0.157	0.539	0.074	0.097	0.133
I1	0.434	0.199	0.069	0.160	0.137
I2	0.204	0.337	0.134	0.119	0.206
I3	0.092	0.285	0.205	0.129	0.290
I4	0.226	0.223	0.204	0.189	0.158
I5	0.124	0.214	0.329	0.179	0.153
I6	0.191	0.181	0.157	0.297	0.175

Among the analyzed metals, only Fe concentrations showed a significant relationship with items assimilated by *A. lacustris*, with this relationship observed only with periphyton ($R^2 = 0.52$, AdjR = 0.44, $p = 0.042$) (Figure 5). No significant relationships were observed between the assimilated items at each sampling site and the respective concentrations of Mn and Hg (Supplementary material Table S3).

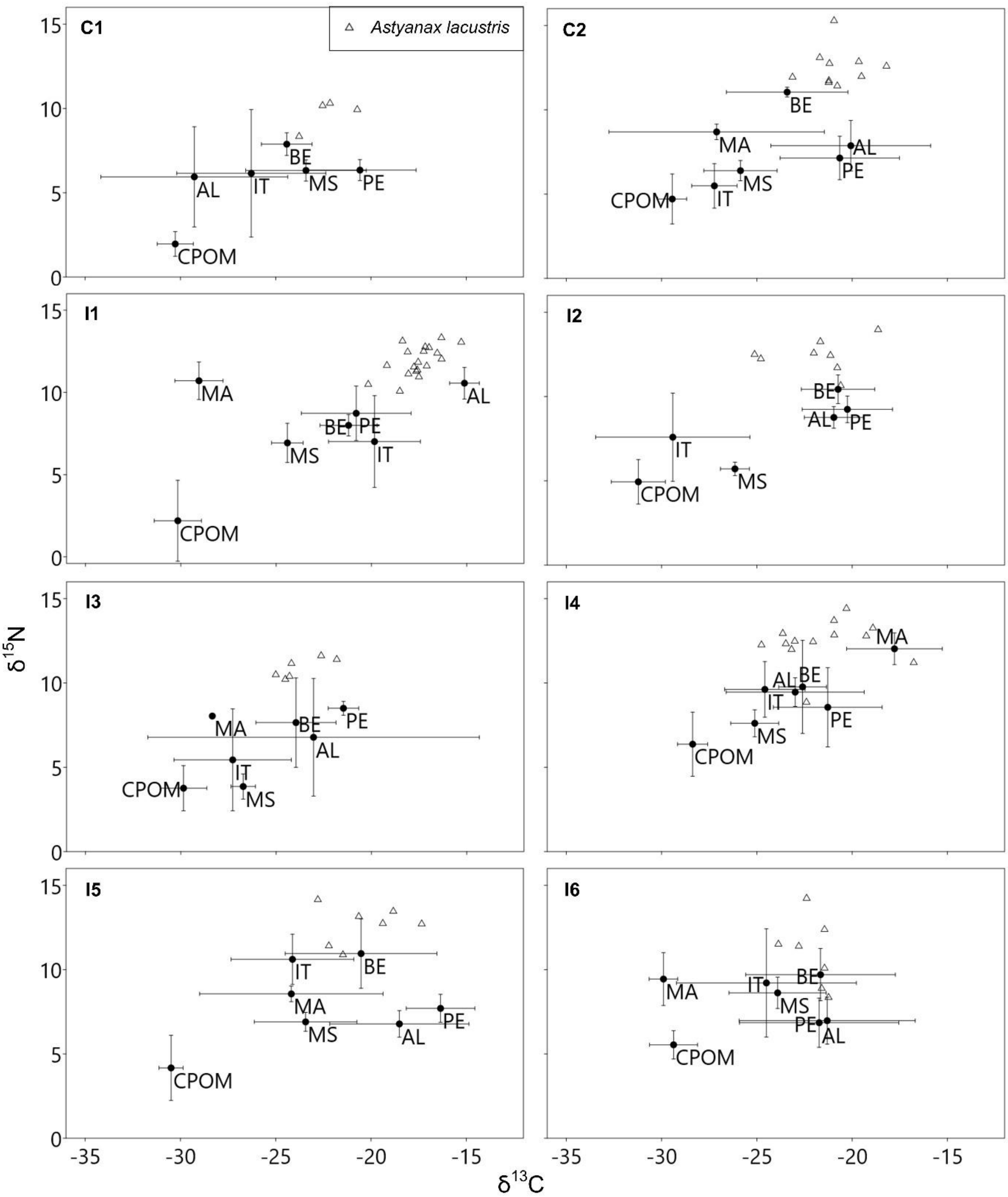

Fig. 4 Bi-plot graph representing the trophic structure of each sampling site, constructed using the isotopic composition values of each *Astyanax lacustris* individual (triangles) and the mean values with standard deviations (error bars) of the isotopic compositions of food resources. AL = filamentous algae; BE = aquatic macroinvertebrates; IT = terrestrial invertebrates; MS = suspended matter; PE = periphyton; MA = macrophytes; CPOM = coarse particulate organic matter. C = control sites; I = sites impacted by the rupture of the ore tailings dam

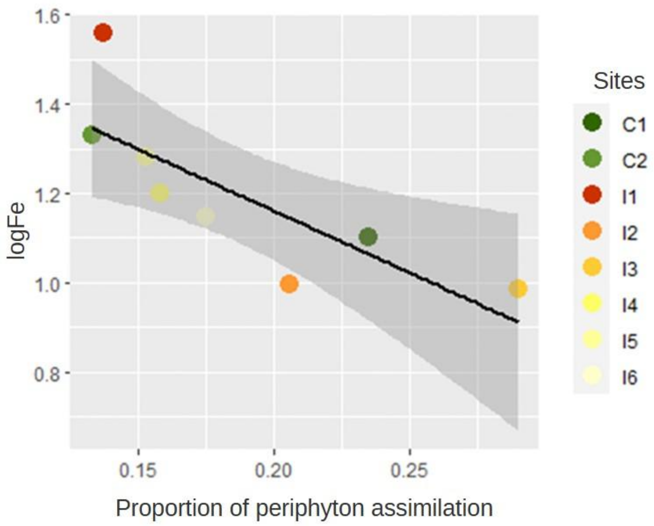

Fig. 5 Relationship periphyton - PE consumption and metal Fe concentrations in *A. lacustris* tissue at the sampling sites in the Doce River basin

4. Discussion

Our results indicate that *A. lacustris* exhibits environmental-dependent variation in its trophic ecology and metal concentrations in tissues in the Doce River basin. *Astyanax lacustris* showed varying Fe, Mn, and Hg concentrations at different sampling sites along the Doce River basin. Although these concentrations did not vary uniformly across sites, they were higher at sites closer to the Fundão dam rupture (especially at site I1). Our results suggested ontogenetic changes in the species' diet based on the perceived inverse relationship between size and the concentration of Fe and Mn. We also observed that individuals with higher $\delta^{15}N$ enrichment showed higher Hg concentrations both locally and among populations. Aquatic invertebrates were the most assimilated item by the specimens, although algae and periphyton were also important resources, depending on the location. The consumption of both animal and plant items by *A. lacustris* confirms its omnivorous habits and highlights its dietary plasticity in response to environmental conditions, as previously documented (Alonso et al. 2019; Bastian et al. 2021; Vidotto-Magnoni et al. 2021). As expected, fish from sites with higher periphyton assimilation (i.e., non-affected sites) had lower metal concentrations, but this relationship was observed only for Fe. Besides being widely distributed in the basin, our results reinforce the bioindicator potential of *A. lacustris*, as it reflects the characteristics of each location.

The passage of iron ore tailings and associated materials caused a disturbance on the Doce river substrate, contributing to increased levels of potentially toxic elements in the water, such as aluminium, arsenic, cadmium, copper, chromium, manganese, and nickel, highlighting lead and mercury with concentrations higher than the legal threshold (IBAMA 2015). In our study, the sampling site accounted for a significant part of the metal concentrations in fish tissues. Results varied across the basin, with the highest concentrations recorded at the site closest to the dam rupture, the Candonga Reservoir (I1). Of the 39.2 million cubic meters of tailings released during the Fundão dam collapse, approximately 24.3 million cubic meters of particles, mainly quartz, and hematite, were deposited along the mud path or retained in the Candonga Reservoir (Sánchez et al. 2018). Additionally, the conditions at this sampling site are distinct from the others, as it is a lentic environment. Thus, explaining the high metal concentrations in the fish tissues. Conversely, metal concentrations were low at the most pristine site, suggesting that the Santo Antônio River could serve as a baseline (control) for future monitoring studies aiming to access the basin's recovery.

The concentration of metals in *A. lacustris* was related to both fish size (Fe and Mn) and $\delta^{15}N$ (Hg). Smaller individuals presented higher concentrations of Fe and Mn, contrary to our expectations, as these elements are typically expected to bioaccumulate over time. This pattern was observed for most sites, including the control ones. Consistent negative relationships in fish size and potentially toxic element concentrations were also found by Merciai et al. (2014) across different fish species, sampling sites, and chemical elements. For *A. lacustris*, this trend might be the result of an increase in the consumption of allochthonous plant resources as fish grow older; such resources typically show lower metal concentrations than those in direct contact with water (such as periphyton, algae, and macrophytes). However, we underscore the need for further studies to understand the mechanisms involved in such complex size-concentration relationships. On the other hand, a positive relationship between fish $\delta^{15}N$ and Hg concentrations was observed. Such a positive relationship is well documented in the literature (e.g., Kidd et al. 1995; Marrugo-Negrete et al. 2018; Cyr et al. 2019), and most of the time, it has been attributed to the trophic position of fishes. Although we intuitively associate fish $\delta^{15}N$ with their trophic position, such relationship is not always straightforward, as the baseline needs to be considered (de Carvalho et al. 2020). For instance, filamentous algae, a basal resource, showed elevated $\delta^{15}N$ values at the site closest to the dam break (I1), with average values of 11‰. Consequently, fish from this site (that consume primarily algae) will also exhibit elevated $\delta^{15}N$ values. In this way, at the site closest to the dam collapse, *A. lacustris* individuals feed at the food web base but show enriched $\delta^{15}N$ values and high metal concentrations. Moreover, a possible effect of the Risoleta Neves Power Plant itself on the Hg concentrations at sites I1 and I2 cannot be ruled out since Hg concentrations tend to be elevated in hydroelectric reservoirs because of the methylation of inorganic Hg by microorganisms, which is stimulated by low oxygen concentration conditions (Mailman et al. 2006).

Because Fe and Mn are considered essential elements for many biological processes, there are no specific limits for these metals in fish under Brazilian legislation. However, excess Fe and Mn can be toxic to fish, leading to physiological and biochemical disruptions (Passos et al. 2022). High levels of Fe can cause histological alterations (Singh et al. 2019) and gill damage (Dalzell and Macfarlane 1999), while excess Mn can affect neurological functions (Costa-Silva et al. 2018). A study conducted in the Doce River basin after the Fundão dam collapse found that *A. lacustris* individuals exposed to the contaminated water exhibited high concentrations of aluminium (Al) and Fe in their gonads, accompanied by histological damage, including latency formation, the presence of atresia, invasion of immature cells into the lumen, and hyperplasia (Merçon et al., 2021). These findings highlight the fact that if the population of *A. lacustris* continues to be subjected to high levels of metals, the harmful effects on their gonads may further compromise the development and reproductive success of this species in the Doce River basin (Merçon et al. 2021). It is also essential to consider that metal concentrations in *A. lacustris* may be even higher since they vary according to the tissue analyzed. For Fe, for example, it has been observed that in the species *Oreochromis mossambicus*, the highest concentrations were found in the intestine, followed by yellow body fat, brain, gills, liver, heart, and finally white body fat (Oberholster et al. 2012).

Mercury, in contrast, is a metal that can be toxic at elevated concentrations. This metal may cause a range of harmful effects in fish at physiological, histological, biochemical, enzymatic, and genetic levels, impacting growth, sexual maturation, and organ development and inducing oxidative stress that damages DNA, proteins, and lipids, potentially leading to cellular death and organ dysfunction (Crump and Trudeau 2009, Morcillo et al. 2017). Contaminated fish can transfer mercury up the food chain, impacting predators such as birds, mammals, and humans through bioaccumulation and biomagnification (Cordoba-Tovar et al. 2022), potentially leading to serious health issues. Mercury, particularly in its methylated form, poses serious health risks to humans, including neurodevelopmental impairments in children, cognitive and motor dysfunction, and cardiovascular problems in adults (Stern 2005, World Health Organization, 2010; Karagas et al. 2012). In Brazil, the legislation establishes mercury limits for certain consumed fish species, such as 1.0 mg/kg for predatory fish and 0.5 mg/kg for other species, such as *A. lacustris* (ANVISA 1998, 2013). Although mercury levels observed in our study are below these regulatory thresholds, it is well established that even low levels can have adverse effects (Karagas et al. 2012), emphasizing the necessity of continuous monitoring to protect human health in contaminated ecosystems.

In addition, the interactions between Fe and Hg may influence mercury mobilization in aquatic systems (Branfireun et al. 2020). This occurs because iron oxides act as important “sinks” for mercury in aquatic environments, but dissolution or transformation of these minerals, especially under anoxic conditions, can release Hg, increasing its risk of transport, methylation, and bioaccumulation in fish and other aquatic organisms (Harris-Hellal et al. 2011). The bioaccumulation of heavy metals in fish, in turn, will be directly related to their diet composition, since some basal resources are considered important sources of Methylmercury in freshwater systems (Branfireun et al. 2020). Despite its tendency towards herbivory recorded in the literature (Vidotto-Magnoni et al. 2021), leaves from riparian vegetation (CPOM) do not appear to be directly assimilated by *A. lacustris* in the Doce River basin, as this resource exhibited C and N isotopic compositions that were quite distinct from those of the fish. In contrast, after invertebrates, periphyton was found to be an essential resource for this species. The highest assimilations of periphyton were observed at sites I3 and C1, which are highly interconnected as the Santo Antônio River (C1) flows into the Doce River in Naque (I3). Despite some anthropogenic interference, the Santo Antônio River is still considered one of the rivers with the best environmental quality in the Doce River basin (Vieira 2009, Fráguas et al. 2025). The periphyton community is strongly related to the concentrations of nutrients and suspended matter in water (Ren et al. 2021). High silicate concentrations, for example, reduce periphyton diversity, while the suspended matter concentration may inhibit the biomass (Ren et al. 2021). Consequently, sites not impacted by the dam break-derived materials (as C1) may present a higher periphyton biomass, explaining the greater consumption of periphyton by *A. lacustris*. As expected, in these environments with greater assimilation of periphyton, the concentration of metals in the fish was lower; however, this relationship was significant only for Fe. The fact that concentrations of Mn and especially Hg do not correlate with the sources assimilated by *A. lacustris* may be due to local contamination affecting all items equally. Therefore, metal concentrations in the fish tissue will be similar regardless of the consumed item. As predicted, this situation would only change with the ingestion of allochthonous items, such as terrestrial insects, but among all tested items, they had a lower assimilation rate by *A. lacustris*.

Despite its preference for certain food items, *A. lacustris* showed variation in the proportion of items consumed across different regions. Whereas at the most preserved site in the basin (C1), *A. lacustris* primarily assimilated aquatic invertebrates and periphyton, likely reflecting its natural diet, at the other sites, other food items became more important. The high trophic plasticity observed in the impaired sites can be explained by environmental changes and the availability of food items (Lobón-Cerviá and Bennemann 2000), which would allow its survival in resource-poor environments. This plasticity is more evident at the Candonga reservoir site, I1, where *A. lacustris* primarily assimilated filamentous algae, contrasting with the other lotic sites, where it primarily assimilated aquatic macroinvertebrates. A similar result was observed in another small characid, *Knodus moenkhausii* (Paiva et al. 2025), suggesting that the change in diet may be a consequence of the low availability of higher-level resources at this site, forcing the fish to rely on primary producers.

Our study demonstrated that the bioindicator potential of *A. lacustris* is particularly relevant, as it integrates information on dietary and ontogenetic variation and local metal contamination, providing a valuable tool for assessing environmental quality in impacted rivers. Moreover, due to its wide distribution across all sections of the River Paraopeba (Sousa et al. 2025), this species could also be used in studies assessing the environmental quality of this River basin, which was severely affected by the 2019 Córrego do Feijão Dam rupture. Although studies assessing metal concentrations in fish following mining dam failures are common worldwide, this is the first study, applied in the context of mining dam ruptures, that combines stable isotope analysis with metal assessments to investigate the mechanisms underlying metal bioaccumulation in fish. Our study is further distinguished from previous research by encompassing multiple regions across the Doce River basin, an approach made possible by the widespread occurrence of *A. lacustris*. Recent studies have shown that the frequency of mining dam failures is increasing, with the pattern of such incidents shifting from developed to developing countries (Islam and Murakami 2021). The most recent events, including the Fundão and Córrego do Feijão Dam collapses, both in Brazil, have caused severe social, economic and environmental damage. Consequently, the development and evaluation of novel methodologies to estimate and monitor the impacts of these disasters have become increasingly critical.

5. Conclusion

Our study highlights the abundant fish species *A. lacustris* as a sensitive bioindicator of ecosystem health, exhibiting dietary plasticity that reflects habitat quality and local metal contamination. Isotopic and metal analyses demonstrate that this species not only adjusts its diet according to environmental conditions but also mirrors the metal content in its tissues. While measured metal concentrations remain within current legal limits for human consumption, even low levels of metals can bioaccumulate and biomagnify through the food web, posing risks to higher predators and human health. Particular attention should be given to areas closest to the dam rupture, where metal concentrations are highest, species have the most dissimilar diet, and ecosystem impacts are most pronounced. Therefore, we strongly recommend regular monitoring of metal concentrations in fish across the Doce River basin, with intensified surveillance near the rupture site, combined with isotopic and trophic analyses to track ecosystem changes. In addition, management practices aimed at mitigating contamination from mining tailings should be implemented to reduce metal exposure in aquatic organisms and prevent further ecosystem degradation. These measures are essential to safeguard both environmental integrity and human health.

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STATEMENTS AND DECLARATIONS

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Authors' Contributions

All authors contributed to the study conception and design. Débora R. Carvalho: Conceptualization, Methodology, Data curation, Investigation, Statistical Analysis, Writing (original draft, review and editing); Isabela Miranda Guimarães: Conceptualization, Methodology, Data curation, Investigation, Writing (original draft, review and editing); Frederico F. Ferreira: Conceptualization, Methodology, Investigation, Writing (review and editing); Jorge A. Dergam: Funding acquisition, Project administration, Writing (review and editing); Marcelo Z. Moreira: Methodology, Writing (review and editing); Paulo S. Pompeu: Conceptualization, Methodology, Funding acquisition, Investigation, Project administration, Writing (review and editing).

Ethics Approval

The sampling, euthanasia, and transportation of organisms were authorized by the Ministério do Meio Ambiente (MMA), Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), Sistema de Autorização e Informação em Biodiversidade (SISBIO, number 80532-1), and the ethics committee of the Federal University of Viçosa (number 7982018).

Consent to Participate

This is not applicable

Consent to Publish

This is not applicable

Competing Interests

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 Statement

Data will be made available on request.