Assessing temporal shifts in trophic diversity in fish assemblages after the Fundão dam collapse

Patrícia Santos Fráguas[a], Débora Reis de Carvalho[b,c], Frederico Fernandes Ferreira[d], Jorge Abdala Dergam[d], Carlos Frankl Sperber[d] e Paulo Santos Pompeu[c]

[a]Programa de Pós-Graduação em Ecologia Aplicada, Departamento de Ecologia e Conservação, Instituto de Ciências Naturais, Universidade Federal de Lavras, 37203-202 Lavras, Minas Gerais, Brazil. psfraguas@yahoo.com.br Corresponding author ORCID: 0000-0002-1340-3488

[b]Lancaster Environment Centre, Lancaster University, Lancaster, United Kingdom; deboracarvalhobio@gmail.com ORCID: 0000-0001-8997-2145

[c]Laboratório de Ecologia de Peixes, Departamento de Ecologia e Conservação, Instituto de Ciências Naturais, Universidade Federal de Lavras, 37203-202 Lavras, Minas Gerais, Brazil. pompeu@ufla.br ORCID: 0000-0002-7938-1517

[d]Departamento de Biologia, Universidade Federal de Viçosa, 36570-000, Viçosa, Minas Gerais, Brazil. ORCID and email: 0009-0006-0341-3830, frederico.fernandes@ufv.br (Frederico Ferreira); 0000-0003-1395-1377, jdergam@gmail.com (Jorge Dergam); 0000-0002-0334-7216, sperber@ufv.br (Carlos Sperber).

Acknowledgements

The authors thank the Fish Ecology Laboratory at Universidade Federal de Lavras - UFLA for the support on laboratory processing. The authors would like to thank the Central of Analysis and Chemical Prospecting of the Universidade Federal de Lavras, and Financiadores de Estudos e Projetos (Finep), Fundação de Amparo à Pesquisa do estado de Minas Gerais (Fapemig), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) e Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes) for supplying the equipment and technical support for experiments involving isotope analyzes. This study was financed by a contract between Fundação de Amparo à Pesquisa do estado de Minas Gerais (FAPEMIG) and RENOVA Foundation – grant term nº 4800028061; and was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) - Finance Code: 32004010017P3. PSP was granted a research fellowship (302328/2022–0) by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Abstract

The rupture of the Fundão dam stands as one of the most significant environmental disasters of its kind on a global scale, profoundly affecting the aquatic ecosystem of Doce River Basin. By employing stable isotopes of carbon and nitrogen, we were able to trace matter and energy flow within ecosystems. In this study, we assessed the spatial and temporal variation, between 2020 and 2022, in species richness and trophic diversity in areas exposed (along a gradient in the main channel of the river) or unexposed (control sites in tributaries systems) to the mine ore tailings in the Doce River Basin. We tested the hypothesis that tailings reduce species richness; and that trophic stability is negatively affected by mining tailings. To estimate trophic stability for each sampling site, we calculated the Standard Ellipse Area (SEA), and six community-wide metrics based on stable isotopes. The three regions studied presented distinct patterns on trophic diversity. Control sites exhibited stability in trophic metrics over time. Affected regions close to the rupture of the dam exhibited significant fluctuations on all six community-wide metrics analyzed than the affected regions farther from the rupture.-Sites close to the rupture exhibited lower species richness, affecting mainly herbivores and piscivores. Our findings show the potential of using the isotopic approach in monitoring the ecological recovery of impacted ecosystems.

Keywords: Ichthyofauna; Stable Isotopes; Carbon; Nitrogen

Introduction

The breach of the Fundão Dam in the city of Mariana, Minas Gerais, Brazil, in November 2015, released approximately 66 million cubic meters of iron ore tailings into the watercourses downstream of its location (Governo do Estado de Minas Gerais, 2016). The iron ore tailing tide flowed into the Gualaxo do Norte, Carmo and Doce rivers, contaminating a stretch of over 650 km of the Doce River until its mouth in the city of Linhares on the coast of Espírito Santo (ANA, 2016). It also affected estuarine, marine, and coastal environments (Carmo et al., 2017). This event is considered one of the largest environmental disasters of its nature worldwide (Garcia et al., 2017; Hatje et al., 2017; Yamamoto, Pauly, et al., 2023). In addition to its impact on aquatic environments, the iron ore tailings and associated sediments affected an area of 1,176 ha (Omachi et al., 2018), suppressing most of the riparian vegetation in the vicinity of the breach (Fernandes et al., 2016) and directly impacting 457.6 ha of the Atlantic Forest (Omachi et al., 2018). The riverbed morphology was abruptly altered by the swift movement of these mining residues (Santos et al., 2019), leading to deposits in floodplains as well (Carmo et al., 2017).

In the aquatic fauna, the impacts of the Fundão Dam breach were immediate and extensive. The tailings caused massive mortality of fish (Governo do Estado de Minas Gerais, 2016), reduced the abundance of native species and increased that of non-native species, as well as altered the composition of fish (Salvador et al., 2022). The tailings further affected the growth of algae and microcrustaceans (Vergilio et al., 2021), microbial communities (Cordeiro et al., 2019), fish larvae (Bonecker et al., 2019), altered the niche of estuarine species (Andrades et al., 2020), reduced the genetic diversity of estuarine and marine fish (Biasi et al., 2023), and induced accumulation and stress responses due to heavy metal exposure in fish (Ferreira et al., 2020; Vergilio et al., 2021; Yamamoto, Onishi, et al., 2023). The disruption of these diverse communities may result in changes in resource availability, impacting the trophic web and energy flow within these systems (de Carvalho et al., 2024). Such changes have been documented in the literature using stable isotopes, albeit for impacts of a different nature, such as domestic and industrial sewage inputs (de Carvalho et al., 2020, 2021), land-use changes (Carvalho et al., 2015, 2017), and gradients of anthropogenic alterations in the environment (Horka et al., 2023; Medina-Contreras et al., 2021; Wang et al., 2021). Stable carbon (^{13}C) and nitrogen (^{15}N) isotopes are valuable tools for elucidating energy flow within trophic webs (Finlay, 2001; Peterson and Fry, 1987). The nitrogen composition of organisms may be used as an indicator of trophic position (Fry, 2006; Post, 2002), as an approximate increase of 2.9‰ in the nitrogen values of consumers at each trophic level is expected (McCutchan et al., 2003). In contrast, the carbon composition exhibits minimal variation across trophic levels, making it a useful indicator of primary energy sources sustaining organisms, given the distinct carbon values of different resources (Fry, 2006; Post, 2002).

Isotopic ecology provides precise means to trace major material and energy flows (Fry, 2006; Zanden and Rasmussen, 2001). However, rivers are environments prone to significant fluctuations (Palmer and Ruhi, 2019; Poff et al., 1997) which may be caused by natural seasonal or interannual changes unrelated to anthropogenic impacts. Therefore, studies assessing temporal changes putatively caused by a disturbance, such as the breach of the Fundão tailings dam, should seek to identify naturally stable patterns of parameters in these environments. These parameters should only undergo alterations in scenarios of ecological restructuring, for instance, during the recovery phase of a river following a major disturbance.

To evaluate trophic diversity changes in communities, metrics proposed by Layman and colleagues (Layman et al., 2007) have been applied, along with the assessment of niches using the standard ellipse areas (Jackson et al., 2011). These tools have been utilized to indicate anthropogenic impacts in watercourses (e.g. de Carvalho et al., 2020). The effects on trophic diversity and niche breadth are unpredictable, as both a decrease (Wang et al., 2021) and an increase (de Carvalho et al., 2020, 2021) have been observed. This variation may be attributed to the magnitude and type of impact to which aquatic environments are exposed.

Following the Fundão Dam breach, differences in the trophic niches of fish were observed in both freshwater (Calais et al., 2024; da Silva et al., 2024; de Carvalho et al., 2024) and estuarine environments (Andrades et al., 2021). Conversely, by using a set of reference conditions within the same basin, it was noted that while certain fish species might exhibit fluctuations in their isotopic signatures, parameters associated with community niche, such as the range of carbon and nitrogen values, as well as the distances between species in the isotopic niche, remained stable (Fráguas et al., unpublished results). Therefore, these parameters are potentially informative to indicate historical changes in river conditions (Fráguas et al., unpublished results).

Previous studies indicate that following the collapse of the Fundão dam, significant transformations in the fish fauna occurred, primarily associated with the presence of non-native species (da Silva et al., 2024; Petesse et al., 2023; Salvador et al., 2022), which can be reflected in the food web structure (de Carvalho et al., 2024) and trophic diversity (da Silva et al. 2024). Assessing the impacts on biota is crucial for the development and implementation of effective recovery strategies for degraded areas. Thus, this study aims to assess the temporal variation in species richness and trophic diversity of the fish assemblage following the breach of the Fundão ore tailings dam in the Doce River Basin. Based on two distinct years of sampling, at sites exposed and unexposed (control sites) to the iron ore tailings, we tested the following hypotheses: 1) tailings reduce species richness. We therefore expect that species richness will be higher in the second year of sampling in impacted regions and remain relatively stable between the two years in control sites. Among the impacted regions, since areas closest to the breach were the most affected, they will exhibit lower species richness. 2) trophic stability is affected by mining tailings. If there are indications of recovery of trophic stability in the Doce

River, these will be more evident further away from the breach site. Therefore, we predict that trophic diversity metrics will remain stable in control regions; impacted regions will be more unstable close to the rupture and less unstable farther from the breach. Through our results, we expect to evaluate whether there was a discernible pattern in the modification of isotopic structure following tailings impact, and thus assess whether trophic diversity metrics can be applied as a tool for monitoring conditions in the Doce River Basin.

Methodology

Study Area

The Doce River Basin encompasses an area of 83,431 km², located entirely within Brazil, spanning the states of Minas Gerais (86%) and Espírito Santo (14%) (ANA, 2016). The main river course is formed by the confluence of the Piranga and Carmo rivers, covering a distance of 888 km until it reaches the estuary at the Atlantic Ocean (ANA, 2005, 2016). The predominant climate in the region is humid tropical (Coelho, 2009). The Doce River basin can be divided into three regional units: Upper, Middle, and Lower Doce River. The Upper Doce River region encompasses the headwaters of the Doce River up to the mouth of the Matipó River. The Middle Doce River segment includes areas downstream from the Matipó River mouth to the border between the states of Minas Gerais and Espírito Santo. The Lower Doce River comprises areas downstream from the Mascarenhas Hydroelectric Plant to the estuary at the Atlantic Ocean (PIRH – Doce, 2010). The rainfall pattern is well-defined, with a dry season from April to September and a rainy season from October to March. The majority of the basin is situated in the Atlantic Forest biome (98%), with the remaining portion in Cerrado areas (ANA, 2016).

Our sampling design includes nine sampling sites distributed across areas exposed (six sites) and unexposed (three sites) to the iron ore tailings from the Fundão dam. The six exposed sites are located in the Doce River gradient, flowing from the area close to the dam breach to the river mouth. Three of them are located in the upper course (U1, U2, and U3) and three are in the middle course (M1, M2, and M3). Due to the widespread impact of tailings on the majority of the Doce River, it is not feasible to select control sites within the main river channel. In the absence of isotopic characterization of the fish fauna prior to the dam failure, baseline data for this river are also unavailable. Consequently, the only viable approach to represent pre-dam failure conditions is through sampling in tributaries. To account for the environmental variability across the river basin, tributaries were strategically selected from different regions, specifically the upper and middle sections of the basin. The three unexposed sites are tributaries and were considered as negative control sites represented by the rivers Piranga (C1), Santo Antônio (C2) and Manhuaçu (C3). The first two sites (C1 and C2) are situated in the upper region of the Doce river basin. The Piranga River basin covers an area of 6,606 km², where livestock farming is the predominant activity (IGAM, 2010b). The Santo Antônio River basin spans an area of 10,429 km², with the majority of it within the Atlantic Forest biome and a small portion in the Cerrado biome. This sub-basin is the best preserved among those that comprise the Doce river basin (IGAM, 2010c). The Manhuaçu River (C3) is located in the middle Doce river region. This basin covers an area of 8,826 km², where agro-pastoral activities are prevalent (IGAM, 2010a). Anthropogenic pressures in these three regions are primarily associated with the inefficient treatment of domestic effluents and the inadequate disposal of solid waste (Figure 1).

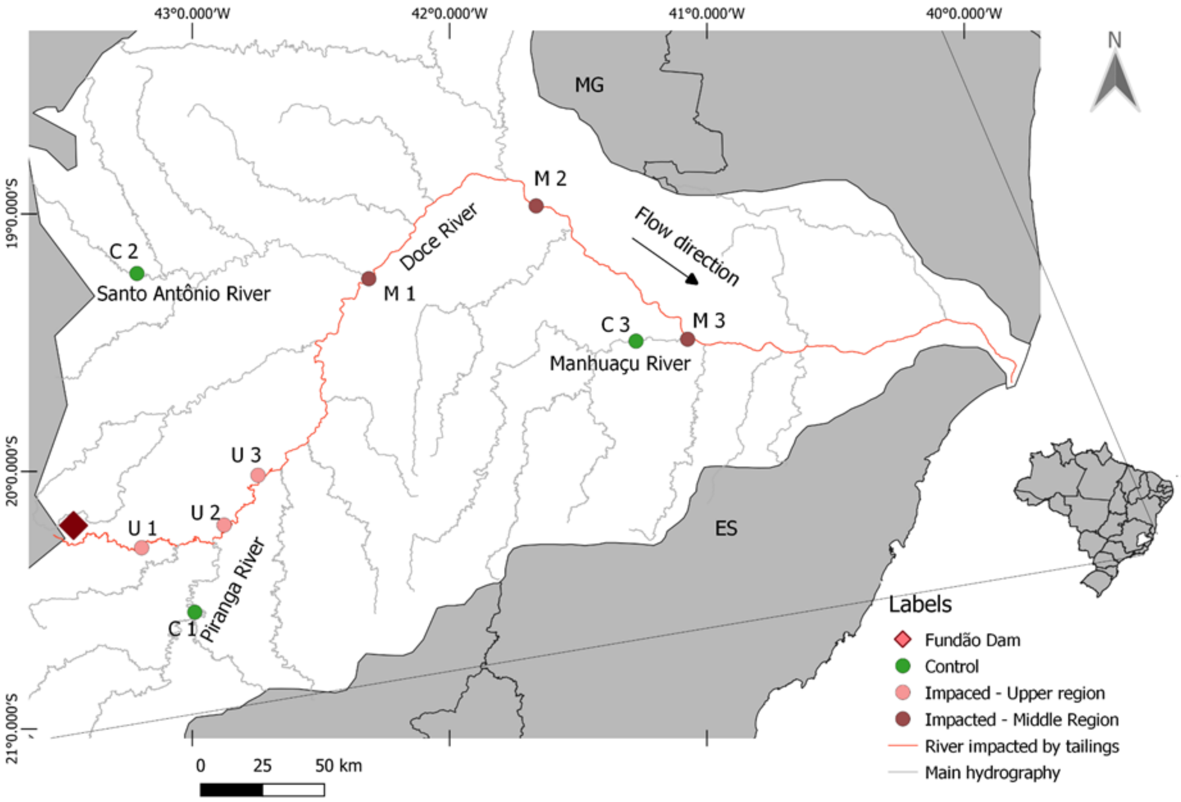

Fig. 1 Study area with nine sampling sites distributed along the Doce River Basin in the state of Minas Gerais, Brazil. C = Control, U= Upper course, M= Middle course.

Data Collection

To evaluate the temporal variability in fish trophic diversity metrics, the nine sites were sampled during two dry seasons, in 2020 (July/August), and in 2022 (July/August/September).

Fish Collection

Fish collection was conducted with complimentary sampling methodologies using gill nets, trawls, and sieve nets. Gill nets (mesh size 3-16 cm, length 20 m) were continuously exposed for 14 hours at each sampling site. Trawls (5 m length, mesh size 1 mm) were employed in shallow and littoral areas. Sieves (diameter 80 cm, mesh size 1 mm) were utilized in macrophyte beds near the shore. Sieves and trawls were deployed for approximately 2 hours in each sampling site. In the field, collected fish were measured, weighed, and identified. A sample of muscle tissue was obtained from each individual in the field and immediately frozen until the laboratory processing to prevent decomposition. At least one individual from each collected species was isotopically analyzed. Stable isotope analyses were conducted for up to five individuals of each species at each sampling site.

All collection procedures were authorized by SISBIO – Biodiversity Authorization and Information System – Nº. 10327-3, and the Animal Ethics Committee of UFLA (CEUA – UFLA), Nº. 034/21.

Sample Processing

In the laboratory, fish tissue samples were lyophilized for 24 hours, ground into a uniform powder, and stored in Eppendorf tubes. Following sample preparation, the samples were sent for isotopic analysis. In 2020, the samples were analyzed at *Centro de Energia Nuclear na Agricultura* (CENA) at the *Universidade de São Paulo* (USP). In 2022, the samples were sent to the *Central de Análises e Prospecção Química* (CAPQ) of the *Universidade Federal de Lavras* (UFLA). The fish samples were weighed (1000 µg) in tin capsules (5 mm x 8 mm). After weighing, to determine the isotopic composition of Carbon and Nitrogen in the samples, the capsules were introduced via an automatic sampler into the elemental analyzer.

Isotopic Analysis

To determine the isotopic ratio in 2020, we used a continuous-flow isotope ratio mass spectrometer (CF-IRMS) with a Carlo Elba analyzer coupled to a Delta Plus mass spectrometer (Thermo Scientific). In the second sampling, in 2022, we used a Delta V Plus Isotope Ratio Mass Spectrometer (Thermo Fisher) coupled with a Flash IRMS elemental analyzer interfaced with ConFlo IV. The results were obtained by using Isodat 3.0 and and are expressed as relative differences in international reference systems, using the delta (δ) notation, in parts per thousand (‰), and calculated using the following formula: $\delta X = [(R\_sample/R\_standard) - 1] \times 10^3$, where X is ^{13}C or ^{15}N, and R is the isotopic ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ (Barrie and Prosser, 1996).

Statistical Analysis

Differences in species richness among the three studied regions and between the collection years for each region were tested using Generalized Linear Models (GLM) with Poisson distribution, overdispersion was checked for the analysis. We ran the models using the MuMIn package (Bartón, 2023). We implemented a hierarchical model classification using the dredge function, and model selection was carried out using the Akaike Information Criterion (AIC). We chose models that had the AIC value of ≤2. We also tested the interaction between years and regions, but since no model had the AIC ≤2, data are not presented here.

To assess differences in isotopic niches between impacted and control sites in the two sampling periods, we employed the Standard Ellipse Area (SEA). SEA represents the isotopic niche and illustrates the richness and uniformity of resources consumed by the fish assemblage (Bearhop et al., 2004). The graphical representation of ellipse areas was performed using the SIBER package (Jackson et al., 2011) in the R program. To increase the accuracy of comparisons, a sample size correction was used, noted by the subscript "c". Using SEA_c, it is possible to compare the niches of populations with different sample sizes (Jackson et al., 2011). The percentage of niche overlap (where 100% represents total overlap), serves as a quantitative metric of diet similarity among species (Hill et al., 2015). Differences in the percentage of niche overlap between 2020 and 2022 were tested using Generalized Linear Models (GLM) with binomial distribution. GLM analyses were conducted in the R software (R Core Team, 2024) using the MuMIn package (Bartón, 2023).

Trophic diversity in the two sampling periods was evaluated using metrics proposed by Layman (2007), which were restructured by the Bayesian method for community comparisons (Jackson et al., 2011). These metrics were then calculated using the SIBER package (Jackson et al., 2011) in R. The first four metrics provide information about the overall community spacing in the biplot space ($\delta^{13}C$ and $\delta^{15}N$). Carbon range (CR): carbon amplitude used by species reflecting the resources that are assimilated by the fish assemblage – measures the distance between the species that have the most enriched and most depleted $\delta^{13}C$ values; Nitrogen range (NR): nitrogen amplitude used by species representing the variation in trophic levels – measures the distance between the species that have the most enriched and most depleted $\delta^{15}N$ values; Total area (TA): is the convex hull area occupied by all the species in isotopic space; Centroid distance (CD): a measure of trophic diversity – it is the average Euclidian distance of each species to the C or N centroid. The last two metrics indicate the relative positions in the niche space and act as proxies for trophic redundancy: Mean nearest neighbor distance (MNND): a measure of trophic redundancy – it is the mean of the Euclidean distances to each species' nearest neighbor in the biplot space; Standard deviation of the nearest neighbor distance (SDNND): represents the distribution of fish community trophic niches and is less influenced by sample size than MNND. We used collections (2020 and 2022) as our temporal scale and sampling regions (control, upper, and middle) as our spatial scale to calculate the trophic diversity metrics. Trophic diversity metrics were calculated for both collections (2020 and 2022). We also present the values of trophic diversity metrics for each region (control, upper, and middle course) in a boxplot graph presenting the subtraction of the values of 2022 by the values of 2020 to assess the variation in metrics over time.

To assess changes in the set of metrics as a means of portraying the trophic diversity of the fish assemblage, we performed a Principal Component Analysis (PCA) combining all metrics. To evaluate data dispersion within each group in the PCA, we performed a Permdisp analysis in the PRIMER program (Clarke and Gorley, 2006).

Results

A total of 953 individuals representing 65 species were analyzed. We sampled 496 individuals from 50 species in 2020, and 457 individuals from 55 species in 2022. Of these, 40 species were present in both samplings, 10 were collected only in 2020, and 15 occurred only in 2022 (Table 1). Species richness differed among the evaluated regions ($z = -2.03$, $p = 0.04$), with the upper region having lower species richness. Even though the middle region showed higher species richness in the second sampling year, there was no variation in richness between sampling years ($p > 0.05$) (Figure 2).

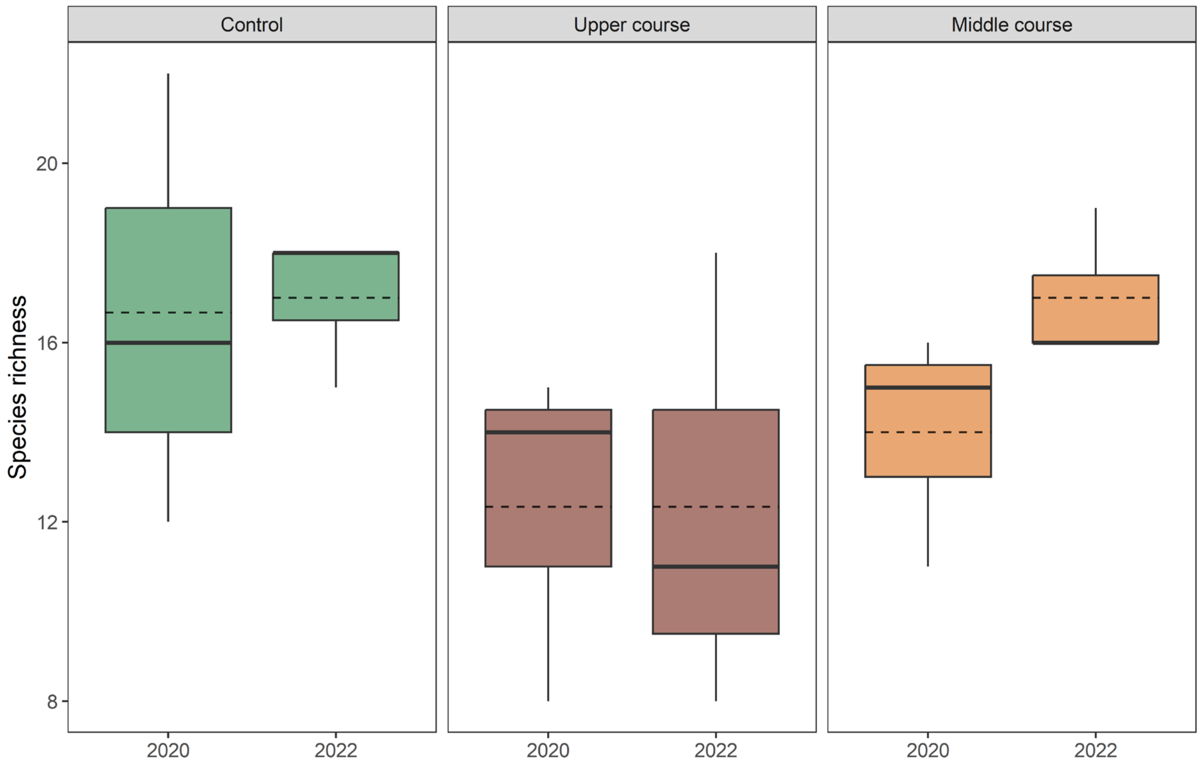

Fig. 2 Species richness in each region of the basin (Control, Upper, and Middle courses) in the two collection years (2020 and 2022). Line inside the box: median (second quartile), lower and upper limits of the box: first and third quartiles, whiskers = 1.5 x IQR (Inter Quartile Range), dashed line = mean.

Table 1: Number of samples of each species collected at each site in the two collection years. The * indicates non-native species in the Doce River basin. C = Control, U= Upper course, M= Middle course.

	2020										2022								
Species	C1	C2	C3	U1	U2	U3	M1	M2	M3		C1	C2	C3	U1	U2	U3	M1	M2	M3
Astyanax lacustris	3	4	10	2	19	8	6	13	7	-	4	2	5	3	4	8	5	5	5
Astyanax sp.										-	6		1						
Astyanax taeniatus										-			6						
Australoheros facetus				1						-									
Brycon dulcis						1				-									
Brycon opalinus		6								-									
Characidium cf. *krenak*										-		1							
Characidium timbuiense		3								-									
Cichla kelberi *			3							-									3
Cichla monoculus *			1							-									
Clarias gariepinus *			1			1				-						1	1		1
Coptodon rendalli *			1		5	5		11		-					5				
Crenicichla lacustris			2							-		1	1						
Crenicichla lepidota *									6	-									2
Cyphocharax gilbert						1				-			2						
Delturus carinotus	1	3	4							-	5	2							
Deuterodon cf. intermedius			7				5			-									
Euryochus thysanos										-		5							
Geophagus brasiliensis	6	11	10	1	2	6		8		-	7	5	5	5	1			4	1
Gymnotus aff. *carapo*										-								1	
Gymnotus sylvius	3		3			1			2	-								1	
Harttia loricaformes										-			1						
Hasemania cf. *nana*				1						-						5			
Henochilus wheatlandii		5								-		2							
Hisonotus sp.										-			3				1	1	

Hisonotus thayeri										-	1	1							
Hoplias intermedius		2	3	1	6	6	4	2	6	-	2	1	3	1	2	1	1	5	5
Hoplias malabaricus										-						1			1
Hoplosternum littorale *			6			1				-			5						
Hyphessobrycon eques *										-	1					5			
Hypomasticus copelandii		1	1							-		2						1	
Hypomasticus mormyrops										-		5							
Hypostomus affinis	4			5						-	2		2	1	1	5	2	3	
Hypostomus luetkeni	3	5	2		1			2	1	-	5	4						2	5
Knodus moenkhausii *	5	5	5		5	5	5	5	5	-	5	5	5	3	5	6	5	5	
Lophiosilurus alexandri *								1		-							2		
Loricariichthys castaneus			2		3		4			-			3		4	1	2		2
Megaleporinus conirostris	2		4		4			1	1	-	2								2
Oligosarcus acutirostris		3					3	7	7	-	5	5				5		3	5
Oligosarcus argenteus	7	4	1		7	6	5	1		-			5		5		3		
Oreochromis niloticus *					7	5	6	13	5	-					5		5		5
Pachyurus adspersus	4	8						1		-	5	5							
Parotocinclus doceanus			1			3				-	1								
Parotocinclus sp.		5	1							-									
Phalloceros cf. *uai*										-	5	1							
Pimelodus maculatus *							2		1	-							3	1	5
Poecilia reticulata *									1	-				9		3			
Poecilia vivipara			3						4	-								5	
Prochilodus argenteus *									1	-									
Prochilodus costatus *							3	5		-						1	5	4	1
Prochilodus vimboides					1	5		1		-						5			1
Psalidodon fasciatus	1				3					-	5	5		1	5	5	5	5	
Psalidodon sp.		5								-									
Pygocentrus nattereri *								3		-								1	
Rhamdia quelen	1			3						-									

Salminus brasiliensis *						1	1		-							2		
Serrapinnus heterodon					2				-			1			5	6	1	
Synbranchus marmoratus								2	-								1	
Trachelyopterus striatulus		2						1	-									4
Trichomycterus alternatus	6								-	5			6				2	
Trichomycterus astromycterus				1					-						6			
Trichomycterus cf. *immaculatus*									-						5	1		
Trichomycterus cf. *ipatinga*									-						5			
Trichomycterus sp.			1	3					-					2				
Trichomycterus tantalus									-	1	1							

When comparing the isotopic niche of each sampling site between the two years, it was observed that the control sites, especially C1 and C2, exhibited little variation. In contrast, several impacted sites, both in the upper (U1 and U2) and middle courses (M1), showed divergent niches between years. However, the differences in niche overlap were not significant among the three evaluated regions (AICc = 10.3 delta = 0.00 weight 1) (Figures 3, 4 and Online resource).

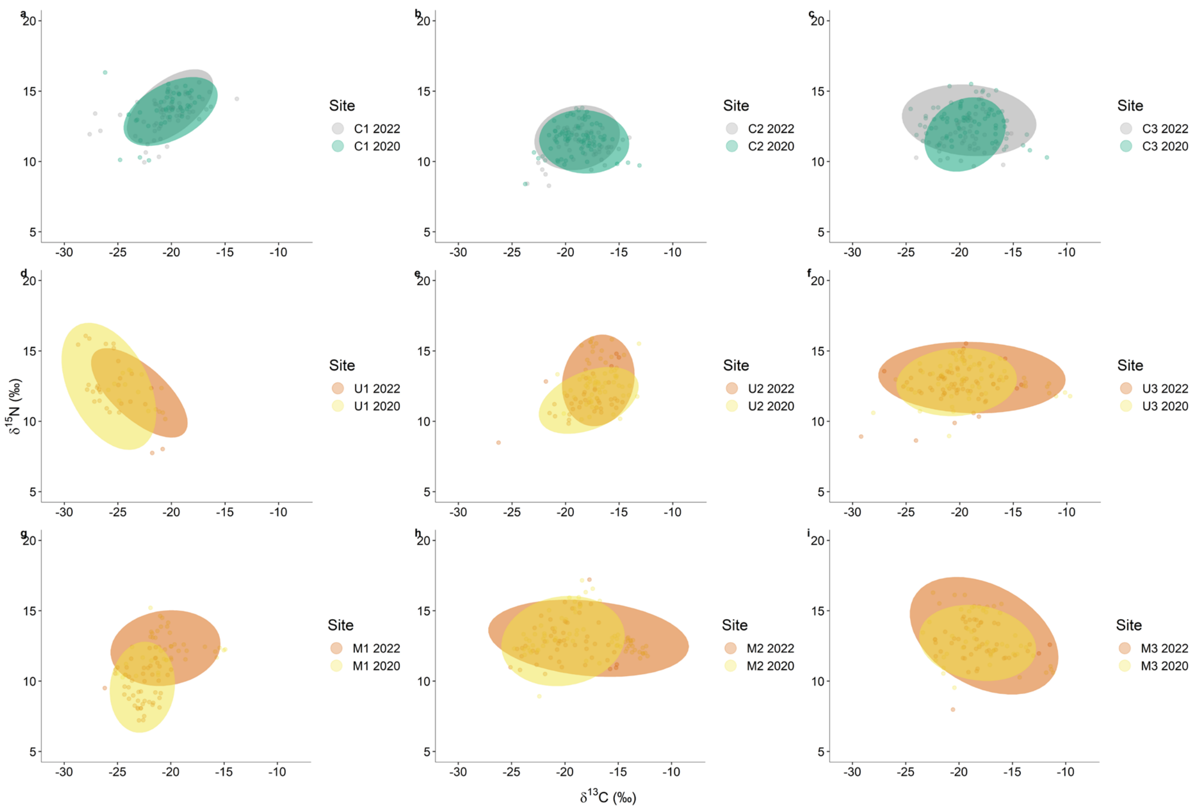

Fig. 3 Ellipse Area (Standard Ellipse Area - SEA - calculated using a 95% confidence interval) representing the niches of fish assemblages at each sampling site in Doce river basin. Light green ellipses represent assemblages from control sites in 2020 and dark green in 2022. Orange ellipses represent assemblages from impacted sites in 2020, and red ellipses represent those in 2022.

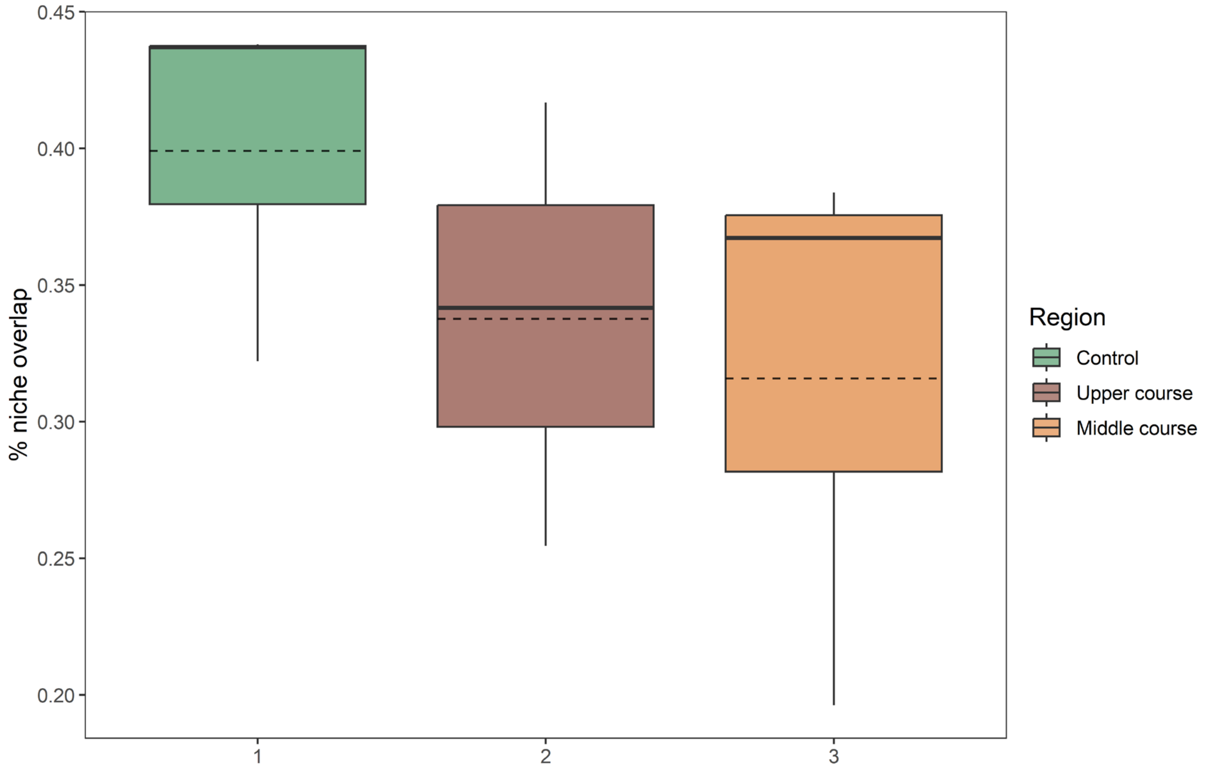

Fig. 4 Boxplot depicting the percentage of niche overlap between 2020 and 2022 for the three analyzed regions. No differences were observed among regions and years. Lines within the box: full line = median (second quartile) and dashed line = mean; lower and upper limits of the box: first and third quartiles, whiskers = 1.5 x IQR (Inter Quartile Range).

The trophic diversity metrics were calculated for both collection years (Table 2). By subtracting the values of the metrics obtained in 2022 from those recorded in 2020, it was possible to assess the variation of each metric over time (Figure 5). For most evaluated metrics, the variation of values between the years in the control region was very close to zero, indicating stability throughout the study assessed period. Meanwhile, the greatest variation in trophic diversity metrics was observed in the upper region (Figure 5).

Table 2: Layman's metrics for the years 2020 and 2022. CR = Carbon range, NR = Nitrogen range, TA = Total area, CD = Centroid distance mean, MNND = Mean nearest neighbor distance, SDNND = Standard deviation of the nearest neighbor distance.

	C1	C2	C3	U1	U2	U3	M1	M2	M3	Year
CR	6.05	5.16	9.72	3.94	3.54	17.23	8.13	10.64	9.15	2020
	9.08	6.46	5.39	4.78	10.97	7.35	4.84	8.57	8.97	**2022**
NR	4.91	3.96	4.26	3.00	3.75	4.04	3.84	4.52	5.74	2020
	3.94	3.67	4.27	5.88	5.45	3.05	4.74	4.49	3.56	**2022**
TA	14.60	11.69	27.00	8.27	8.02	33.94	15.05	26.83	29.94	2020

	C1	C2	C3	U1	U2	U3	M1	M2	M3	Year
	17.95	15.85	11.62	9.79	16.22	12.86	12.93	23.01	18.77	**2022**
CD	2.06	1.62	2.63	1.75	1.48	3.57	1.74	2.91	2.86	2020
	1.90	1.77	1.62	1.77	2.36	1.80	1.43	2.18	2.34	**2022**
MNND	0.82	0.66	0.79	0.93	0.54	1.39	1.27	0.91	1.22	2020
	0.67	0.63	0.66	1.24	1.44	0.57	0.65	0.92	1.07	**2022**
SDNND	0.54	0.61	0.47	0.55	0.28	1.01	1.06	0.43	0.61	2020
	0.76	0.23	0.37	1.21	1.83	0.28	0.54	0.56	0.44	**2022**

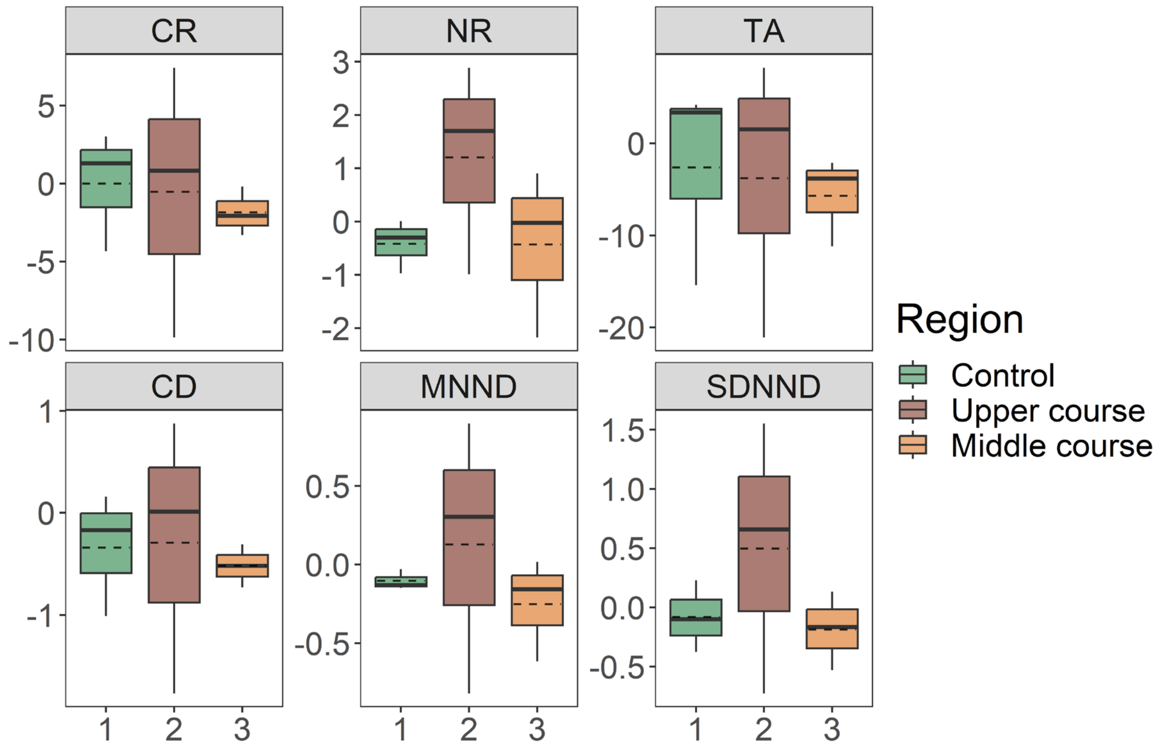

Fig. 5 Boxplot of the subtraction of Layman's metrics values between the years 2022 and 2020 for the fish assemblage sampled in Doce river basin. CR = Carbon range, NR = Nitrogen range, TA = Total area, CD = Centroid distance, MNND = Mean nearest neighbor distance, SDNND = Standard deviation of the nearest neighbor distance. Line inside the box: median (second quartile), lower and upper limits of the box: first and third quartiles, whiskers = 1.5 x IQR (Inter Quartile Range), dashed line = mean.

The first two axes of the PCA explained 84.78% of the variation (58.91% on axis 1 and 25.87% on axis 2) in Layman's metrics. Overall, trophic diversity represented by Layman's metrics was more similar among control sites. The middle course sites were closer to the control sites in terms of metrics compared to the upper course sites. The most affected sites, in the upper course, were more distinct from each other in terms of trophic diversity metrics (Figure

6). Region (p = 0.001) played a more significant role in shaping the trophic diversity of the fish assemblage (Table 3).

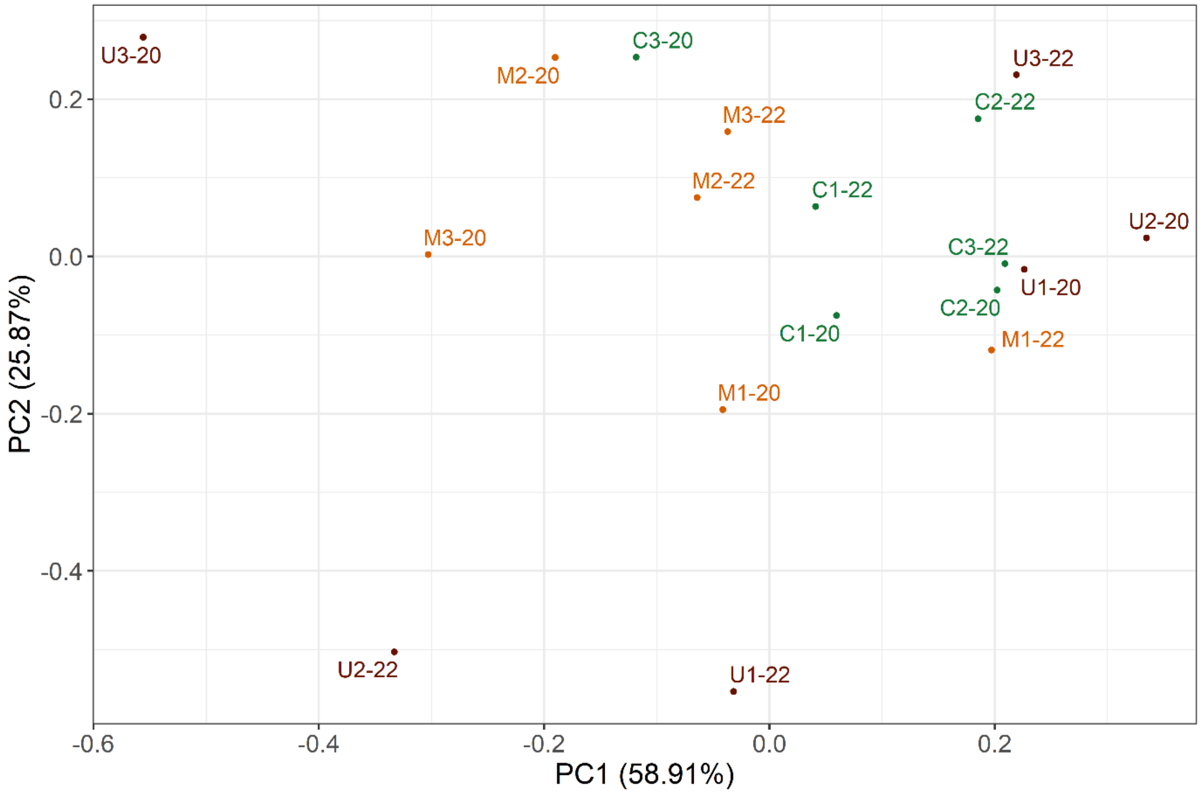

Fig. 6 Principal Component Analysis (PCA) of Layman's metrics between the collection years 2020 and 2022 for the fish assemblage sampled in Doce river basin. Control sites (C1, C2, C3); Upper Course (U1, U2, U3) and Middle Course (M1, M2, M3). Sampling year 2020 was represented by the number 20, while 2022 was represented by the number 22.

Table 3: Comparison of data dispersion related to Layman's metrics between years, regions, and their interaction (PERMDISP).

Variables	F	P
Region	12.11	0.001
Year	0.922	0.434
Region + Year	4.52	0.126

Discussion

Our results provide insight into the spatial variation in both species richness and trophic diversity in a basin significantly impacted by anthropogenic activities, such as the Rio Doce basin. Regarding trophic diversity metrics, our hypothesis was fully confirmed, as our findings highlight distinct patterns across the three regions studied. The control sites remained stable across both years, whereas the upper course region was highly unstable, with great variation on trophic diversity metrics. The overall trophic diversity of the middle course region was similar to that observed in the control sites. Species richness also exhibited spatial variation. The control sites showed a higher average of species richness in both samplings, while sites closest to the tailings dam rupture exhibited lower species richness. Regarding temporal variation, our hypothesis was partially confirmed, as species richness did not increase in the second year of sampling in impacted regions. No significant differences in the temporal niche overlap were observed among the three regions.

Analyzing the trophic diversity metrics of the community reveals distinct trends in the three regions. The control sites, even if geographically distant, exhibit a similar pattern. In contrast, impacted sites, despite being geographically close, are much more unstable over the two years, showing significant variations in trophic diversity metrics. This suggests that even seven years after the dam rupture, the impacts on the trophic structure of the fish assembly are still considerable. Regions closer to the rupture show greater instability in their trophic structure. A study of *Knodus moenkhausii*'s diet in the Doce River Basin also found that the species' diet was also most distinct in the most impacted areas compared to other sites (Paiva et al., 2024). Regions that were farther from the rupture, although also undergoing changes, tend to exhibit smaller variations than those observed in sites closer to the impact. Recent surveys indicate that most non-native species present in the basin before the dam rupture are still found in the Doce River channel, in addition to other non-native species that began to occur in the area after the Fundão dam rupture (Petesse et al., 2023). The growing number of non-native species highlights their capacity for establishment even under adverse conditions. One of the factors associated with invasion success is trophic plasticity (Tonella et al., 2018), in which species can adjust their diet to the availability of feeding resources in the new environment, which may be reflected in the niche space occupied by the fish community. Studies conducted five years after the Fundão dam collapse indicate that non-native fish species occupy broader isotopic niches than native species, often assimilating a range of carbon not exploited by other species (da Silva et al. 2024). Adding this type of impact to the basin, these highly unstable areas may favor non-native species over native species in the region, justified by the greater niche breadth of non-native species. This, in turn, may further increase the pressure on the region's native biota.

The impacted region, particularly the upper course of the Doce River, exhibited substantial variation in all metrics' breadth. Regarding the variation in trophic redundancy (MNND), substantial fluctuations in carbon and nitrogen amplitudes may reflect variations in available resources at these sites, consequently reducing trophic redundancy in these regions. Pollution-affected sites in the neighboring basin, the Velhas River Basin, showed elevated MNND values (indicative of decreased trophic redundancy) (de Carvalho et al., 2020). In contrast, Andrades et al. (2020) observed an opposite pattern in the estuarine regions of the Doce River. However, their study was conducted less than a year after the impact, which may explain the abrupt decline in resource availability, affecting all trophic diversity metrics.

The nitrogen range in the upper course exhibited considerable variations, indicating high instability in the trophic structure of these communities, suggesting variations in trophic levels, or enrichment of basal resources, which would be reflected in fish ^{15}N values (Carvalho et al., 2020). An increase in nitrogen range was observed for estuarine communities in the Doce River following the dam rupture (Andrades et al., 2020). The authors hypothesize that nitrogen-enriched waters due to the use of ester-amines in the treatment to separate hematite from impurities stored in the dam may be responsible for this increase (Andrades et al., 2020; Santos et al., 2019). It is important to note that in the study conducted in estuarine areas, the nitrogen increase was found by comparing communities before and after the arrival of tailings mud. In our study, we assessed two different periods, both with the presence of tailings. Other hypotheses could also be proposed. This variation found in our study, if related to the presence of top predator species, could be associated with a potential recovery process of this part of the basin. The piscivorous species *Oligosarcus argenteus* and *Hoplias intermedius* were recorded in 2020. In 2022, in addition to the previously mentioned species, *Hoplias malabaricus* and *Oligosarcus acutirostris* were also recorded in the upper part of the basin. Moreover, it is not uncommon to observe an increase in nitrogen in regions under the influence of pollutants, consequently showing changes in the isotopic characteristics of their communities (Carvalho et al., 2020; de Carvalho et al., 2021). A wide nitrogen range was also observed in the isotopic niche of fishes in the lower Doce river basin (de Carvalho et al., 2024), that can be related to the presence of pollutants. The impact of tailings on the abundance and diversity of planktonic and zooplanktonic communities (Bonecker et al., 2022) may have, for example, led to the consumption of non-basal organisms in the food web, resulting in an increase in nitrogen range. For these reasons, the mechanism behind this increase deserves investigation.

Differences in species richness along a river are expected, typically exhibiting an increase in richness along a longitudinal gradient from the source to the mouth (Bistoni and Hued, 2002; Oberdorff et al., 1993, 2011). Therefore, the observed differences in richness in the upper and middle course regions and their relation to the dam rupture should be interpreted with caution. Based on several species lists published after the Fundão dam rupture, the richness found in the upper and in the middle courses of the Doce River stretch were estimated as 31, and 66 species respectively (Petesse et al., 2023). In our study, a consistent pattern was observed over both years of sampling: lower species richness in the upper region, 33 species, and higher richness in the middle region with 37 species. The observed difference in the middle section of the basin is likely attributed to the greater number of sampling sites included in the published species lists. It is important to note that control sites showed higher species richness, 44 species, than other regions regardless of the section (upper or middle) in which they were collected.

Tributaries unaffected by the rupture may have served as refuges and, following the disturbance, played a role in the recolonization of species in the main channel of the Doce River (Salvador et al., 2022), which underscores the importance of connected, undammed tributaries in river basins.

Although impacted sites occupied variable isotopic niche spaces between years, no differences in niche overlap were observed. This lack of variation may be due to the aggregation of sampling sites within regions, as these differences become more evident when analyzed separately, particularly for sites U1 and M1. Following the impact of the rupture, changes in the availability of food resources may have influenced the isotopic niche of species. This alteration was observed in the estuarine regions of the Doce River, where six fish species exhibited a reduction in their isotopic niche due to the decrease in available food resources following the arrival of tailings (Andrades et al., 2020). A decline in the diversity of planktonic communities (Ferreira et al., 2020), estuarine benthic macrofauna (Gomes et al., 2017), and marine macrofauna (Nascimento et al., 2022) has already been reported in areas affected by the rupture. Additionally, changes in the richness and composition of zooplankton communities have been observed (Santos et al., 2022). These organisms play a crucial role in transferring energy to higher trophic levels and serve as important prey for fish (Covich et al., 1999). The shift in the abundance, quality, and availability of basal resources may have been reflected in the alteration of isotopic niches in impacted areas. This pattern was observed for *Knodus moenkhausii* studied in the Doce River Basin, where the species consumed more nutritious prey (invertebrates) at control sites compared to impacted sites (Paiva et al., 2024). The increase in the isotopic niche area along the river gradient is also expected according to the River Continuum Concept (Vannote et al., 1980). This pattern was similarly observed in the Rio Doce by Carvalho et al. (2024), underscoring the simplification of the trophic structure and niche breadth at the sites closest to the tailings dam rupture. The substantial variation in isotopic niche compositions across the basin likely reflects the interaction of multiple environmental stressors within the Doce basin (de Carvalho et al., 2024). Other anthropogenic alterations, such as increased nutrient loading in water bodies, also modify species' isotopic niches by altering the availability and quality of food resources (Horka et al., 2023). As observed in streams impacted by a coal mine spill (Medinski et al., 2023), species in the most affected areas exhibited broader isotopic niches. This is likely due to an expansion in the consumption of less nutritious items to compensate for the loss of more nutritious resources that became unavailable following the disturbance (Medinski et al., 2023).

In Brazil, less than four years after the Fundão dam rupture, another failure in a mining tailing dam in Brumadinho, caused severe impacts to the Paraopeba river (Vergilio et al., 2020). In the Neotropical region, impacts such as crude oil spills and tailing dams failure have profound and long-lasting consequences for aquatic ecosystems (Azevedo-Santos et al., 2021). Globally, the frequency of dam failures has been increasing (Islam and Murakami, 2021; Otieno and Shukla, 2023). A recent study estimates that in the last century the rate of tailing dam failure has been approximately 3.45 failures per year (Islam and Murakami, 2021). At least 5300 km of river channels and 4950km² of floodplain areas have been impacted by tailing dam failures (Macklin et al., 2023). Over the past 15 years some of the major tailing dams rupture worldwide took place in Magadan (Russia - 2009); Kolontár (Hungary – 2010); Benguet (Philippines – 2012); Alberta (Canada – 2013); British Columbia (Canada – 2014); Cananea (Mexico – 2014); Vedanta (India- 2017); Tieli (China – 2020). All of these events resulted in extensive social and environmental impacts (Islam and Murakami, 2021; Lumbroso et al., 2019). In the restoration of environments degraded by mining waste, monitoring trophic diversity metrics over time offers valuable insights into community dynamics. The assessment of these metrics can be an important tool in accessing the effectiveness of restoration programs for these environments.

Conclusion

In this study, we highlight important patterns occurring in the upper and middle regions of the basin, considering that most studies related to the impact have so far been carried out in the estuarine region of the Doce River. The assessment of different regions in the Doce River basin highlighted the stability of control areas, with the stability of trophic diversity metrics serving as a good parameter for indicating better environmental quality. In the affected regions, the significant fluctuations observed indicate that the river is undergoing a transformation process, and regions further from the rupture (middle course) appear to be approaching the trophic diversity observed in control sites. The results presented here illustrate an application of the stable isotope technique, demonstrating its effectiveness in identifying trophic structure patterns and their alterations in response to disturbances caused by dam failures. Such an approach proved particularly interesting since expected responses, such as the eventual increase of species richness in impacted regions during the second year, were not detected. Assessing the extent of damage to aquatic biota is a crucial first step in the recovery process. Consequently, this technique proves to be a valuable tool for monitoring and restoring aquatic environments affected by mining waste.

Declarations

All authors have read, understood, and have complied as applicable with the statement on "Ethical responsibilities of Authors" as found in the Instructions for Authors.

Conflicts of interest

The authors have no relevant financial or non-financial interests to disclose.

Funding

This study was financed by a contract between Fundação de Amparo à Pesquisa do estado de Minas Gerais (FAPEMIG) and RENOVA Foundation – grant term nº 4800028061; and was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) - Finance Code: 32004010017P3.

Ethics approval

All collection procedures are authorized by SISBIO – Biodiversity Authorization and Information System – Nº. 10327-3, and the Animal Ethics Committee of UFLA (CEUA – UFLA), Nº. 034/21.

Potential reviewers:

Júlio Guazzelli Gonzalez
Universidade Federal Rural de Pernambuco
julio.gonzalez@ufrpe.br

Alexandra Baeta
University of Coimbra
asbaeta@ci.uc.pt

Erival Gonçalves Prata
Universidade Federal do Pará
erival.gprata@gmail.com

Mojmír Vašek
Biology Centre CAS
mojmir.vasek@hbu.cas.cz

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