PATRÍCIA SANTOS FRÁGUAS

FISH FROM THE DOCE RIVER BASIN SEVEN YEARS AFTER THE FUNDÃO DAM RUPTURE: AN ISOTOPIC APPROACH

LAVRAS – MG
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Tese apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ecologia Aplicada, área de concentração Ecologia e Conservação de Recursos em Paisagens Fragmentadas e Agrossistemas para obtenção do título de Doutora.

Prof. Dr. Paulo dos Santos Pompeu
Orientador

Dra. Débora Reis de Carvalho
Coorientadora

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PATRÍCIA SANTOS FRÁGUAS

PEIXES DA BACIA DO RIO DOCE SETE ANOS APÓS O ROMPIMENTO DA BARRAGEM DO FUNDÃO: UMA ABORDAGEM ISOTÓPICA

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APROVADA em 29 de fevereiro de 2024.
Dra. Alessandra Angélica de Pádua Bueno – UFLA
Dr. Marcelo Passamani – UFLA
Dr. Rafael Pereira Leitão – UFMG
Dr. Gilberto Nepomuceno Salvador – UFMG

Prof. Dr. Paulo dos Santos Pompeu
Orientador

Dra. Débora Reis de Carvalho
Coorientadora

LAVRAS – MG

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INDICADORES DE IMPACTO

Impactos sociais, tecnológicos, econômicos e culturais

Este trabalho de tese, que é parte de um projeto maior, busca avaliar os impactos causados pelo rompimento da barragem de Fundão sobre a comunidade de peixes na bacia do Rio Doce. O rompimento da barragem de rejeitos de mineração em Mariana é considerado um dos maiores desastres ambientais dessa natureza e gerou grande repercussão tanto nacional quanto internacional. Estudos que contribuem para a compreensão desses impactos são de grande interesse para comunidades locais, o público brasileiro e para a comunidade científica. O trabalho utiliza a análise de isótopos estáveis, que permite traçar como ocorre o fluxo de energia no ambiente. Através do uso desta nova técnica de análise, que é nova no Brasil, podemos obter dados mais refinados sobre os recursos alimentares que sustentam a comunidade de peixes. Os resultados obtidos irão contribuir para os esforços de recuperação da bacia do Rio Doce. O projeto se enquadra na categoria de Meio Ambiente nas áreas temáticas da extensão, relacionado ao objetivo de desenvolvimento sustentável da ONU.

Social, technological, economic and cultural impacts

This thesis work, which is part of a larger project, aims to assess the impacts caused by the Fundão dam breach on the fish community in the Rio Doce basin. The breach of the mining tailings dam in Mariana is considered one of the largest environmental disasters of this kind and has generated significant national and international attention. Studies that contribute to understanding these impacts are of great interest to local communities, the Brazilian public, and the scientific community. The work uses stable isotope analysis, which allows tracing how energy flows in the environment. Through the use of this new analysis technique, which is novel in Brazil, we can obtain more refined data on the food resources that sustain the fish community. The results obtained will contribute to the efforts to recover the Rio Doce basin. The project falls under the Environment category in the thematic areas of extension, related to the UN's sustainable development goal.

RESUMO

Os ecossistemas aquáticos estão sobre alto nível de ameaça em decorrência de atividades antrópicas. A bacia do rio Doce apresenta um histórico intenso de exploração e alteração de seus ambientes, relacionados às mais diversas atividades antrópicas. No ano de 2015, houve o rompimento da barragem do Fundão em Mariana, MG, que contaminou o leito do rio Doce com 66 milhões de m^3 de rejeito de mineração, se somando aos outros diversos impactos que incidem sobre esta bacia. O principal objetivo desta tese é avaliar os efeitos da presença de poluentes e do rompimento da barragem em Mariana sobre a estrutura trófica da ictiofauna da bacia do rio Doce, através do uso dos isótopos estáveis de carbono e nitrogênio. No primeiro capítulo focamos em avaliar a estrutura trófica do rio Santo Antônio, um afluente em boa condição ambiental na bacia do Rio Doce. Avaliamos a estabilidade isotópica da comunidade de peixes neste rio, a fim de ter uma base para comparação com os outros pontos que foram impactados pelos rejeitos de mineração. A estrutura trófica se manteve estável isotopicamente ao longo dos anos de coleta. No segundo capítulo, avaliamos a variação temporal na riqueza de espécies e diversidade trófica em pontos impactados e não impactados pelos rejeitos de mineração. As regiões não impactadas apresentaram maior riqueza de espécies e maior estabilidade nas métricas tróficas. A região mais próxima do impacto apresentou grande instabilidade temporal nas métricas tróficas, e regiões impactadas mais distantes tenderam a se aproximar mais das métricas tróficas encontradas nas regiões controle.

Palavras-chave: carbono; ecologia trófica; ictiofauna; isótopos estáveis; nitrogênio; rejeito de mineração; rompimento de barragem.

ABSTRACT

Aquatic ecosystems are under a high level of threat due to human activities. The Doce river basin has an intense history of exploration and alteration of its environments, related to the most diverse human activities. In 2015, there was the rupture of the Fundão dam in Mariana, MG, which contaminated the bed of the Doce River with 66 million m^3 of mining waste, adding to the various other impacts that affect this basin. The main objective of this thesis is to evaluate the effects of the presence of pollutants and the rupture of the dam in Mariana on the trophic structure of the ichthyofauna of the Doce river basin, through the use of stable isotopes of carbon and nitrogen. In the first chapter we focused on evaluating the trophic structure of the Santo Antônio river, a tributary in good environmental condition in the Rio Doce basin. We evaluated the isotopic stability of the fish community in this river, in order to have a basis for comparison with other points that were impacted by mining tailings. The trophic structure remained isotopically stable over the years of collection. In the second chapter, we assessed the temporal variation in species richness and trophic diversity at sites affected and unaffected by mining tailings. Unaffected regions exhibited higher species richness and greater stability in trophic metrics. The region closest to the impact showed significant temporal instability in trophic metrics, while more distant impacted regions tended to approach the trophic metrics found in control regions.

Keywords: carbon; trophic ecology; ichthyofauna; stable isotopes; nitrogen; mining waste; dam break.

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO GERAL

Atividades de mineração alteram profundamente o ambiente e poluem continuamente ecossistemas aquáticos (AFFANDI; ISHAK, 2019). O impacto do rompimento de uma barragem de rejeitos de mineração é ainda mais grave, pois a enorme quantidade de material liberado repentinamente destrói o ambiente e gera impactos que se estenderão a longo prazo (AIRES *et al.*, 2018). Em 2015 com o rompimento da barragem de Fundão, foram liberados cerca de 43 milhões de m³ de rejeitos de minério de ferro (CARMO *et al.*, 2017) no distrito de Bento Rodrigues pertencente ao município de Mariana. O evento causou a morte de 19 pessoas e o deslocamento de toda a população do distrito (FERNANDES *et al.*, 2016). O rejeito atingiu os rios do Carmo, Gualaxo do Norte e Doce, contaminando 650 km de rio até sua foz (ANA, 2016). A área total afetada no oceano compreende toda a costa do estado do Espírito Santo, e partes da costa dos estados do Rio de Janeiro e Bahia (ICMBIO, 2017; MARTA-ALMEIDA *et al.*, 2016). Os ecossistemas de água doce, estuarinos, costeiros e marinhos foram profundamente afetados (CARMO *et al.*, 2017). Áreas de vegetação da Mata Atlântica, região prioritária para a conservação por ser um dos *hotspots* de biodiversidade (MYERS *et al.*, 2000), e unidades de conservação terrestres e marinhas foram atingidas (CARMO *et al.*, 2017; ICMBIO, 2017). Este é considerado um dos maiores desastres ambientais desta natureza no mundo (HATJE *et al.*, 2017).

Após o rompimento da barragem, análises de qualidade da água detectaram níveis acima da média permitida de Alumínio, Ferro, Manganês, Cobre, Chumbo, Arsênio, Cromo, Cádmio, Níquel, Mercúrio e Zinco (SEMAD; IGAM, 2017). Dois anos após o rompimento, os valores de turbidez, sólidos em suspensão e concentração de Ferro e Manganês na água ainda apresentam valores acima da média permitida (SEMAD; IGAM, 2017). O impacto também alterou profundamente o ambiente físico, os rejeitos atingiram 1469 ha de vegetação natural, suprimindo a maior parte da vegetação ripária na área próxima ao rompimento (FERNANDES *et al.*, 2016). A lama se acumulou nas planícies de inundação, em quantidade grande o suficiente para alterar o leito original do rio (CARMO *et al.*, 2017). Devido à grande quantidade de material acumulado, períodos de chuvas intensas carregam os rejeitos para o leito do rio novamente gerando recorrentes interferências no ambiente (HATJE *et al.*, 2017).

As mudanças na estrutura física do ambiente e qualidade da água afetam toda a fauna

aquática.

Os rejeitos tiveram impacto imediato em vários níveis tróficos, na comunidade de peixes, levando à morte de 11 toneladas (GOVERNO DO ESTADO DE MINAS GERAIS, 2016), afetou o crescimento de larvas (BONECKER *et al.*, 2019), e causou acúmulo de metais pesados (FERREIRA *et al.*, 2020; VERGILIO *et al.*, 2021). Outros organismos como algas, microcrustáceos (VERGILIO *et al.*, 2021) e comunidades microbianas (CORDEIRO *et al.*, 2019), tiveram seu crescimento afetado, além de alterar o nicho de espécies estuarinas (ANDRADES *et al.*, 2020). Espera-se que a desestruturação destas comunidades irá refletir em mudanças na disponibilidade de recursos alimentares, que interferem na teia trófica e fluxo de energia nestes sistemas.

O fluxo de energia em um sistema pode ser estudado através da análise de isótopos estáveis de carbono (^{13}C) e nitrogênio (^{15}N) dos recursos e consumidores em uma comunidade (PETERSON; FRY, 1987; SYVÄRANTA *et al.*, 2011). Através da assinatura isotópica de carbono, é possível rastrear quais as fontes de carbono com diferentes composições isotópicas estão sendo incorporadas pelos consumidores, uma vez que a assinatura de ^{13}C é conservativa ao longo da cadeia alimentar (POST, 2002). Já a assinatura isotópica de nitrogênio permite identificar o nível trófico dos organismos, pois há um enriquecimento na assinatura de nitrogênio do consumidor em relação ao seu recurso (POST, 2002). A assinatura de nitrogênio também pode ser utilizada para rastrear impactos por poluição, em processos de eutrofização por exemplo, por refletir a concentração de nitrogênio da água no ^{15}N dos organismos aquáticos (WANG *et al.*, 2016).

A ictiofauna é diretamente afetada pelas atividades de mineração (AFFANDI; ISHAK, 2019). A alteração dos fatores bióticos e abióticos pode levar a alterações na comunidade de peixes (JACKSON; PERES-NETO; OLDEN, 2001). Este grupo possui ampla distribuição e suas espécies ocupam vários níveis tróficos (KARR, 1981), sendo assim, o estudo da estrutura trófica da ictiofauna pode ser utilizado como ferramenta para indicar o estado de integridade de uma bacia (CARVALHO *et al.*, 2017; KARR, 1981).

A dinâmica trófica da ictiofauna pode se alterar em decorrência de diferentes atividades antrópicas (CARVALHO *et al.*, 2015). Uma forma de mensurar o impacto destas alterações, é através da comparação com regiões que possuem pouca ou nenhuma interferência antrópica (MARTINS *et al.*, 2018). Portanto iremos avaliar a estabilidade isotópica temporal na estrutura trófica de um ponto preservado na bacia do Rio Doce para ter uma base de comparação com os pontos afetados rompimento da barragem de Mariana. (Capítulo 1). Também utilizaremos a comparação, através de métricas de diversidade trófica, com regiões menos degradadas para tentar mensurar o processo de recuperação nas áreas atingidas pelo rompimento da barragem de Mariana (Capítulo 2).

2 CONCLUSÃO GERAL

No primeiro capítulo fizemos a descrição isotópica da comunidade de peixes de um ponto do Rio Santo Antônio ao longo de dois anos. Encontramos que mesmo com algumas variações nas espécies da comunidades, as métricas e parâmetros gerais da estrutura isotópica da assembleia de peixes apresentaram estabilidade entre os anos de estudo. Destacamos a importância desta avaliação para o rio Santo Antônio, importante trecho lótico que abriga elevada biodiversidade de espécies nativas, e é um dos rios mais preservados da bacia do Rio Doce. A caracterização dos parâmetros isotópicos deste local preservado poderá ser utilizada como linha de base para estudos em locais afetados pelo rompimento da barragem e também poderá auxiliar no monitoramento das ações de recuperação existentes na bacia.

No segundo capítulo analisamos as características isotópicas da comunidade de peixes em pontos afetados e não afetados pelo rompimento da barragem de rejeitos de mineração do Fundão, no alto e médio curso da bacia do Rio Doce. Corroborando os resultados encontrados no capítulo anterior, verificamos que as regiões não afetadas pelo rompimento, utilizadas aqui como áreas controle, apresentam maior estabilidade nos parâmetros isotópicos ao longo do tempo, sendo a estabilidade de métricas de diversidade trófica um bom parâmetro para avaliação da qualidade ambiental dos pontos analisados. Detectamos padrões importantes ocorrendo nas regiões alta e média da bacia do Rio Doce. As regiões mais próximas ao rompimento (alto curso) apresentaram grande instabilidade e flutuações das métricas de diversidade trófica, indicativo de que esta é uma região que está passando por processos de transformação. Pontos mais distantes do rompimento (médio curso) apresentam uma tendência a se aproximar dos parâmetros encontrados nas regiões controle, por não apresentarem variações tão expressivas quanto as encontradas nas regiões próximas ao rompimento. Desta forma, parâmetros tróficos e métricas de diversidade trófica podem ser utilizados como ferramenta para o monitoramento das condições na bacia do Rio Doce.

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SEGUNDA PARTE - ARTIGOS

MANUSCRITO 1 - Temporal stability in fish assemblage isotopic niches: insights from a reference river

Patrícia Santos Fráguas[1], Débora Reis de Carvalho[2,3], Chayenne Chagas de Castro[3], Frederico Fernandes Ferreira[4], Jorge Abdala Dergam[4], Carlos Frankl Sperber[4] e Paulo dos Santos Pompeu[3]

[1] Programa de Pós-Graduação em Ecologia Aplicada, Departamento de Ecologia e Conservação, Instituto de Ciências Naturais, Universidade Federal de Lavras, 37200-900 Lavras, Minas Gerais, Brazil. psfraguas@yahoo.com.br

[2]Lancaster Environment Centre, Lancaster University, Lancaster, United Kingdom; deboracarvalhobio@gmail.com

[3]Laboratório de Ecologia de Peixes, Departamento de Ecologia e Conservação, Instituto de Ciências Naturais, Universidade Federal de Lavras, 37200-900 Lavras, Minas Gerais, Brazil. pompeu@ufla.br

[4] Departamento de Biologia, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil.

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Highlights

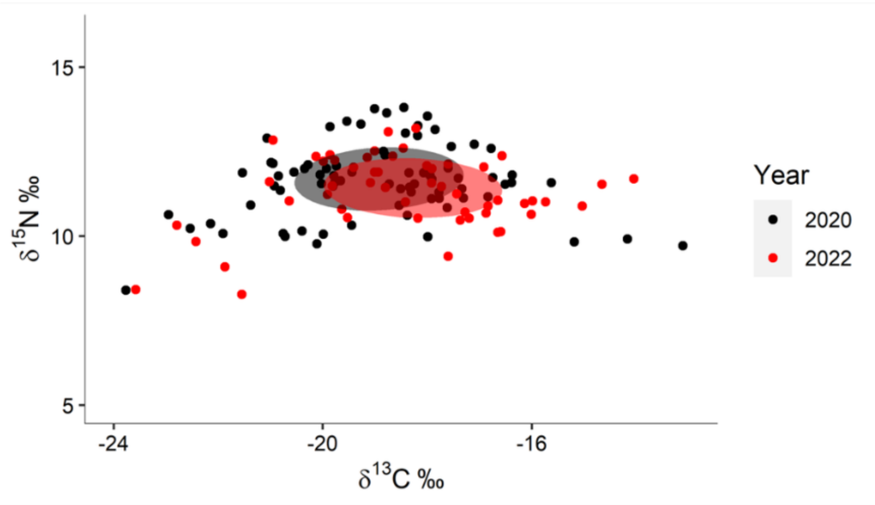
- Isotopic characterization of the fish assemblage in a reference site
- Species composition and principal basal resources varied over time
- Despite the variation, the isotopic niche remained stable temporally
- Isotopic metrics can be used as baseline for impacts and restoration efforts

Abstract

The integrity of aquatic ecosystems is continually threatened by anthropogenic pressures. Thus, the characterization of environments with little or no impact becomes exceedingly important, especially for application in conservation efforts. The Santo Antônio River, a tributary of the Doce River, holds significant biological importance and is a priority area for conservation. This study aims to characterize the trophic structure of the fish assemblage in the Santo Antônio River using stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes. Through collections conducted in two years (2020 and 2022), we assessed the stability of isotopic metrics over time. The community exhibited stability in isotopic parameters, although the species composition varied, totaling 26 species. The overall isotopic niche of the community varied minimally, showing substantial overlap between the two years. The most important resources sustaining the community differed each sampling year. Trophic diversity metrics remained stable. In general, despite some variations in collected species and the assimilated items, the isotopic structure remained stable over time. These parameters can serve as a tool for comparison with other impacted points in the basin and in monitoring recovery efforts within the basin.

Keywords: Doce River; Ichthyofauna; Stable Isotopes; Layman Metrics; Carbon; Nitrogen

Graphical Abstract



Overlap of the trophic niche of the fish community over time

1. Introduction

Water stands as a natural resource of utmost significance for human civilization. Initially employed in agriculture and livestock management, its utilization progressively extended to industrial sectors with the surge in urbanization (Feio et al., 2021; Kummu et al., 2011). Consequently, it is not coincidental that more than half of aquatic ecosystems are currently under a substantial threat due to anthropogenic activities (ICMBio, 2018; Vörösmarty et al., 2010). Several categories of impact have undergone extensive investigation, encompassing the invasion of exotic species, habitat degradation, aquatic pollution, alteration of flow regimes, and overexploitation of communities (Dudgeon et al., 2006; Malmqvist and Rundle, 2002). Recent contributions to this discourse have introduced additional perspectives, such as the cumulative effect arising from the interaction among diverse pressures, and considerations about emerging pollutant sources (Craig et al., 2017; Pelicice et al., 2017; Reid et al., 2019; Torremorell et al., 2021).

Due to the escalating and persistent impact of polluting activities and anthropogenic impacts, it is crucial to comprehend the functioning of environments that still exhibit little or no anthropogenic alteration (Bailey et al., 2004). These environments, defined as reference sites, provide a baseline from which changes can be assessed, aid in discerning the effects of human activities on ecosystems, and serve as models to guide restoration and conservation efforts (Alagona et al., 2012; Dodds and Oakes, 2004; Stoddard et al., 2006). Therefore, the characterization of these areas, taking into account the specific attributes of each studied environment, encompassing physical, chemical, and biological aspects (Bailey et al., 2004), is of paramount importance. However, pristine environments are becoming increasingly rare, leading to the utilization of less disturbed sites as reference areas (Kosnicki et al., 2014; Stoddard et al., 2006).

The Doce River basin, located in the southeastern region of Brazil, has a pronounced history of exploitation and alteration of its environments, attributed to various anthropogenic activities (Vieira, 2009). Intensive land use, deforestation, untreated sewage pollution, eutrophication of watercourses, and river siltation (ANA, 2016; PIRH - Doce, 2010), along with the historical mining exploration (Hatje et al., 2017), have significantly transformed this environment. In the year of 2015, the Fundão dam in Mariana, MG, experienced a huge rupture, resulting in the contamination of the Doce river bed with 66 million m^3 of iron mining tailings (Governo do Estado de Minas Gerais, 2016), adding to the various pre-existing impacts affecting this basin. As a result of this rupture, which was deemed the most significant environmental disaster in Brazil (Hatje et al., 2017), considerable attention has been directed towards the Doce River basin, prompting the initiation of a series of environmental recovery actions. However, the conditions prior to the historical impacts to which the river has been subjected remain unknown, emphasizing the urgent need to identify potential reference conditions, even in less affected tributaries of the basin.

One of the tributaries of the Doce River, the Santo Antônio River holds significant ecological importance in the region and is designated as a priority area for conservation (Drummond et al., 2005), and an important area of free flowing river (IEF, 2021). Recognized for its high biodiversity and as a crucial lotic remnant (Dos Santos and de Britto, 2021; Vieira, 2006), it serves as a habitat for a majority of the native fish fauna in the Doce River basin. Specifically, 57 species, constituting 90% of the total fauna in the basin, are found in this river (Siqueira, 2019; Vieira, 2006). Notably, one species, *Henochilus wheatlandii,* is endemic to this basin (Vieira et al., 2000), while others are endangered species, including *Hypomasticus thayeri*, *Steindachneridium doceanum*, *Brycon dulcis*, and *Phochilodus vimboides* (Biodiversitas, 2021; Sales et al., 2018; Vieira, 2006).

Among the characteristics of a reference site, it is crucial to understand the functioning of trophic dynamics (Zanden et al., 2016), for instance, delineating the primary sources of energy that sustain local communities. The flow of energy within a system can be examined through the analysis of stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes in the resources and consumers of a community (Peterson and Fry, 1987; Syväranta et al., 2011). Through the isotopic signature of carbon, it becomes possible to trace the carbon sources incorporated by consumers, as they will reflect the carbon signature of the consumed food resource (Post, 2002). The nitrogen isotopic signature enables the identification of the trophic level of organisms, as there is an enrichment in the nitrogen signature of the consumer compared to its resource (Post, 2002). The nitrogen signature can also be employed to trace pollution impacts, as changes in nitrogen concentrations in its various forms (nitrate, nitrite, ammonia) in the water may be reflected in the nitrogen signatures of aquatic organisms (de Carvalho et al., 2021; Wang et al., 2016).

Among aquatic organisms, fish constitute a widely distributed group, with species occupying various trophic levels (Karr, 1981). Studies conducted using isotopic signatures of neotropical fish assemblages have indicated that variations in resource signatures can have a significant influence on trophic parameters of the community, particularly in disturbed environments (de Carvalho et al., 2022, 2021). On the other hand, in better-conserved locations, there appears to be greater stability in the overall trophic structure of the community (de Carvalho et al., 2017), allowing for their use as reference conditions.

In this study, the objective was to assess the trophic structure of the fish assemblage in the Santo Antônio River using stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes. Through samplings in two distinct periods (2020 and 2022), we evaluated whether different isotopic metrics are temporally stable, allowing for their use as a baseline. Thus, our specific objectives were a) to characterize the trophic structure of the fish assemblage and the trophic position of each species for each sampling year; b) to analyze the niche occupied by the fish assemblage; c) to identify which resources are most important for the fish assemblage in each year; d) to compare trophic diversity indices between the two years. We will test the hypothesis that, although trophic levels and assimilated items by each species may change between years, parameters of the trophic structure of a fish assemblage in a minimally impacted environment are likely to remain stable. With this information, we aim to isotopically characterize the trophic structure of the fish assemblage in the most significant reference site of the Doce River basin and identify possible metrics that can be used for comparison with degraded regions of the basin.

2. Material and Methods

2.1 Study area

The Santo Antônio River watershed is entirely located in the state of Minas Gerais, covering an area of 10,429.46 km² (PIRH - Doce, 2010). The main left bank tributaries are the Peixe and Guanhães rivers, while on the right bank, the Preto, Itambé and Tanque rivers (PIRH - Doce, 2010). The Santo Antônio River originates in the Serra do Espinhaço, in the municipality of Congonhas do Norte, and flows for approximately 280 km until it joins the Doce River in the municipality of Naque (PIRH - Doce, 2010). The majority of the basin is situated within the Atlantic Forest biome, a biodiversity hotspot and one of the most fragmented biomes globally (de Lima et al., 2020; Myers et al., 2000). The Santo Antônio River was sampled at a location near the municipality of Santo Antônio do Rio Abaixo, Minas Gerais, in two different years (Figure 1). Sampling took place in August/September of 2020 and 2022.

2.2 Data sampling

2.2.1 Fish sampling:

Fish sampling was conducted using gill nets, trawl nets, sieves, and cast nets. Two sets of gill nets (mesh sizes of 3-16 cm and 20m in length) were deployed continuously for a period of 12 hours. Trawl nets (5m in length with a 1mm mesh) were employed for sampling in shallow and littoral areas. Sieves (80cm in diameter with a 1mm mesh) were used in macrophyte banks near the shoreline. Cast nets (3cm mesh) were utilized in deeper regions. After sampling, all individuals were anesthetized in Eugenol solution, measured, weighed, and identified. In the field, a portion of the muscle tissue was excised and immediately frozen until laboratory processing to prevent decomposition. The aim was to sample tissue from at least five individuals of each species for isotopic analysis whenever possible. The sampled species were categorized into trophic guilds (detritivore, herbivore, omnivore, invertivore, and piscivore), according to the literature.

All sampling procedures were authorized by SISBIO – *Sistema de Autorização e informação em Biodiversidade* - License No. 10327-3, and by the Ethics Committee on Animal Use of the Universidade Federal de Lavras (CEUA – UFLA), protocol No. 034/21.

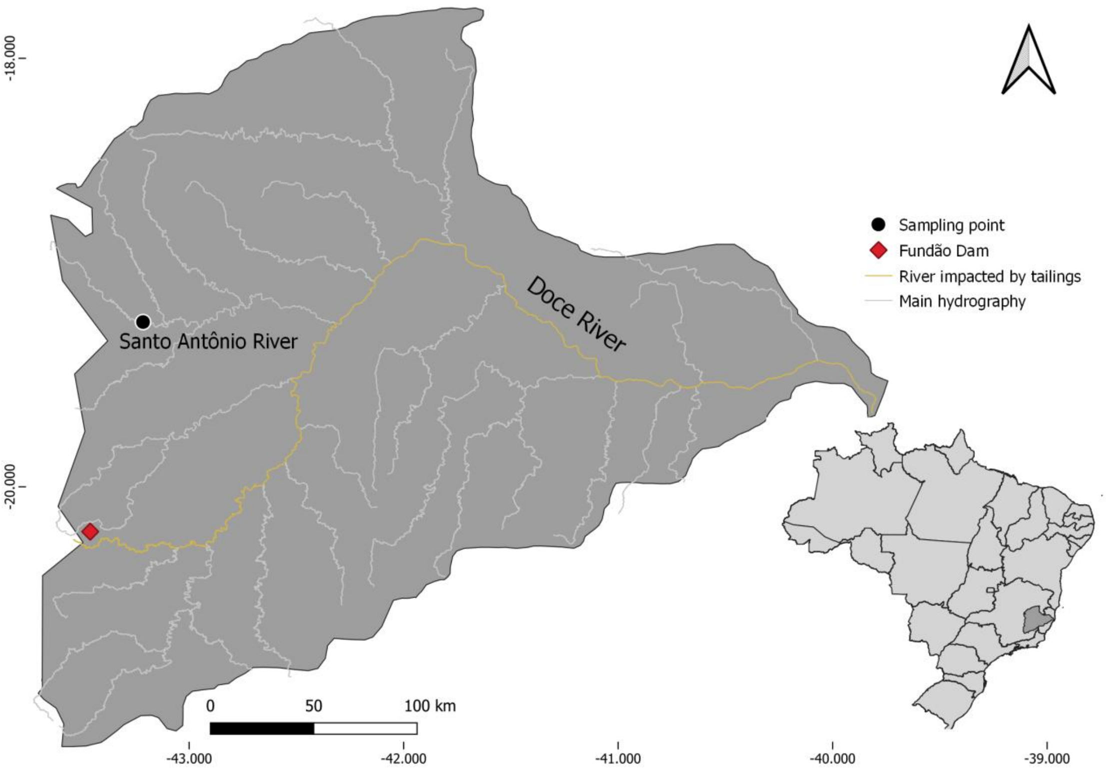


Figure 1: Location of the fish and food resources sampling point in the Santo Antônio River, Minas Gerais, Brazil. The dark-gray area represents the Doce river basin area.

2.2.2 Food Resources Sampling:

Samples of potential fish food items were also sampled for isotopic analysis. Resources sampled in both years included filamentous algae, coarse particulate organic matter (CPOM), terrestrial and aquatic invertebrates, suspended material, and periphyton. To address potential gaps in food resources that were not sampled in 2020, new resources were collected in the second sampling in 2022, including grasses, macrophytes, riparian vegetation, manure, and fine particulate organic matter (FPOM). The collection of five samples for each resource was standardized. Samples of macrophytes, CPOM, terrestrial invertebrates, filamentous algae, grasses, riparian vegetation, and manure were randomly sampled at the sampling point. Aquatic macroinvertebrates were sampled using Surber nets and sieves (80cm in diameter with a 1mm mesh). FPOM samples were collected by resuspending sediment and passing this material through a phytoplankton net (45 μm mesh). Periphyton was sampled by scraping rocks with the aid of a brush, and the collected material was stored in plastic containers with distilled water. Suspended material was sampled using a phytoplankton net (45 μm mesh) placed in the water column for a period of three minutes. The samples were frozen until laboratory processing.

2.3 Isotopic Analysis:

In the laboratory, fish tissue samples were lyophilized for 24 hours, ground into a uniform powder, and stored in Eppendorf tubes. Liquid samples (periphyton, FPOM, and suspended material) were filtered using a filtration apparatus connected to a vacuum pump with pre-burned quartz filters (Whatman® QMA). Subsequently, the filters with the samples were dried in an oven at 40°C for a period of 24 hours. The remaining samples were lyophilized for 24 hours, then ground and stored in Eppendorf tubes for subsequent isotopic analysis. After the preparation of the materials, they were sent for isotopic analysis. In 2020, the samples were analyzed at *Centro de Energia Nucelar na Agricultura* (CENA) at the *Universidade de São Paulo* (USP). In 2022, the analyses were conducted at the *Central de Análises e Prospecção Química* (CAPQ) of the *Universidade Federal de Lavras* (UFLA). For isotopic analysis, 1 mg of fish and resource material was used. For liquid sample resources, 35 mg of material was used.

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 2022, we used a Delta V Plus Isotope Ratio Mass Spectrometer (Thermo Fischer), coupled with a Flash IRMS elemental analyzer interfaced with ConFlo IV. The results were expressed as relative differences in international reference systems, using the delta (δ) notation, in parts per thousand (‰), and calculated using the following formula:

$$\delta X = [\left(\frac{R\ sample}{R\ standard}\right) - 1] \times 10^3$$

where X is ^{13}C or ^{15}N, and R is the isotopic ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ (Barrie & Prosser, 1996).

2.4 Statistical Analyses

The trophic structure of the fish assemblage in the Santo Antônio River was characterized for each year using bi-plot graphs (x-axis: $\delta^{13}C$ and y-axis: $\delta^{15}N$) with the isotopic signatures of potential food resources and sampled fish. The bi-plot graph was represented in two ways: A) Mean values of the isotopic signature for each species and the mean and standard deviation of food resources; and B) Mean values and standard deviation of the isotopic signature of the species. In the first representation, it is possible to observe which basal resources are more assimilated by the fish assemblage, while in the second representation, we can more clearly assess the trophic position of each species and variations in signatures among individuals of the same species.

The trophic position of the species was calculated for each year using the Bayesian package "tRophicPosition" in R (Quezada-Romegialli et al., 2018). The model employed was the "complete two-source baseline model," with the basal resources being benthic macroinvertebrates and periphyton. The isotopic fractionation for muscle tissue was $\Delta^{15}N$: 2.9 ± 0.32‰ and $\Delta^{13}C$: 1.3 ± 0.3‰ (McCutchan et al., 2003).

Differences between the isotopic signatures of resources in the two study years were assessed using T-tests with Welch's correction, or Mann-Whitney tests depending on the normality of the data.

To assess potential differences in the isotopic niche of trophic guilds during the two sampling periods, we employed the Standard Ellipses Area (SEA). SEA was calculated for the whole fish assemblage in each year, and for the trophic guilds of each year. The SEA represents the isotopic niche and illustrates the richness and uniformity of the resources consumed by the population (Bearhop et al., 2004). Graphical representation of the ellipse areas was conducted using the SIBER package (Jackson et al., 2011) in the R program.

The contribution of each food resource to the species' diet was calculated for each year using Bayesian stable isotope mixing models with the MixSIAR package (Stock & Semmens, 2016) in the R program (R Development Core Team, 2015). Food resources selected for the partitioning analysis were based on the feeding habits of each species, as described in the literature. The species were grouped in the following feeding groups: detritivores, herbivores, invertivores, omnivores, and piscivores. For the piscivore guild, the selected resources included fish, terrestrial and aquatic invertebrates. For the omnivore and invertivore guilds, the considered resources were periphyton, terrestrial and aquatic invertebrates, and filamentous algae. Lastly, for the detritivore and herbivore trophic guilds, the considered resources were filamentous algae, coarse particulate organic matter (CPOM), suspended material, and periphyton.

To assess trophic diversity between the two sampling periods, we calculated, for each year, the metrics proposed by Layman et al. (2007): Carbon range (CR): the carbon amplitude used by the species; Nitrogen range (NR): the nitrogen amplitude used by the species; Total area (TA): the area occupied by species in isotopic space; Centroid distance (CD): a measure of trophic diversity; Mean nearest neighbor distance (MNND): a measure of trophic redundancy; Standard deviation of the nearest neighbor distance (SDNND): represents the distribution of trophic niches in fish communities.

3. Results

A total of 129 fish samples, representing 26 species, were analyzed in the Santo Antônio River (Table 1). In 2020, samples from 76 individuals belonging to 17 species were analyzed, and in 2022, a total of 53 samples representing 18 species were analyzed. Among the 26 collected species, nine were present in both sampling years, eight species were collected only in 2020, and nine species were collected only in 2022. Among the 26 species collected over the two sampling years, five were classified as detritivores, two as herbivores, four as invertivores, eleven as omnivores, and four as piscivores, based on the literature.

In 2020, *Hoplias intermedius* and *Oligosarcus solitarius*, piscivorous species, occupied the top trophic position. However, in 2022, the invertivore *Trichomycterus tantalus* took the top position, presenting a carbon signature not explored by other species in the assemblage. The herbivorous species *Henochilus wheatlandii* showed significant intraspecific variation in both years, with individuals exhibiting markedly different carbon signatures. Throughout both years, *Astyanax lacustris* occupied the same basal area on the graph (Figure 2). The assemblages sampled in both years had $\delta^{13}C$ values ranging from -25 to -15‰ and $\delta^{15}N$ values ranging from 8 to 12‰. Species such as *Hisonotus thayeri*, *Phalloceros uai*, and *Trichomycterus tantalus*, collected only in 2022, occupied peripheral positions compared to the rest of the fish assembly in the Santo Antônio River (Figure 3).

Table 1: Means and standard deviations of carbon and nitrogen isotopic signatures of fishes and resources sampled in the Santo Antônio River in 2020 and 2022. Means of resources marked with * were statistically different between years by the T-test. p<0.05.

Year of sampling	2020						2022					
Samples		$\delta^{13}C$		-	$\delta^{15}N$			$\delta^{13}C$		-	$\delta^{15}N$	
	N	Mean	SD	-	Mean	SD	N	Mean	SD	-	Mean	SD
Detritivores	**13**	**-17.38**	**0.57**	-	**11.76**	**0.58**	**12**	**-19.70**	**1.05**	**-**	**11.24**	**0.31**
Delturus carinotus (La Monte, 1933)	3	-17.14	0.38	-	12.66	0.06	2	-20.08	1.33	-	11.97	0.51
Hypostomus luetkeni (Steindachner, 1877)	5	-17.4	0.75	-	11.71	0.2	4	-17.35	0.63	-	10.41	0.20
Parotocinclus sp.	5	-17.5	0.53	-	11.29	0.32				-		
Euryochus thysanos Pereira & Reis, 2017							5	-18.59	1.20	-	12.28	0.23
Hisonotus thayeri Martins & Langeani, 2016							1	-22.79	-	-	10.32	-
Herbivores	**5**	**-18.81**	**3.41**	-	**10.22**	**0.68**	**7**	**-18.4**	**2.94**	**-**	**10.71**	**2.64**
Henochilus wheatlandii Garman, 1890	5	-18.81	3.41	-	10.22	0.68	2	-18.11	4.87	-	9.91	2.31
Hypomasticus mormyrops (Steindachner, 1875)							5	-18.70	1.02		11.51	0.33
Invertivores	**14**	**-18.72**	**1.96**	-	**11.87**	**0.8**	**7**	**-18.61**	**2.11**	**-**	**11.44**	**0.25**
Pachyurus adspersus Steindachner, 1879	8	-19.77	1.8	-	12.14	0.95	5	-17.29	2.11	-	12.08	0.25
Trichomycterus aff. *alternatus* (Eigenmann 1917)	6	-17.32	1.14	-	11.51	0.33				-		
Phalloceros uai Lucinda, 2008							1	-17.60	-	-	9.41	-
Trichomycterus tantalus Reis, Vieira & de Pinna 2022							1	-20.95	-	-	12.84	-
Omnivores	**35**	**-19.62**	**1.93**	-	**11.25**	**0.98**	**20**	**-18.69**	**1.27**	**-**	**10.58**	**0.29**
Astyanax lacustris (Lütken, 1875)	4	-22.3	1.26	-	9.75	0.91	2	-22.73	1.21	-	8.76	0.47
Brycon opalinus (Cuvier, 1819)	6	-17.56	2.36	-	10.17	0.4				-		
Characidium timbuiense Travassos, 1946	3	-20.01	0.31	-	12.03	0.04				-		
Geophagus aff. *brasiliensis* (Quoy & Gaimard, 1824)	4	-17.72	0.92	-	11.55	0.24				-		
Geophagus sp.	7	-19.48	1.29	-	11.83	0.94				-		
Hypomasticus copelandii (Steindachner, 1875)	1	-20.85	-	-	11.78	-	2	-18.06	2.07	-	10.34	0.29
Knodus moenkhausii (Eigenmann & Kennedy, 1903)	5	-20.51	0.63	-	11.52	0.4	5	-16.27	0.87	-	10.78	0.14
Psalidodon sp.	5	-20.29	0.92	-	11.82	0.27				-		
Characidium sp.							1	-18.42	-	-	11.02	-
Geophagus brasiliensis (Quoy & Gaimard, 1824)							5	-16.97	1.56	-	10.96	0.11
Psalidodon fasciatus (Cuvier, 1819)							5	-19.71	0.68	-	11.64	0.45
Piscivores	**9**	**-18.86**	**0.61**	-	**13.16**	**0.98**	**7**	**-18.41**	**1.75**	**-**	**11.89**	**1.37**
Hoplias intermedius (Günther, 1864)	2	-18.89	0.16	-	13.71	0.09	1	-17.91	-	-	12.00	-
Oligosarcus argenteus Günther, 1864	4	-18.67	0.82	-	12.62	1.34	5	-19.60	1.75	-	12.22	1.37

Year of sampling	2020						2022					
Samples		$\delta^{13}C$		-	$\delta^{15}N$			$\delta^{13}C$		-	$\delta^{15}N$	
	N	Mean	SD	-	Mean	SD	N	Mean	SD	-	Mean	SD
Oligosarcus solitarius Menezes, 1987	3	-19.09	0.57	-	13.51	0.26				-		
Crenicichla lacustris (Castelnau, 1855)							1	-17.73	-	-	11.47	-
Feeding resources	**30**	**-25.7**	**4.46**	**-**	**5.76**	**2.62**	**55**	**-24.18**	**3.34**	**-**	**4.48**	**1.71**
Filamentous Algae	5	-29.27	4.9	-	5.94	2.97	5	-19.65	10.22	-	6.17	0.65
CPOM	5	-30.27	0.95	-	1.96	0.73	5	-30.48	0.71	-	2.69	0.88
Terrestrial invertebrates	5	-26.28	3.91	-	6.15	3.78	5	-24.27	5.82	-	6.65	2.15
Aquatic invertebrates	5	-24.42*	1.33	-	7.88	0.67	5	-22.15*	5.48	-	6.47	3.16
Suspended matter	5	-23.41	3.16	-	6.32*	0.63	5	-23.64	2.07	-	3.99*	2.00
Periphyton	5	-20.58	2.95	-	6.34*	0.62	5	-20.4	1.13	-	4.13*	0.26
Grass							5	-26.04	6.59	-	4.58	3.33
Macrophyte							5	-28.02	1.20	-	4.39	1.73
Riparian vegetation							5	-30.51	0.90	-	2.32	2.56
Manure							5	-18.07	1.29	-	3.83	1.76
FPOM							5	-22.85	1.34	-	4.13	0.43

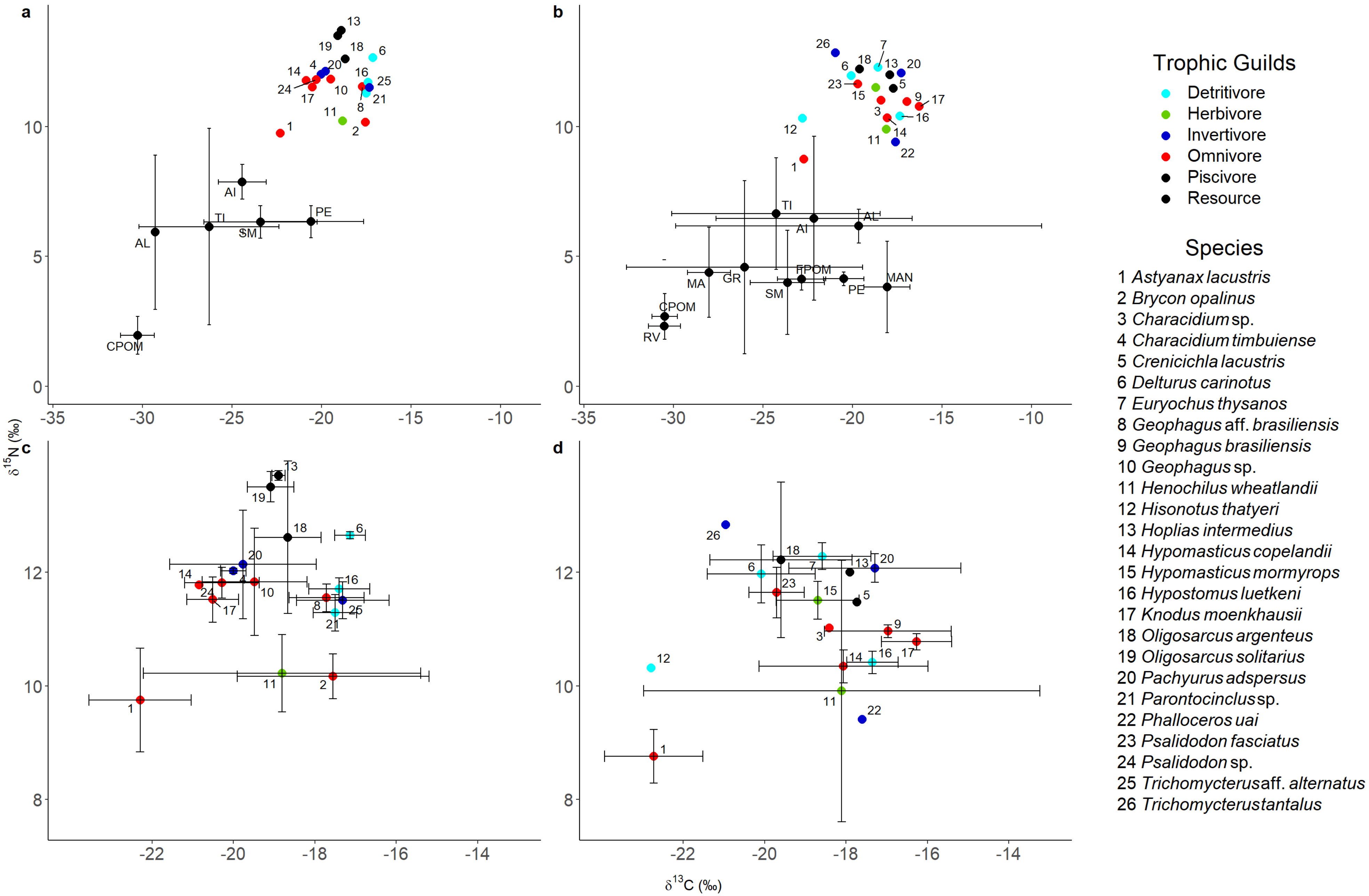

a
b
c
d
δ15N (‰)
δ13C (‰)
Trophic Guilds
Detritivore
Herbivore
Invertivore
Omnivore
Piscivore
Resource
Species
1 Astyanax lacustris
2 Brycon opalinus
3 Characidium sp.
4 Characidium timbuiense
5 Crenicichla lacustris
6 Delturus carinotus
7 Euryochus thysanos
8 Geophagus aff. brasiliensis
9 Geophagus brasiliensis
10 Geophagus sp.
11 Henochilus wheatlandii
12 Hisonotus thatyeri
13 Hoplias intermedius
14 Hypomasticus copelandii
15 Hypomasticus mormyrops
16 Hypostomus luetkeni
17 Knodus moenkhausii
18 Oligosarcus argenteus
19 Oligosarcus solitarius
20 Pachyurus adspersus
21 Parontocinclus sp.
22 Phalloceros uai
23 Psalidodon fasciatus
24 Psalidodon sp.
25 Trichomycterus aff. alternatus
26 Trichomycterus tantalus

Figure 2: Trophic structure of the fish assemblage in the Santo Antônio River in 2020 (a and c) and 2022 (b and d). X-axis: carbon isotopic signature, Y-axis: nitrogen isotopic signature. a) and b) Bi-plot of means and standard deviation of fishes and food resources. c) and d) Bi-plot with means and standard deviation of fishes. Resources: Filamentous algae (AL), periphyton (PE), coarse particulate organic matter (CPOM), aquatic invertebrates (AI), terrestrial invertebrates (TI), and suspended material (SM), manure (MAN), grass (GR), macrophyte (MA), and riparian vegetation (RV).

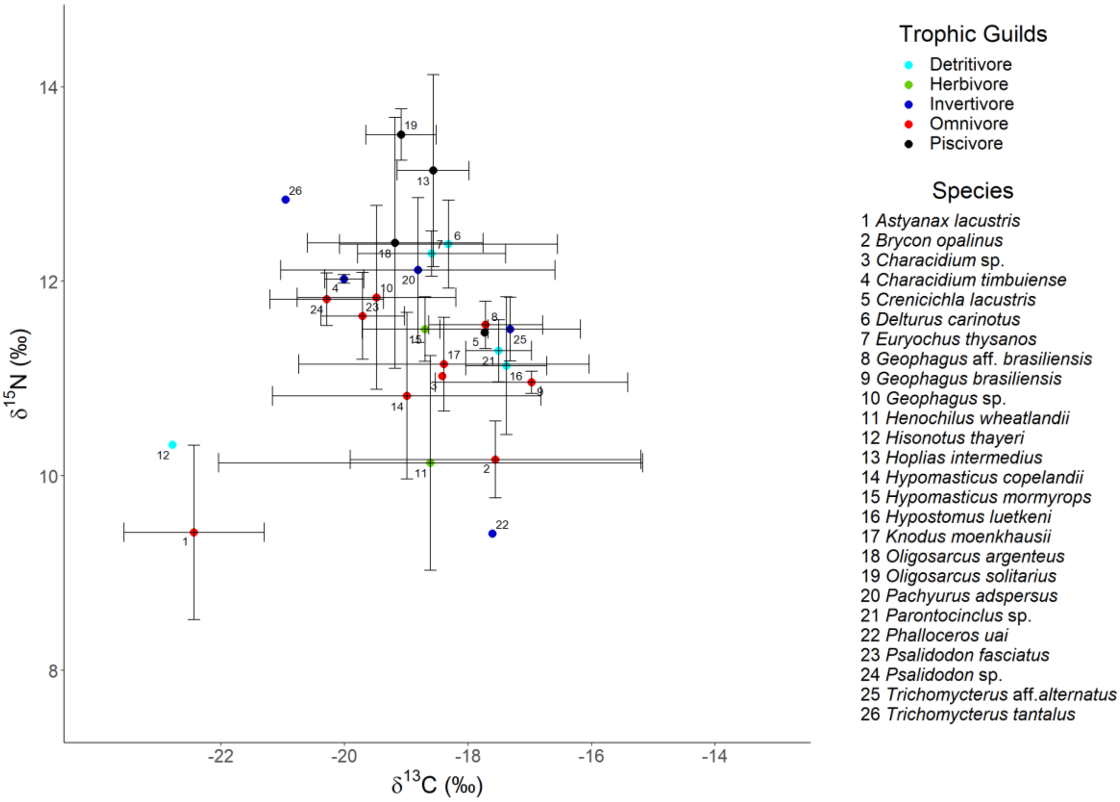


Figure 3: Trophic structure of the fish assemblage in the Santo Antônio River in the years 2020 and 2022 combined. X-axis: carbon isotopic signature, Y-axis: nitrogen isotopic signature. Means and standard deviation of the sampled species.

The species *A. lacustris*, *Henochilus wheatlandii*, and *Hypomasticus copelandii* maintained their basal trophic positions during both years of sampling. The piscivorous species showed a higher trophic position in both years, accompanied by a detritivorous species, *Delturus carinotus*, in 2020 and *H. thayeri* in 2022 (Table 2). The guilds of invertivores, detritivores, and omnivores, representing the majority of the community species, occupied intermediate positions, except for one species in each trophic guild (*Phalloceros uai*, *Hisonotus thayeri*, and *Astyanax lacustris*, respectively), which held lower positions in the trophic chain (Table 2). In 2020, the majority of species occupied intermediate trophic positions, and in 2022 there was an even distribution of trophic positions (Figure 4).

Comparing the trophic niches of the assemblages in the years 2020 and 2022, it is possible to notice a significant niche overlap (Figure 5), with similar amplitude and distribution of $\delta^{13}C$ and $\delta^{15}N$ values in both sampling periods. However, the trophic niche occupied by each guild in 2020 exhibits a more well-defined structure (i.e., more delimited and less overlapping niches). In this year, the herbivores' niche appeared broader and more basal. In the intermediate position are the niches of invertivores, omnivores, and detritivores, with some overlap among them. At the top, there is the more restricted niche of piscivores (Figure 6a). In contrast, in the year 2022, the distinction between guilds is not as clear, with niche overlap among all studied guilds (Figure 6b). When evaluated collectively for both sampling periods, niches still show overlap among trophic guilds. However, the observed pattern of herbivores in the basal regions, omnivores, invertivores, and detritivores in the intermediate position, and piscivores in the top region can still be observed (Figure 6c).

Table 2: Trophic position values for each species in 2020 and 2022.

Trophic Guild	**Species**	**2020**	**2022**
Detritivore			
	Delturus carinotus	4.58	4.58
	Hypostomus luetkeni	4.21	3.78
	Parontocinclus sp.	4.03	
	Euryochus thysanos		5.05
	Hisonotus thayeri		3.88
Herbivore			
	Henochilus wheatlandii	3.47	3.74
	Hypomasticus mormyrops		4.70
Invertivore			
	Characidium timbuiense	4.30	
	Pachyurus adspersus	4.36	4.81
	Trichomycterus aff. *alternatus*	4.16	
	Phalloceros uai		3.62
	Trichomycterus tantalus		4.97
Omnivore			
	Astyanax lacustris	3.16	2.91
	Brycon opalinus	3.53	
	Knodus moenkhausii	4.07	3.68
	Geophagus aff. *brasiliensis*	4.14	
	Hypomasticus copelandii	4.15	4.20
	Psalidodon sp.	4.22	
	Geophagus sp.	4.23	
	Characidium sp.		4.33
	Geophagus brasiliensis		3.75

Trophic Guild	Species	2020	2022
	Psalidodon fasciatus		4.57
Piscivore			
	Hoplias intermedius	4.94	4.86
	Oligosarcus argenteus	4.55	4.76
	Oligosarcus solitarius	4.94	
	Crenicichla lacustris		4.72

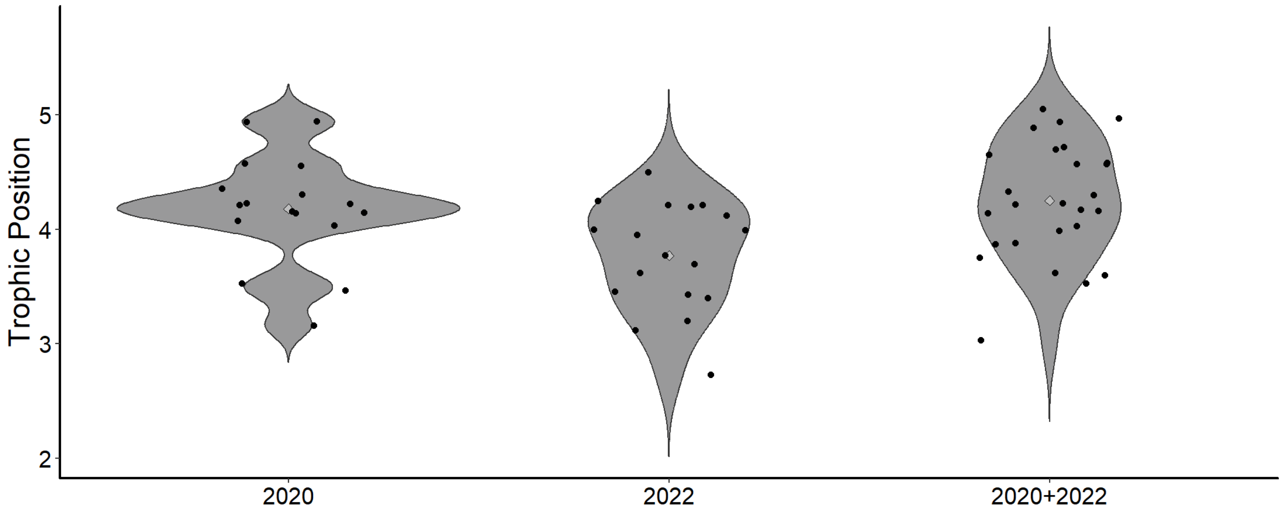


Figure 4: Violin plot illustrating the distribution of trophic positions for the fish communities in a) 2020; b) 2022; c) 2020 and 2022 combined.

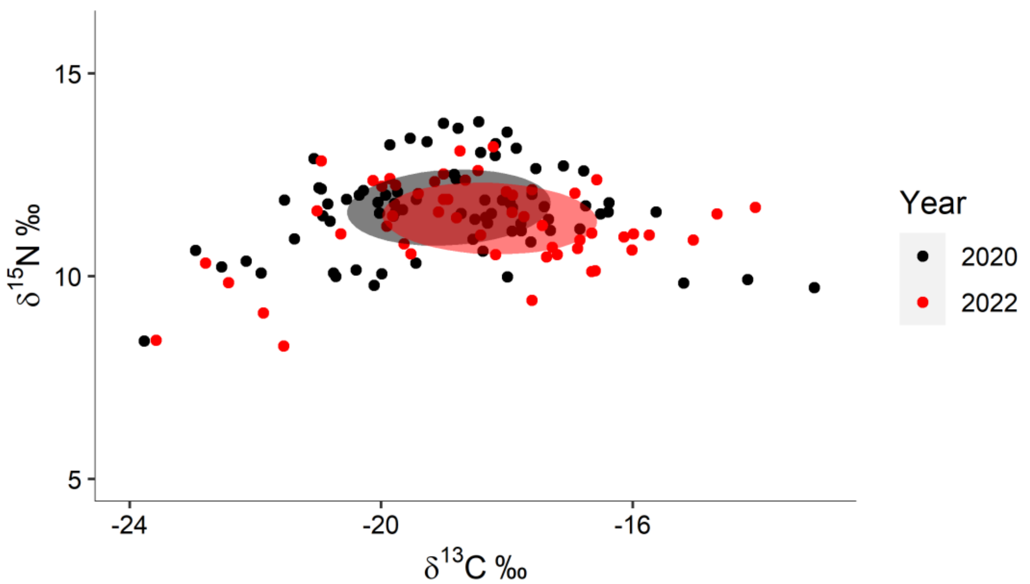


Figure 5: Ellipses (Standard Ellipse Area - SEA - calculated using a 40% confidence interval) representing the trophic niche of the fish assemblage sampled in 2020 and 2022.

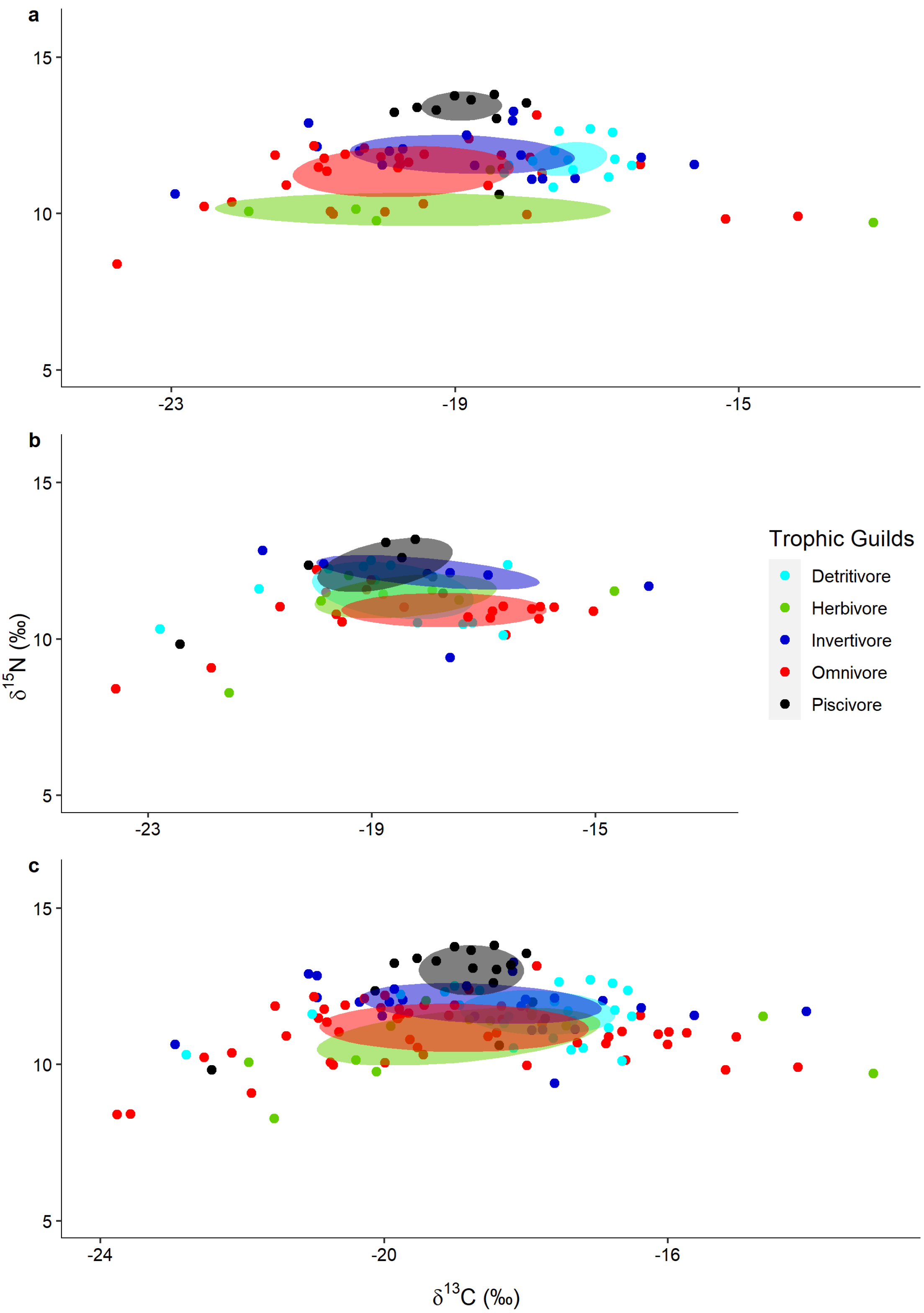


Figure 6: Ellipses (Standard Ellipse Area - SEA - calculated using a 40% confidence interval) representing the trophic niche of fish guilds sampled in the Santo Antônio River: a) 2020; b) 2022; c) 2020 and 2022 combined.

For some of the trophic guilds, variations in the resources assimilated by different species were observed between the sampled years. For the detritivore and herbivore guild, periphyton was the most important resource in 2020, while suspended material took precedence in 2022. In the invertivore and omnivore guilds, aquatic invertebrates were more important in 2020, while terrestrial invertebrates took precedence in 2022. For omnivores, terrestrial invertebrates, which were of little importance in 2020, became significant in 2022. In contrast, for the piscivore guild, fish remained the most important resource in both sampled years (Table 3). Despite the variation in assimilated items by most trophic guilds, different Layman metrics showed similar values in both sampled years (Table 4).

Table 3: MixSIAR partition analysis presenting the mean proportions of assimilated food resources for each fish species sampled in the Santo Antônio River. Resources: Filamentous algae (AL), coarse particulate organic matter (CPOM), aquatic invertebrates (BE), terrestrial invertebrates (IT), periphyton (PE), fish (PX), and suspended material (SM). Resources accounting for more than 40% of assimilation by species are highlighted in bold.

		2020				2022			
Trophic Guild	**Species**	**AL**	**CPOM**	**SM**	**PE**	**AL**	**CPOM**	**SM**	**PE**
Detritivore	*Delturus carinotus*	0.161	0.094	0.270	**0.475**	0.201	0.050	**0.656**	0.093
	Hypostomus luetkeni	0.101	0.053	0.223	**0.623**	0.329	0.038	**0.510**	0.124
	Parotocinclus sp.	0.080	0.051	0.221	**0.649**				
	Hisonotus thayeri					0.195	0.098	**0.592**	0.114
	Euryochus thysanos					0.155	0.027	**0.763**	0.055
Herbivore	*Henochilus wheatlandii*	0.108	0.066	0.315	**0.511**	0.307	0.054	**0.490**	0.150
	Hypomasticus mormyrops					0.193	0.038	**0.688**	0.081
Trophic Guild	**Species**	**AL**	**BE**	**IT**	**PE**	**AL**	**BE**	**IT**	**PE**
Invertivore	*Pachyurus adspersus*	0.060	**0.501**	0.201	0.238	0.094	0.204	**0.640**	0.063
	Trichomycterus aff. *alternatus*	0.061	**0.450**	0.200	0.289				
	Phalloceros uai					0.208	0.308	0.276	0.208
	Trichomycterus tantalus					0.085	0.186	**0.665**	0.065
Omnivore	*Astyanax lacustris*	0.112	**0.416**	0.150	0.322	0.167	0.272	0.381	0.180

		2020				2022			
Trophic Guild	**Species**	**AL**	**BE**	**IT**	**PE**	**AL**	**BE**	**IT**	**PE**
	Brycon opalinus	0.084	0.372	0.104	**0.439**				
	Characidium timbuiense	0.088	**0.412**	0.180	0.319				
	Geophagus aff. *brasiliensis*	0.054	0.309	0.226	**0.412**				
	Geophagus sp.	0.050	**0.409**	0.285	0.256				
	Hypomasticus copelandii	0.072	**0.511**	0.194	0.223	0.167	0.319	0.364	0.149
	Knodus moenkhausii	0.036	**0.609**	0.141	0.214	0.176	0.307	**0.409**	0.107
	Psalidodon sp.	0.068	**0.485**	0.183	0.265				
	Characidium sp.					0.139	0.288	**0.450**	0.123
	Geophagus brasiliensis					0.151	0.294	**0.449**	0.106
	Psalidodon fasciatus					0.095	0.218	**0.605**	0.083
Trophic Guild	**Species**		**BE**	**IT**	**PX**		**BE**	**IT**	**PX**
Piscivore	*Hoplias intermedius*	-	0.175	0.094	**0.730**		0.304	0.181	**0.515**
	Oligosarcus argenteus	-	0.132	0.097	**0.771**		0.204	0.168	**0.628**
	Oligosarcus solitarius	-	0.189	0.077	**0.734**				
	Crenicichla lacustris						0.304	0.201	**0.495**

Table 4: 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, and SDNND = Standard deviation of nearest neighbor distance.

Year	CR	NR	TA	CD	MNND	SDNND
2020	5.155	3.962	11.685	1.615	0.621	0.594
2022	6.520	4.085	17.968	1.787	0.729	0.351

4. Discussion

Despite the variation in species composition sampled each year, the fish assemblage of the Santo Antônio River demonstrated isotopic stability over the two years of sampling, corroborating the central hypothesis of our study. There was substantial niche overlap between the fish assemblage in both years, despite slight variations in trophic levels and assimilated items for each species. The most important resources sustaining the community in 2020 were periphyton and aquatic invertebrates, while in 2022, suspended material and terrestrial invertebrates played a more crucial role. Thus, variations in the most consumed items by each trophic guild were evident each year.

Fish sampling represents an assessment of a subset of local species, especially in large rivers (Argent and Kimmel, 2005; Lapointe et al., 2006). Therefore, it was expected that the sampled community would not be the same between the two years of sampling. Thus, by analyzing both years of sampling together, we obtain a better representation of the fish community in the region, encompassing 45% of all species in the basin (Vieira, 2006). Even with such sampling differences, we observed significant overlap between the isotopic niches of the fish assemblage in both years, as has also been observed in other reference river conditions (de Carvalho et al., 2021). The overlap of niches indicates the stability of trophic conditions in the local fauna, which continues to exploit similar resources over time (Brush et al., 2016). Our findings, however, demonstrated that even with changes on the most assimilated items, it was possible to observe niche overlap between the two years.

The community, in general, has a higher number of species with trophic positions at intermediate levels, with a smaller number of organisms at the base and top of the food chain. This trend was also observed in rivers with good environmental quality in the das Velhas river basin (de Carvalho et al., 2023). Several factors influence the length of the food web in an environment, such as resource availability, ecosystem size, and disturbance (McHugh et al., 2010; Post, 2002; Sabo et al., 2009). These factors tend to vary according to the ecosystem and the interaction of intrinsic and extrinsic factors within the community (Ward and McCann, 2017). Regarding disturbance, regions without constant disturbances, as is the case of the Santo Antônio River, tend to have shorter food chains, as they allow the establishment of species with more predation protection mechanisms (Post, 2002). Rivers classified as having good environmental quality in the Rio das Velhas basin had maximum trophic levels ranging from 4.52 to 4.94 in the analyzed regions (de Carvalho et al., 2023). In the Santo Antônio River, the maximum trophic level ranged between 4.94 and 5.05.

The typical trophic structure for fish communities includes the piscivore guild at the top, followed by omnivores and invertivores in intermediate positions, and herbivores and detritivores in more basal positions (Jepsen and Winemiller, 2002). The same pattern was observed in the Santo Antônio River for the piscivore, invertivore, omnivore, and herbivore guilds. However, detritivores presented more intermediate signatures, along with omnivores and invertivores. The consumption of detritus colonized by microbiota (bacteria, fungi, and protozoa) can increase the trophic position of detritivores (Steffan et al., 2017), thus explaining the observed variation. It is worth noting that species were categorized into trophic guilds based on literature information, which may occasionally deviate from the reality of local species. These studies considered stomach content analyses, providing information on the organism's short-term diet and not taking into account what is absorbed by the organism through long-term diet (Rybczynski et al., 2008). For instance, the omnivorous species

Astyanax lacustris occupied a more basal position than other species in its guild, resembling the signature of herbivorous species. This hypothesis is supported by works indicating the herbivorous tendency of this species (Cassemiro et al., 2002; Pereira et al., 2016).

The isotopic composition of a community also provides information about the niche breadth of species (Newsome et al., 2007). Piscivores and detritivores exhibited a smaller trophic niche area, indicating a more restricted consumption of resources. On the other hand, the guilds of herbivores, omnivores, and invertivores, which are more horizontally elongated, suggest the consumption of resources with more varied carbon signatures. Indeed, these three guilds showed differences in food resources between the sampling years, indicating greater dietary plasticity (Abelha et al., 2001). Despite morphological constraints within these guilds for exploring certain food resources, a change in the most consumed resources is still possible in response to environmental variations (Ibañez et al., 2007). The herbivorous species *Henochilus wheatlandii*, endemic to the Santo Antônio River, deserves special attention for exhibiting trophic plasticity during the two years of sampling, primarily consuming periphyton and suspended material. Despite the species consuming items with a wide variation in carbon signature, it occupies a unique and similar space in the bi-plot graph in both sampling years.

Two food resources were most important in sustaining the detritivore and herbivore guild: suspended material and periphyton. The significance of the latter source deserves special attention. The Santo Antônio River is located in the Rio Doce basin, where one of the largest mining waste dam failures occurred (Hatje et al., 2017). In areas affected by mining waste, periphyton growth was impacted, negatively influenced by the presence of mining tailings in the environment (Zorzal-Almeida and Fernandes, 2021). Moreover, periphyton has been used as a bioindicator in studies indicating changes generated by increased water turbidity (Laird et al., 2021) and the presence of metals (Vidal et al., 2021). Given that periphyton played a significant role in sustaining the fish community in the Santo Antônio River, at least in one of the sampling years, further studies may investigate whether the impact caused by mining tailings on periphyton availability in the rest of the basin alters the energy absorption sources for the fish community. In 2022, higher water turbidity values in the Santo Antônio River (IGAM, 2022) may be related to increased suspended material availability, as turbidity can affect periphyton growth (Laird et al., 2021; Ren et al., 2021).

Invertivorous and omnivorous fish primarily consumed macroinvertebrates, both aquatic and terrestrial. Meanwhile, piscivores showed fish as the most important resource. The consumption of terrestrial invertebrates by fish underscores the importance of preserving riparian vegetation around water bodies (Pusey and Arthington, 2003). Additionally, some terrestrial invertebrates, such as ants and termites, are known to exhibit swarming behavior (Dublon and Sumpter, 2014). The increased availability of terrestrial invertebrates may have influenced the higher consumption of this food item.

Despite some alterations in the resources exploited by the assemblage and the species sampled each year, the overall isotopic niche of the community remained consistent over the two years. There were slight increases in carbon and nitrogen ranges between the years. Total area was the metric that showed the most variation between the years. This change is influenced by species with signatures at extreme values for $\delta^{13}C$ and $\delta^{15}N$. In the 2022 sampling, for example, we have *Hisonotus thayeri*, which exhibited lower carbon signatures, with values close to -23‰; *Phalloceros uai*, showing nitrogen-depleted signatures, with values close to 9.50‰; and *Trichomycterus tantalus*, with a carbon signature close to -20‰. These three species occupied new isotopic spaces in the fish assembly of the Santo Antônio River. In addition to the increase in total area, the slight increase in trophic diversity (CD) reflects greater spacing of species in the second year of sampling, causing a slight decrease in trophic redundancy, as species are exploring different resources. The decrease in the value of

SDNND indicates that niches are more evenly distributed, reinforcing the stability of the community over time. The stability of the isotopic niche of a river under reference conditions was also observed in the Rio das Velhas basin between rainy and dry seasons (de Carvalho et al., 2021).

5. Conclusion

Thus, it is possible to use metrics and general parameters of the isotopic structure of the fish assembly of the Santo Antônio River, which proved to be stable despite isotopic variations in some species. These parameters can be used not only as a baseline in the face of various impacts (PIRH - Doce, 2010) affecting the Doce River basin but also to monitor the effects of basin recovery efforts.

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MANUSCRITO 2 - Assessing temporal shifts in trophic diversity in fish assemblages post-mine tailings dam failure

Patrícia Santos Fráguas[1], Débora Reis de Carvalho[2,3], Frederico Fernandes Ferreira[4], Jorge Abdala Dergam[4], Carlos Frankl Sperber[4] e Paulo dos Santos Pompeu[3]

[1] Programa de Pós-Graduação em Ecologia Aplicada, Departamento de Ecologia e Conservação, Instituto de Ciências Naturais, Universidade Federal de Lavras, 37200-900 Lavras, Minas Gerais, Brazil. psfraguas@yahoo.com.br

[2]Lancaster Environment Centre, Lancaster University, Lancaster, United Kingdom; deboracarvalhobio@gmail.com

[3]Laboratório de Ecologia de Peixes, Departamento de Ecologia e Conservação, Instituto de Ciências Naturais, Universidade Federal de Lavras, 37200-900 Lavras, Minas Gerais, Brazil. pompeu@ufla.br

[4] Departamento de Biologia, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil.

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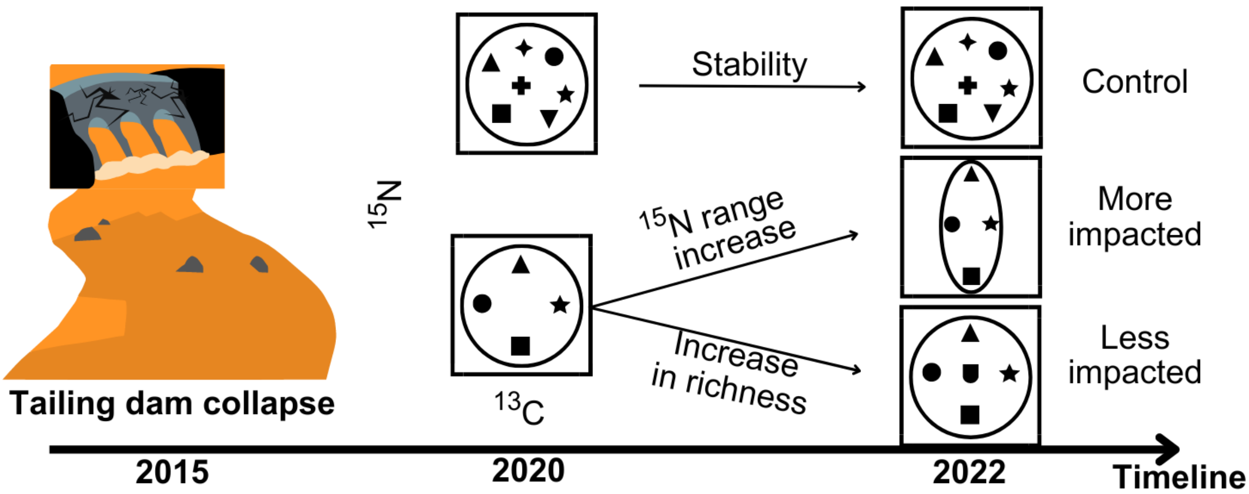
Highlights

- Temporal changes on fish trophic diversity after dam ruptures
- Control sites are stable on trophic diversity metrics
- Impacted sites close to the dam breach are highly unstable

Abstract

The rupture of the Fundão dam stands as one of the most significant environmental disasters of its kind, profoundly impacting aquatic fauna. Employing stable isotopes of carbon and nitrogen, we can delineate the energy flow within ecosystems. In this study, we assess the temporal variation in species richness and trophic diversity in areas affected and unaffected by tailings in the Rio Doce basin. Control regions exhibited higher species richness and stability in trophic metrics over time. Regions in close proximity to the dam rupture demonstrated considerable instability in trophic metrics. Affected regions, farther from the rupture, exhibited an increase in the richness of collected species, and their trophic metrics tended to align more closely with those of the control regions.

Graphical Abstract



Keywords: Ichthyofauna; Stable Isotopes; Carbon; Nitrogen

1. Introduction

The breach of the Fundão Dam in the city of Mariana, Minas Gerais, released approximately 55-62 million cubic meters of iron ore tailings into the watercourses downstream of its location (Governo do Estado de Minas Gerais, 2016). The tailings plume reached the Gualaxo do Norte, Carmo and Doce rivers, contaminating a stretch of over 650 km of the 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 this nature worldwide (Garcia et al., 2017; Hatje et al., 2017; Yamamoto et al., 2023b). In addition to its impact on aquatic environments, the tailings affected an area of 1176 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 rapidly altered by the swift movement of mining residues (Santos et al., 2019), leading to accumulations 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 a 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), 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 (De 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 et al., 2023a).

The disruption of these diverse communities may manifest in changes in resource availability, impacting the trophic web and energy flow within these systems. 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., 2021, 2020), land-use changes (Carvalho et al., 2015; de Carvalho et al., 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 serve as valuable tool for elucidating energy flow within trophic chains (Finlay, 2001; Peterson and Fry, 1987). The nitrogen signature of organisms serves as an indicator of trophic position (Fry, 2006; Post, 2002), as there is an approximate increase of 2.9‰ in the nitrogen signature of consumers at each trophic level incorporated (McCutchan et al., 2003). In contrast, the carbon signature exhibits minimal variation across trophic levels, making it a useful indicator of primary energy sources sustaining organisms, given the distinct carbon signatures of different resources (Fry, 2006; Post, 2002).

Despite isotopic ecology provides precise means to trace major material and energy flows (Fry, 2006; Zanden and Rasmussen, 2001), rivers are environments prone to significant fluctuations (Palmer and Ruhi, 2019; Poff et al., 1997). These fluctuations may result from seasonal or interannual changes unrelated to specific environmental impacts. Therefore, studies assessing temporal changes linked to an impact, such as the breach of the Fundão tailings dam, should seek naturally stable parameters in these environments. These parameters should only undergo alterations in scenarios of restructuring, for instance, during the recovery of a river after a disaster. By evaluating a reference condition river within the same basin, as observed in the preceding chapter, it is noted that while certain fish species may exhibit fluctuations in resource assimilation and, consequently, in their isotopic signatures. Parameters associated with community niche, such as the breadth of carbon and nitrogen signatures, as well as the distances between species in the isotopic niche, remain stable. These parameters possess the potential to indicate potential changes in river conditions.

For the evaluation of trophic diversity 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 impact in watercourses. Isotopic niches and trophic diversity tend to increase longitudinally along rivers, decreasing in locations exposed to anthropogenic impacts, such as sewage inputs and habitat degradation (Wang et al., 2021). In highly polluted basins, an increase in the trophic niche has been observed, likely due to the introduction of carbon sources with distinct isotopic compositions (de Carvalho et al., 2021). Other studies suggest that nutrient enrichment in aquatic environments due to anthropogenic activities can both increase and decrease species niche size (Horka et al., 2023). In the estuarine region of the Doce River after the Fundão Dam breach, a reduction in niches and trophic diversity in predator species was also reported (Andrades et al., 2021).

This study aims to assess the temporal variation in species richness and trophic diversity within the fish assemblage following the breach of the ore tailings dam in the Doce River. Through sampling in two distinct years, at points affected by the breach and at control points, we test the following hypotheses: 1) species richness will be higher at control points and is expected to remain relatively stable between the two years. Among the impacted regions, areas closest to the breach will be most affected, exhibiting lower species richness. If there are indications of recovery in the Doce River, these will be more evident further away from the breach site; 2) Any increases in richness will be reflected in the isotopic niches of the assemblages in the following ways: a) incorporation of species utilizing different resources in the community, resulting in an expansion of the carbon and nitrogen amplitude used by the species; b) an increase in isotopically redundant species, exploring resources already used by the assembly, resulting in a reduction in the spacing of species in the isotopic niche. We aim to evaluate whether there is 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 basin.

2. Methodology

2.1 Study Area

The Doce River basin encompasses an area of 83,431 km², situated entirely within Brazil, spanning the states of Minas Gerais (86%) and Espírito Santo (14%) (ANA, 2016). The primary 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, 2016, 2005). The prevalent 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 headwater 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 in 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).

2.2 Data Collection

We collected data at nine points within the Doce River basin. Six sampling points are situated in regions affected by the tailings breach in Mariana, with three in the upper and three in the middle course of the basin. Three points are located in tributaries of the Doce River and were utilized as control points as they were not influenced by the breach (Santo Antônio River, Piranga River, and Manhuaçu River) (Figure 1, Table 1). Regarding the control points, two of them, Santo Antônio and Piranga Rivers are located on the upper region of the basin and Manhuaçu River is located in the middle region of the basin. The points were sampled during two campaigns, both in the dry season, in the years 2020 and 2022.

Table 1. Sampling points distributed in the Doce River basin, in the state of Minas Gerais, Brazil.

Sampling point	**Coordinates (S)**	**Coordinates (W)**	**River**	**Region**
C1	-20.546203	-42.990349	Piranga river	1 – Control
C2	-19.231225	-43.215228	Santo Antônio river	1 - Control
C3	-19.493909	-41.275483	Manhuaçu river	1 - Control
U1	-20.296162	-43.196883	Gualaxo do Norte river	2 – Upper course
U2	-20.207984	-42.876356	Doce river	2 – Upper course
U3	-20.014662	-42.744474	Doce river	2 – Upper course
M1	-19.251545	-42.314011	Doce river	3 – Middle course
M2	-18.968767	-41.663713	Doce river	3 – Middle course
M3	-19.486436	-41.075063	Doce river	3 – Middle course

2.3 Fish Collection

Sampling was conducted with complementary sampling methodologies using gill nets, trawls, sieves, and cast nets. Gill nets (mesh size 3-16 cm, length 20 m) were continuously exposed for 14 hours at each collection region. 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. Cast nets (elastic mesh 3 cm) were employed in deeper regions. In the field, collected fish were measured, weighed, and identified. A portion of the muscle tissue was extracted from each individual in the field and immediately frozen until the time of analysis to prevent decomposition. At least one individual from each collected species was isotopically analyzed. Stable isotope analyses were conducted for up to 5 individuals of each species for each sampling point.

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.

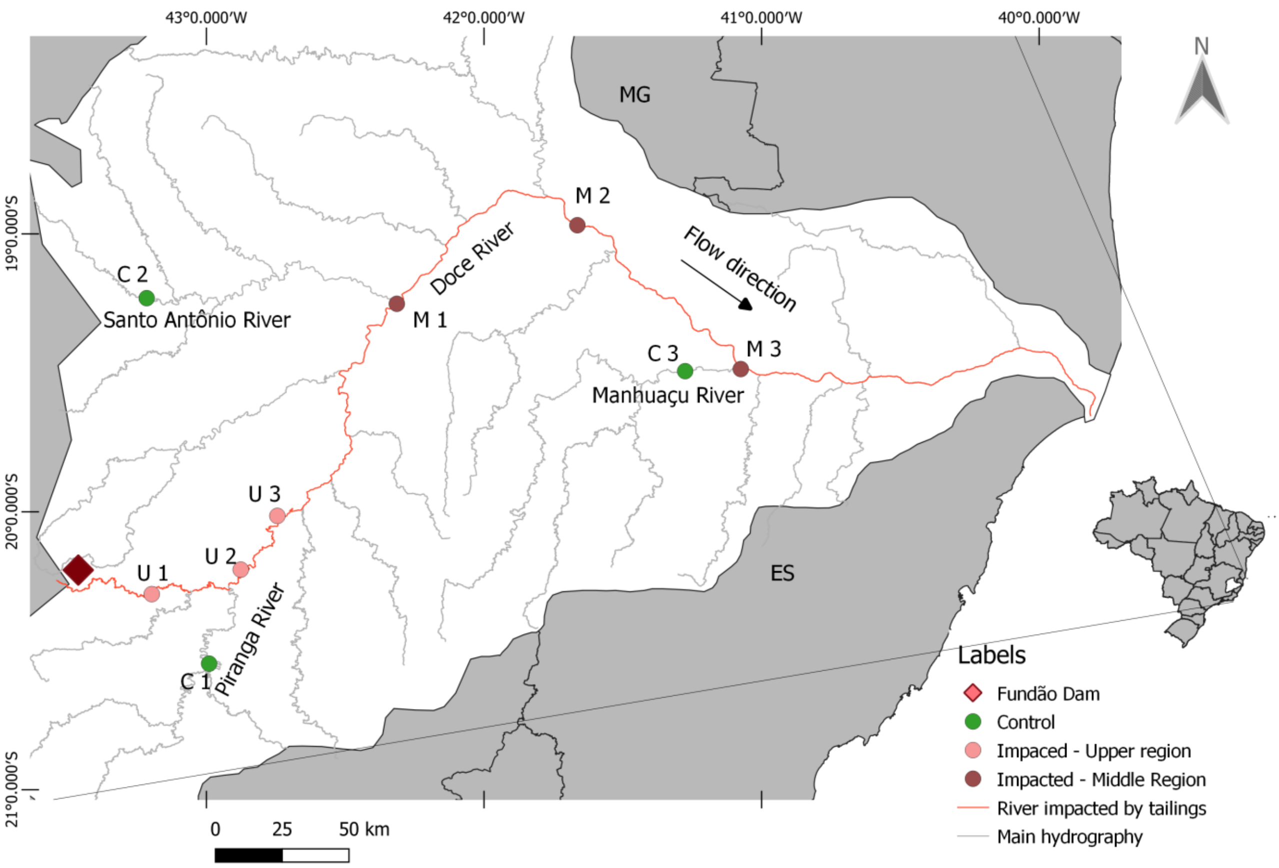


Figure 1. Study Area with nine sampling points distributed along the Doce River basin in the state of Minas Gerais, Brazil. C = Control, U= Upper course, M= Middle course.

2.4 Sample Processing

In the laboratory, fish tissue samples were lyophilized for 24 hours, ground into a uniform powder, and then stored in Eppendorf tubes. Following sample preparation, the samples were sent for isotopic analysis. In 2020, the samples were analyzed at *Centro de Energia Nucelar 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).

2.5 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 Fischer), coupled with a Flash IRMS elemental analyzer interfaced with ConFlo IV. The results 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).

2.5 Statistical Analysis

Differences in species richness among the three studied regions and between the collection years were tested using Generalized Linear Models (GLM) with Poisson distribution. We implemented a hierarchical model distribution, and model selection was carried out using the Akaike Information Criterion (AIC).

To assess differences in isotopic niches between impacted and control points 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 population (Bearhop et al., 2004). The graphical representation of ellipse areas was carried out using the SIBER package (Jackson et al., 2011) in the R program. To increase accuracy of comparisons, a sample size correction was used, noted by subscript “c”. Using SEA_c it is possible to compare the niche of populations with different sample sizes (Jackson et al., 2011). The percentage of niche overlap (where 100% represents total overlap), serves as quantitative metric of diet similarity among species (Hill et al. 2015). Differences in the percentage of niche overlap between the years 2020 and 2022 were tested using Generalized Linear Models.

Trophic diversity in the two sampling periods was evaluated using metrics proposed by Layman (2007), restructured by the Bayesian method allowing for community comparisons (Jackson et al., 2011). 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; Nitrogen range (NR): nitrogen amplitude used by species; Total area (TA): area occupied by species in isotopic space; Centroid distance (CD): a measure of trophic diversity. The last two metrics indicate the relative positions in the niche space and act as proxies for the amount of trophic redundancy: Mean nearest neighbour distance (MNND): a measure of trophic redundancy; Standard deviation of the nearest neighbour 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 spatially we used sampling regions (control, upper and middle) to calculate the trophic diversity metrics. We also present the values of trophic diversity metrics for each region (Control, upper, and middle course) in two boxplot graphs: a) Absolute metric values grouped by the three studied regions for the periods 2020 and 2022, b) subtraction of the values of 2022 by the values of 2020 to assess the variation in metrics over time. The relationship between the variation in Layman metric values over the two collections was analyzed through GLM, with a Gaussian distribution, and model selection was performed using AIC, selecting models with the lowest delta values. All GLM analyses were conducted in the R software (R Development Core Team, 2015) using the MuMIn package (Bartón, 2023).

To assess changes in the set of metrics as a means of portraying the trophic diversity of the fish assemblage, we conducted a 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).

3. Results

A total of 949 individuals belonging to 67 species were analyzed. In the year 2020, 496 individuals from 51 species were collected, and in 2022, 453 individuals from 55 species were collected. Among these, 39 species were recurrent in both samplings, 12 were collected only in 2020, and 16 only in 2022 (Table 2). Species richness did not vary between the collection years but differed among the evaluated regions (p = 0.5583, AICc = 102.3, Delta = 1.67, Weight = 0.232) (Figure 2).

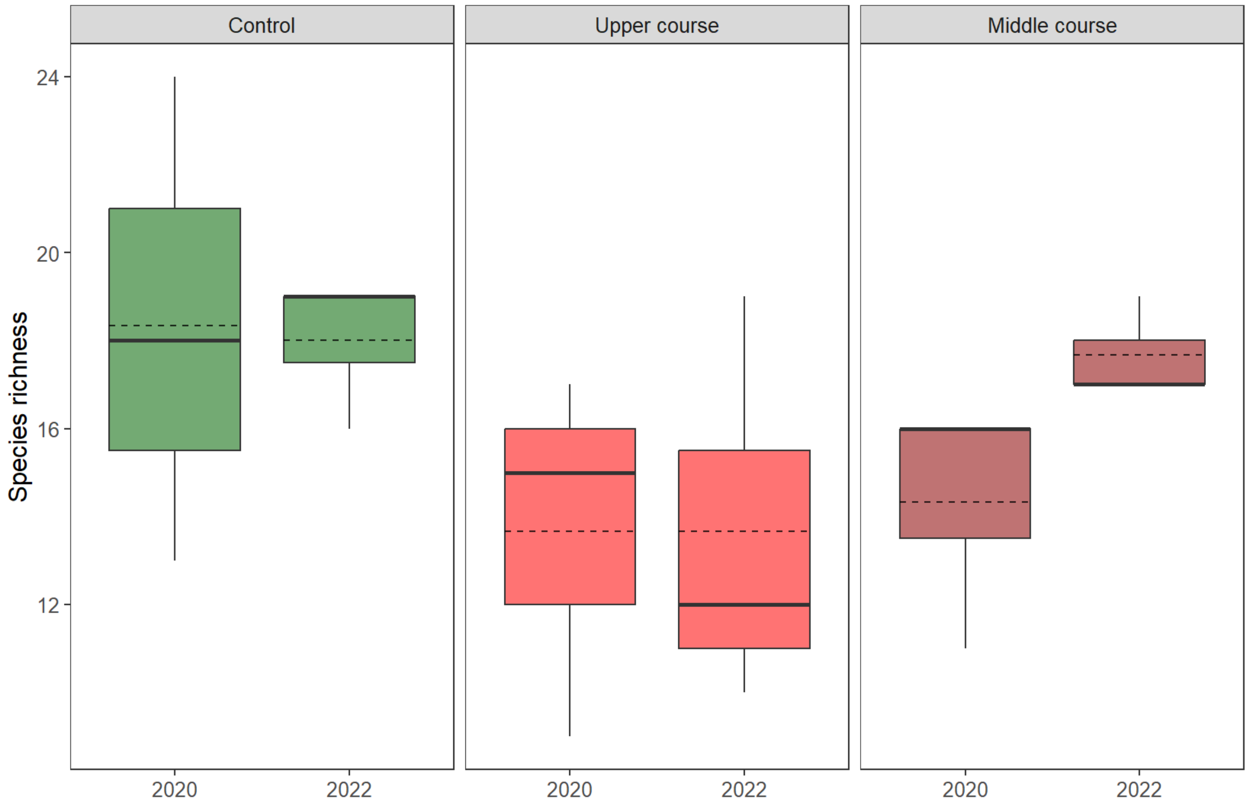


Figure 2: Species richness in each region of the basin (Control, Upper, and Middle courses) in the two sampling years (2020 and 2022). Line inside the box: median (second quartile), lower and upper limits of the box: first and third quartiles, outer lines = 1.5 x IQR, dashed line = mean.

Table 2: Number of samples of each species collected at each point in the two sampling 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 sp.										-		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 intermedius			7				5			-									
Euryochus thysanos										-		5							
Geophagus brasiliensis	6	4	5	1	2	4		8		-	7	5	5	1	1			4	1
Geophagus sp.		7	5			2				-				4					
Gymnotus carapo										-								1	
Gymnotus sylvius	3		3			1			2	-								1	
Harttia loricaformes										-			1						
Hasemania nana				1						-						5			
Henochilus wheatlandii		5								-		2							

					2020										2022				
Species	C1	C2	C3	U1	U2	U3	M1	M2	M3	-	C1	C2	C3	U1	U2	U3	M1	M2	M3
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 auroguttatus										-									5
Hypostomus luetkeni	3	5	2		1			2	1	-	5	4						2	
Knodus moenkhausii *	5	5	5		5	5	5	5	5	-	5	5	5	3	5	6	6	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 argenteus	7	4	1		7	6	8	8	7	-	5	5	5		5	5	3	3	5
Oligosarcus solitarius		3								-									
Oreochromis niloticus *					7	5	6	13	5	-					5		5		5
Pachyurus adspersus	4	8						1		-	5	5							
Parotocinclus doceanus			1			3				-	2								
Parotocinclus sp.		5	1							-									
Phalloceros uai										-	7	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	1	1

	2020										2022								
Species	C1	C2	C3	U1	U2	U3	M1	M2	M3	-	C1	C2	C3	U1	U2	U3	M1	M2	M3
Prochilodus vimboides					1	5		1		-						5			1
Psalidodon fasciatus	1				3					-	5	5		1	5	5	5	5	
Psalidodon sp.		5								-									
Pygocentrus nattereri *								3		-									
Rhamdia quelen	1			3						-									
Salminus brasiliensis *							1	1		-							2		
Serrapinnus heterodon						2				-			1			4	7	1	
Synbranchus marmoratus									2	-								1	
Trachelyopterus striatulus			2						1	-									4
Trichomycterus alternatus		6								-	2			6				2	
Trichomycterus astromycterus					1					-						6			
Trichomycterus cf. *immaculatus*										-						5	1		
Trichomycterus cf. *ipatinga*										-						4			
Trichomycterus sp.				1	3					-					2				
Trichomycterus tantalus										-	1	1							

When comparing the isotopic niche of each sampling point between the two surveyed years, it was observed that the control points, especially C1 and C2, exhibited little variation. However, some of the impacted points, both in the upper (U1 and U2) and middle courses (M1), showed minimal overlap between years, and the differences in overlap were not significant among the three evaluated regions ($p > 0.05$) (Figures 3 and 4).

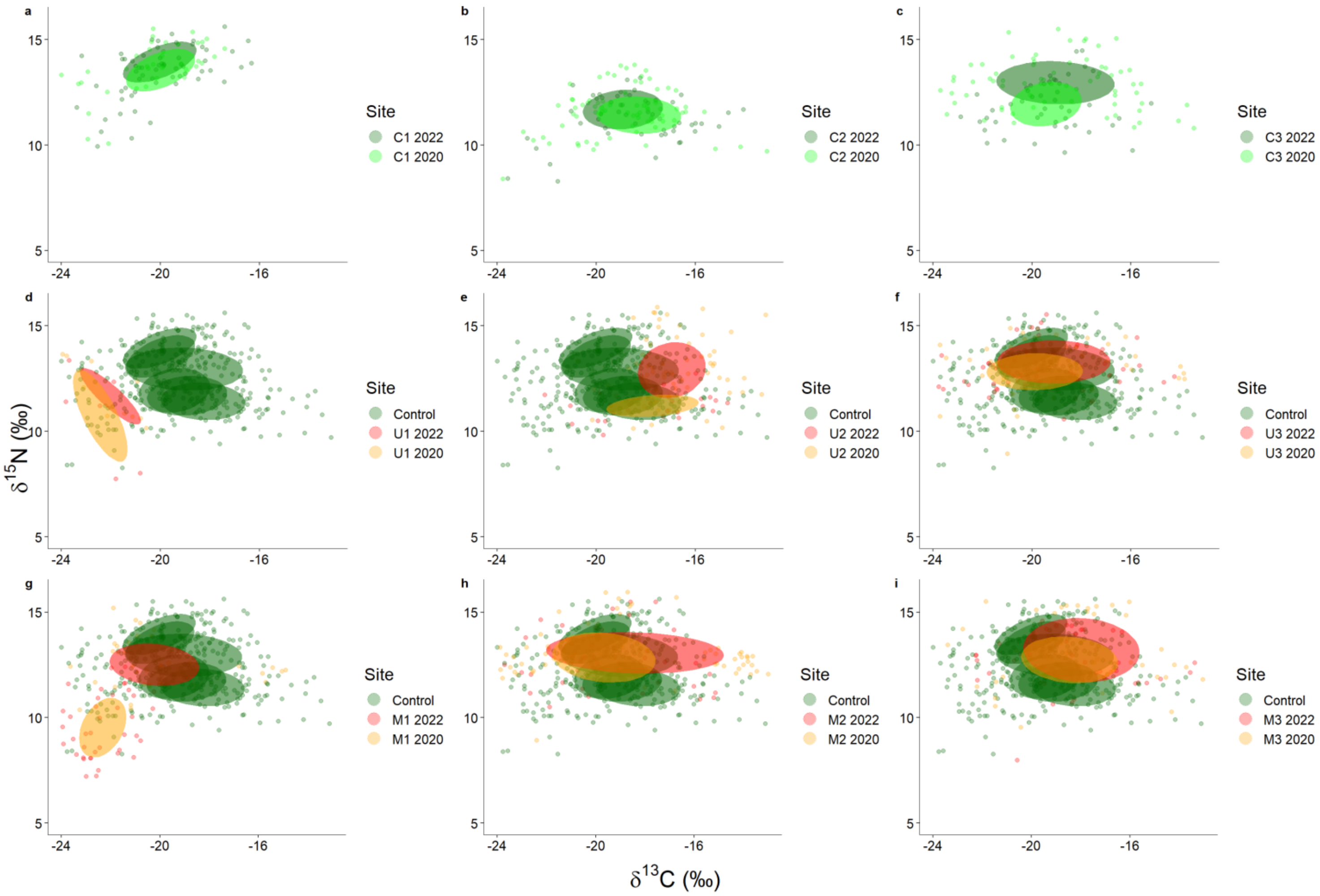


Figure 3: Ellipse Area (SEA) representing the niches of fish assemblages at each sampling point in Doce river basin. Green ellipses represent assemblages from control points. Orange ellipses represent assemblages from impacted points in 2020, and red ellipses represent those in 2022.

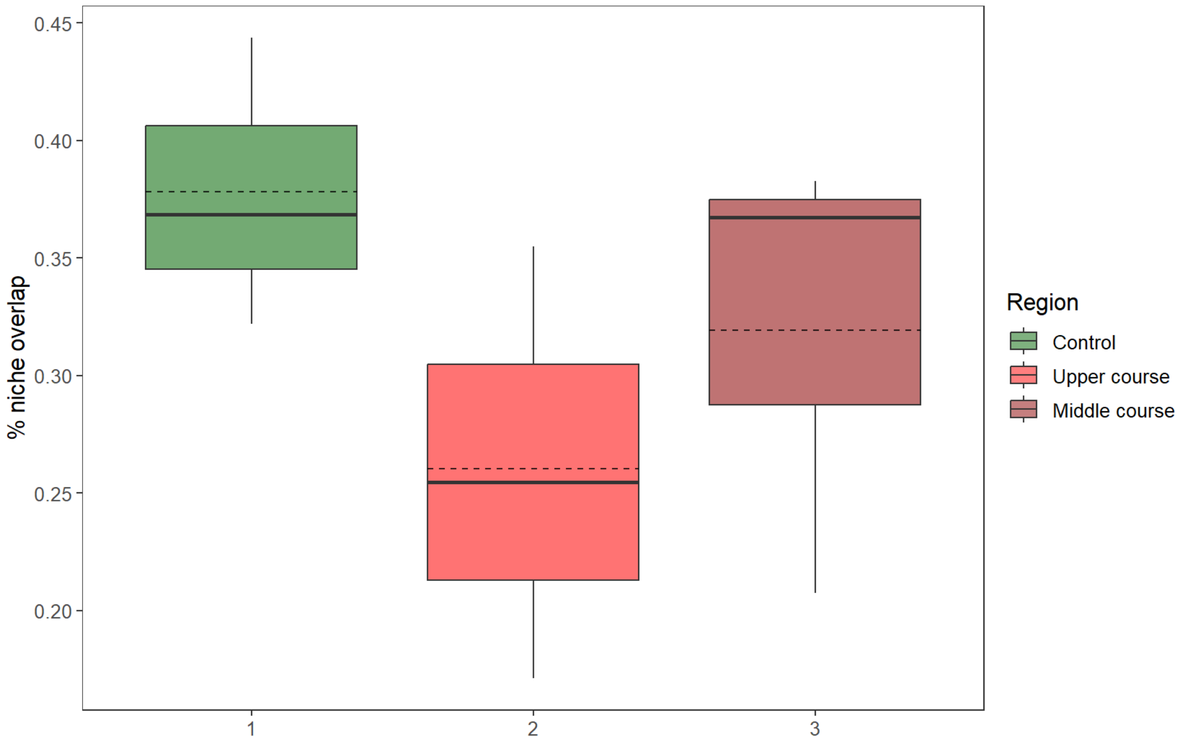


Figure 4: Boxplot depicting the percentage of niche overlap between 2020 and 2022 for the three analyzed regions. Line within the box: median (second quartile), lower and upper limits of the box: first and third quartiles, outer lines = 1.5 x IQR, dashed line = mean.

The set of trophic diversity metrics for the control points shows more homogeneity between the two years of sampling, even though they are in geographically distinct regions within the basin. Among the impacted areas, the region closest to the breach exhibits the greatest variation in metrics, despite being geographically closer points (Figure 5).

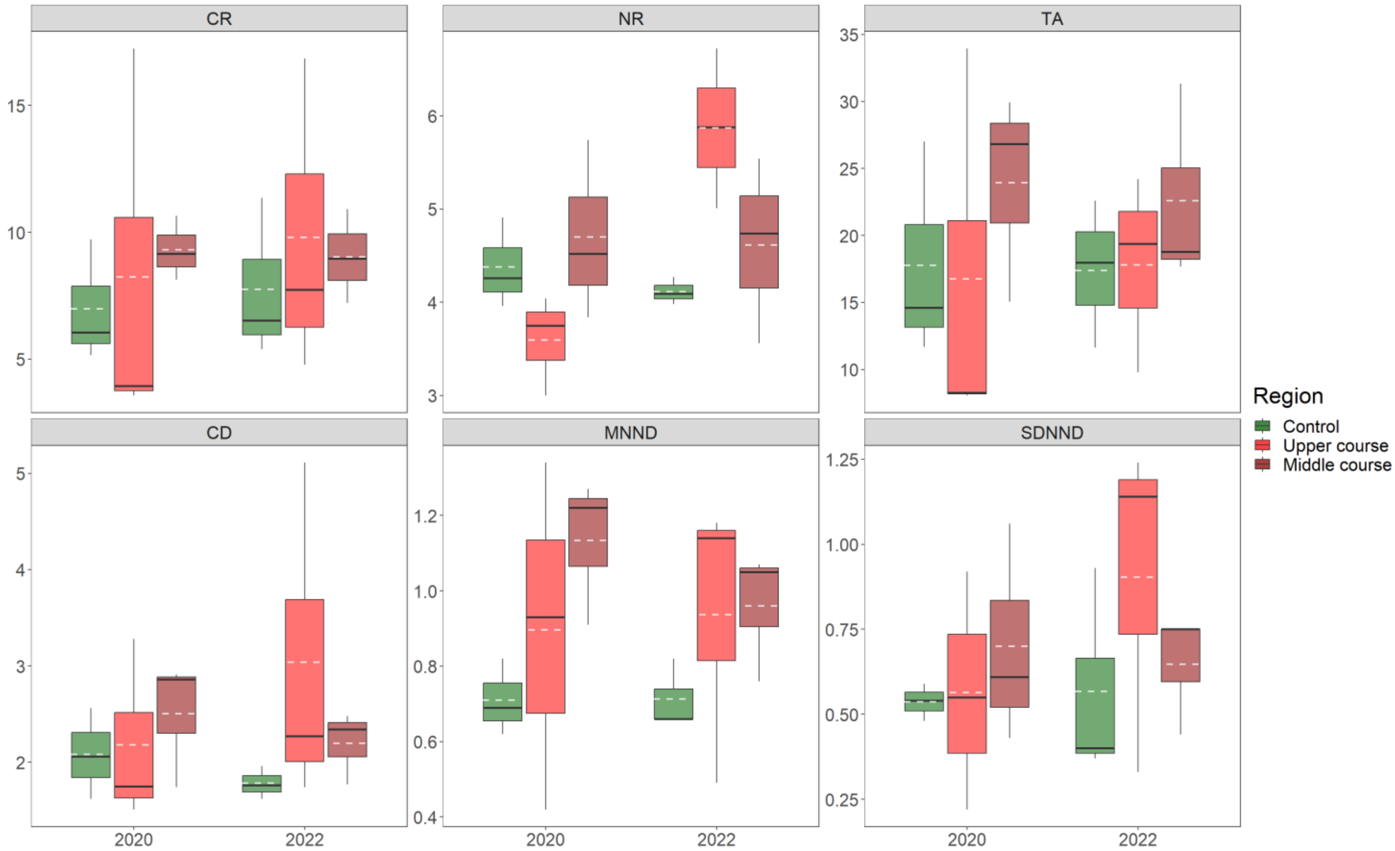


Figure 5: Boxplot of Layman's metrics for the three sampled regions in the years 2020 and 2022 for the fish assemblage sampled in Doce river basin. CR = Carbon range, NR = Nitrogen range, TA = Total area, CD = Centroid distance mean, MNND = Mean nearest neighbor distance, SDNND = Standard deviation of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

By subtracting the values of the metrics obtained in 2022 from those found in 2020 (Table 3), we were able to assess the variation of each metric over time (Figure 6). For most evaluated metrics, the variation of values between the years in the control region is very close to zero, indicating stability during the assessed period.

Carbon range (CR), mean trophic diversity (CD), and the uniformity of the distribution of trophic niches (SDNND) showed little variation in the three regions, with fluctuations around zero (Figure 6), exhibiting no influence from either the year or region (Table 4). The communities in the upper and middle courses showed an elongation in nitrogen range (NR) in the second year of sampling (Figure 6), with the collection year being related to this difference (Table 4). The region (control, upper, and middle courses) influenced both the niche breadth (TA) and trophic redundancy (MNND) (Table 4).

Table 3: 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	3.57	17.23	8.13	10.64	9.15	2020
NR	4.91	3.96	4.26	3	3.75	4.04	3.84	4.52	5.74	2020
TA	14.6	11.69	27	8.27	8.06	33.94	15.05	26.83	29.94	2020
CD	2.06	1.62	2.56	1.75	1.51	3.28	1.74	2.91	2.86	2020
MNND	0.82	0.62	0.69	0.93	0.42	1.34	1.27	0.91	1.22	2020
SDNND	0.54	0.59	0.48	0.55	0.22	0.92	1.06	0.43	0.61	2020
CR	11.35	6.52	5.39	4.78	16.85	7.74	7.22	10.91	8.97	2022
NR	3.98	4.09	4.27	5.88	6.72	5.01	4.74	5.54	3.56	2022
TA	22.59	17.97	11.62	9.79	24.21	19.37	17.69	31.32	18.77	2022
CD	1.96	1.76	1.62	1.74	5.11	2.27	1.77	2.48	2.34	2022
MNND	0.82	0.66	0.66	1.18	1.14	0.49	0.76	1.05	1.07	2022
SDNND	0.93	0.4	0.37	1.14	1.24	0.33	0.75	0.75	0.44	2022

Table 4: Model selection assessing the variation of Layman's metrics at each sampling point according to the region and collection year.

Metric	Year	Region	df	logLik	AICc	Delta	Weight
CR			2	-49.256	103.9	0.00	0.633
NR	0.6411		3	.22.774	53.3	0.56	0.333
TA		2.844	3	-61.928	131.6	1.29	0.299
CD			2	-22.521	49.8	0.00	0.634
MNND		0.1675	3	0.915	5.9	0.00	0.674
SDNND			2	-3.203	11.2	0.00	0.617

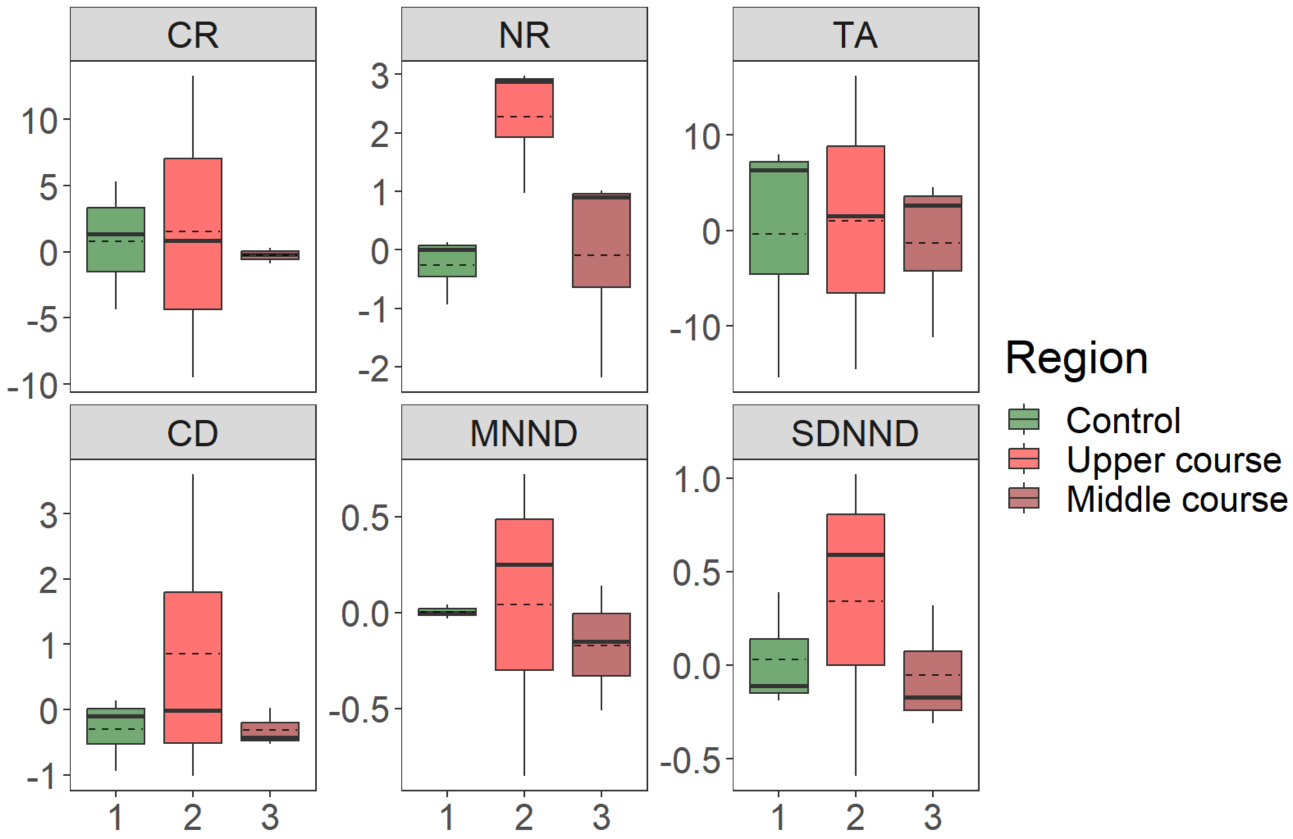


Figure 6: Boxplot of the subtraction of Layman's metrics values between the years 2022 and 2020 for the fish assemblage sampled in Doce river basin. CR = Carbon range, NR = Nitrogen range, TA = Total area, CD = Centroid distance, MNND = Mean nearest neighbor distance, SDNND = Standard deviation of the nearest neighbor distance. Line inside the box: median (second quartile), lower and upper limits of the box: first and third quartiles, outer lines = 1.5 x IQR, dashed line = mean.

The first two axes of the PCA explained 77.49% (60.20 axis 1 and 17.29 axis 2) of the variation in Layman's metrics. Overall, trophic diversity represented by Layman's metrics is more similar among control points, showing less variation. The points from the middle course are closer to the control point in terms of metrics than the upper points. The most affected points, in the upper course, are more distinct from each other in terms of trophic diversity metrics. The region ($p = 0.004$) presented greater importance in shaping the trophic diversity of the fish assemblage (Table 5).

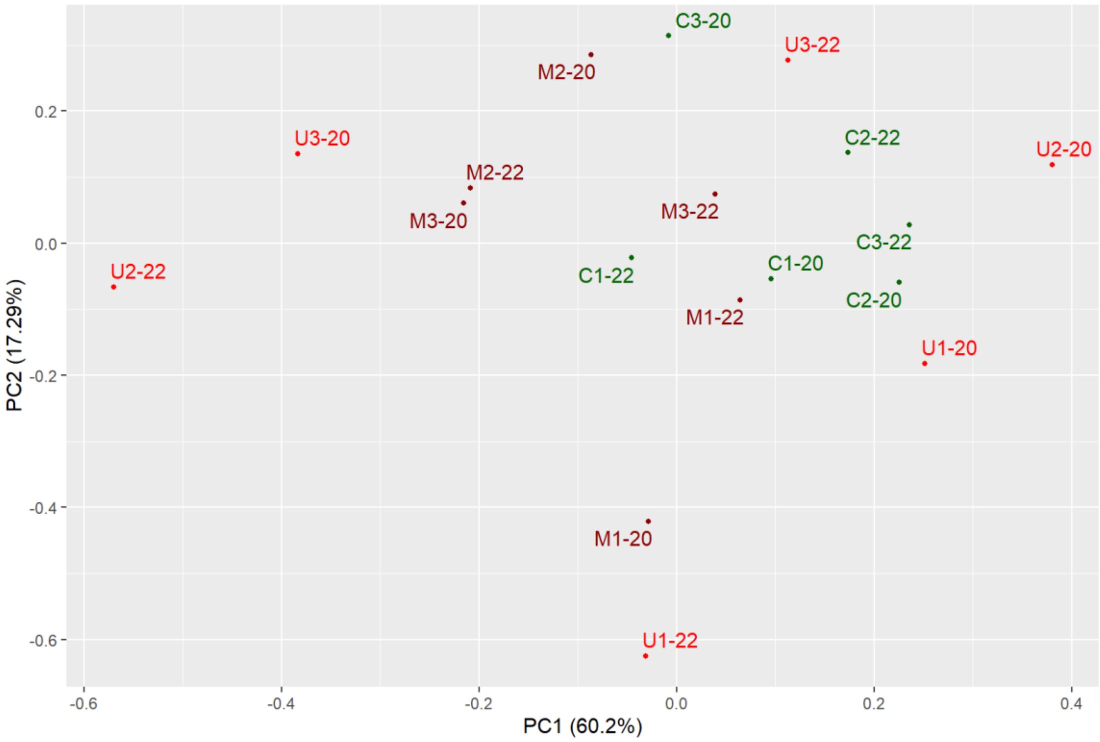


Figure 7: Principal Component Analysis (PCA) of Layman's metrics between the collection years 2020 and 2022 for the fish assemblage sampled in Doce river basin. C1, C2, C3 – Controls 1, 2, and 3; U1, U2, U3 – Upper Course 1, 2, and 3; M1, M2, M3 – Middle Course 1, 2, and 3; 20 – collection year 2020; 22 – collection year 2022.

Table 5: Comparison of data dispersion related to Layman's metrics between years, regions, and their interaction (PERMDISP).

Variables	F	P
Region	9.59	0.004
Year	2.89	0.131
Region + Year	2.23	0.297

4. Discussion

The region indeed influences the species richness sampled in the Rio Doce basin. The control points showed a higher average species richness in both samplings, while points closest to the tailing dam rupture exhibited lower species richness. No significant differences in niche overlap were observed among the three regions. Regarding trophic diversity metrics, the three regions had different patterns. The control region remained stable, the upper course region showed an increase in nitrogen range over the years, and there was substantial variation in the other metrics, indicating the instability of this region. The overall structure of the middle course region approached that observed in the control points.

Differences in species richness along a river are expected, typically showing an increase in richness along a longitudinal gradient from the source to the mouth (Bistoni and Hued, 2002; Oberdorff et al., 2011, 1993). Therefore, the observed differences in richness in the upper and middle course regions and their relation to the dam rupture should be approached with caution. Based on several species lists after the Fundão dam rupture, the richness found in the upper Rio Doce stretch was 31 species, and 66 for the middle course region (Petesse et al., 2023). It is important to highlight that control areas showed higher species richness regardless of the region in which they were collected. Tributaries unaffected by the rupture may have acted as refuge areas and, after the impact, assisted in the recolonization of species in the main channel of the Rio Doce (Salvador et al., 2022). Which highlights the importance of undammed tributaries in river basins.

Although not statistically significant, notable changes in the overlap of isotopic niche ellipses between years were observed among impacted points. Some sites do not present niche overlap between years, reflecting the instability of these regions. Previous studies demonstrated that the impact caused by the rupture altered the niche of estuarine fish species in the Rio Doce (Andrades et al., 2020). Impacts caused by an increase in metal concentration in the water due to the tailings have been shown for some organisms (Vergilio et al., 2021). Changes in richness and composition of zooplankton communities have been reported, for example (Santos et al., 2022); stress responses to metal presence (Marques et al., 2022), a decrease in diversity after the passage of tailings in the marine planktonic community (Fernandes et al., 2020); and a decrease in richness, diversity, and abundance of marine benthic macrofauna (Nascimento et al., 2022). Moreover, the accumulation of various metals in tissues of shrimp and fish has also been observed (Costa et al., 2022), which occupy higher trophic levels. Other anthropogenic alterations, such as increased nutrient load in water bodies, also alter the isotopic niche of species by modifying the availability and quality of food resources (Horka et al., 2023). Alterations caused by the tailings contribute to changes in the environment, which result in shifts in the trophic niche of the communities.

The region of the river significantly pointed out differences in two evaluated metrics: trophic redundancy (MNND) and niche breadth (TA). In our study, impacted points exhibited higher values of MNND. Similar findings of elevated MNND values (indicative of decreased trophic redundancy) were also reported for pollution-affected points in the Rio das Velhas river basin (de Carvalho et al., 2020). The impacted region, particularly the upper course of the Doce river, displayed variation in the metrics' breadth. Substantial fluctuations in carbon and nitrogen amplitudes may reflect variations in available resources at these points, consequently reducing trophic redundancy in these regions. In the estuarine regions of the Doce River, Andrades et al. (2020) observed the opposite. Their assessment, conducted less than a year after the impact, may explain the abrupt decline in resource availability, affecting all trophic diversity metrics. We found that total area (TA), indicative of niche breadth, showed temporal increases, particularly in the mid-course region, suggesting potential recovery, or predominance of generalist species.

The nitrogen range (NR) metric varied across sampling years. The most affected region (the upper course) exhibited significant variations in its values, an indication of high instability in the trophic structure of these communities, suggesting an increase in trophic levels in the second sampling year, or enrichment of basal resources, which would be reflected on fish ^{15}N. An increase in nitrogen range was also observed for estuarine communities in the Rio Doce after 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 worth noting 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 be raised. This increase, if related to the presence of top predator species, could be associated with a potential recovery process of this part of the basin. However, only one piscivorous species, *Hoplias malabaricus*, was recorded in the upper course of the basin only in 2022. 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 (de Carvalho et al., 2021). 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 chain, resulting in an increase in nitrogen range. For these reasons, the mechanism behind this increase deserves investigation. There were no changes in the diversity of resources sustaining the fish assembly (CR); trophic diversity (CD) and the uniformity of niches distribution (SDNND) since these metrics showed no changes between years and among regions.

Analyzing the trophic diversity metrics of the community reveals trends in the three regions. The control points, even if geographically distant, exhibit similar pattern. On the other hand, impacted points, despite being geographically close, are much more unstable over the two years, showing significant variations in metrics. This fact shows 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. Regions farther from the rupture, although also undergoing changes, tend to exhibit smaller variations than those observed in points closer to the impact. Recent surveys indicate that most non-native species present in the basin before the dam rupture are still observed in the Rio Doce channel, in addition to other non-native species that began to occur in the area after the dam rupture (Petesse et al., 2023). The growing number of non-native species points to their establishment capacity 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, and it can reflect changes in the niche space occupied by the fish community. Adding this type of impact to the basin, these highly unstable areas may end up favoring non-native species over native species in the region. Further increasing the pressure on native species in the region.

5. 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 mouth region of the Rio Doce. The assessment of different regions in the Rio Doce basin points to the stability of control areas, with the stability of trophic diversity metrics serving as a good parameter for indicating 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 structure observed in control points. Thus, trophic parameters of the community can be used as a tool for monitoring in the basin.

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