Non-native fish community occupies broader isotopic niche than native fish community in an impaired river system

Daniel Azarias Rezende da Silva[a]; Débora Reis de Carvalho[ab]; Frederico Fernandes Ferreira[c]; Jorge A. Dergam[d]; Marcelo Zacharias Moreira[e]; Paulo Santos Pompeu[a].

[a]Laboratório de Ecologia de Peixes, Programa de Pós-Graduação em Ecologia Aplicada, Departamento de Ecologia e Conservação, Universidade Federal de Lavras, Campus Universitário, Caixa Postal 3037, CEP 37200-000 Lavras, MG, Brazil. daniel.rezendde@gmail.com, pompeu@ufla.br

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

[c]Programa de Pós-Graduação em Ecologia, Universidade Federal de Viçosa, Viçosa, MG, Brazil; frederico.bio@gmail.com,

[d]Laboratório de Sistemática Molecular Beagle, Departamento de Biologia Animal, Universidade Federal de Viçosa, Viçosa, MG, Brazil; dergam@ufv.br

[e]Laboratório de Ecologia Isotópica, Centro de Energia Nuclear na Agricultura - CENA, Universidade de São Paulo, Av. Centenário, 303, Caixa Postal 96, CEP 13400-970, Piracicaba, SP, Brazil. mmoreira@cena.usp.br

***Corresponding author:** pompeu@ufla.br

ORCID: Daniel Azarias Rezende da Silva (0009-0001-7855-034X), Débora R. de Carvalho (0000-0001-8997-2145), Frederico F. Ferreira (0009-0006-0341-3830), Jorge A. Dergam (0000-0003-1395-1377); Marcelo Z. Moreira (0000-0001-6769-5570) and Paulo S. Pompeu (0000-0002-7938-1517).

STATEMENTS AND DECLARATIONS

Competing Interests

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

Funding

This study was funded by Fundação Renova (grant number 005836/2019). Paulo S. Pompeu was awarded a research productivity grant (grant number 302328/2022-0) by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Data availability

Data will be made available on request.

Ethics approval

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

Credit authorship contribution statement

Daniel A. R. Silva: Conceptualization, Methodology, Data curation, Investigation, Writing (original draft, review & editing); Débora R. Carvalho: Conceptualization, Methodology, Data curation, Statistical Analysis, Investigation, Writing (review & editing); Frederico F. Ferreira: Conceptualization, Methodology, Investigation, Writing (review & editing); Jorge A. Dergam: Funding acquisition, Project administration, Writing (review & editing); Marcelo Z. Moreira: Methodology, Writing (review & editing); Paulo S. Pompeu: Conceptualization, Methodology, Funding acquisition, Investigation, Project administration, Writing (review & editing).

Acknowledgments

We thank the Fundação Renova for the project financial support (grant number 005836/2019) and CNPq for the PSP research productivity grant (grant number 302328/2022-0). Thanks also to the Laboratory of Fish Ecology (UFLA) and Laboratory of molecular systematics Beagle (UFV) who assisted in the processing of samples and infrastructure, and to the Centre for Nuclear Energy in Agriculture (CENA) for their support and partnership in the isotopic analysis.

Abstract

This study aimed to compare the trophic ecology of native and non-native fish species in the Doce River basin, which has been subjected to various anthropogenic impacts, including Brazil's largest environmental disaster: the rupture of the Fundão iron ore tailings dam. Using carbon and nitrogen stable isotopes, we evaluated the isotopic niche and trophic position occupied by all fish species sampled at eight sampling points along in the upper, middle, and lower reaches of the Doce River. Currently, non-native species exhibit a broader isotopic niche than the native assembly, occupying all trophic levels. Their establishment seems to have been favored both by "vacant" niche positions and by the reduction of native species populations. The historically most impacted points, which also received the tailings from the dam breach, presented a higher percentage of non-native species. The higher this percentage, the greater the observed isotopic overlap with native species. Non-native species occupied 'vacant' isotopic niches in most disturbed regions, represented by more enriched $\delta^{13}C$ signatures. However, locally, their range of $\delta^{13}C$ compared to native species was not different among least and most disturbed sites. Our results underscore the urgent need for strategies to control non-native species populations in the basin.

Keywords: Stable isotopes; Introduced species; Trophic position; Trophic niche; Fundão dam.

Introduction

The introduction of species is a longstanding practice (Casimiro et al., 2010), and with the process of globalization, it has been intensifying over time (Rahel, 2007; Ricciardi, 2007; Leprieur et al., 2008; Vitule et al., 2012). Due to its significant impact on biodiversity (Azevedo-Santos et al., 2015), species introduction is considered the second leading cause of fish species extinction worldwide (Miller et al., 1989; Clavero & García-Berthou, 2005; Seebens et al., 2021). The proliferation of non-native species in aquatic environments harms the entire native biota, leading to negative impacts on both humans (Casimiro et al., 2010), and the established fish community (Vitule et al., 2009). This, in turn, results in various economic, social, and environmental complications (Becker & Grosser, 2003; Ricciardi et al., 2017). Global annual costs of species introductions have been estimated exceeding 423 US billion dollars, where 92% correspond to losses in ecosystem function affecting people and their quality of life, and only 8% are related to management of these species (IPBES, 2023).

Estimating ecological modifications resulting from the introduction of non-native fish is a complex process (Underwood, 1992), as impacts can vary from genetic changes to changes in the trophic structure of aquatic ecosystems (Vitule & Prodocimo, 2012; Garcia et al., 2021) giving rise to what are known as "cascading interactions" (Pinto-Coelho et al., 2008; Gozlan et al., 2010; Flood et al., 2020). Concerning the most frequently observed damage to fish fauna, there is a decrease in the diversity and richness of native species, alterations in population structure, the spread of pathogens, pests, and parasites, stunting, ecosystem disturbances, hybridization, competition, predation, biotic homogenization, changes in energy pathways, and possible extinction of native species (Charles & Dukes, 2007; Vitule et al., 2009; Pelicice et al., 2023). Numerous factors may be associated with the exclusion of native species due to the introduction of non-native species, such as increased predation on juveniles and adults (de Souza et al., 2021), competition for resources, and the overlap of trophic niches (Zaia Alves et al., 2020).

Identifying the main factors behind global invasions is essential for developing effective conservation strategies (Hulme, 2003). Elton (1958) suggested that successful invaders are frequently linked to habitats altered by human activity, and that those habitats susceptible to invasion are typically experiencing human-induced disturbances. When species share similar environmental needs, native species can inhibit the spread of nonnative species. However, nonnative fishes that spread quickly usually fill "vacant" niche positions in the life-history spectrum, a phenomenon linked to "niche opportunities" created by human-induced environmental changes (Olden et al., 2006). In this scenario, disturbances are believed to free up resources and create opportunities for invaders (Davis et al., 2000). Particularly when disturbances happen too swiftly, many native species are unable to adapt, resulting in their decreased numbers, local extinctions, and the creation of unoccupied niches that nonnative species can then exploit (Havel et al., 2005; Clavero et al., 2013). The "human activity" hypothesis, although only one of several explanations for the invasion process, has been supported by research on freshwater fish invasions (e.g., Leprieur et al., 2008; Anas & Mandrack, 2021; Milardi et al., 2022). Therefore, one of the potential ways to understand the drivers of invasion, and estimate the effects resulting from the introduction of non-native fish species is through the analysis of trophic niches (Pennock et al., 2021). This approach has also shown promise in understanding trophic ecology in areas directly impacted by anthropogenic influences (e.g. de Carvalho et al., 2019a, 2019b).

Through the use of stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes, it is possible to calculate the isotopic niche, which can be considered a proxy for the trophic niche (Layman et al., 2007; Jackson et al., 2011). The $\delta^{13}C$, due to its small variation from one trophic level to another (between 0‰ to 1‰) (Peterson & Fry, 1987; Manetta & Benedito-Cecilio, 2003), can provide information about the dietary resources assimilated by consumers (Fry, 2006). On the other hand, $\delta^{15}N$ exhibits an enrichment of approximately 3‰ between trophic levels (DeNiro & Epstein, 1981; Post, 2002; Kaymak et al., 2018) and can be used to estimate the trophic positions of consumers (Minagawa & Wada, 1984; Albrecht et al., 2021). Therefore, since the isotopic composition of fish reflects their feeding habits in a given system and season it is expected that generalist species will exhibit greater variation in $\delta^{13}C$ and $\delta^{15}N$ values, while specialist species will show little variation in isotopic compositions (Bearhop et al., 2004). Consequently, these variations will be reflected in the isotopic niche occupied by species, and stable isotopes of C and N provide an alternative to estimate not only the breadth of these niches but also to assess their overlap (Shipley & Matich, 2020).

The Doce River basin, due to their history of degradation and proliferation of non-native species, provide opportunities to investigate the relationship between attributes of the trophic niche of the native species pool and the local invasive species. The basin is notable for its high incidence of species introduction, with 25.5% of its fish fauna (39 species) classified as non-native (Bueno et al., 2021). The introduction of non-native species in this basin began in the 1970s with the aim of improving the fishing system in the region (Alves et al., 2007; Marques et al., 2013). These introductions were, for example, responsible for the local extinction of native species (e,g, *Oligosarcus solitarius* Menezes 1990) in lakes within the most important conservation unit in the basin, the Doce River State Park (Latini, 2001; Fragoso-Moura et al., 2016). The Doce River basin is also noteworthy for its long history of environmental degradation (Sánchez et al., 2018), which was exacerbated by the rupture of the Fundão iron ore tailings dam on November 5th 2015, in Mariana, Minas Gerais . Considered the largest environmental disaster in Brazil, this event had significant effects on the aquatic ecosystems of the region (Brasil, 2015; Espindola et al., 2016), due to, among other factors, water contamination with tailings, riverbed siltation, and destruction of riparian and aquatic vegetation.

We aimed to test the hypothesis that human-induced environmental changes in the Doce River basin have favored the establishment of non-native fish species by the release of resources through both "vacant" niche positions and the reduction of native species populations. We characterized the isotopic niche of the pool of native and non-native species, and their respective trophic levels in regions of the Doce River with different levels of degradation. We expect that: (i) the historically most impacted sites in the basin, which also received the tailings from the dam rupture, have a higher percentage of non-native species; and the higher this percentage, the greater the isotopic overlap with native species; (ii) if the non-native species occupied 'vacant' niches in most disturbed regions, their pool will use more resources diversity (wider range of $\delta^{13}C$) compared to non-native species. Information regarding the trophic ecology of fish in this basin, which has been going through from environmental degradation for decades, is still limited. Therefore, we hope that the information obtained will contribute to understanding the role that potential measures for controlling non-native species will have in the basin's recovery.

Material and Methods

Study area

This study was conducted in the Doce River basin, a river entirely located in the southeastern region of Brazil, spanning 225 municipalities in the states of Minas Gerais (86% of the territory) and Espírito Santo (14% of the territory). The Doce River flows for 853 kilometers from its headwaters in the Mantiqueira Mountains to its mouth at the Atlantic Ocean (in the district of Regência Augusta, Linhares, Espírito Santo) (Brasil, 2016) (Fig 1). The basin covers a drainage area of 84.000 Km^2 and is mainly situated within the Atlantic Forest biome (Brasil, 2016). Additionally, the Doce River basin plays a crucial role in the economy of eastern Minas Gerais and northwestern Espírito Santo by providing essential water resources for domestic, agricultural, industrial, and energy generation activities (Brasil, 2016). The basin features rugged terrain and is divided into three regional units (referred to as upper, middle, and lower Doce River) and has ten hydroelectric power plants (UHEs) installed, being four of them, located on the Doce River channel itself and six on its tributaries (Brasil, 2016).

The Doce River basin has undergone various anthropogenic modifications resulting from industrial activities, particularly in the Vale do Aço region in the Middle Doce River, as well as from agriculture, livestock farming, forestry, mining, energy production, discharge of untreated domestic sewage from cities in its vicinity, and the introduction of non-native fish species (Coelho, 2009; Brasil, 2016). In addition to these factors, in November 2015, the Fundão dam in the municipality of Mariana, MG, which contained iron ore tailings from the SAMARCO company, collapsed. Approximately 43 million m^3 of tailings, possibly contaminated with metals such as Iron (Fe), Arsenic (As), Mercury (Hg), and Manganese (Mn), were discharged into the Doce River basin (Brasil, 2015, 2016). The mud carried from the upper part of the basin to its mouth directly impacted the local fish community, not only by altering water quality, but also by destroying breeding, nesting, and resting habitats and altering food availability, which, in turn, was detrimental to the local population dependent on fishing (Brasil, 2015, 2016).

Sample design

Eight sampling points were distributed along the Doce River, covering the upper, middle, and lower sections of the basin (Fig. 1, Table 1). Among these points, five were located along the main channel of the Doce River and were affected by the rupture of the Fundão dam (M1-M5). These sites were considered the most disturbed. The other three points were considered least disturbed points (L1-L3) as they were not impacted by the dam breach and were also historically less impacted. However, only one of them, the Santo Antônio River (L2), might be considered a reference site (Vieira, 2006). Thus, the upper Doce River region included one most disturbed point (M-1) and one least disturbed point (L1 - Piranga River), the middle course had two most disturbed points (M2 and M3) and one least disturbed point (L2 - Santo Antônio River), and the lower course had two most disturbed points (M4 and M5) and one least disturbed point (L3 - Manhuaçu River). Sampling was conducted during the dry season between the months of August and September 2020.

Sampling

For the stable isotope analysis, the collection was standardized to include at least five samples per point (whenever possible) from each of the following compartments of the food web: I) available food resources in the environment (filamentous algae, aquatic, and terrestrial invertebrates, aquatic macrophytes, suspended matter, coarse particulate organic matter - CPOM, and periphyton); and II) all fish species (including native and non-native ones). The collection and processing of each compartment occurred as described below.

Fish - Collection and Processing

Fish sampling was conducted using two sets of gillnets with different mesh sizes (15, 20, 25, 30, 35, 40, 50, 60, 70, and 80 mm), totaling 20 nets at each point. The gillnets were set in the water column during the night for 12 hours and retrieved at dawn. Additionally, in order to complement the sampling of the fish assemblage and overcome the selectivity of gillnets (Šmejkal et al., 2015), trawl nets (3 m and 10 m in length, with a 5 mm mesh) and hand sieves (80 cm in diameter with a 1 mm mesh) were also employed for a 2-hour sampling period at each point. All captured individuals were anesthetized in a Eugenol solution and were identified to the lowest possible taxon. Samples of muscle tissues intended for isotopic analysis were taken in the field and kept frozen until processing in the laboratory to prevent degradation. After tissue removal, the collected specimens were fixed in formalin (10% formaldehyde) and sent to the laboratory, where they underwent identification confirmation. In the laboratory, fish samples intended for isotopic analysis were lyophilized for a minimum of 24 hours, ground using a mortar and pestle to obtain a fine and homogeneous powder and stored in Eppendorf-type tubes for isotopic analysis.

The collection, euthanasia, and transportation of organisms were authorized by the Ministry of the Environment (MMA), Chico Mendes Institute for Biodiversity Conservation (ICMBio), and the Biodiversity Authorization and Information System (SISBIO - number 80532-1) and the ethics committee of the Federal University of Viçosa (number 7982018).

Food Resources - Collection and Processing

Filamentous algae (AL) were randomly collected from the riverbed using forceps. Aquatic/benthic invertebrates (BE) were sampled using Kick-net and sieves (80 cm in diameter with a 1 mm mesh) near the banks, in aquatic macrophytes, leaf banks, and rapids. Terrestrial invertebrates (IT) were manually sampled at different points in the riparian forest and riverbanks. Macrophytes (MA) were randomly collected from the riverbed, with a preference for collecting distinct species whenever possible. Suspended matter (SM) was collected by towing a phytoplankton net (45 µm mesh) in the water column for a period of 3 minutes at each location. Coarse particulate organic matter (CPOM), consisting of decomposing leaves deposited on the riverbed, was randomly collected from the riverbed. Periphyton (PE), the biofilm that grows on rocks, was collected by scraping rocks with brushes, which were then washed with distilled water, and the obtained content was stored in plastic tubes (samples containing distilled water + periphyton).

All samples collected in the field were stored in plastic containers or bags and kept in a cooler with ice until subsequent freezing in the laboratory. In the laboratory, solid samples (filamentous algae, aquatic and terrestrial invertebrates, aquatic macrophytes, and coarse particulate organic matter - CPOM) were lyophilized for at least 24 hours, ground into a fine and homogeneous powder, stored in Eppendorf-type plastic microtubes, and sent for isotopic analysis. Liquid samples (suspended matter and periphyton) were filtered using a quartz filter (Whatman® QMA quartz filters) and a filtration apparatus connected to a vacuum pump. Subsequently, the filtered samples were dried in an oven at 40°C until their weight stabilized, ground into a fine and homogeneous powder using a mortar and pestle, stored in Eppendorf-type plastic microtubes, and sent for isotopic analysis.

Data Analysis

Isotopic Analysis

A total of 744 samples were submitted for stable isotope analysis of C and N, including 478 fish samples from 54 species, 40 filamentous algae samples, 40 aquatic invertebrate samples, 40 terrestrial invertebrate samples, 26 aquatic macrophyte samples (the only resource not found at all points), 40 suspended matter samples, 40 coarse particulate organic matter (CPOM) samples, and 40 periphyton samples.

Stable isotopes of carbon and nitrogen were analyzed at the Center for Nuclear Energy in Agriculture (CENA) at the University of São Paulo. For the determination of isotopic ratios, a continuous-flow isotope ratio mass spectrometer (CF-IRMS) system was used with a Carlo Erba elemental analyzer (CHN 1110) coupled to a Thermo Scientific mass spectrometer (Delta Plus). The results were expressed as the relative difference from internationally recognized reference standards for ^{13}C (Pee Dee Belemnite) and ^{15}N (atmospheric nitrogen), using the delta (δ ‰) notation, and calculated based on the following formula: $\delta X = [(R_{sample}/R_{standard}) - 1] \times 10^3$, where X corresponds to ^{13}C or ^{15}N, and R represents the isotopic ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$. (Barrie & Prosser, 1996).

Statistical Analyses

To assess the trophic structure of the fish community at each sampled point in the Rio Doce basin, bi-plot graphs were constructed using the isotopic compositions of the fish and resources (x-axis: δ^{13}C and y-axis: δ^{15}N). Each collected individual had its δ^{13}C and δ^{15}N signature represented on the graph, while the resources were represented by the mean and standard deviation.

The trophic position occupied by individuals of each species at each sampling point was estimated using the method proposed by Vander Zanden et al. (1997): $TP_{fish} = [(\delta^{15}N_{fish} - \delta^{15}N_{resources}) \div 2.9] + 1$, where $\delta^{15}N_{fish}$ = δ^{15}N values of each fish individual, $\delta^{15}N_{resources}$ = mean values of δ^{15}N of baselines (we considered CPOM and filamentous algae, since these baselines represents allochthonous and autochthonous sources, respectively), 2.9 represents the fractioning per trophic level according to McCutchan et al., (2003) (muscle tissue Δ^{15}N: 15 values with an average of 2.9 ± 0.32‰) and 1 is the position of producers within the food chain.

At each sampling site, and for the entire basin, the isotopic niches occupied by of native and non-native fish species sets were estimated using the SIBER package (SEA, SEAc, and SEAb – expressed in ‰²). The standard ellipse area (SEA) represents the central isotopic niche space and is a proxy for the richness and uniformity of resources consumed by the population (Bearhop et al., 2004). Small sample size correction (indicated by the letter "c") was applied to SEA to increase the precision of comparisons, allowing the comparison of niche widths in communities with different sample sizes. Bayesian estimates of SEAb (bootstrapped n = 10000 – indicated by the letter “b”) were generated to test significant differences in the width of the isotopic niche of native and non-native fish species, comparing their confidence intervals (Jackson et al., 2011). Additionally, using the same package (SIBER), the degree of niche overlap (expressed in percentage, where 100% indicates complete overlap) was estimated, representing a quantitative measure of dietary similarity between native and non-native species sets.

The primary sources of carbon supporting fish communities at each site sampled along the Doce River were inferred through a visual assessment of the similarities between the isotopic compositions of the fish and their resources (i.e., fish exhibit ^{13}C isotopic compositions similar to those of the resources they consume). We did not employ mixing model analysis, such as MixSIAR (Stock & Semmens, 2016), because we found that the assumptions required by mixing models to determine consumers' diets were not met at all sites, i.e., the isotope values of consumers did not fall within the resource polygon (Phillips et al., 2014).

The sampled points are located in different regions of the river, where the richness and composition of fish fauna are expected to be naturally different. Thus, we focused on compare among least and most disturbed point local differences in attributes of the native and non-native species pools. The percentage of non-native species in the fish assemblage was compared between least disturbed and most disturbed points using the Kruskal-Wallis test. The difference between the trophic position width of the pool of native and non-native species (Δ TP native – Δ TP non-native) was compared between least disturbed and most disturbed points using the T test. The relationship between the percentage of non-native species and niche overlap between native and non-native fish assemblages was tested through simple linear regression. The difference between the width of resources used by the pool of native and non-native species (Δ^{13}C native - Δ^{13}C non-native) was compared between least disturbed and most disturbed points using the Kruskal-Wallis test. All the statistical analyses were performed in RStudio 1.3.959 (R Core Team, 2021).

Results

Out of the 54 species collected, 36 were native to the Rio Doce basin, whereas 18 were non-native (Table 2). The least disturbed points L1 and L2 had a higher percentage of native species compared to non-native ones, with L2 (Santo Antônio River) having the highest relative richness of native species (94.1%). Among the most disturbed points, the proportion of non-native species ranged from 37% to 47%, significantly higher than in the least disturbed points (KW = 5.01; p = 0.03). Among the native species, *Astyanax lacustris* (Lütken 1875) was the most widely distributed, being present in all sampling points. On the other hand, among the non-native species, *Knodus moenkhausii* (Eigenmann & Kennedy 1903) was the most widely distributed, found in seven out of the eight sampled points (Table 2).

For most of the sites, a significant isotopic similarity was observed between individuals of native and non-native species. At the least disturbed sites, primarily aquatic invertebrates, but also periphyton and filamentous algae, appear to be the most consumed resources by both native and non-native species (Fig 2; Online Resource 1). These resources also appear to be the most relevant at the most disturbed sites. However, in some points (M1, M2, and M3), non-native species appeared to be exploiting not sampled more enriched ^{13}C sources (Fig. 2).

The estimated trophic positions occupied by each fish species reveal that non-native species occupy positions from the basal to the top of the food web (Fig.3, Online Resource 2, 3). Among them, *Poecilia reticulata* and *Pterigoplichthys pardalis* exhibited the lowest trophic levels, while *Cichla kelberi* and *Crenicichla lepidota* exhibited the highest (Table 3). Moreover, similarly to native species, most non-native species predominantly occupy intermediate trophic positions (TP between 2 and 4) (Fig. 3). However, locally, native species occupied a broader range of trophic levels at all sites (Fig. 4A). The greater diversity of trophic levels of native species was more pronounced at some least disturbed sites (L1 and L2), but the differences in the range of trophic levels between the native and non-native species pools, across most disturbed and least disturbed sites, were not significant ($F = 4.18$; $P = 0.09$).

The niche overlap between non-native and native species was directly related to the percentage of non-native species in the assemblage ($r^2 = 0.71$; $p = 0.02$) (Fig. 5; Table 4). Moreover, for the entire basin, the set of non-native species (SEAc = 24.20‰²) explores a wider isotopic niche than native species (SEAc = 18.87‰²) (Fig. 6; Table 4). Such a difference is due to the exploration of a broader range of ^{13}C sources by the non-native species (Fig. 6). Nevertheless, the difference between the breadth of resources used by the pool of native and non-native species (Δ^{13}C native - Δ^{13}C non-native) when compared between the least disturbed and most disturbed points was not significant ($KW = 2.69$; $p = 0.10$). However, only at some of the most disturbed sites (i.e. M4 and M5) does the range of carbon signatures of the pool of non-native species exceed that of the pool of native species, and the closer to the dam rupture region (i.e. M1, M2 and M3), the greater the difference in favor of non-native species (Fig. 4B, Online Resource 4).

Discussion

The main hypothesis of our study was supported, since non-native fish fauna currently occupies a considerable portion of the isotopic niches in the Doce River basin, and their establishment seems to have been favored both by "vacant" niche positions and by the reduction of native species populations. Non-native species pool presented an isotopic niche wider to that of the native assembly, occupying all trophic levels. Most predictions were also confirmed. The historically most impacted points in the basin, which also received the tailings from the dam breach, presented a higher percentage of non-native species. The higher this percentage, the greater the observed isotopic overlap with native species. Non-native species occupied 'vacant' isotopic niches in most disturbed regions, represented by more enriched $\delta^{13}C$ signatures. However, locally, their range of $\delta^{13}C$ compared to native species was not different among least and most disturbed sites.

Introduced species often exhibit greater success in invading degraded environments (Hermoso et al., 2011; de Carvalho et al., 2017; de Moraes et al., 2017). In fact, the Doce is among the southeastern Brazilian basins with the highest number of invasive fish species (Bueno et al., 2021). Dominance of non-native species and high abundance of hyper-tolerant species were observed in the Guadiamar River (Spain), affected by the dam breach at the Los Frailes mine in 1998 (De Miguel et al., 2014). Phenotypic plasticity of non-native species has been considered as a key trait for invasion success, allowing them to establish in habitats distinct from their place of origin (Kaymak et al., 2023). Such seems to be the case of *Knodus moenkhausii*, which was captured in most locations in the Rio Doce basin, and for which opportunistic feeding behavior and high trophic plasticity has already been reported (de Carvalho et al., 2019b).

The patterns of isotopic niche occupation observed for the fish fauna of the Doce River basin suggest that its historical degradation process, exacerbated by the tailings dam rupture, may have favored invasion processes through both local species extinction and the weakening of local communities. The broader $\delta^{13}C$ signature range of non-native species is a strong indication that there are resources exclusively consumed by some of these species. Thus, it is possible that non-native species has occupied "niche opportunities" provided by human-created environmental conditions, as proposed by the biotic resistance hypothesis (Olden et al., 2006). Moreover, the closer to the rupture area, the greater the difference in $\delta^{13}C$ signature amplitude in favor of non-native species. However, both the removal of species from a community and the removal of resources seem to have similar effects on increasing invasion success (Byers & Noonburg, 2003). The high overlap between the two groups of species at all evaluated points, coupled with the fact that non-native species occupy all trophic levels of the food web, suggests the utilization of the same trophic resources with significant potential for competition with non-native species.

Competition with native species has been widely reported in the literature (Pilger et al., 2010; Lima & Chagas, 2019; Britton, 2022), and is likely to be particularly important in a basin with a high degree of degradation. The tailings released by the dam breach in Mariana, for example, could potentially have reduced the input of locally important energy sources, such as periphyton and filamentous algae (Brasil, 2015), both by siltation and increased turbidity. The importance of these items as autochthonous primary producers that sustain fish assemblages has also been reported for rivers with different conservation status, draining the southeastern Brazil (de Carvalho et al., 2020; 2023). This recent impact has also accrued the historical degradation of riparian forests in the basin and its negative impacts on the input of allochthonous energy sources (Coelho, 2009).

Predation by non-native species is another crucial factor in modifying the trophic structure (Pelicice & Agostinho, 2009; de Souza et al., 2021), often leading to cascading effects. In this study, we recorded 15 piscivorous species (27.7% of the total richness) in the Doce River basin, including six non-natives (*Cichla kelberi*, *Cichla monoculus*, *Lophiosilurus alexandri*, *Pygocentrus nattereri*, *Salminus brasiliensis* e *Serrasalmus brandtii*). These non-native piscivorous are reported to negatively impact original native communities, causing caudal fin mutilation (Andrade et al., 2018) and even local extinctions (Vitule et al. 2009; Brito et al., 2020; de Souza et al., 2021), raising concerns about the potential intensification of competitive exclusion (Pompeu & Lima Godinho, 2001; Britton et al., 2019; Mofu et al., 2019). Therefore, it is likely that non-native species are not only a consequence but also a cause of the simplified trophic structure in the most disturbed points, as indicated by previous studies (de Carvalho et al., 2023).

Our experimental design has a hierarchical structure, since most disturbed sites are located in the main channel, and the least disturbed (control sites) in tributaries. We sought to control the known effects of the hierarchical structure of river networks on biodiversity distribution, by comparing only local differences in trophic attributes of the pool of native and non-native species. Nevertheless, the causal relationship between the effects of the dam breach and the results of the comparison between least and most disturbed impacted sites should be addressed with caution. Especially important is to consider that of the three least disturbed sites, only one can be considered a true reference regarding the history of basin degradation. The Santo Antônio River (L2), in its upper stretch, has high richness and harbors most of the threatened and endemic species of the Doce River Basin (Vieira, 2006). This river not only had the lowest percentage of non-native species but also is the one where these species consume the least variety of resources. On the other hand, the other two least disturbed sites, for some of the parameters evaluated, showed values comparable to the most disturbed sites. While the Piranga River (L1) receives a significant load of domestic and pig farming sewage (De Melo et al., 2017), the Manhuaçu River (L3) has several dams along its course, besides flowing into the reduced flow section of the Aimorés Hydroelectric Plant (Marques et al., 2013). Thus, in addition to pointing out possible effects of the rupture of the tailings dam, our results also indicate that the current invasion status is also the result of the historical degradation process of the basin.

Conclusion

Despite the well-known presence of non-native species in the Rio Doce basin (Alves et al., 1999; Barros et al., 2012; Jankowsky et al., 2021), our results point to the potential impact of this biological invasion process on the use of available trophic resources. Both local extinctions and the weakening of the community through the change and reduction of resources may have favored their success, especially in the more degraded areas of the basin. In these points, non-native species not only overlap with the isotopic niches of native species and occupy the same trophic levels, but also explore resources that are not currently utilized by the pool of native species. Therefore, considering the impacts of the Fundão tailings dam disaster, along with the long history of degradation and species introductions in the Rio Doce basin, our results emphasize the urgent need for population control strategies for non-native species to preserve the integrity and conservation of the native fish community in the basin.

References

Albrecht, M. P., A. Da Silva Reis, V. Neres-Lima, & E. Zandonà, 2021. Stable isotopes and other tools in trophic studies of tropical stream fish. Oecologia Australis Universidade Federal do Rio de Janeiro 25: 283–300.

Alves, C. B. M., F. Vieira, A. L. B. Magalhães, & M. F. G. Brito, 2007. Impacts of non-native fish species in Minas Gerais, Brazil: present situation and prospects. Ecological and Genetic Implications of Aquaculture Activities 291–314.

Alves, C. B. M., V. Vono, & F. Vieira, 1999. Presence of the walking catfish Clarias gariepinus (Burchell) (Siluriformes, Clariidae) in Minas Gerais state hydrographic basins, Brazil. Revista Brasileira de Zoologia 16: 259–263.

Anas, M. M., & Mandrak, N. E. 2021. Drivers of native and non-native freshwater fish richness across North America: Disentangling the roles of environmental, historical and anthropogenic factors. Global Ecology and Biogeography 30: 1232-1244.

Andrade, F. R., L. D. Silva, I. Guedes, A. M. Santos, & P. S. Pompeu, 2018. Non-native white piranhas graze preferentially on caudal fins from large netted fishes. Marine and Freshwater Research CSIRO 70: 585–593.

Azevedo-Santos, V. M., F. M. Pelicice, D. P. Lima-Junior, A. L. B. Magalhães, M. L. Orsi, J. R. S. Vitule, & A. A. Agostinho, 2015. How to avoid fish introductions in Brazil: Education and information as alternatives. Natureza e Conservacao Elsevier 13: 123–132.

Barrie, A., & S. J. Prosser, 1996. Automated analysis of light-element stable isotopes by isotope ratio mass spectrometry In Boutton, T. W., & S.-I. Yamasaki (eds), Mass Spectrometry of Soils. Marcel Dekker, Inc.: 1–46.

Barros, L. C., U. Santos, J. C. Zanuncio, & J. A. Dergam, 2012. Plagioscion squamosissimus (Sciaenidae) and Parachromis managuensis (Cichlidae): A threat to native fishes of the Doce River in Minas Gerais, Brazil. PLoS ONE 7.

Byers, J. E., & Noonburg, E. G. 2003. Scale dependent effects of biotic resistance to biological invasion. Ecology 84: 1428-1433.

Bearhop, S., C. E. Adams, S. Waldron, R. A. Fuller, & H. Macleod, 2004. Determining Trophic Niche Width: A Novel Approach Using Stable Isotope Analysis. Journal of Animal Ecology 73: 1007–1012.

Becker, F. G., & K. M. Grosser, 2003. Piscicultura e a introdução de espécies de peixes não-nativas no RS. Fundação Zoobotânica RS 1–29.

Brasil, 2015. Laudo Técnico Preliminar - Impactos ambientais decorrentes do desastre envolvendo o rompimento da barragem de Fundão, em Mariana, Minas Gerais. Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis - Ibama. Brasília, pages 1–38.

Brasil, 2016. Encarte Especial sobre a Bacia do Rio Doce - Rompimento da Barragem em Mariana/MG. Agência Nacional de Águas. Brasília, pages 1–50.

Brito, M. F. G., V. S. Daga, & J. R. S. Vitule, 2020. Fisheries and biotic homogenization of freshwater fish in the Brazilian semiarid region. Hydrobiologia Springer Science and Business Media Deutschland GmbH 847: 3877–3895.

Britton, J. R., 2022. Contemporary perspectives on the ecological impacts of invasive freshwater fishes. Journal of Fish Biology John Wiley and Sons Inc.

Britton, J. R., C. Gutmann Roberts, F. Amat Trigo, E. T. Nolan, & V. De Santis, 2019. Predicting the ecological impacts of an alien invader: Experimental approaches reveal the trophic consequences of competition. Journal of Animal Ecology Blackwell Publishing Ltd 88: 1066–1078.

Bueno, M. L., A. L. B. Magalhães, F. R. Andrade Neto, C. B. M. Alves, D. de M. Rosa, N. T. Junqueira, T. C. Pessali, P. S. Pompeu, & R. D. Zenni, 2021. Alien fish fauna of southeastern Brazil: species status, introduction pathways, distribution and impacts. Biological Invasions Springer Science and Business Media Deutschland GmbH 23: 3021–3034.

Casimiro, A. C. R., F. Y. Ashikaga, G. Kurchevski, F. S. Almeida, & M. L. Orsi, 2010. Os impactos das introduções de espécies exóticas em sistemas aquáticos continentais. Boletim da Sociedade Brasileira de Limnologia. pages 1–12.

Charles, H., & J. S. Dukes, 2007. Impacts of Invasive Species on Ecosystem Services. Ecological Studies 193: 217–237.

Clavero, M., & E. García-Berthou, 2005. Invasive species are a leading cause of animal extinctions. Trends in Ecology and Evolution Elsevier Ltd 20: 110.

Clavero, M., Hermoso, V., Aparicio, E., & Godinho, F. N. 2013. Biodiversity in heavily modified waterbodies: native and introduced fish in Iberian reservoirs. Freshwater Biology 58: 1190-1201.

Coelho, A. L. N., 2009. Bacia hidrográfica do rio doce (MG/ES): uma análise socioambiental integrada. Geografares 7: 131–146.

Davis, M. A., Grime, J. P., & Thompson, K. 2000. Fluctuating resources in plant communities: a general theory of invasibility. Journal of ecology 88: 528-534.

de Carvalho, D. R., D. M. P. de Castro, M. Callisto, A. J. de M. Chaves, M. Z. Moreira, P. S. Pompeu, & a, 2019a. Stable isotopes and stomach content analyses indicate omnivorous habits and opportunistic feeding behavior of an invasive fish. Aquatic Ecology Springer Netherlands 53: 365–381.

de Carvalho, D. R., D. M. P. de Castro, M. Callisto, M. Z. Moreira, P. S. Pompeu, & a, 2017. The trophic structure of fish communities from streams in the Brazilian Cerrado under different land uses: an approach using stable isotopes. Hydrobiologia Springer International Publishing 795: 199–217.

de Carvalho, D. R., A. S. Flecker, C. B. M. Alves, J. P. Sparks, P. S. Pompeu, & b, 2019b. Trophic responses to aquatic pollution of native and exotic livebearer fishes. Science of the Total Environment Elsevier B.V. 681: 503–515.

de Carvalho, D. R., Alves, C. B. M., Moreira, M. Z., & Pompeu, P. S. 2020. Trophic diversity and carbon sources supporting fish communities along a pollution gradient in a tropical river. Science of the Total Environment 738: 139878.

de Carvalho, D. R., Alves, C. B. M., & Pompeu, P. S. 2023. Trophic structure of fish assemblages from oligotrophic tropical rivers: evidence of growing assimilation of autochthonous primary producers with the increase in river dimensions. Aquatic Ecology 57: 405-419.

de Carvalho, D. R., Ferreira, F. F., Dergam, J. A., Moreira, M. Z., & Pompeu, P. S. 2024. Food web structure of fish communities of Doce River, 5 years after the Fundão dam failure. Environmental Monitoring and Assessment 196: 300.

De Melo, M. C., de Mendes, R. S., da Fonseca, M., Aquino, F. A., & Rodrigues, A. F. (2017). Environmental monitoring planning in river basins: the case of pig farming in river Piranga basin. Caminhos Geogr 18: 454-471.

De Miguel, R. J., F. J. Oliva-Paterna, L. Gálvez-Bravo, & C. Fernández-Delgado, 2014. Fish composition in the Guadiamar River basin after one of the worst mining spills in Europe. Limnetica Asociacion Iberica de Limnologia 33: 375–384.

de Moraes, M. B., C. N. M. Polaz, S. dos S. Júnior, E. P. Caramaschi, G. Souza, & F. L. Carvalho, 2017. Espécies Exóticas e Alóctones da Bacia do Rio Paraíba do Sul: Implicações para a Conservação. Biodiversidade Brasileira 7(1): 34–54.

de Souza, C. P., C. A. de S. Rodrigues-Filho, F. A. R. Barbosa, & R. P. Leitão, 2021. Drastic reduction of the functional diversity of native ichthyofauna in a neotropical lake following invasion by piscivorous fishes. Neotropical Ichthyology Sociedade Brasileira de Ictiologia 19: 1–18.

DeNiro, M. J., & S. Epstein, 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochimica et Cosmochimica Actu 45: 341–351.

Elton, C. S. 1958. The ecology of invasions by animals and plants. John Wiley and Sons, New York

Espindola, H. S., R. B. F. Campos, K. C. C. Lamounier, & R. S. Silva, 2016. Desastre da Samarco no Brasil: Desafios para a conservação da biodiversidade. Fronteiras OpenJournals Publishing 5: 72–100.

Flood, P. J., A. Duran, M. Barton, A. E. Mercado-Molina, & J. C. Trexler, 2020. Invasion impacts on functions and services of aquatic ecosystems. Hydrobiologia Springer 847: 1571–1586.

Fragoso-Moura, E. N., L. T. Oporto, P. M. Maia-Barbosa, & F. A. R. Barbosa, 2016. Perda de biodiversidade em uma unidade de conservação da mata atlântica brasileira: Efeitos da introdução de espécies não nativas de peixes. Brazilian Journal of Biology Instituto Internacional de Ecologia 76: 18–27.

Fry, Brian., 2006. Stable isotope ecology. Springer.

Garcia, D. A. Z., F. M. Pelicice, M. F. G. de Brito, M. L. Orsi, & A. L. B. Magalhães, 2021. Non-native fishes in brazilian streams: State of the art, gap of knowledge and perspectives. Oecologia Australis Universidade Federal do Rio de Janeiro 25: 565–587.

Gozlan, R. E., J. R. Britton, I. Cowx, & G. H. Copp, 2010. Current knowledge on non-native freshwater fish introductions. Journal of Fish Biology 76: 751–786.

Havel, J. E., Lee, C. E., & Vander Zanden, J. M. 2005. Do reservoirs facilitate invasions into landscapes? BioScience 55: 518-525.

Hermoso, V., M. Clavero, F. Blanco-Garrido, & J. Prenda, 2011. Invasive species and habitat degradation in Iberian streams: an analysis of their role in freshwater fish diversity loss. Ecological Applications 21: 175–188.

Hulme, P. E. 2003. Biological invasions: winning the science battles but losing the conservation war? Oryx 37: 178-193.

IPBES, 2023. Summary for Policymakers of the Thematic Assessment Report on Invasive Alien Species and their Control. Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services, https://doi.org/10.5281/zenodo.7430692.

Jackson, A. L., R. Inger, A. C. Parnell, & S. Bearhop, 2011. Comparing isotopic niche widths among and within communities: SIBER - Stable Isotope Bayesian Ellipses in R. Journal of Animal Ecology 80: 595–602.

Jankowsky, M., R. M. de Carvalho, V. A. do P. Gomes, & R. R. de Freitas, 2021. Peixes e pesca na bacia do rio doce, uma análise bibliométrica. Brazilian Journal of Production Engineering - BJPE Universidade Federal do Espirito Santo 14–40.

Kaymak, N., N. Emre, F. B. Yalim, C. Toslak, Y. Emre, & Ş. Akin, 2023. Seasonal variation in trophic niches and niche overlap between native and introduced cyprinid fishes. Spectroscopy Letters Taylor and Francis Ltd.

Kaymak, N., K. O. Winemiller, S. Akin, Z. Altuner, F. Polat, & T. Dal, 2018. Spatial and temporal variation in food web structure of an impounded river in Anatolia. Marine and Freshwater Research CSIRO 69: 1453–1471.

Latini, A. O., 2001. Estado Atual e Perspectivas para a Ictiofauna da Região do Parque Estadual do Rio Doce, MG. Instituto Estadual de Florestas, pages 1–49.

Layman, C. A., D. Albrey Arrington, C. G. Montan˜a, M. Montan˜a, & D. M. Post, 2007. Can stable isotope ratios provide for community-wide measures of trophic structure?. Ecology 88: 42–48.

Leprieur, F., O. Beauchard, S. Blanchet, T. Oberdorff, & S. Brosse, 2008. Fish invasions in the world’s river systems: When natural processes are blurred by human activities. PLoS Biology 6: 0404–0410.

Lima, M. A. T., & R. J. Chagas, 2019. Dieta e Sobreposição Alimentar entre Espécies de Peixes Nativas e Introduzidas no Reservatório da Barragem da Pedra, Bahia. Uniciências Editora e Distribuidora Educacional 23: 89–94.

Milardi, M., Iemma, A., Waite, I. R., Gavioli, A., Soana, E., & Castaldelli, G. 2022. Natural and anthropogenic factors drive large-scale freshwater fish invasions. Scientific Reports 12: 10465.

Manetta, G. I., & E. Benedito-Cecilio, 2003. Aplicação da técnica de isótopos estáveis na estimativa da taxa de turnover em estudos ecológicos: uma síntese. Acta Scientiarum: Biological Sciences 25: 121–129.

Marques, B., F. Belei, & W. M. S. Sampaio, 2013. Ictiofauna do baixo rio Manhuaçu (Bacia do baixo rio Doce). Evolução e Conservação da Biodiversidade Araucaria Comunicacao Integrada 4: 32.

McCutchan, J. H., W. M. Lewis, C. Kendall, & C. C. McGrath, 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. Oikos 102: 378–390.

Miller, R. R., J. D. Williams, & J. E. Williams, 1989. Extinctions of North American Fishes During the past Century. Fisheries Wiley 14: 22–38.

Minagawa, M., & E. Wada, 1984. Stepwise enrichment of "N along food chains: Further evidence and the relation between 615N and animal age. Geochmica et Cosmochimica 48: 1135–1140.

Mofu, L., J. South, R. J. Wasserman, T. Dalu, D. J. Woodford, J. T. A. Dick, & O. L. F. Weyl, 2019. Inter-specific differences in invader and native fish functional responses illustrate neutral effects on prey but superior invader competitive ability. Freshwater Biology Blackwell Publishing Ltd 64: 1655–1663.

Olden, J. D., Poff, N. L., & K. R. Bestgen. 2006. Life-history strategies predict fish invasions and extirpations in the Colorado River Basin. Ecological Monographs 76: 25-40.

Pelicice, F. M., & A. A. Agostinho, 2009. Fish fauna destruction after the introduction of a non-native predator (Cichla kelberi) in a Neotropical reservoir. Biological Invasions 11: 1789–1801.

Pelicice, F. M., A. A. Agostinho, C. B. M. Alves, M. S. Arcifa, V. M. Azevedo-Santos, M. F. G. Brito, P. S. de Brito, P. M. G. de Castro Campanha, F. R. Carvalho, G. C. da Costa, M. A. Cozzuol, A. M. Cunico, F. C. P. Dagosta, R. M. Dias, R. Fernandes, A. C. S. Franco, D. A. Z. Garcia, T. Giarrizzo, É. A. Gubiani, E. C. Guimarães, L. Ikeda, A. M. Katz, A. L. B. Magalhães, L. F. de A. Montag, M. A. M. de P. Nogueira, M. L. Orsi, F. P. Ottoni, C. S. Pavanelli, T. G. Peixoto, A. C. Petry, P. S. Pompeu, T. P. A. Ramos, L. R. R. Rodrigues, J. Sabino, W. M. S. Sampaio, V. L. M. dos Santos, W. S. Smith, G. Souza, L. H. Tonella, & J. R. S. Vitule, 2023. Unintended consequences of valuing the contributions of non-native species: misguided conservation initiatives in a megadiverse region. Biodiversity and Conservation Springer Science and Business Media B.V. 32: 3915–3938.

Pennock, C. A., Z. T. Ahrens, M. C. McKinstry, P. Budy, & K. B. Gido, 2021. Trophic niches of native and nonnative fishes along a river-reservoir continuum. Scientific Reports Nature Research 11.

Peterson, B. J., & B. Fry, 1987. Stable Isotopes in Ecosystem Studies. Annual Review of Ecology and Systematics 18: 293–320.

Phillips, D. L., Inger, R., Bearhop, S., Jackson, A. L., Moore, J. W., Parnell, A. C., ... & Ward, E. J. 2014. Best practices for use of stable isotope mixing models in food-web studies. Canadian Journal of Zoology 92: 823-835.

Pilger, T. J., K. B. Gido, & D. L. Propst, 2010. Diet and trophic niche overlap of native and nonnative fishes in the Gila River, USA: Implications for native fish conservation. Ecology of Freshwater Fish 19: 300–321.

Pinto-Coelho, Bezerra-Neto, F. Miranda, T. G. Mota, R. Resck, M.-B. Santos, N. D. Mello, M. O. Campos, & Barbosa, 2008. The inverted trophic cascade in tropical plankton communities: Impacts of exotic fish in the Middle Rio Doce lake district, Minas Gerais, Brazil. Brazilian Journal of Biology 68: 1025–1037.

Pompeu, P. dos S., & A. Lima Godinho, 2001. Mudança na dieta da traíra Hoplias malabaricus (Bloch) (Erythrinidae, Characiformes) em lagoas da bacia do rio Doce devido à introdução de peixes piscívoros. Revista Brasileira de Zoologia 18: 1219–1225.

Post, D. M., 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83: 703–718.

Rahel, F. J., 2007. Biogeographic barriers, connectivity and homogenization of freshwater faunas: It’s a small world after all. Freshwater Biology 52: 696–710.

Ricciardi, A., 2007. Are modern biological invasions an unprecedented form of global change?. Conservation Biology 21: 329–336.

Ricciardi, A., T. M. Blackburn, J. T. Carlton, J. T. A. Dick, P. E. Hulme, J. C. Iacarella, J. M. Jeschke, A. M. Liebhold, J. L. Lockwood, H. J. Macisaac, P. Pyšek, D. M. Richardson, G. M. Ruiz, D. Simberloff, W. J. Sutherland, D. A. Wardle, & D. C. Aldridge, 2017. Invasion Science: A Horizon Scan of Emerging Challenges and Opportunities. Trends in Ecology & Evolution 32: 464–474.

Sánchez, L. E., K. Alger, L. Alonso, F. A. R. Barbosa, M. C. W. Brito, F. V Laureano, P. May, H. Roeser, & Y. Kakabadse, 2018. Os impactos do rompimento da Barragem de Fundão O caminho para uma mitigação sustentável e resiliente. UICN. Gland, Suiça.

Seebens, H., S. Bacher, T. M. Blackburn, C. Capinha, W. Dawson, S. Dullinger, P. Genovesi, P. E. Hulme, M. van Kleunen, I. Kühn, J. M. Jeschke, B. Lenzner, A. M. Liebhold, Z. Pattison, J. Pergl, P. Pyšek, M. Winter, & F. Essl, 2021. Projecting the continental accumulation of alien species through to 2050. Global Change Biology Blackwell Publishing Ltd 27: 970–982.

Šmejkal, M., Ricard, D., Prchalová, M., Říha, M., Muška, M., Blabolil, P., ... & J. Kubečka, J. 2015. Biomass and abundance biases in European standard gillnet sampling. PLoS One 10: e0122437.

Shipley, O. N., & P. Matich, 2020. Studying animal niches using bulk stable isotope ratios: an updated synthesis. Oecologia Springer 193: 27–51.

Stock, B. C., & Semmens, B. X. 2016. Unifying error structures in commonly used biotracer mixing models. Ecology 97: 2562-2569.

Underwood, A. J., 1992. Beyond BACI: the detection of environmental impacts on populations in the real, but variable, world. Journal of Experimental Marine Biology and Ecology 161: 145–178.

Vander Zanden, M. J., Cabana, G. & Rasmussen, J. B. 1997. Comparing trophic position of freshwater fish calculated using stable nitrogen isotope ratios (δ15N) and literature dietary data. Canadian Journal of Fisheries and Aquatic Sciences 54: 1142–1158.

Viera, F. 2006. A ictiofauna do rio Santo Antônio, bacia do rio Doce, MG: proposta de conservação. Belo Horizonte: Universidade Federal de Minas Gerais. Umpublished Ph.D. Theses.

Vitule, J. R. S., C. A. Freire, & D. Simberloff, 2009. Introduction of non-native freshwater fish can certainly be bad. Fish and Fisheries. 98–108.

Vitule, J. R. S., C. Freire, D. P. Vázquez, & M. Andres Nuñez, 2012. Revisiting the Potential Conservation Value of Non-Native Species Sequencing the fungal environment: from populations to communities View project Morphophysiological effects of capture on elasmobranchs View project. Conservation Biology 26, https://www.researchgate.net/publication/260139492.

Vitule, J. R. S., & V. Prodocimo, 2012. Introdução de espécies não nativas e invasões biológicas. Estudos de Biologia Pontificia Universidade Catolica do Parana - PUCPR 34.

Zaia Alves, G. H., V. M. Cionek, G. I. Manetta, L. H. R Pazianoto, & E. Benedito, 2020. Stable isotopes reveal niche segregation between native and non-native Hoplias in a Neotropical floodplain. Ecology of Freshwater Fish 29: 602–610, https://onlinelibrary.wiley.com/doi/10.1111/eff.12536.

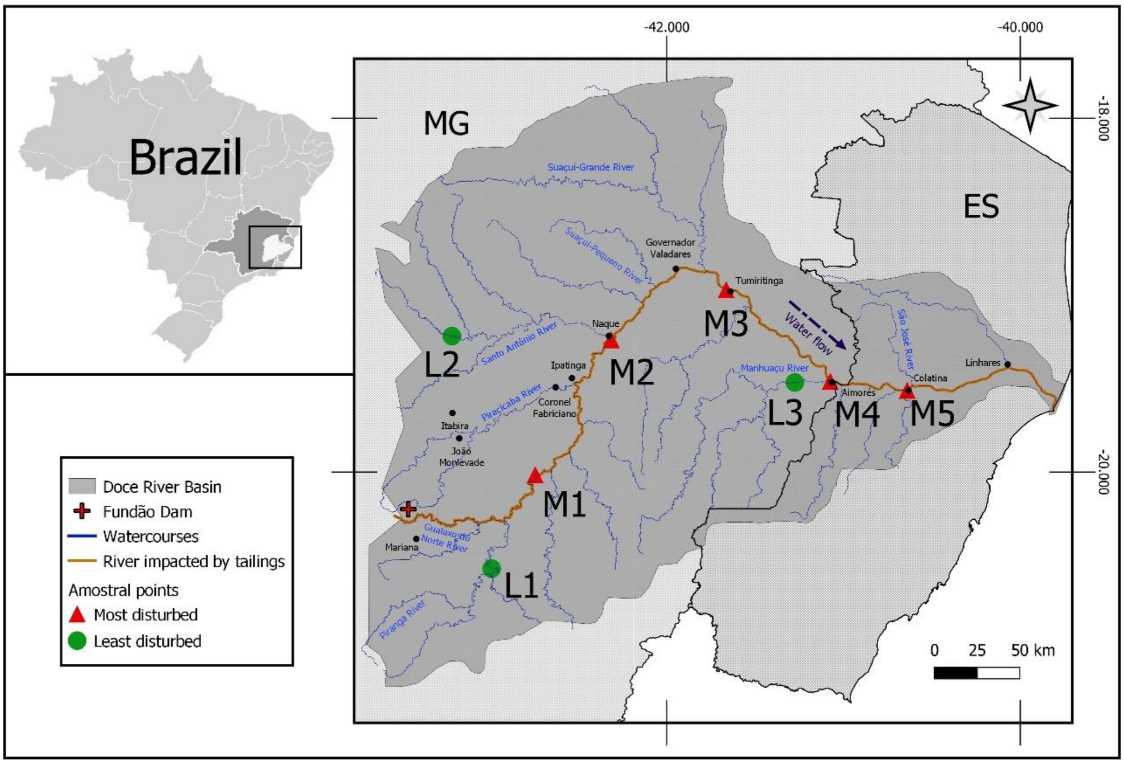

Fig. 1 Least disturbed points (L1 to L3) and most impacted points (M1 to M5) sampled along the Doce River basin. MG = Minas Gerais. ES = Espírito Santo. L1 = Piranga River; L2 = Santo Antônio River; L3 = Manhuaçu River; M1 = Doce River (UHE Risoleta Neves); M2 = Doce River (Naque); M3 = Doce River (Tumiritinga); M4 = Doce River (Aimorés); M5 = Doce River (Colatina)

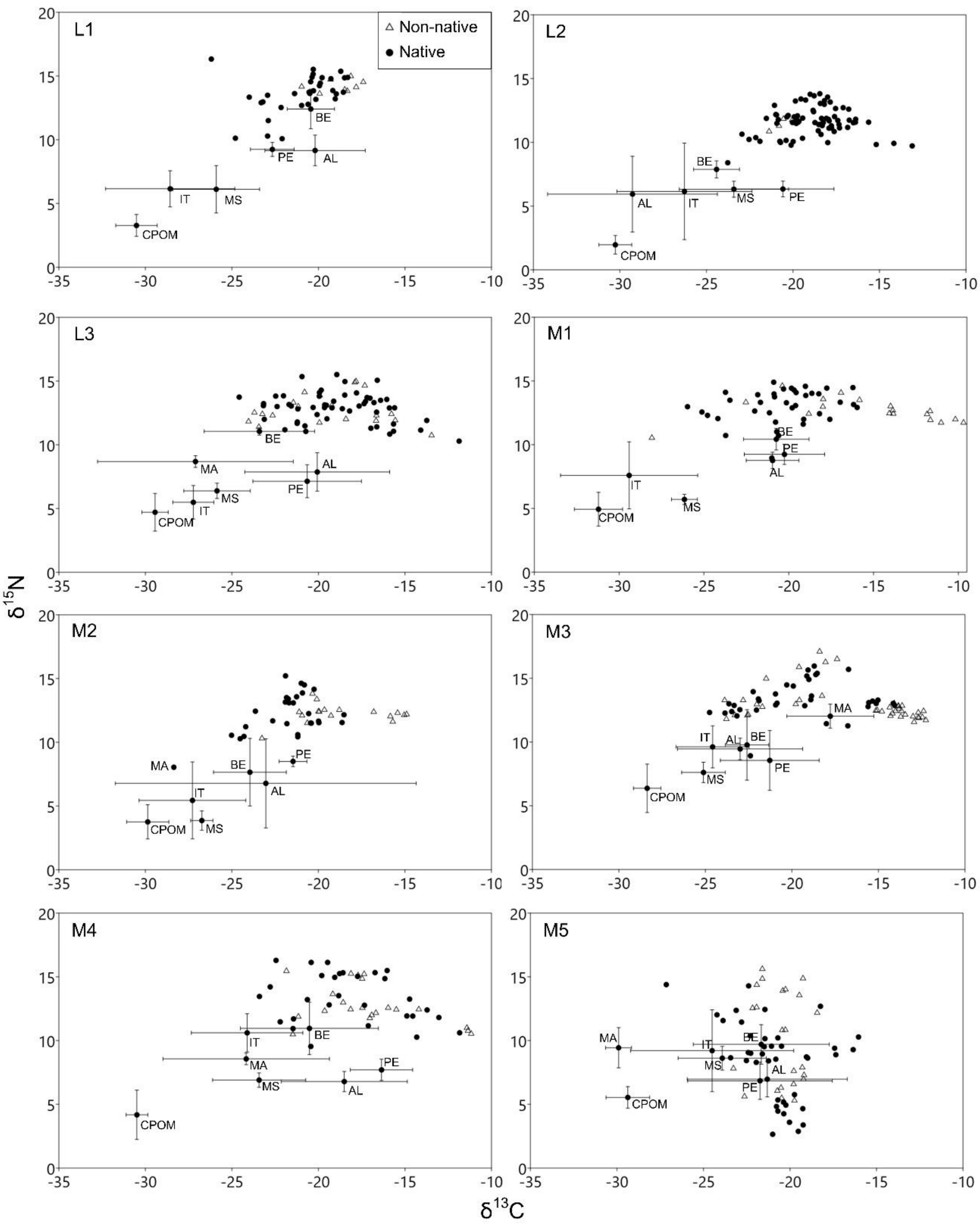

Fig. 2 The trophic structure (represented by the bi-plot graph) of the fish community from the eight sampled points in the Rio Doce basin. Least disturbed sites: L1 = Piranga River; L2 = Santo Antônio River; L3 = Manhuaçu River. Most disturbed sites: M1 = Doce River (Risoleta Neves Dam); M2 = Doce River (Naque); M3 = Doce River (Tumiritinga); M4 = Doce River (Aimorés); M5 = Doce River (Colatina); AL = Filamentous algae; BE = Aquatic invertebrates; CPOM = Coarse particulate organic matter; IT = Terrestrial invertebrates; MA = Aquatic macrophytes; MS = Suspended matter; PE = Periphyton.

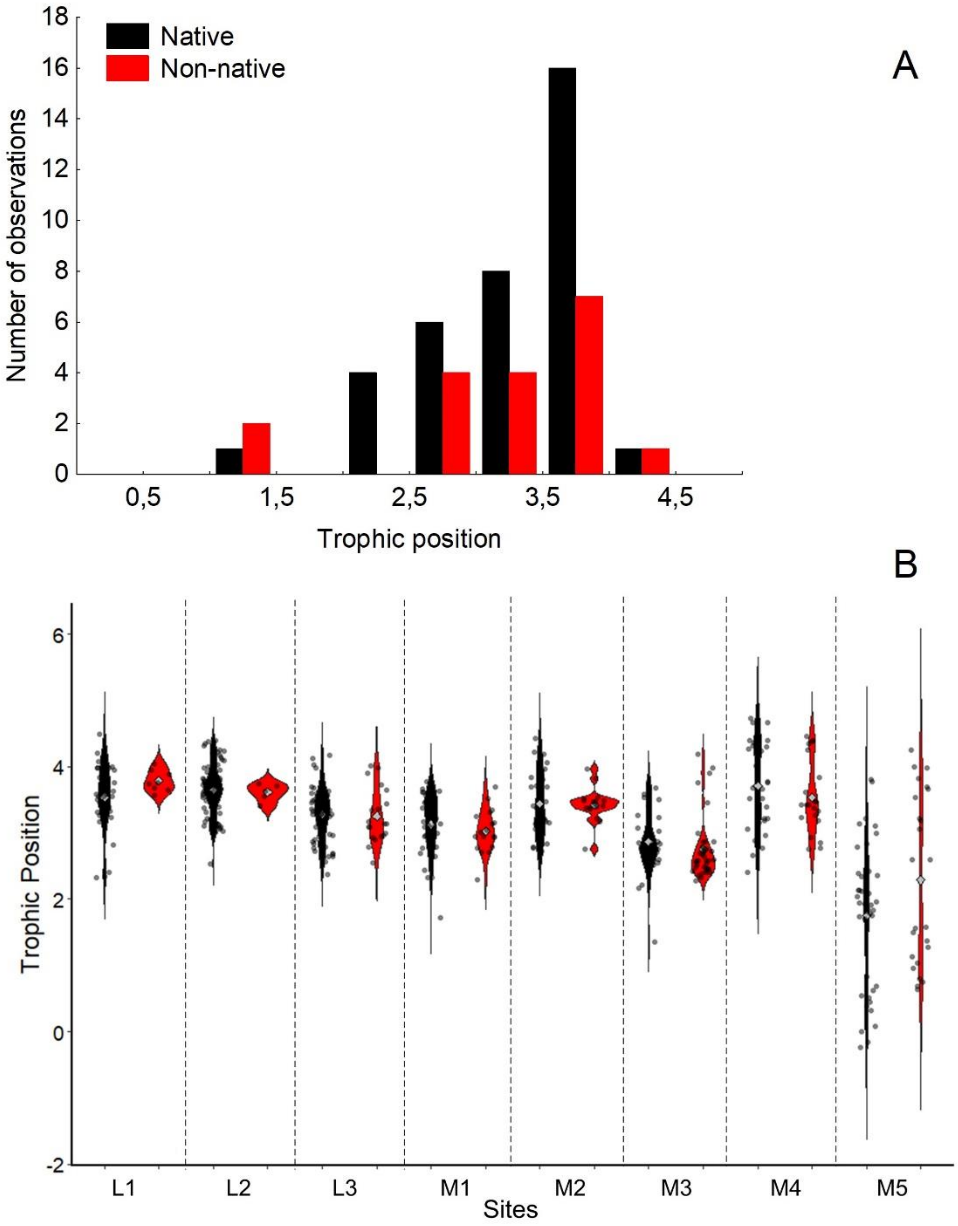

Fig. 3 Trophic position occupied by native and non-native species in the Rio Doce considering all sampling points together (A) and separately (B). L1 = Rio Piranga; L2 = Rio Santo Antônio; L3 = Rio Manhuaçu; M1 = Rio Doce (UHE Risoleta Neves); M2 = Rio Doce (Naque); M3 = Rio Doce (Tumiritinga); M4 = Rio Doce (Aimorés); and M5 = Rio Doce (Colatina).

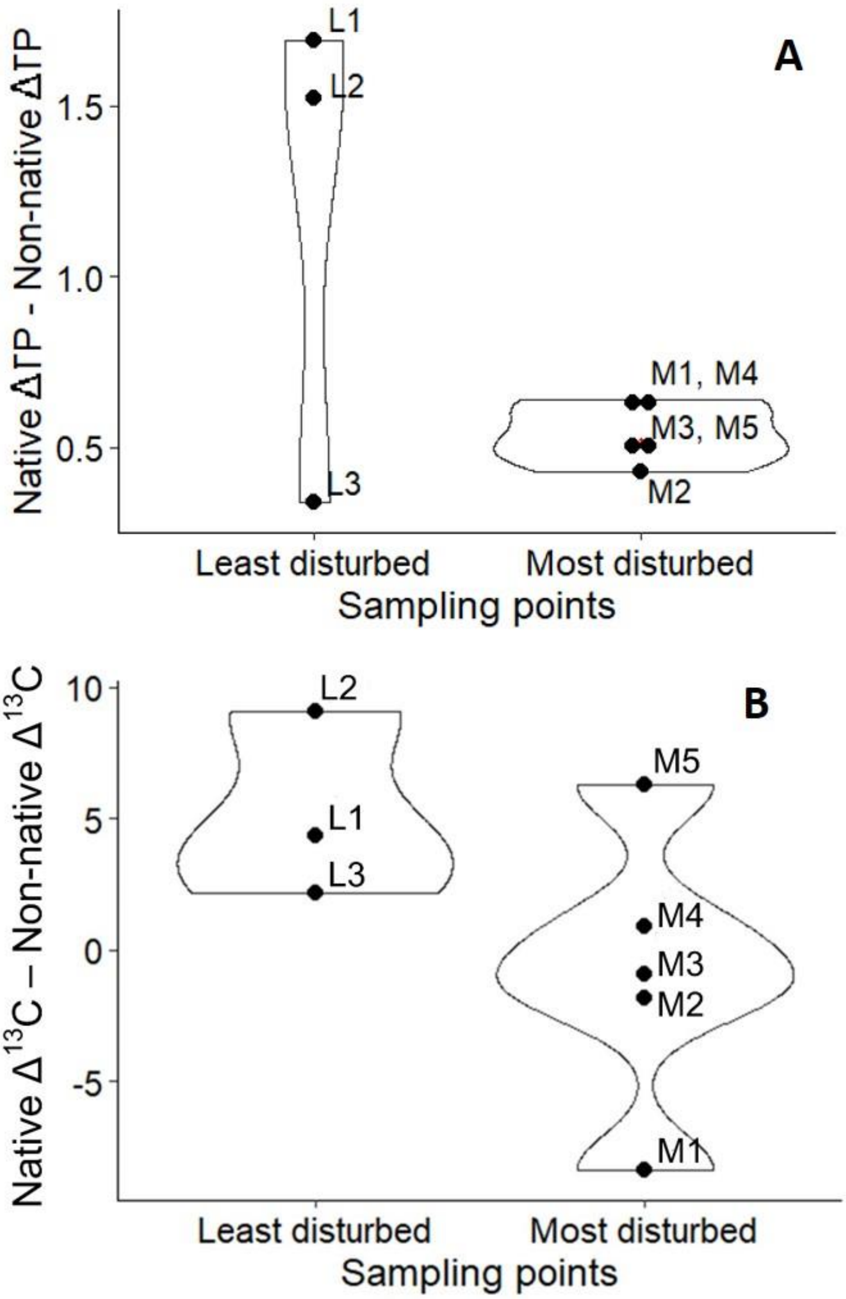

Fig. 4 Difference between the trophic position width of the pool of native and non-native species (Δ TP native – Δ TP non-native) (A), and difference between the width of resources used by the pool of native and non-native species (Δ^{13}C native - Δ^{13}C non-native) (B), in least disturbed and most disturbed points. L1 = Rio Piranga; L2 = Rio Santo Antônio; L3 = Rio Manhuaçu; M1 = Rio Doce (UHE Risoleta Neves); M2 = Rio Doce (Naque); M3 = Rio Doce (Tumiritinga); M4 = Rio Doce (Aimorés); and M5 = Rio Doce (Colatina)

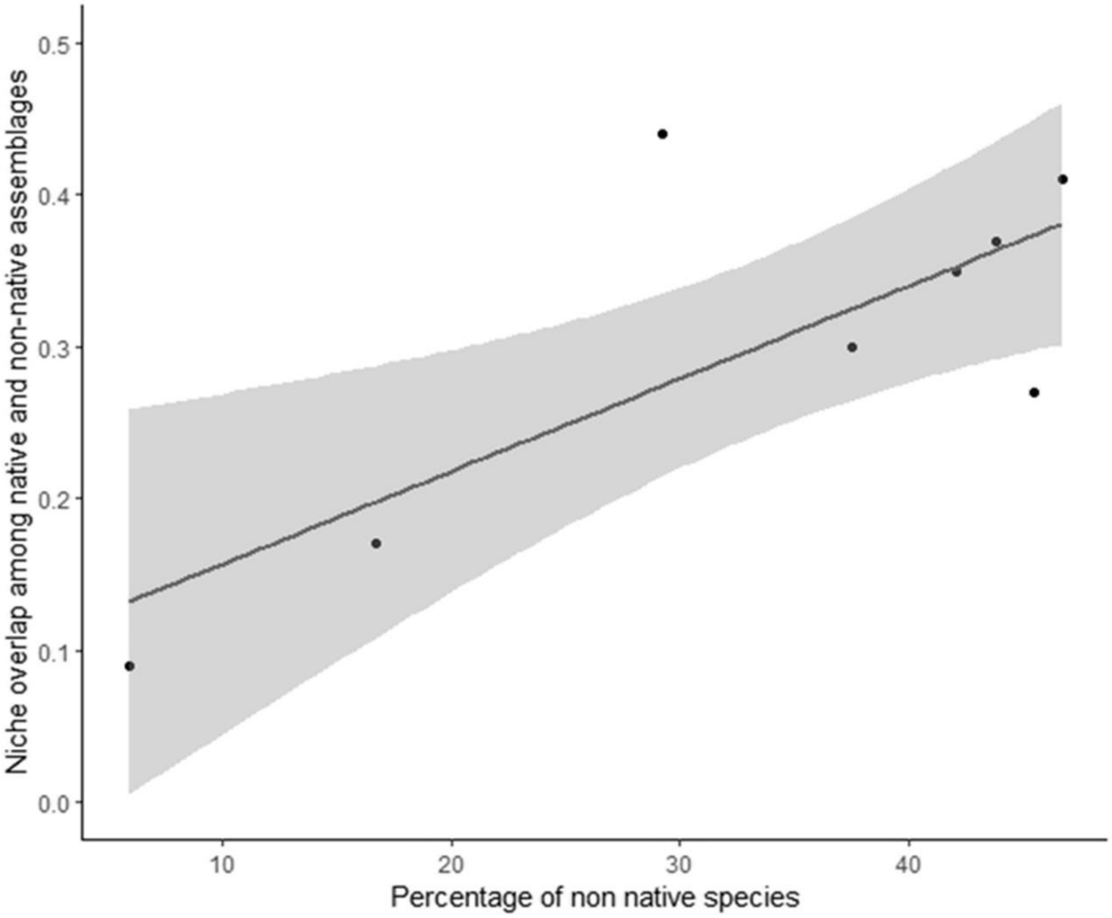

Fig. 5 Relationship between the percentage of non-native species in the assemblages and the overlap of isotopic niches between native and non-native species in the Rio Doce basin.

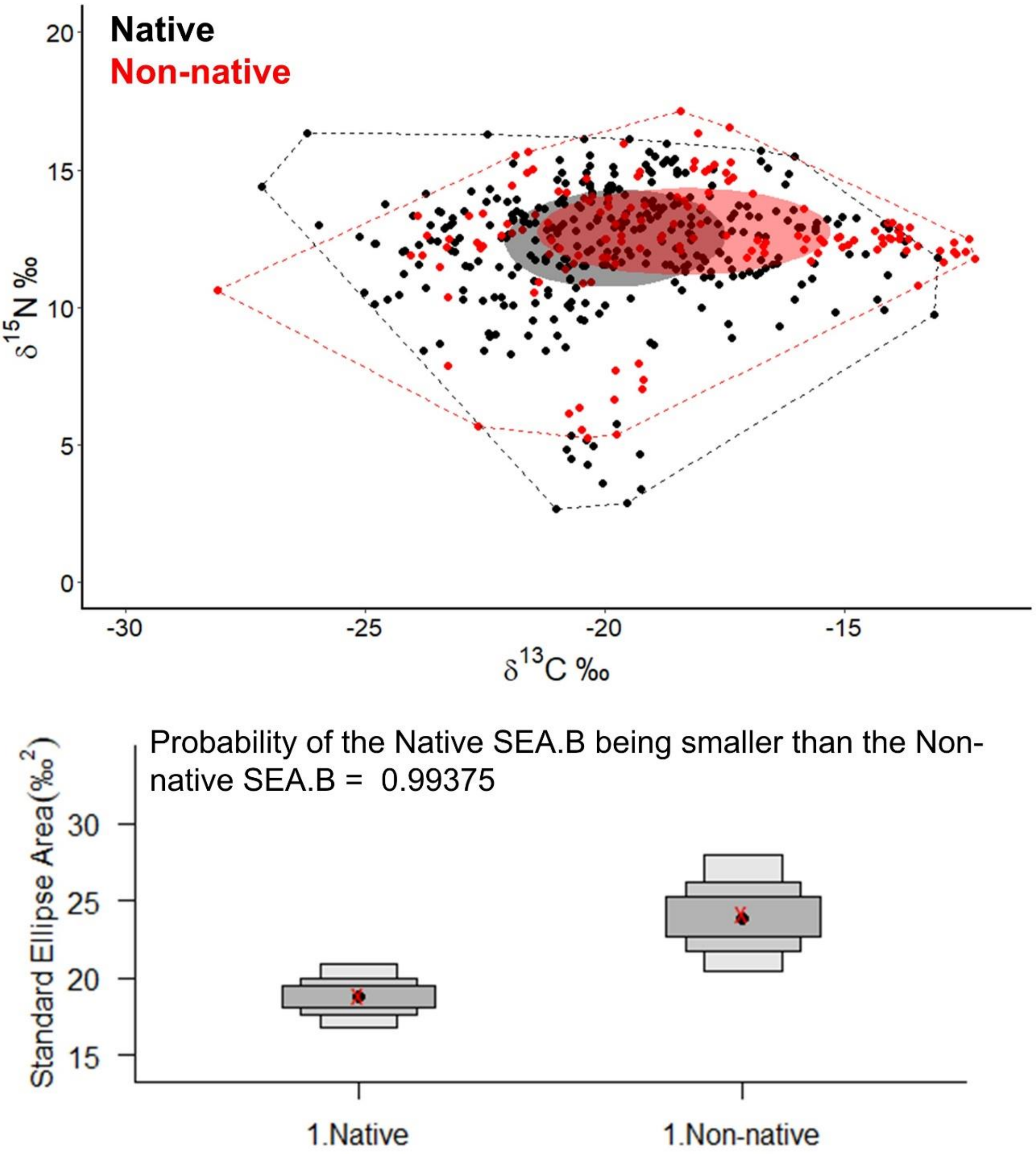

Fig. 6 Ellipses (standard ellipse area - SEA, in ‰²) calculated using a 40% confidence interval representing the isotopic niche of the native and non-native fish community considering all sampled points together

Table 1 Geographic locations of the eight sampled points in the Doce River basin

Region	Point	Condition	Drainage	State	UTM	Coord. (E)	Coord. (S)
Upper	L1	Least disturbed	Piranga River	MG	23K	709518	7726784
Middle	L2	Least disturbed	Santo Antônio River	MG	23K	687610	7872625
Lower	L3	Least disturbed	Manhuaçu River	MG	24K	261172	7842938
Upper	M1	Most disturbed	Gualaxo do Norte River	MG	23K	688284	7754717
Middle	M2	Most disturbed	Doce River	MG	23K	782354	7869156
Middle	M3	Most disturbed	Doce River	MG	24K	219512	7900508
Lower	M4	Most disturbed	Doce River	MG	24K	282205	7844032
Lower	M5	Most disturbed	Doce River	ES	24K	327949	7838898

Table 2 Number of samples isotopically analyzed for each native and non-native fish species at the sampled points in the Rio Doce basin. L1=Rio Piranga; L2=Rio Santo Antônio; L3=Rio Manhuaçu; M1= Rio Doce (UHE Risoleta Neves); M2=Rio Doce (Naque); M3=Rio Doce (Tumiritinga); M4=Rio Doce (Aimorés); M5=Rio Doce (Colatina). Non-native species list was based on Bueno et al. 2021

Species	**L1**	**L2**	**L3**	**M1**	**M2**	**M3**	**M4**	**M5**	**Total**
Native	**32**	**71**	**53**	**38**	**27**	**36**	**29**	**40**	**326**
Astyanax lacustris (Lütken 1875)	3	4	10	8	6	13	7	7	58
Awaous tajasica (Lichtenstein 1822)								3	3
Brycon dulcis Lima & Vieira 2017				1					1
Brycon opalinus (Cuvier 1819)		6							6
Characidium timbuiense Travassos 1946		3							3
Crenicichla lacustris (Castelnau 1855)			2						2
Cyphocharax gilbert (Quoy & Gaimard 1824)				1					1
Delturus carinotus (LaMonte 1933)	1	3	4						8
Deuterodon cf. *intermedius*			5		5				10
Deuterodon intermedius (Eigenmann 1908)			2						2
Eleotris pisonis (Gmelin 1789)								1	1
Geophagus aff. *brasiliensis* (Quoy & Gaimard 1824)	6	4	5	4		8		5	32
Geophagus sp.		7	5	2					14
Henochilus wheatlandii Garman 1890		5							5
Hoplias intermedius (Günther 1864)		2	3	6	4	2	6		23
Hypomasticus copelandii (Steindachner 1875)		1	1						2
Hypostomus affinis (Steindachner 1877)	4							6	10
Hypostomus luetkeni (Steindachner 1877)	3	5	2			2	1		13
Loricariichthys castaneus Castelnau 1855)			2		4				6
Megaleporinus conirostris (Steindachner 1875)	2		4			1	1	2	10
Microphis lineatus (Kaup 1856)								5	5
Oligosarcus acutirostris Menezes 1990					3	7	7		17
Oligosarcus argenteus Günther 1864	7	4	1	6	5	1			24
Oligosarcus solitarius Menezes 1990		3							3
Pachyurus adspersus Steindachner 1879	4	8				1		2	15
Parotocinclus doceanus (Miranda Ribeiro 1918)			1	3					4
Parotocinclus sp.		5	1					2	8

Species	L1	L2	L3	M1	M2	M3	M4	M5	Total
Poecilia vivipara Bloch & Schneider 1801			3				4	5	12
Prochilodus vimboides Kner 1859				5		1			6
Psalidodon aff. *fasciatus* (Cuvier 1819)	1								1
Psalidodon sp.		5							5
Rhamdia quelen (Quoy & Gaimard 1824)	1								1
Serrapinnus heterodon (Eigenmann 1915)				2					2
Synbranchus marmoratus Bloch 1795							2	2	4
Trachelyopterus striatulus (Steindachner 1877)			2				1		3
Trichomycterus aff. *alternatus* (Eigenmann 1917)		6							6
Non-native	**8**	**5**	**20**	**18**	**17**	**39**	**21**	**24**	**152**
Cichla kelberi Kullander & Ferreira 2006			3						3
Cichla monoculus Spix & Agassiz 1831			1						1
Clarias gariepinus (Burchell 1822)			1	1					2
Coptodon rendalli (Boulenger 1897)			1	5		11			17
Saxatilia lepidota (Heckel 1840)							6	1	7
Gymnotus sylvius Albert & Fernandes-Matioli 1999	3		3	1			2	2	11
Hoplosternum littorale (Hancock 1828)			6	1					7
Knodus moenkhausii (Eigenmann & Kennedy 1903)	5	5	5	5	5	5	5		35
Lophiosilurus alexandri Steindachner 1876						1			1
Oreochromis niloticus (Linnaeus 1758)				5	6	13	5	5	34
Pimelodus maculatus Lacepède 1803					2		1	2	5
Poecilia reticulata Peters 1859							1	5	6
Prochilodus argenteus Spix & Agassiz 1829							1		1
Prochilodus costatus Valenciennes 1850					3	5			8
Pterygoplichthys pardalis (Castelnau 1855)								2	2
Pygocentrus nattereri Kner 1858						3		6	9
Salminus brasiliensis (Cuvier 1816)					1	1			2
Serrasalmus brandtii Lütken 1875								1	1
Total number of samples	**40**	**76**	**73**	**56**	**44**	**75**	**50**	**64**	**478**
Total richness	**12**	**17**	**24**	**16**	**11**	**16**	**15**	**19**	**54**
Relative richness – Native (%)	83.3	94.1	70.8	62.5	54.5	56.3	53.3	57.9	66.7
Relative richness – Non-native (%)	16.7	5.9	29.2	37.5	45.5	43.8	46.7	42.1	33.3

Table 3 Estimates of the trophic positions (mean) occupied by native and non-native fish in the Rio Doce basin at each sampling point. L1 = Rio Piranga; L2 = Rio Santo Antônio; L3 = Rio Manhuaçu; M1 = Rio Doce (UHE Risoleta Neves); M2 = Rio Doce (Naque); M3 = Rio Doce (Tumiritinga); M4 = Rio Doce (Aimorés); and M5 = Rio Doce (Colatina)

Sites	L1	L2	L3	M1	M2	M3	M4	M5	Mean TP species
Native									
Astyanax lacustris	2.36	3.00	3.17	2.94	2.96	2.58	3.49	2.65	2.91
Awaous tajasica								2.01	2.01
Brycon dulcis				3.11					3.11
Brycon opalinus		3.14							3.14
Characidium timbuiense		3.79							3.79
Crenicichla lacustris			3.63						3.63
Cyphocharax gilbert				2.79					2.79
Delturus carinotus	3.98	4.00	3.54						3.77
Deuterodon cf. *intermedius*			3.43		3.21				3.32
Deuterodon intermedius			3.68						3.68
Eleotris pisonis								2.14	2.14
Geophagus aff. *brasiliensis*	3.58	3.62	2.84	3.15		2.79		0.55	2.74
Geophagus santosi		3.72	3.37	3.18					3.52
Henochilus wheatlandii		3.16							3.16
Hoplias intermedius		4.36	3.22	3.37	3.72	2.90	3.65		3.53
Hypomasticus copelandii		3.70	3.34						3.52
Hypostomus affinis	3.69							1.08	2.13
Hypostomus luetkeni	3.98	3.68	3.42			3.54	3.68		3.69
Loricariichthys castaneus			3.03		3.07				3.05
Megaleporinus conirostris	3.24		3.54			2.89	4.32	2.47	3.28
Microphis lineatus								1.28	1.28
Oligosarcus acutirostris					4.28	3.28	4.34		3.89
Oligosarcus argenteus	3.58	3.99	4.02	3.27	3.84	3.41			3.63
Oligosarcus solitarius		4.30							4.30
Pachyurus adspersus	4.05	3.82				2.77		2.10	3.59
Parotocinclus doceanus			2.69	3.41					3.23
Parotocinclus sp.		3.53	2.84					2.53	3.20
Poecilia vivipara			2.93				3.01	1.99	2.56
Prochilodus vimboides				2.62		2.50			2.60
Psalidodon aff. *fasciatus*	2.82								2.82
Psalidodon sp.		3.71							3.71
Rhamdia quelen	3.59								3.59
Serrapinnus heterodon				3.68					3.68
Synbranchus marmoratus							3.05	1.74	2.40
Trachelyopterus striatulus			3.32				4.73		3.79
Trichomycterus aff. *alternatus*		3.61							3.61
Non-native	3.79	3.61	3.26	3.03	3.43	2.75	3.53	2.29	3.04
Cichla kelberi			3.97						3.97

Sites	**L1**	**L2**	**L3**	**M1**	**M2**	**M3**	**M4**	**M5**	**Mean TP species**
Cichla monoculus			3.72						3.72
Clarias gariepinus			3.64	3.13					3.39
Coptodon rendalli			2.56	3.00		2.64			2.74
Crenicichla lepidota							4.11	3.82	4.07
Gymnotus sylvius	3.76		3.20	3.26			3.31	2.60	3.27
Hoplosternum littorale			3.03	2.29					2.93
Knodus moenkhausii	3.80	3.61	3.10	3.36	3.42	2.87	3.39		3.37
Lophiosilurus alexandri						3.77			3.77
Oreochromis niloticus				2.80	3.38	2.47	3.03	1.37	2.60
Pimelodus maculatus					3.89		4.46	3.20	3.73
Poecilia reticulata							2.75	0.82	1.14
Prochilodus argenteus							3.61		3.61
Prochilodus costatus					3.21	2.49			2.76
Pterygoplichthys pardalis								1.18	1.18
Pygocentrus nattereri						4.02		3.75	3.84
Salminus brasiliensis					3.47	3.46			3.47
Serrasalmus brandtii								3.69	3.69
Mean TP site	3.59	3.64	3.27	3.10	3.44	2.81	3.64	1.95	3.14

Table 4 Comparison between the isotopic niche size of native and non-native species, and their respective overlaps in all sampled points in the Rio Doce River. L1 = Rio Piranga; L2 = Rio Santo Antônio; L3 = Rio Manhuaçu; M1 = Rio Doce (UHE Risoleta Neves); M2 = Rio Doce (Naque); M3 = Rio Doce (Tumiritinga); M4 = Rio Doce (Aimorés); and M5 = Rio Doce (Colatina)

	L1	**L2**	**L3**	**M1**	**M2**	**M3**	**M4**	**M5**
Probability of the Native SEA being smaller than the Non-native SEA	0.00	0.00	0.76	0.91	0.28	0.81	0.15	0.11
Overlap SEA Native with SEA Non-native	0.21	0.10	0.98	0.75	0.50	0.82	0.71	0.61
Overlap SEA Non-native with SEA Native	0.97	1.00	0.80	0.50	0.59	0.68	0.95	0.83
Overlap with each other	0.17	0.09	0.44	0.30	0.27	0.37	0.41	0.35