Convergent responses of fish belonging to different feeding guilds to sewage pollution

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This study aimed to evaluate if the presence of pollutants promotes changes in feeding habits of fish species from different trophic guilds: the detritivorous species, *Hypostomus francisci*, and the piscivorous, *Hoplias intermedius*. Both species were sampled at 12 sites (with different degrees of pollution) in the Rio das Velhas basin, which is heavily polluted by domestic and industrial sewage from the Metropolitan Region of Belo Horizonte (MRBH). Stable isotope analyses of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) of fish tissue and the main food resources were performed. Fishes from both trophic guilds altered their diets in degraded environments, but the detritivorous species showed greater trophic plasticity. The isotopic niche of both trophic guilds was broadest in unpolluted sites and more $\delta^{15}N$ enriched in polluted regions. The detritivorous species presented high niche-breadth in unpolluted sites, probably due to the greater variety of resources consumed. In addition, the $\delta^{15}N$ of the detritivorous was more enriched than the piscivorous species in polluted sites. In conclusion, fishes from both trophic guilds presented similar isotopic responses to environmental pollution. However, the detritivorous species was more sensitive to these alterations and therefore, is likely a better indicator of environmental condition than the piscivorous.

Keywords: Detritivorous, *Hoplias intermedius*, *Hypostomus francisci*, Piscivorous, Stable isotopes.

Este estudo teve como objetivo avaliar se a presença de poluentes promove mudanças nos hábitos alimentares de espécies de peixes de diferentes guildas tróficas: a espécie detritívora, *Hypostomus francisci*, e a piscívora, *Hoplias intermedius*. Ambas espécies foram amostradas em 12 locais (com diferentes níveis de poluição) na bacia do Rio das Velhas, que é altamente poluída por esgoto doméstico e industrial da Região Metropolitana de Belo Horizonte (RMBH). Foram realizadas análises de isótopos estáveis de carbono (δ^{13}C) e nitrogênio (δ^{15}N) dos tecidos dos peixes e dos principais recursos alimentares. Espécies de ambas guildas tróficas alteraram suas dietas em ambientes degradados, mas a espécie detritívora apresentou maior plasticidade trófica. O nicho isotópico de ambas as espécies foi mais amplo em locais menos perturbados e mais enriquecido em δ^{15}N em regiões poluídas. A espécie detritívora apresentou grande amplitude em seu nicho isotópico em locais menos perturbados, provavelmente devido à maior variedade de recursos consumidos. Além disso, o δ^{15}N da espécie detritívora foi mais enriquecido que a espécie piscívora em locais poluídos. Em conclusão, ambas as espécies apresentaram respostas isotópicas semelhantes à poluição ambiental. No entanto, a espécie detritívora foi mais sensível a essas alterações e, portanto, é provavelmente uma melhor indicadora de condição ambiental do que a espécie piscívora.

Palavras-chave: Detritívoros, *Hoplias intermedius*, *Hypostomus francisci,* Isótopos estáveis, Piscívoros.

INTRODUCTION

Fish are good indicators of environmental quality and are used to assess the integrity of aquatic environments (Karr, 1981; Pompeu *et al*., 2005; de Carvalho *et al*., 2017b). The selection of these organisms for environmental biomonitoring occurs because changes in habitat can be reflected in multiple dimensions among ichthyofauna, for example, by changes in feeding habit (Carvalho *et al*., 2015; de Carvalho *et al*., 2017a), reproduction (Schulz, Martins-Junior, 2001), and community composition (Fausch *et al*., 1990; Cunico *et al*., 2006). In addition, fishes are represented at several trophic levels in aquatic food webs (Freitas, Siqueira-Souza, 2009), and many species have long-life cycles (Karr, 1981; Smith *et al*., 1997). Therefore, assessment of ichthyofauna to understand the effects of anthropogenic impacts on aquatic ecosystems is very important.

The discharge of domestic and industrial sewage without proper treatment into the rivers is considered as one of the main anthropogenic impacts in aquatic systems (Camargo *et al*., 1995; Soares *et al*., 2016). One of the main consequences of sewage pollution is the increased nutrient load in aquatic ecosystems, which can lead to a rapid decrease in oxygen levels through eutrophication and have catastrophic impacts on fish diversity (Smith, 2003; Silva, Fonseca, 2016).

More subtle impacts, such as changes in the food webs of aquatic ecosystems,

can also be observed in polluted environments (Goulart, Callisto, 2003), related to changes in primary productivity (Delitti, 1995). In response, fish species may seek alternative resources to complement their diets in modified habitats and improve survival probability, a behaviour known as trophic plasticity (Abelha *et al.*, 2001). Such changes in the feeding preferences are related to both seasonal and spatial differences of the supply of food (Uieda, Pinto, 2011). Although morphology may set limits to patterns of resource use, since ecomorphological traits are specific to each trophic guild (Albouy *et al.*, 2011), these limits are broad enough to allow fishes changing their choice of prey resources to respond to local biotic and/or abiotic conditions (Ibañez *et al.*, 2007). Detritivorous for instance, are expected to consume more algae in polluted sites and more periphyton in undisturbed sites, as the presence and abundance of these resources are related to water quality (Moschini-Carlos, 1999).

A common underlying assumption is that environmental degradation leads to the decline of the proportion of trophic specialists and carnivores, while the proportion of omnivores increases (Fausch *et al.*, 1990). Several studies have also suggested that certain anthropogenic activities may alter the trophic niche of aquatic organisms (*e.g.*, Crook *et al.*, 2015; Castro *et al.*, 2016; de Carvalho *et al.*, 2017a; Alonso *et al.*, 2019). Large ranges of carbon sources exploited by benthic macroinvertebrates and fishes have been reported, for instance, in streams under influence of pasture (Castro *et al.*, 2016; de Carvalho *et al.*, 2017a). However, little is known about the impact of sewage pollution on the trophic niche of fish species. Furthermore, it is not known whether species of different guilds respond in the same way to stressors, such as pollutants.

The stable isotopes of carbon (δ^{13}C) and nitrogen (δ^{15}N) are key tools for accessing information on feeding habits and trophic structure, since the isotopic composition of the consumers reflects the isotopic composition of their diet, providing long-term feeding information (Manetta, Benedito-Cecilio, 2003). Isotopic niche has been used as a proxy of the trophic niche, and represented with isotopic values (δ^{15}N and δ^{13}C) as coordinates, because an animal's chemical composition is directly influenced by what it consumes as well as the habitat in which it lives (Newsome *et al.*, 2007). A positive factor in using isotopes is that, unlike stomach contents, it is possible to analyse the main food sources of consumers over weeks (Sacramento *et al.*, 2016; Winter *et al.*, 2019), not just what they fed on in the moments prior to capture. Therefore, from the isotopic composition of consumers and its main food sources it is possible to evaluate the diet of captured organisms, energy flow, and the trophic dynamics of aquatic environments (Manetta, Benedito-Cecilio, 2003).

Considering that pollution can affect biota in different ways, the main objective of this work was to evaluate if the presence of sewage in aquatic environments alters the feeding habits and isotopic niche of fish species from different trophic guilds. For this, the carbon and nitrogen isotopic composition of two species — the piscivorous *Hoplias intermedius* (Günther, 1864) and the detritivorous *Hypostomus francisci* (Lütken, 1874) — were evaluated in different regions of a highly polluted Brazilian river basin. These species were selected because they represent different trophic guilds with resident habit (non-migratory) and because they were widely distributed and abundant throughout the studied basin. *Hypostomus francisci* (Siluriformes, Loricariidae), inhabit rocky or sandy bottoms in places with running water, and are considered detritivorous, as they ingest large amounts of organic matter from sediments (Cardone *et al.*, 2006).

As a consequence, these fish are import for the recycling of nutrients in aquatic environments (Pereira, Resende, 1998; Flecker, Taylor, 2004). *Hoplias intermedius* (Characiformes, Erythrinidae) are of predatory habit, feeding preferentially on other fishes. In addition, this species is economically important and a source of food for the local community. The following hypotheses were tested: 1) Species of different trophic guilds respond similarly to the presence of pollution in the aquatic environment; 2) piscivorous species are more sensitive to pollution, since negative effects strengthen across trophic levels; and 3) both trophic guilds will present larger isotopic niches in unpolluted habitats due to a greater availability of different resources.

MATERIAL AND METHODS

Study area. The study was carried out in the main channel and main tributaries of the Rio das Velhas sub-basin, that belongs to the Rio São Francisco basin, located in the Brazilian state of Minas Gerais (Fig. 1). The headwaters of the rio das Velhas are located in the municipality of Ouro Preto, and has an overall extension of approximately 801 km, making it the largest tributary of the Rio São Francisco (Machado *et al.*, 2008). The Rio das Velhas basin encompasses 51 municipalities of Minas Gerais, totalling almost five million inhabitants (IBGE, 2000). The river is responsible for most of the water supply in this region, presenting significant economic and social importance. Annual average temperature varies between 19 and 23 °C, with precipitation levels between 900 and 2000 mm, and well-defined seasons - dry (winter) and rainy (summer) (PDRH, 2015). The Cerrado (Brazilian Savanna) is the predominant natural vegetation in the watershed, however; approximately 90% has already been modified by human activities. Due to the diverse human activities developed in the region, this basin is in an advanced state of degradation. Main sources of pollution along the river's course include waste products from iron ore mining, and the discharge of sewage and other pollutants from the Metropolitan Region of Belo Horizonte (MRBH) in its middle portion. Despite this scenario, it is still possible to find relatively unpolluted tributaries, such as the Rio Cipó, which has its headwaters in the Serra do Cipó National Park.

Sampling design. Samples were taken at five sites along the main channel of the Rio das Velhas (RV), which were later grouped into three regions: one sampling site in the upper section of Rio das Velhas – "Upper RV" (region with the best water quality); two sample sites in the middle section of Rio das Velhas – "Middle RV" (most impacted) and two sampling sites in the lower section of Rio das Velhas – "Lower RV" (region farthest from the MRBH and near the river's mouth, presents a partial improvement in the water quality as the distance from MRBH increases). In addition, seven sites were sampled in six of the main tributaries of the Rio das Velhas basin, which were considered as controls due to the absence of human disturbance (Rio Jabuticatubas, Rio Taquaraçu, Rio da Onça, Rio Bicudo, Rio Curimataí and two sites in Rio Cipó). Two sewage treatment plants (STP), Arrudas and Onça, were also sampled to obtain complementary samples of the suspended material to determine the isotope signature of sewage (Tab. 1; Fig. 1). Fish collections were carried out over three campaigns in the main channel (two in the dry season and one in the rainy
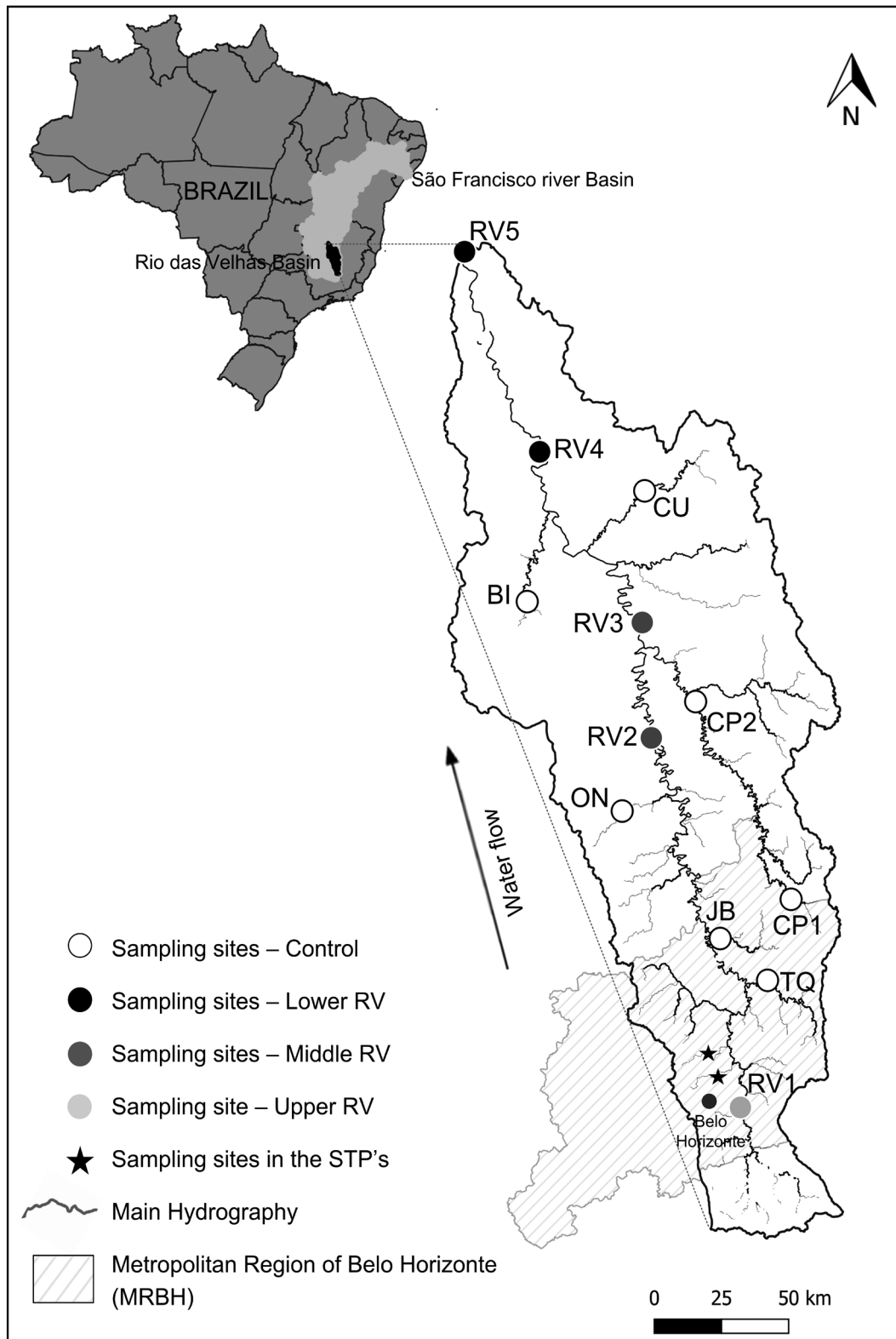
FIGURE 1 | Sampling network in the rio das Velhas basin, Minas Gerais, Brazil. Sampling sites at rio das Velhas main stem (RV-01 to RV-05), rio Taquaraçu (TQ); rio Jaboticatubas (JB); rio Cipó (CP1 and CP2); rio da Onça (ON); rio Bicudo (BI); rio Curimataí (CU); and Sewage Treatment Plants (STP's).

season) and two campaigns in tributaries (one in the dry season and one during the rainy season) (Tab. 1).

Information about the level of degradation in sampling sites (excluding Rio Taquaraçu that did not have a corresponding point) was obtained from previously published data (Feio *et al.*, 2015). Degradation levels ranged from I (undisturbed/ unpolluted) to IV (degraded/polluted) (Tab. 1).

Data on water quality, hypereutrophic condition, toxic contamination and pressure factors acting in studies sites were accessed through Instituto Mineiro de Gestão das Águas website (IGAM, 2018), which publishes quarterly monitoring reports from several points across the Rio das Velhas basin. Values of conductivity, dissolved oxygen, ammoniacal nitrogen and total phosphorous presented in this study correspond to average values obtained from the IGAM measurements during 2015 and 2016. The hypereutrophic condition, toxic contamination and pressure factors acting in sampling sites were obtained from the report of the year 2017. Based on their geographic proximity, the IGAM monitoring sites BV010; BV136; BV139, BV141, BV144, BV147, BV149; BV150; BV151; BV162; SC33 were considered as the correspondents of CP-01, JB-01, RV-01, RV-02, ON-01, BI-01, RV-05, RV-03, RV-04, CP-02 and CU-01, respectively (Tab. 1).

Fish sampling. Trophic guilds were determined from stomach content analysis previously conducted by the authors (Tab. S1, available only in the online version). The specimens of *H. intermedius* and *H. francisci* were collected with gillnets (20 m long, with 3–16 cm stretch measure mesh), seines (5 m long, 1 mm mesh), cast nets (3 cm stretch measure mesh), and mosquito nets (80 cm in diameter, 1 mm mesh). Gill nets were deployed in the water column for 14 h overnight. Seines were used in shallow areas or littoral zones, mosquito nets were used in near-shore aquatic macrophytes (both shorelines), undercut banks and in riffles, and cast nets were used in habitats too deep to wade. The three latter methods were employed for 1–3 h. In the field, muscle samples from adult specimens were removed and were kept frozen until laboratory processing. In laboratory, these samples were lyophilized for 24 hours and ground to a fine and homogeneous powder using a pestle and mortar and subsequently stored in *eppendorf* tubes.

Collected species were deposited in the Coleção Ictiológica da Universidade Federal de Lavras (CI-UFLA) and the Museu de Zoologia da Universidade de São Paulo (MZUSP), with the following catalogue numbers: *H. francisci* (CI-UFLA 1029, MZUSP 73724); *H. intermedius* (MZUSP 73655, MZUSP 73735, MZUSP 73839).

Resource sampling. Food resources usually consumed by *H. francisci* and *H. intermedius* were identified by analysing the stomach contents of individuals of both species (see Tab. S1, available only in the online version) and through information in the literature (*e.g.*, Cardone *et al.*, 2006). We collected five replicates (at each site) of the following resources: periphyton, filamentous algae, grasses, riparian vegetation, suspended material (raw sewage) and fish (due to piscivorous habit of *H. intermedius*) (see Tab. S2, available only in the online version).

Samples of algae and vegetation (grasses and riparian vegetation) were collected

TABLE 1 | Geographic location (in degrees/minutes/seconds and UTM, date, altitude and municipality) and water quality of the sampling sites sampled in the main channel and tributaries of rio das Velhas. Cond.= Condutivity (iS/cm); D.O.= Dissolved oxygen (mg/l); Am. Nitr. = Ammoniacal nitrogen (mg/l); Phosp. = Total phosphorus (mg/l); Tox. Contam. = Toxic contamination; Deg. Level = degradation level ranging from I (undisturbed/unpolluted) to IV (degraded/polluted) (Feio *et al.*, 2015). *Sites with hypereutrophic condition according IGAM.

	Characteristics of sampling sites						Water Quality						
Region	Sampling sites	Date of sampling	Coordinates	Altitude (m)	Municipality	Cond.	D.O.	Am. Nitr.	Phosp.	Tox. Contam.	Deg. Level	Pressure Factors
Upper RV	RV-01	19/08/2015 20/01/2016 9/06/2016	20°01'10.7"S 43°49'45.4"W 23K 622454 E 7785916 W	729	Nova Lima	73.21	7.54	0.12	0.08		I	
Middle RV	RV-02	10/08/2015 11/01/2016 31/05/2016	18°48'19.2"S 44°09'09.2"W 23K 589298 E 7920498 W	567	Curvelo	287.20	7.25	0.92	0.41	Arsenic and total ammoniacal nitrogen	III*	Gold metallurgy and discharge of domestic sewage
Middle RV	RV-03	11/08/2015 12/01/2016 1/06/2016	18°25'33.2"S 44°11'10.9"W 23K 585926 E 7962502 W	552	Corinto	203.23	7.30	0.23	0.22	Arsenic	II	Agriculture
Lower RV	RV-04	13/08/2015 13/01/2016 3/06/2016	17°51'55.4"S 44°32'57.4"W 23K 547752 E 8024649 W	495	Lassance	162.21	7.63	0.21	0.17	Arsenic	II	Discharge of domestic sewage and agriculture (sugar cane)
Lower RV	RV-05	12/08/2015 14/01/2016 2/06/2016	17°12'25.9"S 44°48'49.8"W 23K 519793 E 8097515 W	464	Várzea da Palma	153.00	8.35	0.14	0.11	Arsenic	II	Discharge of domestic sewage
Control	Rio Taquaraçu (TQ)	19/06/2015 23/10/2015	19°36'03.1"S 43°44'09.9"W 23K 632551 E 7832196 N	677	Taquaraçu de Minas	67.55	7.49	0.20	0.08		-	
Control	Rio Jaboticatubas (JB)	16/06/2015 22/10/2015	19°27'51.4"S 43°54'18.0"W 23K 614931 E 7847433 N	651	Jaboticatubas	102.20	7.55	0.14	0.06		I	
Control	Rio Cipó (CP1)	15/06/2015 21/10/2015	19°20'01.1"S 43°39'17.0"W 23K 641315 E 7861707 N	757	Santana do Riacho	18.13	6.70	0.13	0.03		I	
Control	Rio da Onça (ON)	11/07/2015 18/10/2015	19°02'48.1"S 44°15'15.9"W 23K 578454 E 7893841 N	632	Cordisburgo	312.75	7.85	0.22	0.05		II	
Control	Rio Cipó (CP2)	10/07/2015 13/10/2015	18°41'07.0"S 43°59'48.4"W 23K 605789 E 7933699 N	567	Presidente Juscelino	68.18	6.90	0.13	0.05		I	
Control	Rio Bicudo (BI)	06/07/2015 17/10/2015	18°21'30.7"S 44°35'30.8"W 23K 543117 E 7970099 N	564	Corinto	72.77	6.22	0.11	0.07		II	
Control	Rio Curimataí (CU)	08/07/2015 15/10/2015	17°59'33.4"S 44°10'47.5"W 23K 586829 E 8010438 N	518	Augusto de Lima	55.10	6.75	0.12	0.06		I	
Sewage - MRBH	STP Arrudas	20/07/2016 25/01/2017			Sabará							
Sewage - MRBH	STP Onça	20/07/2016 18/01/2017			Belo Horizonte							

randomly at all sites where present, stored in plastic pots and kept frozen until laboratory processing. Periphyton were collected by scraping stones and collected material stored in plastic bottles with distilled water. To obtain the isotope signature of sewage, suspended matter in the water was sampled using a phytoplankton net (45 μm mesh) for a period of three minutes in the sewage treatment plants (STPs) Arrudas and Onça. After collection, the liquid samples (periphyton and suspended matter) were immediately frozen for preservation of the material. In the laboratory, samples were filtered using a filtration apparatus attached to a vacuum pump using calcined quartz fiber filters (Whatman® QMA quartz filters). All basal resource samples were then dried in an oven at 60°C for a minimum period of 48 hours. Afterwards, dried samples were ground to a fine and homogeneous powder using pestle and mortar and

stored in *eppendorf* tubes.

Other fish species (see Tab. S2, available only in the online version), which make up the assembly of each sample point and are the primary food source for *H. intermedius,* were collected with the same fishing equipment and sampling effort mentioned in the previous section. Sample processing was also done in the same manner; however, small fish individuals (with less than 3cm) were processed as a whole, without the digestive tract. To avoid contamination, all equipment and material used were washed in distilled water along the processing.

Isotopic analysis. After laboratory processing, a total of 129 fish samples (66 *H. francisci* and 63 *H. intermedius*) and 1725 resources samples (including fish from other species that served as potential resources for *H. intermedius*) were sent to the Centre for Nuclear Energy in Agriculture (CENA) at the University of São Paulo for isotopic analysis. Approximately 2–5 mg of dry material from animal tissue, and about 5–10 mg of basal resources were selected for analysis. To determine the isotopic ratio, a mass spectrometer system in the Continuous–flow (CF-IRMS) mode was used with a Carlo Erba elemental analyzer (CHN 1110) coupled to a Delta Plus mass spectrometer (Thermo Scientific). Results were expressed as the relative difference of international reference standards (air nitrogen and PeeDee Belemnite), in the delta notation (δ‰), 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" represents the isotopic ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ (Barrie, Prosser, 1996).

Statistical Analysis. Differences in the isotopic ratios of $\delta^{13}C$ and $\delta^{15}N$ of consumers and resources between the four regions were tested using one-way analyses of variance (ANOVAs) where assumptions of normality and homoscedasticity were met. The nonparametric Kruskal-Wallis test was used for data with non-normal distributions. When significant differences ($p<0.05$) were observed, the means were compared using the post-hoc Tukey's test. These analyses were performed in the software Statistica 6.0.

To evaluate the trophic structure of the detritivorous and piscivorous populations, the individuals of two species were plotted in the *bi-plot* space according to the isotopic values of carbon (x-axis) and nitrogen (y-axis) in each region. Source contributions to the detritivorous and piscivorous diet were estimated for the four regions (Upper RV, Middle RV, Lower RV, and Control) based on stable isotope data analysed through Bayesian stable isotope mixed models (Moore, Semmens, 2008; Parnell *et al.*, 2010), using the MixSIAR package in R (Stock, Semmens, 2016b). A Markov chain Monte Carlo sampling was conducted based on the following parameters: number of chains = 3; chain length = 100,000; burn in = 50,000; thin = 50 and model 4 (Resid*Process) error structure (Stock, Semmens, 2016a). Diagnostic tests (Gelmin–Rubin, Heidelberger– Welch and Geweke) and trace plots were used to examine models. The fractionation values used for consumers were 0.4 ± 1.3‰ for C and 2.54 ± 1.27‰ for N (Post, 2002; Vanderklift, Ponsard, 2003). Both the graphical representation and the partition analysis were done using the MixSIAR package using the R software.

The isotopic niches of the detritivorous and piscivorous in both regions (Upper RV, Middle RV, Lower RV and Control) were quantified based on standard ellipse areas (SEA - expressed in ‰²) through use of the Stable Isotope Bayesian Ellipses package in R – SIBER (Jackson *et al.*, 2011). The standard ellipse area (SEA) represents the core isotopic niche space and it is a proxy of the richness and evenness of resources consumed by the population (Bearhop *et al.*, 2004).

RESULTS

Individuals of the detritivorous (and/or algivorous) *Hypostomus francisci* and the piscivorous *Hoplias intermedius* were collected at all sampling sites. Both the detritivorous and piscivorous showed significant variation in their carbon and nitrogen isotopic composition between study regions. For both trophic guilds, the $\delta^{13}C$ and $\delta^{15}N$ were enriched in regions under the influence of heavy pollution (middle and lower RV) (Fig. 2). Basal resources also presented extensive variation in isotopic composition of $\delta^{13}C$ and $\delta^{15}N$ between study regions, except for riparian vegetation that did not vary in $\delta^{13}C$ composition between the four sampled regions (Tab. 2).

The two trophic guilds varied in their distribution in bi-plot space in unpolluted (upper RV and control) and polluted regions (middle and lower RV). In unpolluted regions, the detritivorous presented greater amplitude in $\delta^{13}C$ and smaller amplitudes of $\delta^{15}N$ when compared to the piscivorous (Figs. 3A, D). However, in polluted regions, the detritivorous presented more enriched $\delta^{15}N$ values than the piscivorous (Figs. 3B, C).

Partition analyses revealed that the proportions of different resources assimilated by fishes varied between sample regions. The most assimilated resource by the piscivorous was fish. However, in the upper section of the Rio das Velhas, such guild also assimilated basal resources (filamentous algae and periphyton), as well as allochthonous items from the forest in the surroundings (riparian vegetation and grasses) (Fig. 4, see Tab. S3, available only in the online version).

The highest variation in the proportion of assimilated resources was observed for the detritivorous. Filamentous algae were the main resource, except in lower RV sites, where the main source of carbon came from grasses. The importance of grasses as a carbon source was also observed in the upper section of the Rio das Velhas (approximately 22%). Periphyton were the second most important resource for the detritivorous in all regions (mainly in the middle section) (Fig. 4, Tab. S4, available only in the online version).

Comparing the isotopic niche occupied by these species in different regions, we observed that the two fish species, despite being members of different trophic guilds, presented similar responses to the presence of pollutants (Fig. 5). In regions under high influence of sewage (middle and lower RV), both trophic guilds presented isotopic niches with values more enriched in nitrogen. In addition to influencing nitrogen levels, the presence of pollutants also altered the type and range of assimilated resources, with fishes from both trophic guilds feeding on resources with depleted $\delta^{13}C$ in unpolluted regions. Such variation in the assimilated resources was more visible for the detritivorous, as the amplitude of the x axis (carbon) was much wider in unpolluted environments.
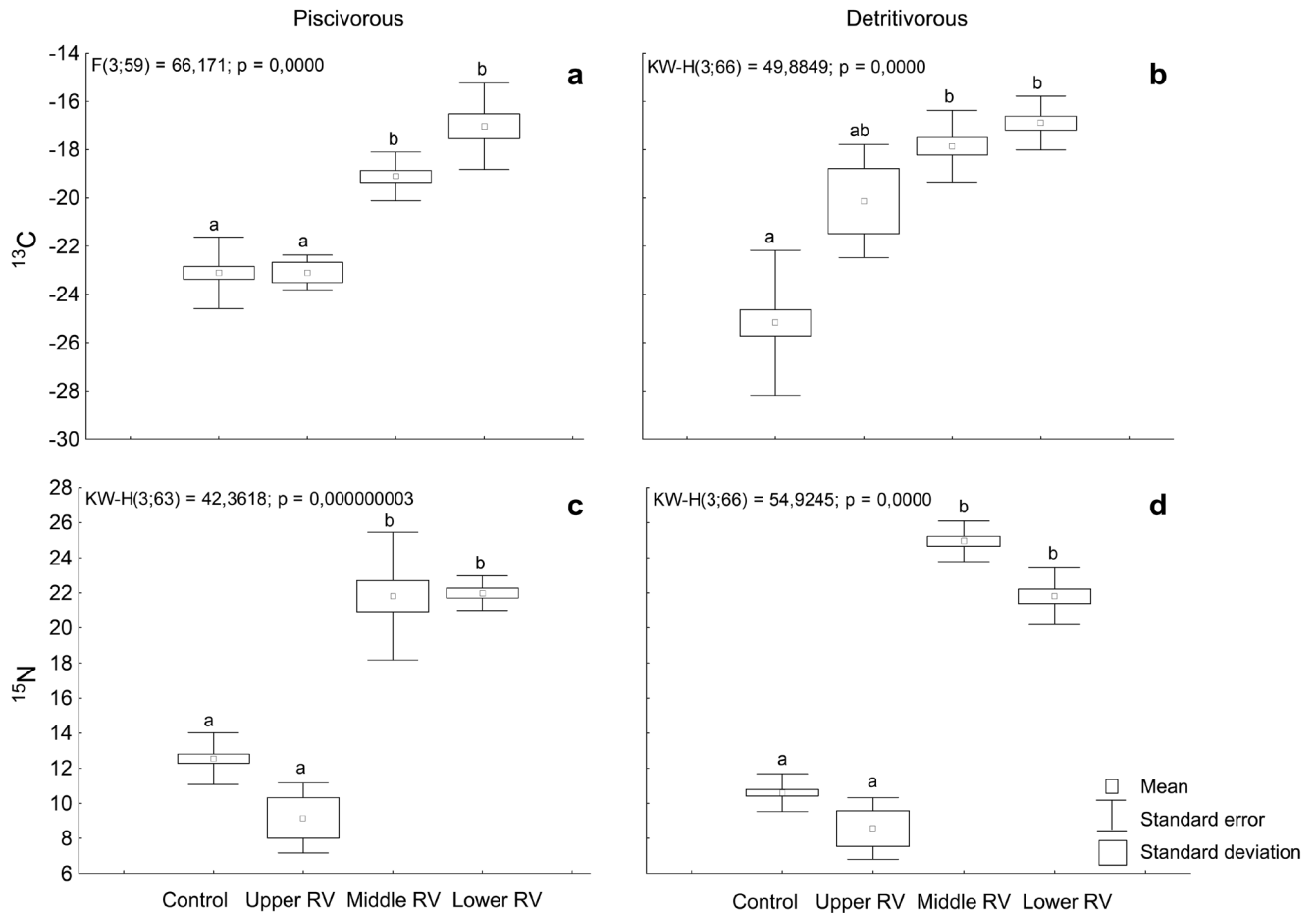
FIGURE 2 | Variation in the isotopic composition of carbon (**A.** and **C.**) and nitrogen (**C.** and **D.**) in the piscivorous species *Hoplias intermedius* (**A.** and **C.**) and the detritivorous species *Hypostomus francisci* (**B.** and **D.**) among the studied regions.

DISCUSSION

Using isotopic analyses, we confirm the hypothesis that fish from different trophic guilds respond similarly to the presence of pollution from domestic and industrial sewage discharge in aquatic environments. It was also possible to confirm our third hypothesis that isotopic niches occupied by fish species are wider in unpolluted environments, probably due to the consumption of a greater variety of resources. However, the greater trophic plasticity and higher nitrogen enrichment of the detritivorous diets in polluted sites, suggests that such guild may be more sensitive to variation in environmental conditions and, therefore, a better bioindicator of water quality.

We found striking evidence of nitrogen enrichment in fish species and basal resources sampled in polluted regions. Such enrichment has also been observed in aquatic environments under different anthropogenic impacts (*e.g.*, de Carvalho *et al.*, 2015; Loomer *et al.*, 2015; Castro *et al.*, 2016; Orlandi *et al.*, 2017). Although δ15N values above 25‰ are rarely recorded, they have been consistently observed in fishes

Marina R. Prado, Débora R. Carvalho, Carlos B. M. Alves, Marcelo Z. Moreira and Paulo S. Pompeu

TABLE 2 | Variation in the carbon and nitrogen isotopic composition of the resources sampled in the four regions of the rio das Velhas basin, Minas Gerais, Brazil. Letters **a, b, c** and **d** indicate significant differences according to post-hoc Tukey's test.

|	REGIONS	ALGAE			PERIPHYTON			GRASSES			RIPARIAN VEGETATION			FISH			SEWAGE		
		N	MEAN	SD	N	MEAN	SD	N	MEAN	SD	N	MEAN	SD	N	MEAN	SD	N	MEAN	SD
δ¹³C	Control	53	-25.02	±6.33a	44	-25.85	±1.88a	70	-22.09	±8.31a	70	-29.52	±1.75a	474	-24.68	±2.62a			
	Upper RV	5	-21.78	±2.16ab	15	-24.09	±2.22ab	5	-14.33	±0.99ab	5	-30.94	±1.00a	109	-20.65	±2.32b			
	Middle RV	18	-11.64	±8.47b	30	-21.62	±3.45b	10	-15.25	±4.51b	10	-29.99	±1.45a	278	-20.16	±2.18b	40	-23.16	±0.40
	Lower RV	15	-6.41	±1.21b	30	-18.91	±3.68c	10	-17.46	±6.71ab	10	-29.27	±1.51a	280	-18.24	±2.82c			
	p		<0.01			<0.01			0.02			0.24			<0.01				
δ¹⁵N	Control	53	7.05	±2.13a	44	6.34	±1.06a	70	3.65	±2.23a	70	2.40	±2.18a	474	10.23	±2.11a			
	Upper RV	5	8.63	±0.69ac	15	6.12	±1.10a	5	-0.93	±0.78b	5	0.50	±2.33a	109	9.19	±1.30b			
	Middle RV	18	22.64	±3.73b	30	20.45	±5.28b	10	5.95	±2.97c	10	5.17	±2.75b	278	22.82	±3.33c	40	1.50	±0.45
	Lower RV	15	13.26	±2.71bc	30	14.21	±3.26b	10	9.76	±3.05d	10	7.94	±2.32b	280	19.43	±3.35d			
	p		<0.01			<0.01			<0.01			<0.01			<0.01				
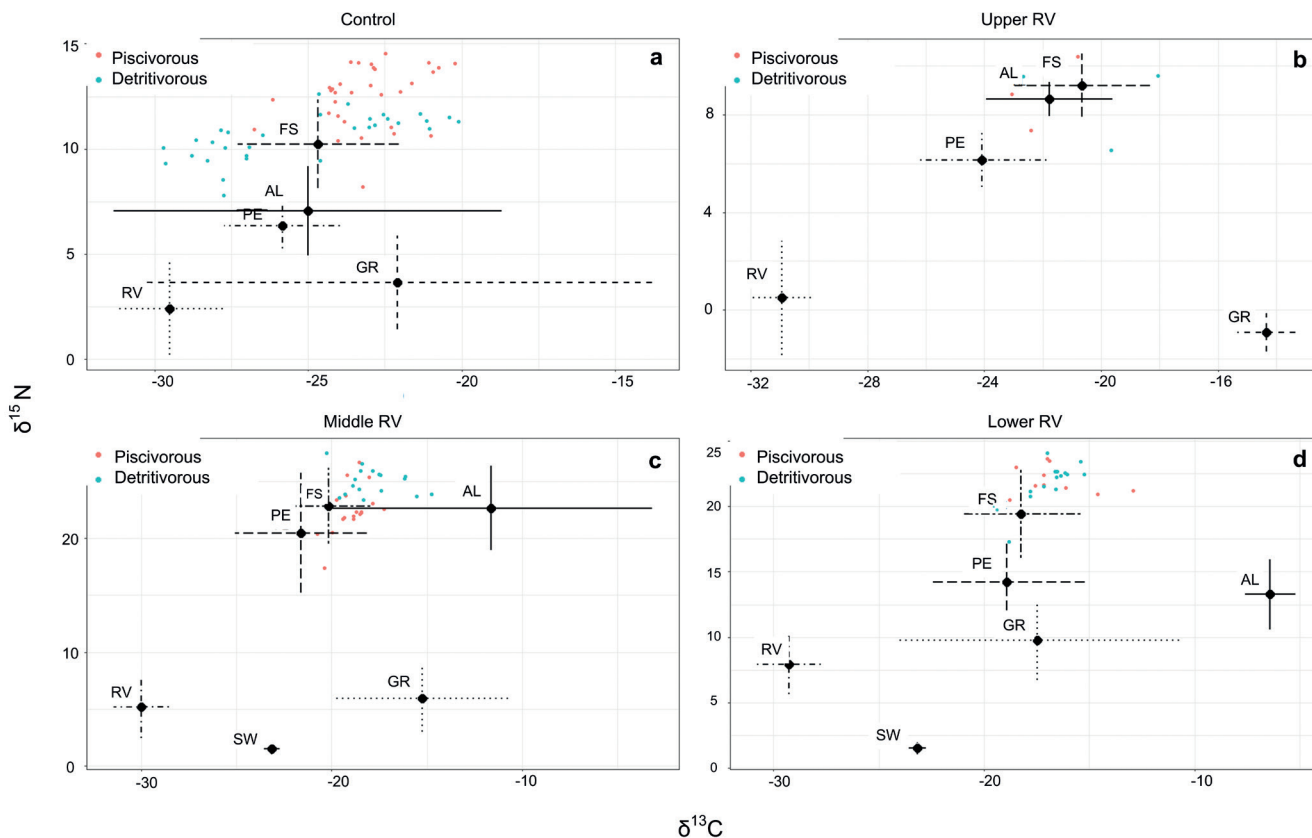
FIGURE 3 | Distribution of the piscivorous species *Hoplias intermedius* (red points) and the detritivorous species *Hypostomus francisci* (blue points) in the bi-plot space by study regions: **A.** Upper RV, **B.** Middle RV, **C.** Lower RV and **D.** Control sites. Resources: GR = Grasses; RV= Riparian vegetation; PE = periphyton; SW = Sewage (before treatment); AL = filamentous algae and FS = Fish.
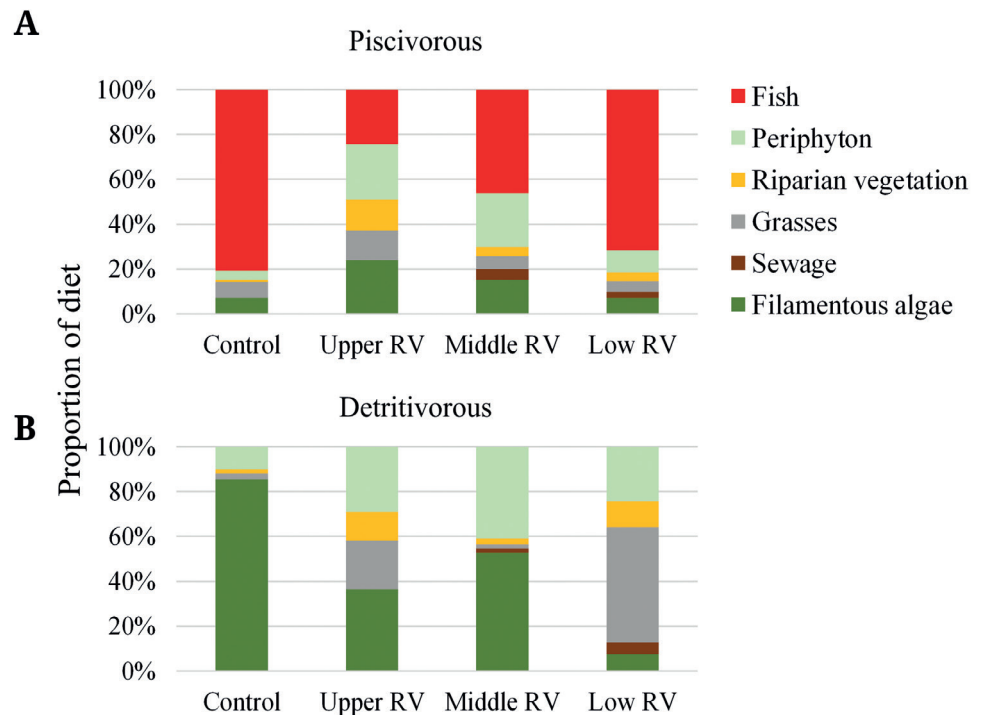
FIGURE 4 | Mean proportion of each resource assimilated by **A.** *Hoplias intermedius* (piscivorous) and **B.** *Hypostomus francisci* (detritivorous) at each study region (MixSIAR results). AL = filamentous algae; SW = Sewage (before treatment); GR = Grasses; RV= Riparian vegetation; PE = periphyton and FS = Fish.

and resources from the Rio das Velhas basin (Alonso *et al.*, 2019). The most extreme $\delta^{15}N$ values were observed in the region where the high discharge of pollutants occurs (middle RV) and downstream of this region (lower RV). At lower RV, despite the high $\delta^{15}N$ of fishes, the $\delta^{15}N$ was slightly less enriched than in the region close to MRBH (middle RV) likely due the sewage dilution. Such high values are probably related to eutrophication processes (Silva, Fonseca, 2016), and were detected in all components of trophic webs, from autotrophic organisms (*e.g.*, algae and periphyton) to the consumers.

In the most polluted regions (middle and lower RV), $\delta^{15}N$ of detritivorous fish were more enriched than piscivorous fish. This pattern is not expected, since, by theory, the piscivorous fishes should occupy the top of the food web. The reason why the $\delta^{15}N$ enrichment of piscivorous fishes is lower than that of detritivores is not clear. Both piscivorous and detritivorous species sampled in this study are resident, so it is unlikely piscivorous fishes are not feeding on fish from the same site where they were sampled. As detritivorous fishes usually presents bottom habits and feed in the resources located in this portion of the water bodies, probably they may be consuming $\delta^{15}N$ enriched resources (not sampled in the present study) that are not being consumed by fishes from other guilds. In addition, the detritivorous from the genus *Hypostomus* are barely predated due its morphology and adoption of defence strategies (Bruton, 1996; Kirchheim, Goulart, 2010), what could explain why the $\delta^{15}N$
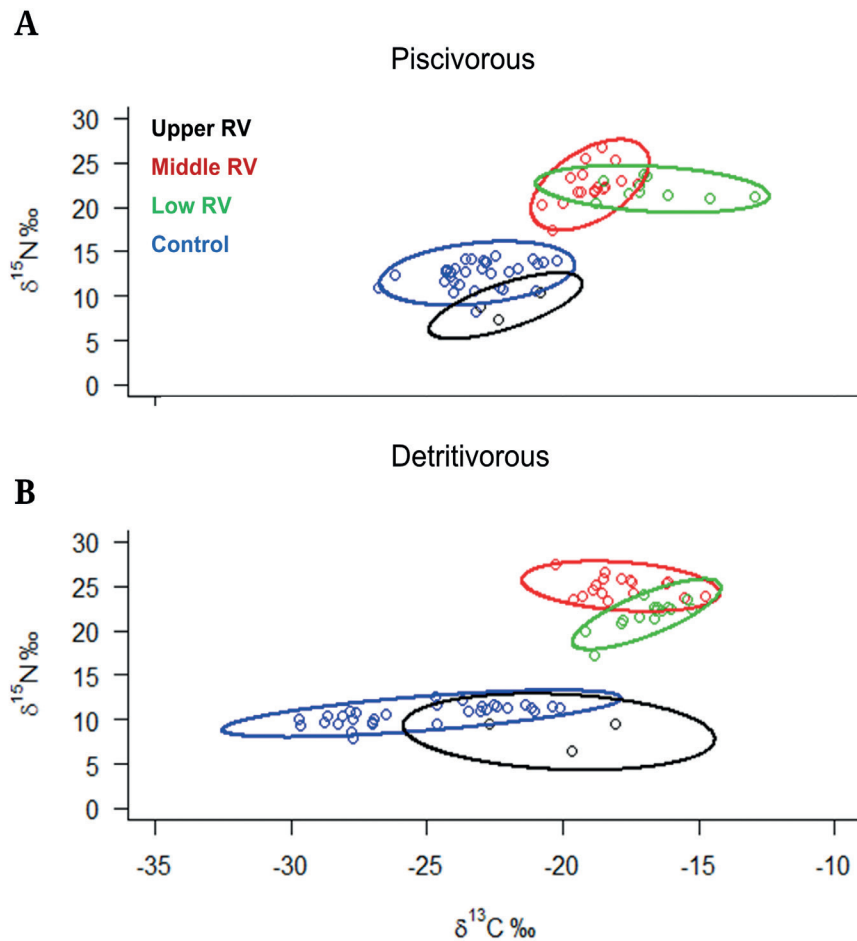
FIGURE 5 | Isotopic niche (measured by ellipse area with 95% confidence interval) occupied by **A.** *Hoplias intermedius* (piscivorous) **B.** *Hypostomus francisci* (detritivorous) in different regions of the rio das Velhas basin, Minas Gerais, Brazil.

enrichment is not propagating to piscivorous fishes. Therefore, the consume of $\delta^{15}N$ enriched unsampled resources together with the lack of predation of detritivorous fishes by piscivorous fishes (otherwise the piscivorous should be more enriched than the detritivorous) seems to be the main hypothesis to explain why detritivorous fishes are more $\delta^{15}N$ enriched than the piscivorous fishes.

The different carbon isotopic compositions between control sites and the polluted ones, suggests that fishes from both trophic guilds are modifying their feeding habits in response to changes in the aquatic environment (Abelha *et al.*, 2001), despite differences in their trophic plasticity. Such changes were reflected in the isotopic niches occupied by the consumers, and similar patterns of variation in niche amplitude, even though these species belong to different trophic guilds. Moreover, a clear homogenization in the isotopic composition of available resources in polluted areas may also explain the narrower isotopic niches of consumers in such regions (Abelha *et al.*, 2001; Pusey, Arthington, 2003).

Fish were the most consumed resources by the piscivorous, as expected for species of the genus *Hoplias* (Pompeu, Godinho, 2001; Montenegro *et al.*, 2013). However, other

items were also assimilated by this species, which probably influenced the variation of the isotopic composition of this species between the regions. Vegetable remnants and algae/periphyton, may have been ingested together with the prey during predation events, as they are not normally part of the diet of this species (Corrêa, Piedras, 2009; Beliene *et al.*, 2014). However, in some regions (Upper and Middle RV), this accounted for over 20% of assimilated resources, which weakens the hypothesis of accidental consumption. Another hypothesis is that these resources may have been indirectly assimilated through the ingestion of aquatic insects and their larvae, which may be herbivores/detritivorous (feeding on small algae and organic matter), or small particulate filter-feeders suspended in water. As approximately two thirds of sampled individuals were juveniles reinforces this hypothesis, as juveniles predominantly feed on aquatic invertebrates, and adults may do so also in presence of other piscivorous species (Pompeu, Godinho, 2001).

The detritivorous species assimilated a range of basal resources, including algae, periphyton and C4 grasses among the sampled regions, which consequently was reflected in the variation of the $\delta^{13}C$ isotopic composition. The detritivore/ herbivore habit has been described to representatives of the genus *Hypostomus* (Pessoa *et al.*, 2013) and was confirmed through stomach content analysis, which explains the consumption of allochthonous items, such as plant remnants (grasses). The high consumption of algae and periphyton was expected, as this genus is also considered algivorous (Cardone *et al.*, 2006). In addition, as this species adheres to the substrate during foraging, some items outside their normal diet can be ingested (Ross, 1986; Villares-Junior *et al.*, 2016), such as insect larvae and pupae that are buried in sediments. Therefore, some items reflected in the isotopic composition of the detritivorous may also have been assimilated indirectly, through the accidental consumption of insects and other aquatic organisms.

Greater algal growth is expected in more eutrophic sites, where there is sewage discharge (Tundisi, Matsumura, 2008). On the other hand, periphyton are more associated with undisturbed environments, where water transparency (Cetto *et al.*, 2004), and the presence of free surfaces, such as rocks and/or submerged vegetation, favours their development (Tundisi, Matsumura, 2008). Nevertheless, the opposite was observed in the control region, with algae and periphyton contributing approximately 86% and 10% of assimilated resources, respectively. This could reflect the specific characteristics of the studied tributaries, which may have favoured the proliferation of algae rather than periphyton. However, the abundance of each resource in each sampled region was not evaluated in the present study. In addition, detritivorous may exhibit selectivity and preference for algae over periphyton, even when both resources are abundant (Bozza, Hahn, 2010).

It is interesting to note that carbon from grasses contributed 22% of the isotopic composition of detritivorous in the upper RV and 51% in the lower RV. This high assimilation of grasses was not expected, as it is not considered as a primary energy source for detritivorous fish (Araujo-Lima *et al.*, 1986). In addition, C4 grasses are generally consumed by fish in small quantities because they contain fewer nutrients and are difficult to digest. However, recent studies have suggested that the contribution of C4 plants can be substantial to aquatic communities (Hoeinghaus *et al.*, 2007; Ferreira *et al.*, 2012), and herbivorous fish species can feed on this resource in impacted

environments (de Carvalho *et al.*, 2015). In addition, we also need to consider the indirect assimilation of C4 sources through debris consume (Garcia *et al.*, 2016), since it is composed by a mix of autochthonous (*e.g.*, algae, macrophytes and periphyton) and allochthonous material (*e.g.*, leaves from trees and grasses) provided by river banks.

Through the results it was possible to observe that the detritivorous species showed great variation in the proportion of assimilated resources in different regions and high enrichment in the isotopic composition of nitrogen in polluted sites. Detritivorous species are important for the functioning of aquatic ecosystems (Pereira, Resende, 1998) and usually comprise a significant portion of the total community (Vasconcelos-Filho *et al.*, 2009). Therefore, environmental degradation, and subsequent alterations in resources abundance and in the feeding habits of this trophic group, may have implications for ecosystem functioning.

We can conclude that the use of carbon and nitrogen isotopes contributes greatly to our understanding of the trophic ecology of fish species in impacted aquatic environments. We observed that fishes from both trophic guilds exhibited high food plasticity, varying the proportion of resources consumed among regions. Moreover, pollution promoted nitrogen enrichment in resources and consumers which, consequently, was reflected in the isotopic niches occupied by them. Such changes may have major impacts on ecosystem functioning in aquatic systems, especially when important changes are observed at different levels of trophic webs (*e.g.*, top predators and detritivorous). In addition, for future biomonitoring and assessment of the biotic integrity, we suggest the use of first-level consumers rather than species that occupy higher trophic levels (predators), as they could be more sensitive to changes in environmental conditions, especially where domestic sewage is the main stressor. Finally, it is important to emphasise that our results are based on the evaluation of only two species in a large area. Therefore, whole fish community evaluations should be encouraged.

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AUTHOR CONTRIBUTIONS ⓒ

Marina Rodrigues do Prado: Formal Analysis, Investigation, Methodology, Writing (original draft).

Débora Reis de Carvalho: Conceptualization, Data curation, Formal Analysis, Writing (original draft).

Carlos Bernardo Mascarenhas Alves: Conceptualization, Formal Analysis, Methodology, Writing (original draft).

Marcelo Zacharias Moreira: Conceptualization, Formal Analysis, Writing (review & editing).

Paulo Santos Pompeu: Conceptualization, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Writing (original draft), Writing (review & editing).

ETHICAL STATEMENT

This project was conducted under the approval of the Animal Ethics Committees (CEUA - UFLA 070/15) and Collection License (ICMBio 10327-2).

COMPETING INTERESTS

Not applicable.

ADDITIONAL INFORMATION

Supplementary information accompanies this paper at http://www.sbi.bio.br/ni.

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