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Tracing sewage-derived organic matter in fish trophic pathways in the lower Doce River basin, Brazil

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Untreated wastewater discharges into freshwater systems threaten aquatic environments and disrupt food web dynamics. This study assessed the contribution of sewage to the fish food web in the lower Doce River using stable carbon and nitrogen isotopes. We tested the following hypotheses: 1) sewage assimilation is higher during the dry season; 2) fish species that assimilate more sewage are associated with lower trophic positions, and 3) detritivorous and omnivorous fish guilds assimilate more sewage-derived carbon. Samples of 29 fish species, potential food resources, and sewage were collected during the dry and wet seasons of 2022. Sewage samples exhibited depleted nitrogen isotopic compositions in both seasons, while sewage assimilation by fishes was similar between seasons. Species occupying the lowest trophic positions showed greater assimilation, reflecting the isotopic composition of sewage. Omnivorous species assimilated particularly high proportions of sewage-derived resources, with *Oreochromis niloticus* exhibiting the highest assimilation, up to 90% of its diet. Our study shows that sewage inputs have been incorporated into the local food web during both seasons. This highlights the need to improve wastewater treatment, as untreated effluents are readily assimilated, ultimately affecting riverine fauna.

Keywords: Aquatic food web, Aquatic pollution, Ichthyofauna, Stable isotopes, Wastewater.

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O aporte de esgoto não tratado em sistemas de água doce ameaça os ambientes aquáticos e perturba a dinâmica da teia trófica. Este estudo avaliou a contribuição do esgoto para a teia trófica de peixes no baixo rio Doce, utilizando isótopos estáveis de carbono e nitrogênio. Nós testamos as seguintes hipóteses: 1) a contribuição do esgoto é maior durante a estação seca; 2) espécies que assimilam mais esgoto estão associadas a posições tróficas mais baixas; e 3) guildas tróficas de onívoros e detritívoros assimilam mais carbono derivado do esgoto. Amostras de 29 espécies de peixes, prováveis recursos alimentares, e esgoto foram coletadas nas estações seca e chuvosa de 2022. As amostras de esgoto apresentaram assinaturas isotópicas de nitrogênio empobrecidas em ambas as estações, enquanto a assimilação de esgoto pelos peixes foi similar entre as estações. Espécies que ocupam posições tróficas mais baixas apresentaram maior assimilação, refletindo a composição isotópica do esgoto. Espécies onívoras assimilaram proporções particularmente elevadas de recursos derivados do esgoto, com *Oreochromis niloticus* apresentando a maior assimilação, chegando a até 90% de sua dieta. Nosso estudo mostra que os aportes de esgoto foram incorporados à teia alimentar local em ambas as estações. Isso reforça a necessidade de aprimorar o tratamento de esgoto já que efluentes não tratados são prontamente assimilados, afetando a fauna dos rios.

Palavras-chave: Águas residuais, Ictiofauna, Isótopos estáveis, Poluição aquática, Rede trófica aquática.

INTRODUCTION

The input of untreated sewage into watercourses is a growing threat to freshwater ecosystems worldwide (Dudgeon *et al*., 2006; Malmqvist, Rundle, 2002; Wear *et al*., 2021). In addition to posing serious risks to public health (Akpor, Muchie, 2011; Sojobi, Zayed, 2022), it also affects associated fauna (Malik *et al*., 2020; Rangel-Buitrago *et al*., 2024). The presence of these effluents can alter water quality, including shifts in salinity and increased concentrations of nitrates and phosphates (Malik *et al*., 2020). The increased nutrient availability, in turn, stimulates excessive primary production, which leads to reduced dissolved oxygen levels in the water (Malik *et al*., 2020; Rangel-Buitrago *et al*., 2024), negatively impacting aquatic fauna (Devlin, Brodie, 2023; Feng *et al*., 2023).

These disruptions in primary production and aquatic fauna reflect trophic web changes, as fish feeding habits shift in response to alterations in the availability and quality of food resources (Horka *et al*., 2023; Neves *et al*., 2024). In polluted environments, omnivorous species may gain a competitive advantage, as their opportunistic feeding strategies enable them to tolerate fluctuations in resource availability (de Carvalho *et al*., 2019a, 2020a; Neves *et al*., 2024). Notably, the omnivorous species *Poecilia reticulata* has been reported to feed directly from sewage in polluted sites (de Carvalho *et al*., 2019b). Detritivorous species may also be favored by sewage pollution (de Carvalho *et al*., 2020a), as the discharge of organic matter increases detrital food sources (Terra *et al*., 2013), and they are capable of adjusting their diets according to pollution (Prado *et al*., 2020). Seasonality also influences food availability. For instance, sewage may become more diluted during the wet season due to increased rainfall, reducing its influence. In contrast, during the dry season, reduced water flow can lead to higher sewage concentrations and greater ecological impacts (Yunus, Nakagoshi, 2004; Kütter *et al*., 2023).

In this context, carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes provide valuable insights into the feeding ecology of fish in polluted environments. Nitrogen isotopes reflect the organism's trophic position, as $\delta^{15}N$ increases at each trophic transfer (Fry, 2006; Anderson, Cabana, 2007). In addition, $\delta^{15}N$ can serve as a tracer of anthropogenic nitrogen inputs, since sewage and other pollution sources often elevate nitrogen concentration in aquatic systems, resulting in distinct isotopic compositions (Wang *et al*., 2016; de Carvalho *et al*., 2021). Studies show that, depending on the type of sewage treatment, impacted systems may exhibit depleted $\delta^{15}N$ compositions (Loomer *et al*., 2015; de Carvalho *et al*., 2019b, 2020b). Carbon isotopes, in turn, are more conservative across trophic levels and are used to identify the primary basal sources that support the food web (Fry, 2006; Anderson, Cabana, 2007). As sewage-derived carbon becomes an additional basal energy source for aquatic communities, it can be traced through the food web (Loomer *et al*., 2015; de Carvalho *et al*., 2019b, 2020b).

The Doce River basin has historically been affected by multiple anthropogenic pressures, including mining activities, deforestation, untreated sewage discharge, intensive land use, and industrial development (ANA, 2016; Hatje *et al*., 2017; Macêdo *et al*., 2024). These cumulative impacts were further intensified in 2015 by one of Brazil's most severe environmental disasters: the collapse of the Fundão tailings dam in Mariana, Minas Gerais (Garcia *et al*., 2017; Vergilio *et al*., 2021; Yamamoto *et al*., 2023b). This event drew national and international attention to the basin, prompting numerous studies focused on assessing the impacts of the disaster on the river's ichthyofauna (Bonecker *et al*., 2019; Andrades *et al*., 2020, 2021; Ferreira *et al*., 2020; Yamamoto *et al*., 2023a; de Carvalho *et al*., 2024; Paiva *et al*., 2024; da Silva *et al*., 2024; Fráguas *et al*., 2025). Human occupation continues to exert pressure on the basin. The lower portion of the Doce River basin lies in the state of Espírito Santo and includes two major cities: Colatina, with a population of 120,033, and Linhares, with 166,786 inhabitants (IBGE, 2022). Colatina, situated upstream in the lower basin, treats only 14.45% of its sewage before discharging it into the Doce River (SNIS, 2023). Although the presence of sewage pollution has been documented in the literature for the Doce River basin (Fraga *et al*., 2021; Kütter *et al*., 2023; Yamamoto *et al*., 2023b), few studies have investigated its impacts on the trophic ecology of fish assemblages.

This study aimed to assess the incorporation of urban sewage-derived carbon into the aquatic food web of the lower Doce River basin. Additionally, we aimed to identify which trophic guilds and species assimilate the highest proportion of sewage-derived carbon. We tested the following hypotheses: 1) during the dry season, sewage has a greater assimilation by the fish assemblage, 2) species that assimilate more sewage usually occupy lower trophic positions, 3) Detritivorous and omnivorous fish are those that most assimilate sewage-derived carbon.

MATERIAL AND METHODS

Study area. This study was conducted in the lower Doce River basin, within the municipality of Colatina. Colatina has a population of 120,033 inhabitants (IBGE, 2022) and is located 132 km from Vitória, the capital of Espírito Santo State, Brazil. The main economic activities in the region include commerce, services, and a strong industrial and agricultural sector (INCAPER, 2023). The city lies within the Atlantic Forest biome, although most of its area has been altered to support agriculture and livestock activities (INCAPER, 2023). The region receives an average annual rainfall of approximately 1,100 mm, with the rainy season extending from October to April, and the dry season from May to September (INCAPER, 2023). In addition to land-use changes, another anthropogenic pressure is the discharge of untreated sewage into the river. According to data from 2022, only 14.45% of the sewage generated in the city is treated before being discharged into the Doce River (SNIS, 2023). Sampling of fish and potential food sources was conducted once during the dry (September) and wet (February) seasons of 2022.

Fish sampling. Seines, gill nets, and mosquito nets were used for fish sampling during wet and dry seasons in the Doce River. Shallow and littoral zones were sampled using seines (5 m long and 1mm mesh). We used two sets of gillnets, with 10 nets in each set (10 m long, with mesh sizes ranging from 3 to 16 cm stretched mesh). Nets were exposed for 14 h overnight. Near-shore macrophyte banks were sampled using mosquito nets (80 cm in diameter and 1mm mesh) for a period of approximately 1 h. After capture, fish were measured, weighed, and identified. Samples for isotopic analysis were collected for up to five individuals per species. Larger fish had a portion of the muscle tissue removed for isotopic analysis, while smaller fish were analyzed whole, with only the digestive tract removed. Samples were frozen until subsequent laboratory processing. Specimens were deposited at the Ichthyological Collection of Universidade Federal de Lavras (CI-UFLA) (Tab. **S1**).

Resource sampling. We collected five samples per food resource in each season: aquatic macrophytes (MA), fine particulate organic matter (FPOM), coarse particulate organic matter (CPOM), periphyton (PE), filamentous algae (AL), terrestrial invertebrates (TI), aquatic invertebrates (AI), suspended matter (SM), C3 vegetation (VC3), C4 vegetation (VC4), sewage after treatment (SEWA), and sewage before treatment (SEWB). D-frame nets were used to sample AI, while FPOM was sampled by passing the same net through resuspended sediment. SM was collected by placing a phytoplankton net (45μm mesh) in the water for three minutes. Stones were scraped with a small brush in order to collect PE, and the material was stored with distilled water in plastic bottles. FPOM, MA, TI, AL, VC3 and VC4 were randomly collected in the sampling site. Samples of SEWA and SEWB were collected at the wastewater treatment plant in Colatina, Espírito Santo, with authorization from the Environmental Sanitation Company of Colatina (SANEAR). All samples were kept frozen until laboratory processing.

In the laboratory, fish muscle and dry food resources were lyophilized for 24 hours. Liquid samples (PE, FPOM, SM, SEWA and SEWB) were filtered through calcined quartz fiber filters (Whatman® QMA), using a filtration apparatus attached to a vacuum pump. Subsequently, all samples were dried and ground to a homogeneous powder and stored in Eppendorf tubes for further analysis. Samples were sent for isotopic analysis at the Central de Análises e Prospecção Química (CAPQ) of the Universidade Federal de Lavras (UFLA).

Isotopic analysis. A total of 114 samples of fish and 115 samples of food resources were analysed isotopically. Isotopic ratios were determined using a Delta V Plus Isotope Ratio Mass Spectrometer (Thermo Fisher) coupled with a Flash IRMS elemental analyzer interfaced with ConFlo IV. Results were expressed as relative differences from the international reference standard, with delta notation (δ ‰), according to the following formula: $\delta X = [(R\ sample/R\ standard) - 1] \times 10^3$, where X is ^{13}C or ^{15}N, and *R* is the isotopic ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$ (Barrie, Prosser, 1996). We applied a mathematical correction to isotopic ratios, to account for lipid variation in fish tissues. The correction formula proposed by Post *et al.* (2007) was used: $\Delta\delta^{13}C = -3.32 + 0.99 \times C{:}N$.

Statistical analysis. The trophic structure was seasonally characterized using bi-plot graphs (x axis: $\delta^{13}C$ and y axis: $\delta^{15}N$). The bi-plot displays mean values for all species alongside the means and standard deviations for the food resources. This plot allows the visualization of the most important food resources for the community.

Trophic position of each species was estimated for each season using the Bayesian package "tRophicPosition" (Quezada-Romegialli *et al*., 2018). We employed the oneBaseline model, using periphyton as the baseline source. Isotopic fractionation factors were set at $\Delta^{15}N$: 2.9 ± 0.32‰ and $\Delta^{13}C$: 1.3 ± 0.3‰ based on values reported by McCutchan *et al.* (2003).

To evaluate the resource contribution for each species, we used Bayesian mixing models, with the simmr package (Govan *et al*., 2023). Isotope mixing models included concentration dependence of basal sources to account for differences in %C and C:N ratios. Models were run with 3 Markov chains of 100.000 iterations each, a burn-in of 50.000 iterations and a thinning interval of 50, resulting in 3.000 posterior samples per model. Convergence was assessed using the Gelman-Rubin diagnostic ($\hat{R}$) (Gelman, Rubin, 1992). Trophic enrichment factors (TEF) were set at $\Delta^{15}N$: 2.9 ± 0.32‰ and $\Delta^{13}C$: 1.3 ± 0.3‰ (McCutchan *et al*., 2003). Two types of priors were used. For models with ≥4 individuals, we used the default uninformative Dirichlet prior implemented in simmr. For models with ≤3 individuals, informative priors were specified using the *simmr_elicit* function to improve parameter and identifiability under small sample sizes. Food resources included in the model were defined for each feeding guild. The food resources selected for detritivores were: PE, SM, SEWA, and SEWB. For herbivores, we selected MA, PE, VC4, and CPOM. For omnivores, we selected AI, SEWA, SEWB, and TI. For invertivores, we selected AI and TI. For the piscivore guild, we selected AI, TI, and Fish. The Fish used as a resource comprised small-sized species and species documented in the literature as potential prey of the piscivorous species analyzed in this study. The selected species were *Astyanax lacustris*, *Astyanax* sp., *Geophagus brasiliensis*, *Oreochromis niloticus*, *Hoplias malabaricus*, *Oligosarcus argenteus*, *Knodus moenkhausii*, *Poecilia vivipara*, and *Deuterodon taeniatus.* To estimate the sewage contribution through all compartments of the food web, we also applied the mixing models for FPOM, SM, AI and TI. For FPOM and SM, the resources used in the model were: CPOM, PE, and SEWB. The TEF for these two food resources was set at Δ^{15}N: 0.5 ± 0.4‰ and Δ^{13}C: 0.5 ± 0.4‰ to account for potential isotopic shifts associated with microbial processing. For aquatic invertebrates, the resources used in the model were FPOM, PE, SEWB, and SM. For terrestrial invertebrates, the selected resources were VC3 and VC4. TEF for aquatic and terrestrial invertebrates was the same as that used for fish.

To visualize trophic interactions between food resources and feeding guilds, we constructed flow diagrams with the DiagrammeR package (Iannone, Roy, 2016), using the grViz() function. The flow diagram represents directed relationships, where arrows indicate the relative contribution of each food resource for the feeding guilds and the contribution of sewage to food resources. The width of the arrows is scaled proportionally to indicate the estimated contribution of each food resource summarized by feeding guilds. All analyses were performed using the R software (R Development Core Team, 2024).

RESULTS

We sampled a total of 114 individuals from 29 species (Tab. 1), 46 individuals from 15 species in the dry season and 68 individuals from 20 species in the wet season. Six species were sampled in both seasons, whereas nine species were exclusive to the dry season and 14 to the wet season (Fig. 1). Detritivore fish were only sampled during the wet season (Fig. 2A; Tab. **S2**), while herbivores were only sampled during the dry season (Fig. 2B; Tab. **S3**).

Mixing model diagnostics indicated satisfactory performance. All parameters across all models showed R <1.004, indicating adequate convergence and mixing of chains. Among models including three or more sources, most posterior correlations were moderate. However, five out of 35 cases showed strong negative correlations (r ≤ -0.85; Tab. **S4**). Two-source models were excluded from this assessment because perfect negative correlation is mathematically constrained in such models. Posterior median source contributions and their 95% credible intervals (2.5%–97% quantiles) for all species are reported in Tabs. **S2**, **S3**.

Among invertivorous fish, *Pimelodus maculatus* showed greater assimilation of aquatic invertebrates in the wet season (Fig. 2C). Sewage-derived assimilation through aquatic invertebrates was higher for *Pachyurus adspersus* in the dry season (Tabs. **S2**, **S3**). Among the omnivores, sewage (both before and after treatment) accounted for approximately half of the species assimilation (Fig. 2D). *Oreochromis niloticus* showed high sewage assimilation in both seasons, especially during the wet season, which accounted for up to 90% of its assimilated diet. For piscivores, the assimilation of food resources had similar ranges between seasons, and *Pygocentrus nattereri* had higher assimilation of aquatic invertebrates in both seasons (Fig. 2E).

Trophic positions ranged from 2.02 (95% CI: 2.00–2.82) to 5.07 (95% CI: 2.04–9.47) in the dry season, and from 2.21 (95% CI: 2.00–3.66) to 5.67 (95% CI: 2.14–9.55) in the wet season (Tab. **S5**). The highest trophic positions were observed in piscivorous species, except *Hoplias intermedius* and *O. argenteus* in the dry and wet seasons, respectively. Among the six species sampled in both seasons, *Oreochromis niloticus* consistently had

TABLE 1 | Mean and standard deviation of $\delta^{13}C$ and $\delta^{15}N$ in fish species and feeding resources in the dry and wet seasons.

Season	Dry					Wet				
Species/Resource	N	$\delta^{13}C$		$\delta^{15}N$		N	$\delta^{13}C$		$\delta^{15}N$	
		Mean	SD	Mean	SD		Mean	SD	Mean	SD
Detritivore										
Hoplosternum littorale						5	-24.33	3.17	10.8	1.17
Hypoptopomatinae sp.						4	-24.64	3.08	8.33	2.34
Hypostomus affinis						1	-26.25	0	12.51	0
Prochilodus argenteus						1	-17.67	0	12.26	0
Prochilodus costatus						1	-16.14	0	12.58	0
Herbivore										
Metynnis lippincottianus	2	-21.12	3.19	9.5	3.08					
Invertivore										
Pimelodus maculatus	1	-18.61	0	11.99	0	5	-23.13	2.83	13.7	1.34
Saxatilia lepidota						2	-20.39	1.46	11.73	1.29
Microphis lineatus	5	-17.26	1.07	5.73	1.12					
Pachyurus adspersus	5	-20.19	3.56	12.91	0.53					
Phalloceros sp.	1	-17.83	0	5.46	0					
Omnivore										
Astyanax lacustris						5	-21.81	1.90	11.05	1.83
Awaous tajasica						5	-18.05	1.90	11.98	0.96
Characidium sp.						1	-24.12	0	13.51	0
Clarias gariepinus						1	-25.54	0	13.44	0
Deuterodon cf. *taeniatus*						5	-21.40	2.03	12.69	1.06
Geophagus brasiliensis	5	-16.36	2.35	8.69	3.63	1	-20.56	0	12.48	0
Knodus moenkhausii	5	-16.77	1.38	10.19	0.73	5	-19.37	1.13	11.81	1.62
Oreochromis niloticus	5	-14.12	1.22	6.07	0.36	10	-22.80	1.62	7.92	2.7
Poecilia vivipara	2	-16.61	1.05	6.24	1.06	5	-22.88	3.25	10.44	2.12
Astyanax sp.	2	-16.36	0.17	4.75	0.75					
Gymnotus cf. *sylvius*	2	-25.83	0.74	11.49	3.00					
Piscivore										
Centropomus sp.						1	-26.27	0	16.17	0
Cichla kelberi						1	-19.24	0	14.04	0
Oligosarcus argenteus						1	-20.96	0	11.13	0
Pygocentrus nattereri	6	-20.05	0.58	13.48	0.69	8	-20.21	0.39	13.62	0.99
Hoplias intermedius	3	-18.59	0.15	9.09	1.85					
Hoplias malabaricus	1	-20.63	0	13.96	0					
Salminus brasiliensis	1	-19.50	0	14.04	0					
Feeding Resources										
Aquatic Invertebrates	5	-22.31	1.15	9.49	2.04	5	-21.51	1.29	10.75	1.15
CPOM	5	-30.53	1.87	4.98	3.05	5	-30.47	0.32	2.62	1.04
FPOM	5	-23.05	0.52	5.18	0.98	5	-23.75	0.71	4.37	0.77
Macrophyte	5	-29.26	0.96	8.21	0.57	5	-26.54	0.64	9.07	0.99
Periphyton	5	-18.59	2.98	7.07	0.71	5	-23.23	0.52	7.23	1.69
Sewage – After treatment	5	-21.59	0.25	0.79	0.27	5	-23.47	0.28	1.46	0.78
Sewage – Before Treatment	5	-24.91	0.53	2.41	0.52	5	-23.86	0.24	3.58	0.23
Suspended Matter	5	-26.6	3.7	7.32	1.18	5	-25.6	2.07	5.86	0.5
Terrestrial Insect	5	-25.07	2.85	5.97	4.22	5	-21.5	6.08	6.99	1.1
Vegetation C3	8	-29.75	0.89	3.86	2.69	8	-29.5	1.59	6.83	1.34
Vegetation C4	2	-13.21	1.64	6.76	2.05	2	-13.83	0.18	3.6	0.91
Algae	5	-23.41	4.45	6.31	1.33					

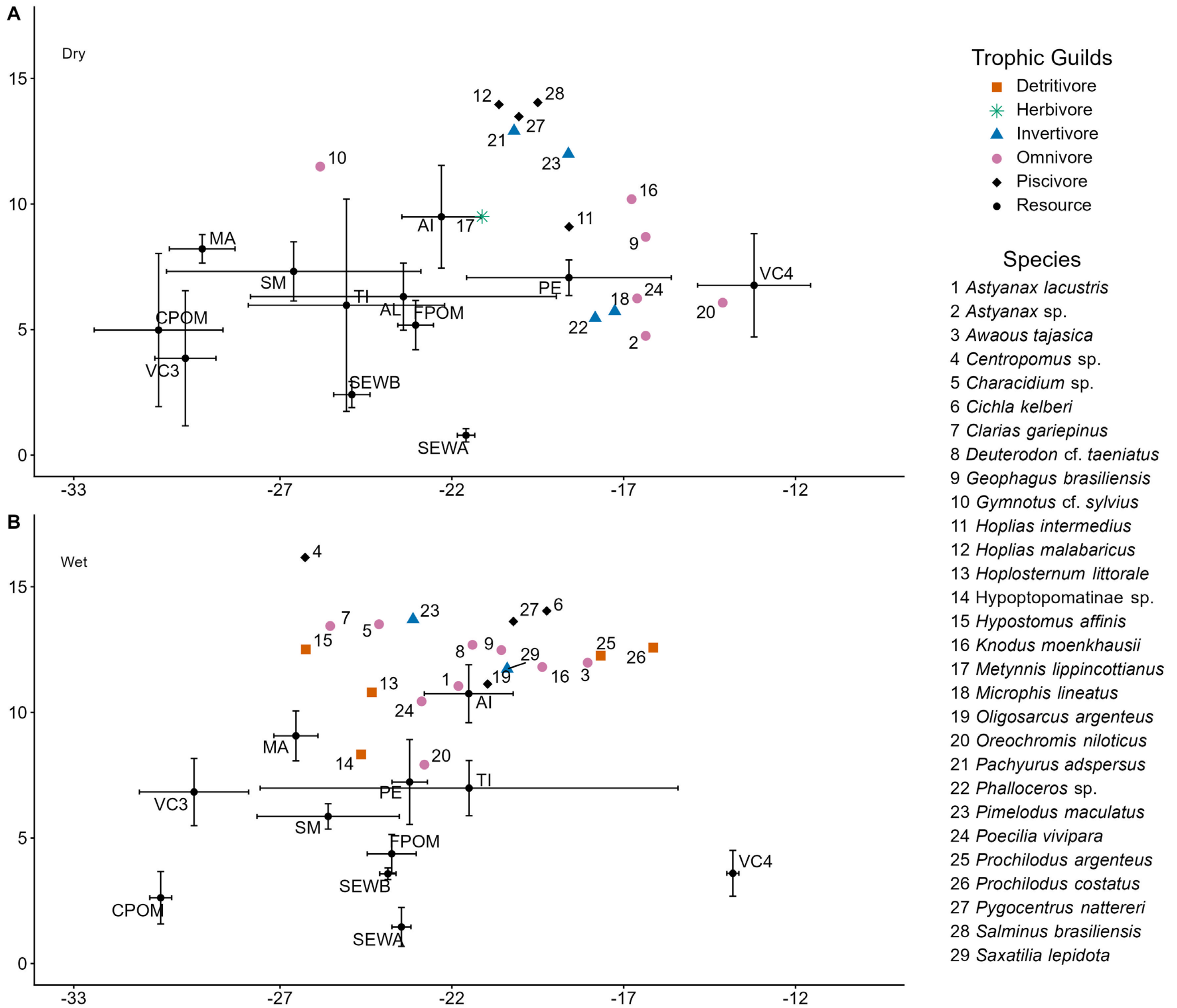

FIGURE 1 | Trophic structure of fish assemblage in Colatina in the dry (**A**) and wet (**B**) seasons. Graphics **A**, **B** show the mean values for each fish species and the mean and standard deviation for food resources. Food resources: Filamentous algae (AL), periphyton (PE), coarse particulate organic matter (CPOM), aquatic invertebrates (AI), terrestrial invertebrates (TI), suspended matter (SM), macrophyte (MA), fine organic particulate matter (FPOM), C3 vegetation (VC3), C4 vegetation (VC4), sewage before treatment (SEWB), sewage after treatment (SEWA).

the lowest values. *Geophagus brasiliensis* and *Poecilia vivipara* had lower values in the dry season compared to the wet season. The other three species (*Knodus moenkhausii*, *Pimelodus maculatus*, and *Pygocentrus nattereri*) presented similar values in both seasons (Fig. 3).

Sewage-derived carbon contributed to multiple trophic levels in both seasons. Assimilation was similar between seasons within each guild: invertivores (~22%), omnivores (~60%), and piscivores (~30%). Among guilds, the omnivores had the highest assimilation, accounting for more than 60% in both seasons (Fig. 4). Regarding sewage contributions to food resources, FPOM showed the highest values in both seasons, ranging from 44% in the dry season and 60% in the wet season (Fig. 5).

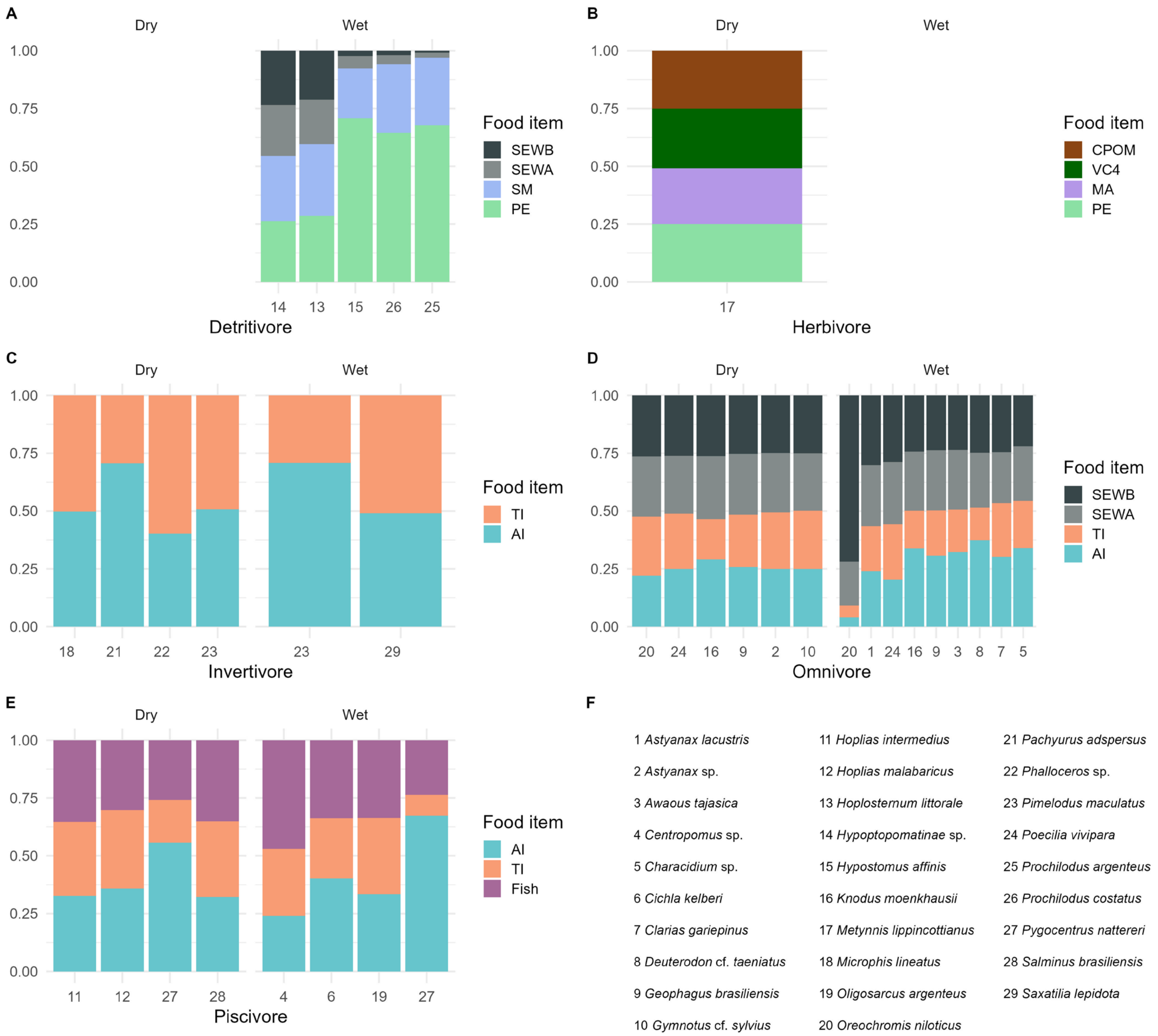

FIGURE 2 | Percentage of food resources sustaining each feeding guild during wet and dry seasons in Colatina. **A.** Detritivore, **B.** Herbivore, **C.** Invertivore, **D.** Omnivore, **E.** Piscivore. Food resources: Aquatic invertebrates (AI); Coarse particulate organic matter (CPOM); Fish; Macrophytes (MA); Periphyton (PE); Sewage before treatment (SEWB); Sewage after treatment (SEWA); Suspended matter (SM); Terrestrial invertebrates (TI) and C4 Vegetation (VC4).

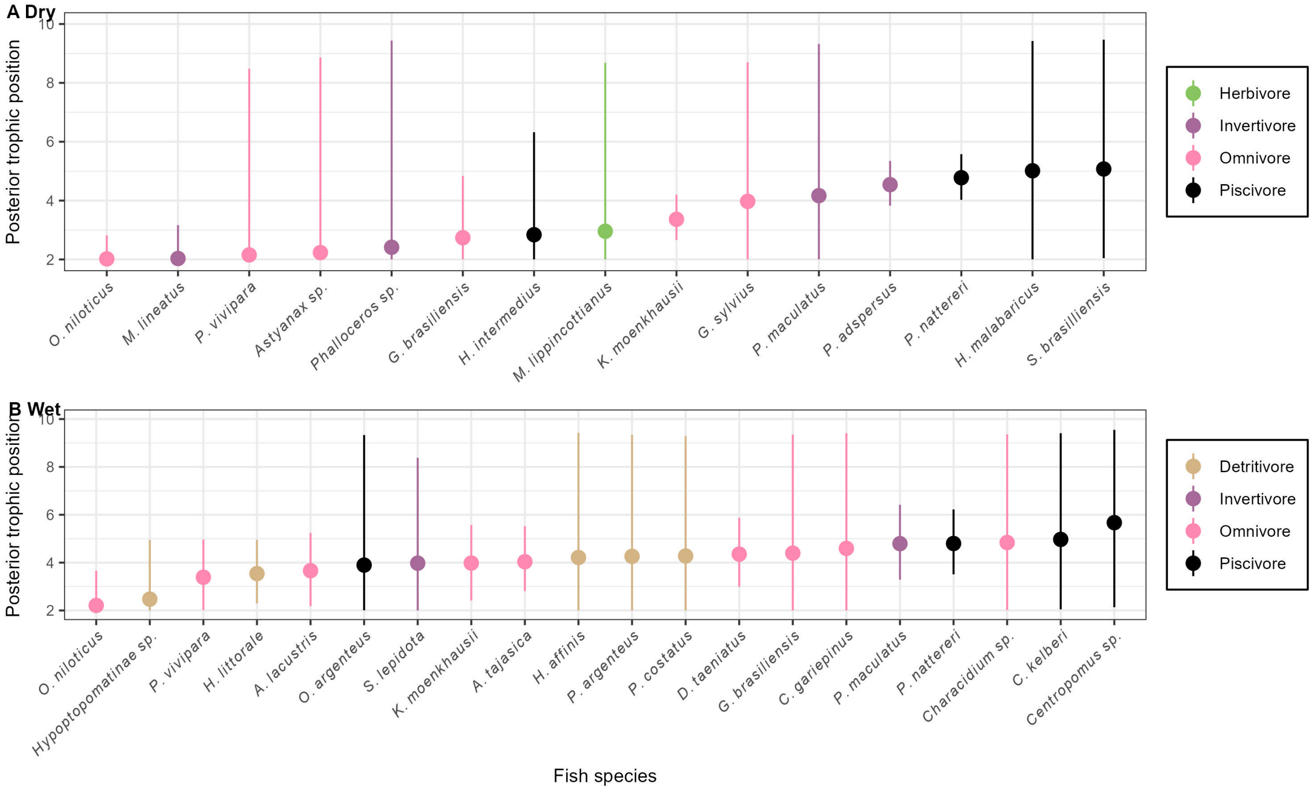

FIGURE 3 | Trophic position occupied by each species in the dry (**A**) and wet (**B**) seasons in Colatina, lower Doce River, Brazil.

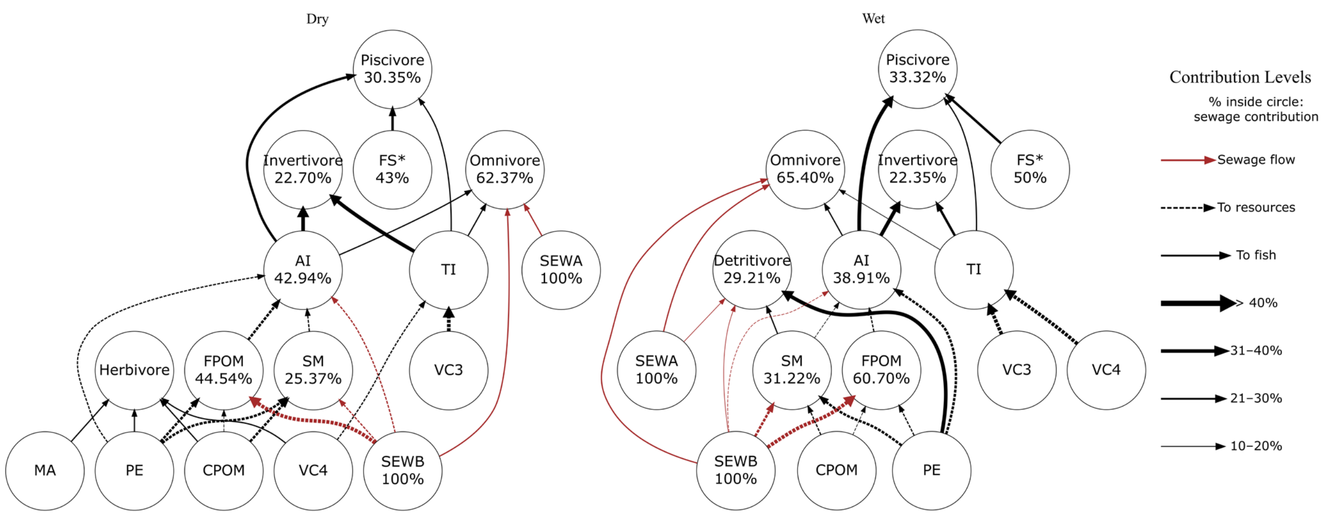

FIGURE 4 | Food resources that sustain the fish community in Colatina, lower Doce River, Brazil. Food resources: Aquatic invertebrates (AI); Coarse particulate organic matter (CPOM); Fine particulate organic matter (FPOM); FS* (specific fish species that are common preys to the piscivores); Macrophytes (MA); Periphyton (PE); ewage before treatment (SEWB); Sewage after treatment (SEWA); Suspended matter (SM); Terrestrial invertebrates (TI); C3 Vegetation (VC3), and C4 Vegetation (VC4). The percentages shown inside the circles indicate the proportion of sewage-derived material contributing to each component of the trophic web, where 100% corresponds to untreated and treated sewage samples.

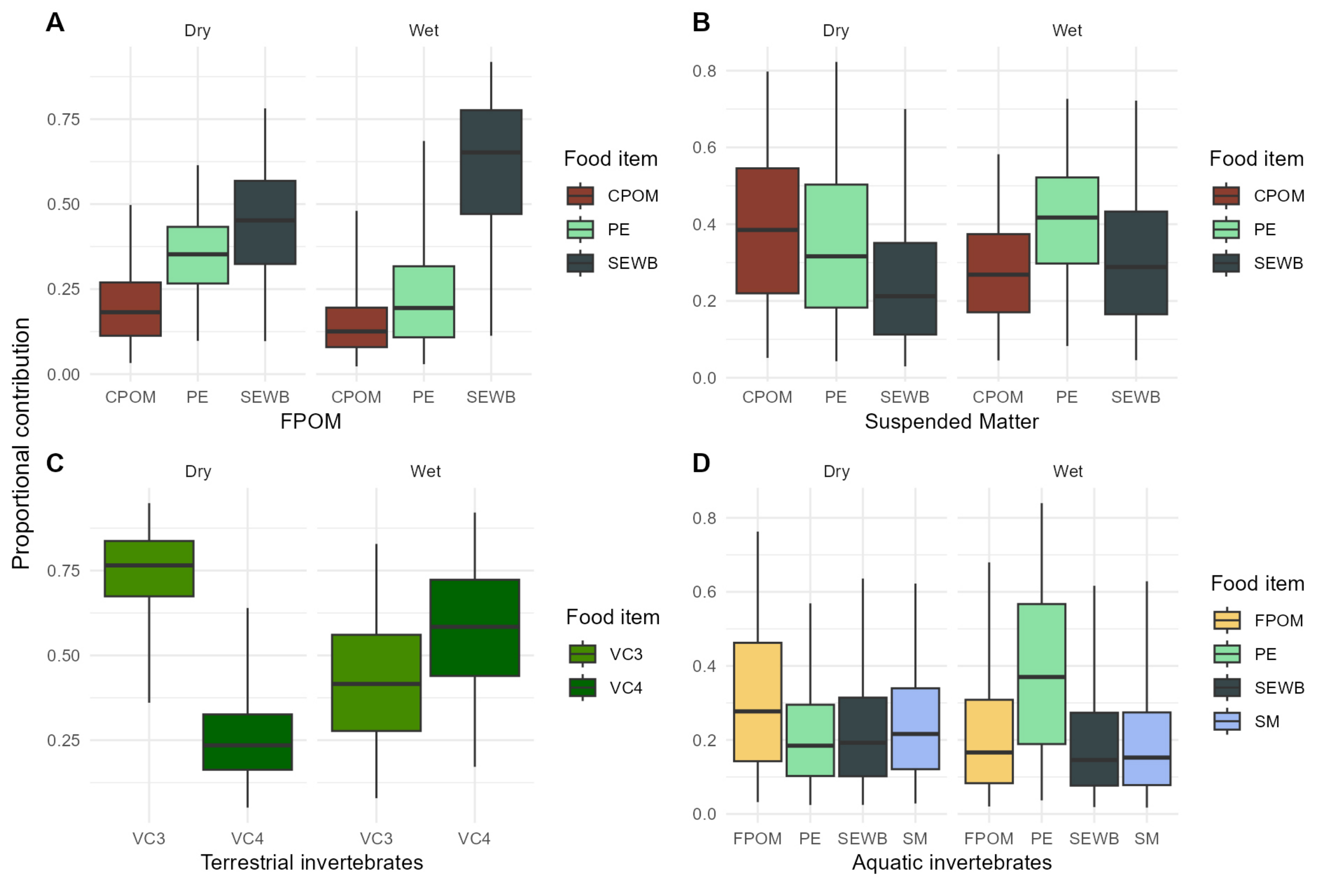

FIGURE 5 | Estimated sewage contribution for fine particulate organic matter (FPOM) (**A**), suspended matter (SM) (**B**), terrestrial invertebrates (TI) (**C**) and aquatic invertebrates (AI) (**D**). Food resources considered in the partitioning model: Coarse particulate organic matter (CPOM); Fine particulate organic matter (FPOM); Periphyton (PE); Sewage before treatment (SEWB); Suspended matter (SM); C3 Vegetation (VC3) and C4 Vegetation (VC4). Boxplots represent posterior distributions of source contributions from mixing models. Central line indicated the median, boxes represent the interquartile range (25–75%), and whiskers indicate the 95% credible interval (2.5–97.5%).

DISCUSSION

Our results indicate that sewage–derived resources were assimilated at similar levels across seasons, rejecting our first hypothesis. Species with higher assimilation of sewage-derived resources occupied the lowest trophic positions within the assemblage, supporting our second hypothesis. The third hypothesis was partially confirmed, as omnivorous species assimilated high proportions of sewage-derived resources, but detritivorous species were not sampled in the dry season.

Although sewage assimilation did not differ between seasons in our dataset, the influence of sewage inputs is often expected to be higher during the dry season, due to reduced rainfall, which makes sewage less diluted than in the wet season (Yunus, Nakagoshi, 2004; Kütter *et al*., 2023). The absence of detritivores in the dry season limits a full evaluation, as detritivores could increase sewage-derived carbon assimilation and potentially enhance its importance during the period.

In our study, untreated sewage samples showed depleted $\delta^{15}N$ values, averaging 3.5‰ in the wet season and 2.4‰ in the dry season, which were reflected in the fish isotopic values. Sewage was also reflected in the resources, since we found that sewage-derived carbon accounts for 25% to 60% of key basal resources such as suspended matter and FPOM. These low $\delta^{15}N$ values likely reflect strong sewage influence, given that over 80% of local sewage is discharged untreated (SNIS, 2023). Effluents receiving only primary treatment, or none, typically carry elevated ammonia concentrations, with depleted $\delta^{15}N$ values (Gaston, Suthers, 2004; Hicks *et al*., 2017), whereas secondary treatment tends to enrich $\delta^{15}N$ through nitrification and denitrification, which preferentially remove ^{14}N (Heaton, 1986; Loomer *et al*., 2015; Alldred *et al*., 2023). The depleted $\delta^{15}N$ values observed in Colatina are consistent with a neighboring basin reporting values around 1.5‰ (de Carvalho *et al*., 2019b, 2020a) and with Canadian studies documenting depleted $\delta^{15}N$ in fish downstream of poor-quality (Loomer *et al*., 2015). Furthermore, the isotopic compositions of fish in our study closely mirror those recorded in the same area two years earlier where $\delta^{15}N$ ranged from 5 to 15‰ (de Carvalho *et al*., 2024), indicating that sewage assimilation remains a persistent trend.

Several species assimilated a greater proportion of sewage and occupied the lowest trophic positions. Among them, the omnivorous *O. niloticus* deserves special attention, as it assimilated the highest proportion of sewage-derived carbon in both seasons. During the wet season, when sewage assimilation was more pronounced, this resource accounted for up to 90% of the species' diet. *Oreochromis niloticus* is non-native to the Doce River basin (Bueno *et al*., 2021), and its success may be partly attributed to its trophic plasticity (Abelha *et al*., 2001), which allows it to shift its primary food items according to environmental availability. Similar dietary shifts have been observed in other non-native species, such as *Poecilia reticulata*, which has been reported feeding directly on sewage in the Rio das Velhas River basin (de Carvalho *et al*., 2019b). These patterns highlight the ability of invasive species to establish themselves under adverse environmental conditions and also increase pressure on native fauna. Most omnivorous species assimilated at least 50% of sewage-derived carbon, confirming that this feeding guild is particularly adaptable to environmental changes (de Carvalho *et al*., 2019a,b; Neves *et al*., 2024).

Detritivorous species were only sampled during the wet season. Two species, Hypoptomatinae sp. and *Hoplosternum littorale*, showed higher assimilation of sewage-derived carbon, with values of 35% and 28%, respectively. For the remaining species, the contribution of sewage-derived carbon was below 10%, with periphyton and suspended matter being the main food sources. In Colatina, the Doce River flows through a broad channel. Therefore, the influence of riparian vegetation on shading is reduced, allowing greater light penetration and potentially stimulating periphyton growth (McCall *et al*., 2017). However, during the wet season, increased water flow contributes to higher levels of suspended organic material, an important food resource for fish; however, the resulting turbidity can negatively affect periphyton growth (Ren *et al*., 2021). It is important to note that the consumption does not necessarily reflect resource availability, which was not quantified in this study. Although detritivores can adjust their diets to environmental conditions (Prado *et al*., 2020), they are often specialized due to morphological constraints (Bowen, 1983) and may continue to consume periphyton even when it is less abundant.

Beyond freshwater species, sewage inputs also affect estuarine species. In our sampling, three estuarine species were recorded: *Awaous tajasica*, *Centropomus* sp., and *Microphis lineatus*. These species move into the lower Doce River, where they use the area for feeding, shelter, and nursery functions (Sarmento-Soares, Martins-Pinheiro, 2022). Some, such as *Centropomus* sp., also have economic importance (Sarmento-Soares, Martins-Pinheiro, 2022). The invertivore *Microphis lineatus* assimilated sewage-derived carbon, representing 11% of its diet during the dry season. Considering that sewage also introduces additional pollutants (Strauch, Erzinger, 2021; Roveri *et al*., 2025), the assimilation of sewage-derived carbon by estuarine species may still lead to broader impacts on estuarine species and the marine environment.

Stable isotope analysis revealed that sewage inputs into Colatina have been incorporated into the local food web during both seasons. This is consistent with fish being effective indicators of nitrogen sources in freshwater (de Carvalho *et al*., 2020b) and estuarine systems (Schlacher *et al*., 2005), as their distribution across trophic levels enables the tracking of isotopic signals across the food web (Schlacher *et al*., 2005).

Domestic sewage also introduces multiple contaminants into the ecosystem, including pathogenic microorganisms, detergents, heavy metals, and pharmaceutical compounds (Strauch, Erzinger, 2021; Gomes *et al*., 2022; Roveri *et al*., 2025). Their presence alongside sewage raises concern about their movement through the food web and potential impacts on both ecosystem integrity and public health. In addition, industrial effluents may also contain high metal concentrations (Strauch, Erzinger, 2021), and, together with the 2015 Fundão dam collapse, have intensified metal pollution in the region (Queiroz *et al*., 2018), and its impacts have already been documented (Ferreira *et al*., 2020; Vergilio *et al*., 2021; Yamamoto *et al*., 2023a).

Although our findings are restricted to Colatina, the Doce River flows through other major urban centers, such as Ipatinga, Governador Valadares, and Linhares. As these cities are also influenced by sewage discharge (SINISA, 2025), future research should evaluate pollution gradients along the river and test whether the patterns observed here are consistent across the basin. At the local scale, public policies in Colatina should prioritize increasing the proportion of treated sewage and expanding treatment infrastructure capacity to reduce pollutant inputs and mitigate their ecological impacts.

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AUTHORS' CONTRIBUTION

Patrícia Santos Fráguas: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing-original draft, Writing-review and editing.
Débora Reis de Carvalho: Conceptualization, Formal analysis, Investigation, Methodology, Writing-review and editing.
Frederico Fernandes Ferreira: Investigation, Writing-review and editing.
Carlos Frankl Sperber: Funding acquisition, Project administration, Resources, Writing-review and editing.
Paulo Santos Pompeu: Conceptualization, Formal analysis, Methodology, Project administration, Resources, Supervision, Writing-review and editing.

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ETHICAL STATEMENT

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

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author, Patrícia Fráguas, upon reasonable request.

AI STATEMENT

ChatGPT (OpenAI, free version) was used solely for English language editing to improve clarity and grammar. No scientific content, analyses, interpretations, or conclusions were generated by AI.

COMPETING INTERESTS

The authors declare no competing interests.

SUPPLEMENTARY MATERIAL

Supplementary Material File

PEER REVIEW FILE

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