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Diet composition of a native fish species in a neotropical lentic environment

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ABSTRACT. Native species are important to the dynamics of aquatic environments. Studies that seek to understand the basic biology of these species provide information on the species and the dynamics of the natural environment. We characterized the diet, feeding habits, and trophic niche breadth of the native species *Hoplosternum littorale* (Hancock, 1828) in neotropical lentic environments. Collections were carried out in July 2018 at two sampling points (lagoons) located in the São Francisco Falso River and the Corvo River using gill nets. Stomach content was analyzed, and food items were separated, identified, and quantified using the volumetric method. PERMANOVA analysis was performed to evaluate possible differences in diet between the lagoons. Furthermore, PERMDISP was used to test the trophic niche breadth. Fifteen food items were recorded in the São Francisco lagoon, with the most consumed items being detritus, Diptera (larvae and pupa), and Odonata (nymph). Seven food items were recorded in the Corvo lagoon, with detritus being the most consumed. There was no difference in diet composition and trophic niche breadth between the evaluated lagoons. For both lagoons studied, the species was determined to be a detritivore feeding habit, given its diet's predominant consumption of detritus. The detritivore classification may be related to environmental conditions, food availability, and functional morphology. We also observed the presence of microplastics in the stomachs of some specimens, demonstrating anthropic influence on aquatic environments. Our study contributes to bettering knowledge on this species, and consequently the conservation of the species. It can also serve as a basis for conducting future scientific studies, along with developing the area of study related to Brazilian fish ecology.

Keywords: Actinopterygii; food; freshwater fish; microplastic; wild fish; Upper Paraná River basin.

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Introduction

The freshwater fish fauna of South America is one of the richest and most diverse on the planet (Albert, Tagliacollo, & Dagosta, 2020; Reis et al., 2016; Rezende & Mazzoni, 2003), and Brazil contributes to this diversity with approximately 3,513 species (Froese & Pauly, 2023). This high diversity occurs due to large hydrographic systems in the country, such as the Amazon and Paraná-Paraguay basins, which have morphologically distinct habitats and make up different biomes (Albert et al., 2020; Rosa & Lima, 2005). Among such hydrographic systems, the Upper Paraná River basin has 211 fish species in its floodplain (Ota, Deprá, Graça, & Pavanelli, 2018). Species richness of about 80% has been recorded for Siluriformes and Characiformes, comprising the dominant groups in most lotic environments in the Upper Paraná Basin (Frota, Ota, Deprá, Ganassin, & Graça, 2020; Langeani et al., 2007).

Seven thousand two hundred and seventy-eight freshwater and marine species distributed among 40 families have been registered as Siluriformes (Fricke, Eschmeyer, & Van der Laan, 2023). Among these, the Callichthyidae family is naturally distributed in tropical and subtropical fresh and brackish waters (Nico, Walsh, & Robins, 1996). Callichthyidae has at least 22 genera and 223 valid species (Fricke et al., 2023) and inhabit benthic regions of water bodies (Nico et al., 1996). The number of species and their great diversity in tropical and subtropical watersheds of this Neotropical Region have also shown a constant and significant increase in studies on Brazilian ichthyofauna (Buckup, Menezes, & Ghazzi, 2007).

Hoplosternum littorale (Hancock, 1828), Callichthyidae, is a native species that is widely distributed over South America and has been recorded in the Upper Paraná, Ribeira de Iguape, Paraíba do Sul, and coastal basins (Ota et al., 2018; Oyakawa & Menezes, 2011). This species inhabits shallow, swampy, and lentic environments (Caldeira, Silva, Sá, & Silva, 2007). Morphologically, the species has a small, ventral, and terminal mouth that projects forward, and a pair of barbels on each side of the mouth (Santos, Jégu, & Merona, 1984). These characteristics influence their feeding habits, limiting the size of the food ingested, enabling them to live in benthic environments (Caldeira et al., 2007). In other studies, *H. littorale* has shown to be omnivorous (Caldeira et al., 2007; López-Rodríguez et al., 2019), invertivores (Santos, Ferreira, & Amadio, 2008) and planktivorous (Echevarría, Rodríguez, & Machado-Allison, 2018; López- Rodríguez et al., 2019). However, most of the work carried out with the species has been in rivers (Caldeira et al., 2007; López-Rodríguez et al., 2019; Oliveira, Corrêa, & Smith, 2020), with little information on its biological aspects (diet and feeding habits) in lentic environments, e.g., lagoons. Lentic environments are characterized as having water that is almost still or with variable flows (lakes, lagoons, dams, reservoirs), low current strength, and with mainly autochthonous energy (Oliveira & Goulart, 2000).

Considering the wide distribution of *H. littorale*, its feeding habits, its opportunistic character, and since the feeding behavior of fish can change according to its environment, studies that evaluate diets are relevant for understanding the relationships between species and habitats (Esteves, Aranha, & Albrecht, 2021). Furthermore, knowledge on the biological aspects of native species is fundamental for understanding the trophic dynamics in their natural aquatic environments (Santos, Oliveira, & Moralles, 2009). Thus, these studies, in addition to providing information on diets, allow us to understand the behavior of fish in the face of environmental variations in food availability (Esteves et al., 2021; Gerking, 1994) offering necessary information for preserving natural environments (Adam, Burkepile, & Ruttenberg, 2015; Barros, Almeida, Figueiredo, Nunes, & Neta, 2021). We, therefore, sought to characterize the diet composition, feeding habits, and trophic niche breadth of *H. littorale* in Neotropical lentic environments.

Material and methods

Study area

Collections were made at two marginal lagoons on the São Francisco Falso River (SFFR): São Francisco lagoon (SFL) (24°53'35.80" S - 54°13'30.79" W), with an entrance to the SFFR; and the Corvo lagoon (COL) (24°53'17.11" S - 54°13'02.30" W), with an entrance to the Corvo River and lateral connections with the SFFR (Figure 1).

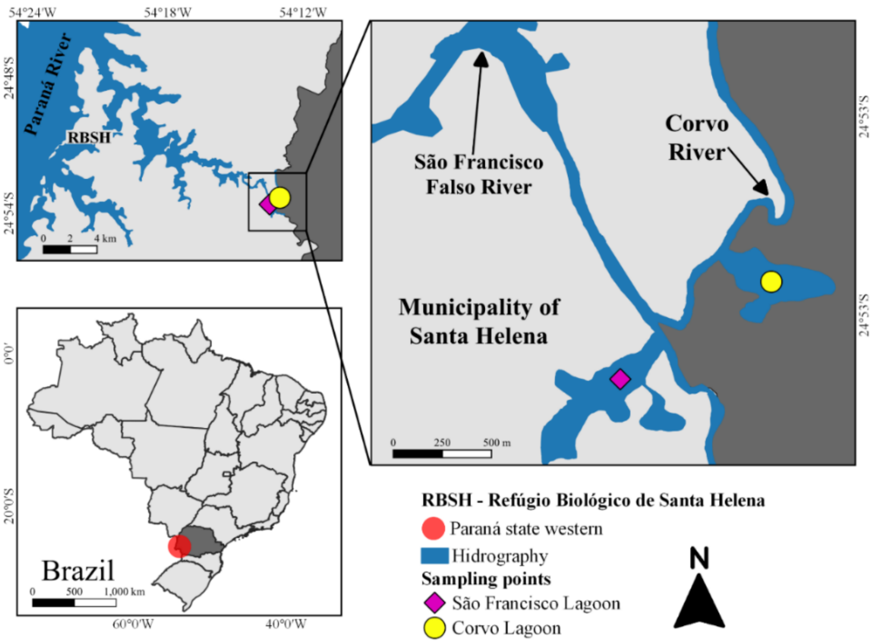

Figure 1. Study area with location of the São Francisco lagoon of the São Francisco Falso River and Corvo lagoon of the Corvo River, of Paraná III River Basin, Brazil.

Details on both lagoons are given in Table 1. The São Francisco Falso River is a tributary of the left bank of the Paraná River, formed by two arms, with its mouth located in the Paraná River where the *Refúgio Biológico de Santa Helena* (RBSH) - Area of Relevant Ecological Interest (ARIE), a Protected Area for Sustainable Use) is located (Kliver, 2010). The SFFR does not receive discharges from urban-industrial waste into its waterways, but is marked by the presence of livestock and agriculture activities (Silva, Miola, Silva, & Sousa, 2010). The Corvo River, also known as the São Francisco Braço Norte River, is one of the 12 sub-basins of the Paraná Basin 3. Its source is located near the city of Cascavel/PR. Subsequently, it meets the São Francisco Braço Sul River, flowing into the Itaipu Reservoir, Paraná State, Brazil, on the Paraná River (Rocha & Bade, 2018).

Table 1. Physiographic characteristics of the São Francisco lagoon of the São Francisco Falso River and Corvo lagoon of the Corvo River, Paraná III River Basin, Brazil.

Physiographic characteristics	Corvo Lagoon	São Francisco Lagoon
Type of aquatic vegetation	Presence of floating and submerged macrophytes in small quantities in the coastal region	Presence of floating and submerged macrophytes in small quantities in the coastal region
Land use	Presence of grass and area of agricultural occupation	Presence of grass and area of agricultural occupation
Riparian Zone	Ciliary Forest	Ciliary Forest
Environmental gradient	Lentic	Lentic
Water transparency	1.1 meters	1.1 meters

Collecting biological material

Collections of fish specimens were carried out using gill nets (3 - 7 cm between non-adjacent nodes) in July 2018 (Authorization SISBIO nº 57181-1, and Register SisGen A186700). The fish were euthanized via an overdose of Eugenol at the following concentration: 3000 mg L^{-1} (Lucena, Calegari, Pereira, & Dallegrave, 2013; Authorization CEUA-UTFPR 2016/031). In the laboratory, the standard length of the specimens was measured using an ichthyometer (0.1 cm) along with their total mass (g) using an analytical balance (0.01 g). Subsequently, the specimens were dissected, and the stomachs were removed and fixed in a 4% formaldehyde solution and preserved in 70% alcohol.

Laboratory analysis

Stomach content was examined under a stereomicroscope or an optic microscope when necessary. Food items were separated and identified to the lowest taxonomic level, using identification keys (Bicudo & Bicudo, 1970 for algae and Mugnai, Nessimian, & Baptista, 2010 for invertebrates). Subsequently, the food items were quantified according to the volumetric method, measuring the displacement caused by each food item in a graduated measuring utensil (Hyslop, 1980). In this case, a gridded Petri dish was used for smaller food items and a graduated beaker for larger food items, as proposed by Hellawell and Abel (1971). We visually identified microplastic material using an optical microscope based on the following criteria: i) No cellular or organic structure visible in the microplastics; and ii) Equally thick fibers along their entire length (Norén, 2007).

Data analysis

All analyses were performed in the R programming environment (RStudio Team, 2022) and the vegan package (Oksanen et al., 2022), considering a level of statistical significance at $p < 0.05$.

To verify the sample sufficiency (SFL - 12 specimens; COL - 19 specimens), cumulative diversity curves were drawn (x-axis: specimens; y-axis: cumulative number of foods) (Figure S1) using the Specaccum function (Oksanen et al., 2022).

To characterize the diets, data on the food composition of the species was expressed as a percentage of the volume of each food item relative to the total volume of all the grouped items. Furthermore, the food items were grouped according to their trophic origin, as allochthonous (originating from terrestrial environments), autochthonous (originating from aquatic environments; Silva, Gubiani, Neves, & Delariva, 2017) or indeterminate, for each lagoon.

To determine the feeding habits, a matrix of food items (in ml) was used, with classifications defined by the predominance of a certain type of food resource in the diet of the population in each lagoon (> 51% of the total volume) (Corrêa, Albrecht, & Hahn, 2011): detritivores, ⩾ 51% detritus in stomachs; herbivore, ⩾ 51% plant items in stomachs; carnivore, ⩾ 51% animal items in stomachs.

To compare the diet of *H. littorale* between SFL and COL, one-way Permutational Multivariate Analysis of Variance (PERMANOVA) was used with the aid of the adonis function (Oksanen et al., 2022). This non-parametric test evaluated significant differences between groups based on the measured distance, in this case the Bray-Curtis distance, with 999 random permutations (Anderson, 2001).

To evaluate the trophic niche breadth, multivariate permutational analysis (PERMDISP) (Anderson, 2006) was used with the aid of the Bray-Curtis dissimilarity measure, using the betadisper function (Oksanen et al., 2022). The trophic niche breadth was measured via the spatial dispersion of the individual diet. The assumption was that differences in distance between lagoons indicate that the species' diet in one lagoon is more restricted or broader than in the other (Silva et al., 2017). In this case, the centroid distance (D) corresponds to the trophic niche breadth. To test the null hypothesis, which was that the individual intraspecific dispersion and trophic niche breadth did not differ between groups, an *F* statistic was calculated to compare the mean distance of each sample to the group's centroid. Subsequently, the p-value was obtained via 999 permutations of the least square residuals (Anderson, 2006) using the permutest.betadisper function (Oksanen et al., 2022). *Post-hoc* pairwise comparisons were made using Tukey's significant difference method with the aid of the TukeyHSD.betadisper function (Oksanen et al., 2022).

Results

The food spectrum in the SFL comprised 15 food items, while the food spectrum in the COL comprised seven food items. The most consumed food items in the SFL were detritus (66.5%), Diptera (larvae and pupa) (17.9%), and Odonata (nymph) (8.8%) (Table 2). For COL, detritus almost entirely composed the diet of *H. littorale*, and was the most consumed item (99.7%) (Table 2). The species at both sites were characterized by detritivore feeding habits (Detritus consumption > 51% of total volume).

Table 2. Food composition in percentage for *Hoplosternum littorale* in the São Francisco lagoon (SFL) of the São Francisco Falso River and Corvo lagoon (COL) of the Corvo River, Paraná III River Basin, Brazil. * percentage < 0.01. Sl (Standard length); Wt (Weight), ME (Mean); SD (Standard deviation), and min–max (Minimum and maximum values).

Area	SFL	COL
N	12	19
Sl (cm) – ME ± SD (min–max)	15.36 ± 1.08 (14–17)	15.20 ± 2.03 (12–21.8)
Wt (g) – ME ± SD (min-max)	153.86 ± 28.13 (114.5–222.75)	145.12 ± 25.35 (100.31–192.77)
	Food item (%)	
AUTOCHTHONOUS	28	0.14
Filamentous algae	0.01	*
Microcrustacean	0.15	0.07
Mollusca	*	
Cladocera	*	*
Hydracarina	*	*
Aquatic insect	1.83	
Diptera (larvae e pupa)	17.96	0.07
Trichoptera (larvae)	0.01	
Hymenoptera (larvae)	*	
Odonata (nymph)	8.84	
Nematoda	0.01	
ALLOCHTHONOUS	5	0.07
Diptera (adult)	0.02	
Terrestrial plant	4.56	0.07
UNDETERMINED	67	99.79
Detritus	66.52	99.79
Animal fragment	0.08	
Microplastic	*	*

No differences were observed in the diet between the lagoons (PERMANOVA, D = 1; F = 1.63; p = 0.121). The trophic niche breadth and the individual intraspecific dispersion also did not differ between the evaluated lagoons (PERMDISP, D = 1; F = 3.38; p = 0.08).

The presence of microplastics in the stomachs of some specimens (SFL – 5 specimens; COL – 3 specimens) was also recorded (Table 2; Figure 2). In total, 16 microplastics were identified, presenting characteristics with lengths between one and three millimeters, with rigid and fibrous structures, with no visible cellular structure, and with a blue coloration.

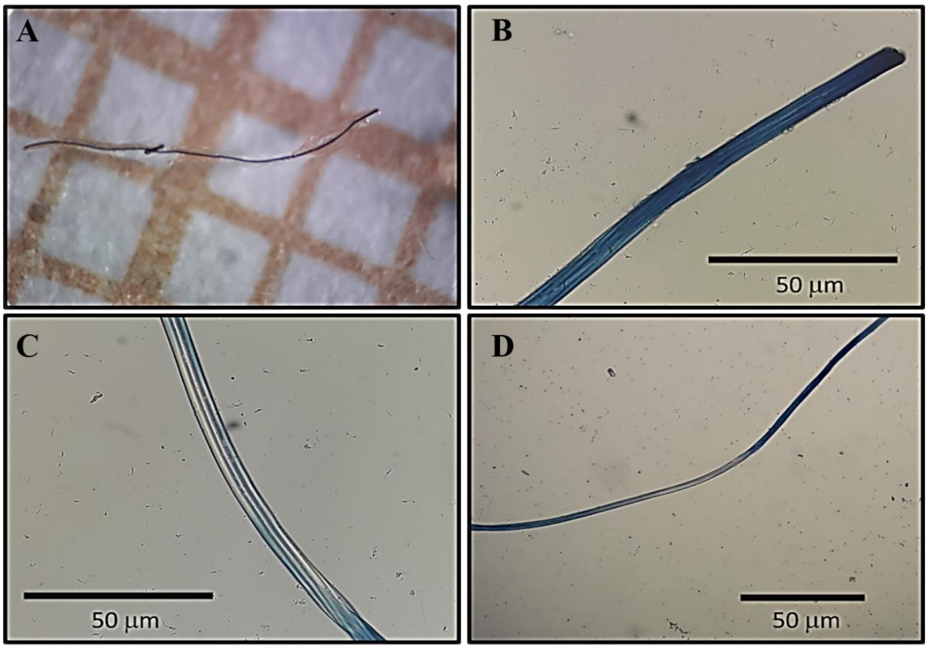

Figure 2. Microplastics in the stomach of *Hoplosternum littorale* collected in the São Francisco lagoon of the São Francisco Falso River and Corvo lagoon of the Corvo River, Paraná III River Basin, Brazil. (A) Fragment in stereomicroscope on graph paper. (B-C) Photomicrograph using a stereoscopic microscope at 400x magnification. (D) Photomicrograph using a stereoscopic microscope with a 200x magnification.

Discussion

The diet of the native species *H. littorrale* in a lentic environment reflected and corroborated the influences of the environment on the fish diet. Studies in a lotic environment have demonstrated iliophagous (Oliveira et al., 2020) and omnivore-planktivore feeding habits for the species (Caldeira et al., 2007; López-Rodríguez et al., 2019). However, here, the species demonstrated detritivore feeding habits with the consumption of detritus and aquatic insects in both lagoons, confirming this species' opportunism and trophic plasticity. In the case of a native species, this opportunism and trophic plasticity are important for the maintenance of the species itself in its natural environment, as well as the preservation of the ecosystem (Balassa, Fugi, Hahn, & Galina, 2004).

The composition and abundance of available resources are directly related to the conditions and type of environment and can interfere with the feeding spectrum of fish (Santos, Medeiros, Rocha, Dias, & Severi, 2014). We observed no differences in the diet of *H. littorale* between the lagoons nor in the detritivore feeding habits in both lagoons. The same characteristics of both lagoons corroborate this result, indicating that the food availability in the areas was similar. Thus, we suggest that the lagoons' overall characteristics favored the food habits of *H. littorale*. In a lentic environment flows are slower, favoring organic matter sedimentation (Fantin-Cruz, Oliveira-Loverde, & Girard, 2008). Although we did not measure the flow velocity in the evaluated lagoons, the consumption of detritus and the transparency of the water may corroborate higher sedimentation levels. The water transparency observed here (Table 1) in both lagoons, associated with slow waters, flow indicates that suspended particulate matter is quickly deposited, as per Fantin-Cruz et al. (2008).

Feeding habits can be related to the morphology of the mouth, limiting food items that can be consumed (Esteves et al., 2021). *Hoplosternum littorale* has a sub-inferior mouth and a body covered by bone plates that are ventrally flattened (Hahn, Almeida, & Luz, 1997). These characteristics functionally aided in obtaining benthic resources, allowing it to consume organic compounds in the sediment (Thomaz, Lansac Tôha, Roberto, Esteves, & Lima, 1992). These organic compounds come from accumulating autochthonous and allochthonous organic matter (Azevedo, Mizukawa, Teixeira, & Pagioro, 2008). Thus, lagoons are lentic environments, so the organic matter is deposited easily and consumed by fish (Oliveira & Goulart, 2000).

Another food resource in the diet of *H. littorale*, mainly for the SFL specimens, was aquatic insects. This was also shown to be a staple food item for the species in other studies (Winemiller, 1987; Hahn et al., 1997; Caldeira et al., 2007) but was little consumed by the species in this study. In this case, the most consumed items were Diptera larvae and pupa, insects that, during their development phase, are usually found in the sediment (Arimoro, Auta, Odume, Keke, & Mohammed, 2018). Possibly, the consumption of these aquatic insects may be associated with detritus consumption since both are available resources in the lagoon sediment.

The consumption of detritus and aquatic insects is evidenced by observing the representativeness of the origin of the food. Food items of autochthonous origin were most consumed by *H. littorale* in both lagoons. However, allochthonous resources were less present in the diet, probably due to less environmental availability. In tropical regions, subject to wide seasonal variations in water levels, fish diets are strongly influenced by changes in the hydrological regime (Pinto, Rocha, Santos, Medeiros, & Severi, 2011). In the flood season, allochthonous resource contributions are at their greatest (Pinto et al., 2011). Thus, the lower levels of these resources can be explained by the fact that they are not as abundant, especially since collections were carried out in a period with less rainfall.

We should also highlight the presence of microplastics in the stomachs of some of the analyzed fish. This can occur due to the increasing level of plastic products discarded into aquatic environments (Roda, Lauer, Risso, & Martinez, 2020). Consequently, fish ingest these microplastics, given their small size and attractive characteristics, e.g., coloring and buoyancy, and can be mistaken for food and are thus easily consumed (Jovanović, 2017; Pinheiro, Oliveira, & Vieira, 2017). Furthermore, microplastics efficiently adsorb organic pollutants in the water (Jovanović, 2017). Thus, toxic substances adhered to microplastics that are ingested by fish can cause direct damage to the health of these animals (Oliveira, Ribeiro, Hylland, & Guilhermino, 2013; Jovanović, 2017).

We emphasize that the presence of microplastics in the stomachs of native fish is worrying. In addition to causing possible damage to the animal, microplastic consumption can also cause damage along the trophic chain since these substances can accumulate in the trophic chain (Batel, Linti, Scherer, Erdinger, & Braunbeck, 2016; Jovanović, 2017). Furthermore, the lagoons studied here are located on the Corvo and São Francisco Falso Rivers, the mouths of which are located within a protected area. This shows that the excessive use of plastics by humans and their improper disposal can affect places close to protected areas or even the ichthyofauna of protected areas, as has already been observed (Ramos et al., 2022). Our observations demonstrate the anthropic impacts on native species and natural aquatic ecosystems.

Conclusion

Our study adds new information on the trophic ecology of the native species *H. littorale* and is the first report on this species for the studied lagoons. The species proved to be detritivores in both lagoons. We reiterate our concern regarding the anthropic impacts demonstrated by the presence of microplastics in lagoon substrates, making it possible to use our study for qualitative assessments of pollution in aquatic ecosystems and future actions for species conservation.

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