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Characterization of wild fish diet and trophic guild in a protected area

Caracterização da dieta e guilda trófica de peixes silvestres em área protegida

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Abstract: Aim: We characterize the diet and trophic guild to the fish community in a protected area to contribute information about the trophic ecology. **Methods:** The collection was carried out at three sampling points, in 2017 and 2018, with the help of gill nets. The collected specimens were euthanized in a 0.5% benzocaine solution. In the laboratory, all specimens' stomachs were removed, fixed in a 4% formalin solution, and preserved in 70% alcohol. Stomach contents were examined, and the food items were identified to the lowest possible taxonomic level. Food items were quantified according to the volumetric method. The characterization of the diet was presented through the percentage volume of each food item consumed. To determination of the trophic guild of each species, the predominance of a type of food resource (> 51% of the total volume) in the population's diet was considered. **Results:** The species' diets were distributed in 16 food items (predominance of decapods, fish fragments, terrestrial insects, and aquatic plants), and four trophic guilds were observed (carcinophage, piscivore, terrestrial insectivore, and herbivore). Furthermore, there was the occurrence of microplastic in the diet of three species. **Conclusions:** the fish community evaluated here demonstrates the wide range of resources that can make up the fish diet and demonstrates the different trophic guilds that can be observed in a protected area. As it is a conservation unit, knowing the diet and trophic guilds that make up the fish community can contribute to understanding the dynamics of food chains, the structure of the community, and the functioning of the ecosystem. Thus, this work can contribute information about the biology of the species evaluated for future work and conservation programs.

Keywords: diet, freshwater fish, Itaipu Reservoir, Neotropical fish, Santa Helena Biological Refuge.

Resumo: Objetivo: Caracterizamos a dieta e a guilda trófica da comunidade de peixes em uma unidade de conservação para contribuir com informações sobre a ecologia trófica. **Métodos:** As coletas foram realizadas em três pontos de amostragem, em 2017 e 2018, com o auxílio de redes de espera. Os espécimes foram eutanasiados em solução de benzocaína a 0,5%. Em laboratório, os estômagos foram retirados, fixados em formol 4% e preservados em álcool 70%. O conteúdo estomacal foi examinado e os itens alimentares foram identificados até o menor nível taxonômico possível e quantificados de acordo com o método volumétrico. A caracterização da dieta foi apresentada por meio do volume percentual de cada alimento consumido. Para a determinação da guilda trófica de cada espécie, foi considerada a predominância de um determinado tipo de recurso alimentar (> 51% do volume total) na dieta da população. **Resultados:** A dieta das espécies foi distribuída em 16 itens alimentares (predominância de decapoda, fragmento de peixe, inseto terrestre e vegetal aquático), e quatro guildas tróficas foram observadas (carcinófago, piscívoro, insetívoro terrestre e herbívoro). Além disso, houve a ocorrência de microplástico na dieta de três espécies. **Conclusões:** A comunidade de peixes aqui avaliada demonstra uma ampla gama de recursos que podem compor a dieta dos peixes e demonstra as diferentes guildas tróficas que podem ser observadas em uma área protegida. Por se tratar de uma unidade de conservação, conhecer a dieta e as guildas tróficas que compõem a comunidade de peixes pode contribuir para o entendimento da dinâmica das cadeias alimentares, da estrutura da comunidade e do funcionamento do ecossistema. Assim, este trabalho pode contribuir com informações sobre a biologia das espécies avaliadas para futuros trabalhos e programas de conservação.

Palavras-chave: dieta, peixes de água doce, Reservatório de Itaipu, Peixes Neotropicais, Refúgio Biológico de Santa Helena.

1. Introduction

Freshwater ecosystems are considered the most threatened habitats in the world (Reid et al., 2019). In this case, conservation units are essential areas for protecting natural resources, guaranteeing the maintenance of the landscape and local biodiversity (Hassler, 2005; Henry-Silva, 2005). The development of research on these protected areas is necessary for the protection, conservation, and management of these units (Luz & Elias, 2014). Knowing which species inhabit the conservation units and their ecology makes it possible to understand the influences that conservation units have on the community (Ferreira et al., 2020; Henry-Silva, 2005).

In aquatic protected areas, the ecological study of fish is a good tool for biomonitoring, contributing to the management and conservation of these areas (Carvalho et al., 2020; Chase & Leibold, 2004). The fish are fundamental organisms in food chains, whether as consumers or food sources, maintaining the balance among the various species that make up these webs and acting as an important parameter in the trophic ecosystem characterization (Behn & Baxter, 2019; Dias et al., 2005). With the study of the fish diet, it is possible to understand various aspects of the life of these animals, such as growth, reproduction, and adaptation, knowledge of the trophic organization of the ecosystem, effects of space, time variation, and biotic and abiotic factors (Esteves et al., 2021; Esteves & Aranha, 1999). In addition to understanding food resource sharing strategies, and how all these purposes reflect on the animals' diet and the place where they live (Esteves et al., 2021).

Fish feeding may reflect the relationship between the aquatic environment and the surrounding areas (Carvalho et al., 2020), allowing evaluation to directly of the influence of river surroundings on aquatic environments (Gerking, 1994; Silva et al., 2017). The forests around the rivers are transition zones between terrestrial and aquatic ecosystems, acting in processes that involve the transfer of energy and matter between these environments (Pusey & Arthington, 2003). Also, act as an important food source for fish - trunks, branches, leaves, fruits, seeds, and invertebrates (Gonçalves et al., 2018).

Assessing fish feeding, it is also possible to organize species into trophic guilds according to the exploited food resources (Gerking, 1994). Species that belong to the same trophic guild play trophic similarity in a community (Root, 1967). Thus, knowing the trophic guilds that make up the community can elucidate the dynamics of food chains and the structure of the community (Liu et al., 2019). Therefore, when it comes to protected areas, understanding the diet and trophic guilds of the fish community is of paramount importance. This information can contribute as a monitoring tool for these sites, ensuring the protection of native species and the resources necessary for their survival and development (Hassler, 2005; Luz & Elias, 2014). That said, we characterized the diet and trophic guild to the fish community in a conservation unit, seeking to answer the composition of the diet and trophic guild of wild fish in a conservation unit. Such a response may provide information about the trophic ecology of a poorly studied protected area.

2. Material and Methods

2.1. Study area

The Paraná River constitutes the second-largest hydrographic basin in South America (Hales & Petry, 2019). From its origin, at the confluence of the Paranaíba and Grande rivers, to the estuary of the Rio da Prata, it travels approximately 4.700 km, covering 2.8 million km^2 that drain about 10% of the Brazilian territory (Agostinho et al., 2007; Agostinho & Gomes, 2005; Hales & Petry, 2019). The upper Paraná Basin is the most intensively dammed among the basins of South America, drains an area with large urban, industrial, and agricultural centers, and constitutes the most explored region in the country (Agostinho & Gomes, 2005).

The study area comprises the Refúgio Biológico de Santa Helena (RBSH) in the Paraná Hydrographic Basin III (Figure 1). The RBSH is a private environmental protection area belonging to the company Itaipu Binacional, part of the Permanent Preservation Area of Lake Itaipu. The RBSH has an area of 1.482 ha, classified as an Area of Relevant Ecological Interest, a Conservation Unit for Sustainable Use. These areas aim to conserve natural ecosystems of regional/local importance and regulate the use of these areas, allowing for research activities, environmental monitoring, inspection, and restricted visitation. The area is considered a priority for conservation by the Ministry of the Environment, as it is an integral part of the Iguaçu-Paraná Biodiversity Corridor (Santa Helena, 2010).

The sampling areas comprise three points on the Paraná River, inserted in the conservation area (RBSH 01 [24°48'31.82"S; 54°21'0.24"W], RBSH 02 [24°49'39.84"S; 54°21'32.87"W] and RBSH 03 [24°51'14.54"S; 54°21'24.27"W]) (Figure 1). Details about the sampling points can be found in Table 1.

2.2. Collection of biological material

The collections of the fish specimens were carried out in the years 2017 and 2018, with the help of gill nets (3, 4, 5, 6, 7, 8, 10, 12, 14 cm between knots not adjacent). The species collected were: *Trachelyopterus galeatus* (Linnaeus, 1766), *Hoplias malabaricus* (Bloch, 1794), *Serrasalmus maculatus* (Kner, 1858), *Serrasalmus marginatus* (Valenciennes, 1837), *Schizodon borellii* (Boulenger, 1900), and *Plagioscion squamosissimus* (Heckel, 1840).

The collected specimens were euthanized in a 0.5% benzocaine solution, individualized, and stored in polyethylene thermal boxes (Authorization SISBIO nº 57181 -2, CEUA-UTFPR 2016/031 and SisGen A186700). The standard length (cm) and total weight (g) of all specimens were measured, the stomachs were removed, fixed in a

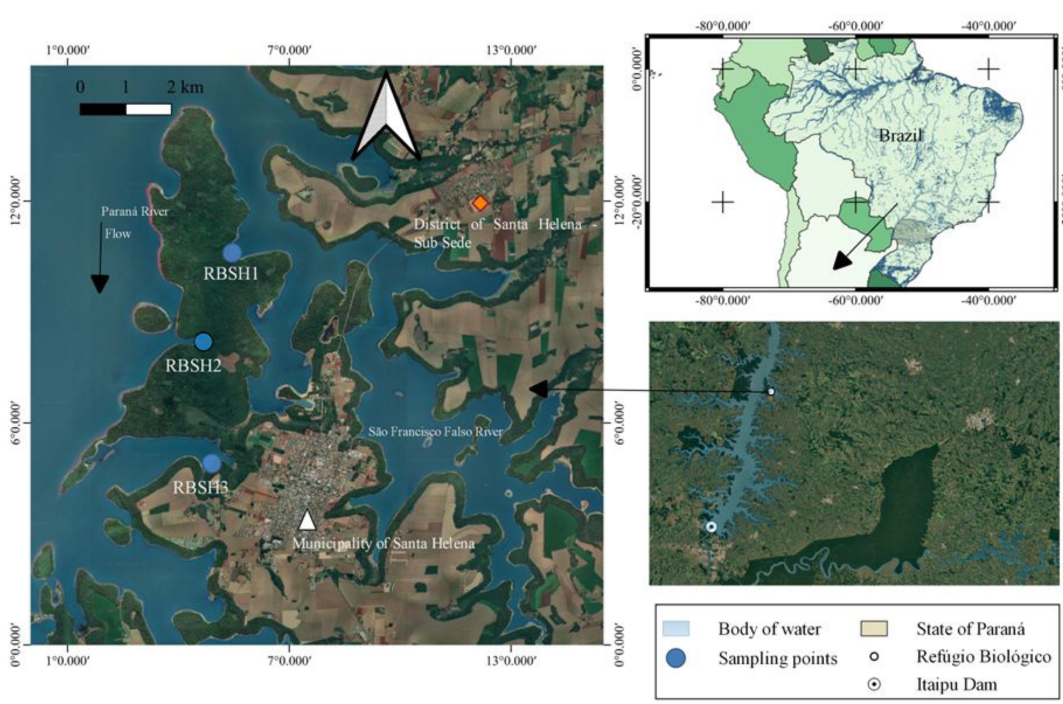

Figure 1. Study area with the location of sampling points in the Refúgio Biológico de Santa Helena, Itaipu Reservoir, Paraná III River Basin, Brazil.

Table 1. Physiographic characteristics of the sampling points around Refúgio Biológico de Santa Helena, Itaipu Reservoir, Paraná III River Basin, Brazil, in which, RBSH = Refúgio Biológico de Santa Helena.

Sampling points	Type of floating vegetation	Vegetation of the coastal zone	Riparian Forest	Environmental gradient
RBSH1	Large areas of *Eichhornia* sp., *Salvinia* sp., *Pistia stratiotes* and *Egeria* sp.	Formation of grasses that extend at the intersection of land and water.	Ciliary Forest	Lentic
RBSH2	Presence of *Eichhornia* sp. and *Egeria* sp.	Presence of grasses that extends towards the Itaipu water protection strip	Ciliary Forest	Semi lentic
RBSH3	Presence of *Eichhornia* sp. and *Egeria* sp.	Presence of grasses that extends from land to water	Ciliary Forest	Semi lentic

4% formaldehyde solution, and preserved in 70% alcohol.

2.3. Laboratory analysis

The contents of the stomachs were examined in a stereomicroscope and microscope when needed, and the food items were identified to the lowest possible taxonomic level, using identification keys Bicudo & Bicudo (1970) for algae, Mugnai et al. (2010) for invertebrates and Ota et al. (2018) for fish. Food items were quantified according to the volumetric method (the displacement by each measured food item in some graduated measuring utensil) (Hyslop, 1980). In this case, used were a gridded Petri dish for smaller food items and a graduated beaker, for larger food items, as proposed by Hellawell & Abel (1971).

2.4. Data analysis

A minimum of seven specimens per species was used as the criteria for the inclusion of the species in all analyses. The characterization of the diet was presented through the percentage volume of each food consumed calculated in the Excel program (Microsoft). The items were classified as autochthonous (originating from the aquatic environment), allochthonous (originating from the terrestrial environment), and undetermined (Silva et al., 2017). To determination of the trophic guild of each species, the predominance of a type of food resource (≥ 51% of the total volume) in the population's diet was considered (adapted from Corrêa et al., 2011): herbivore, ≥ 51% vegetable items in the stomachs; terrestrial insectivore, ≥ 51% terrestrial insects in the stomachs; piscivore, ≥ 51% fish fragments in the stomachs and carcinophage, ≥ 51% decapods.

Specimens were deposited in the Ichthyological Collection of Federal University of Technology – Paraná State, UTFPR, Santa Helena Campus (*P. squamosissimus* CISH 148TB), as well as in the Ichthyological Collection of Núcleo de Pesquisa em Limnologia, Ictiologia e Aquicultura, NUPÉLIA (*H. malabaricus* NUP 23044, *S. borellii* NUP 23037, *S. marginatus* NUP 23028, *S. maculatus* NUP 23030 and *T. galeatus* NUP 23107).

3. Results

The stomach contents of 138 individuals belonging to six species and five families (Table 2), were analyzed. *Trachelyopterus galeatus*, *H. malabaricus* and *S. maculatus* are native species, and *S. marginatus*, *S. borellii,* and *P. squamosissimus* are non-native species (Table 2).

The species diets were composed of allochthonous and autochthonous resources. We observed a predominance in the consumption of decapods, fish fragments, terrestrial insects, and aquatic plants (Table 3; Figure 2).

We also report the occurrence of microplastics in the diet of *T. galeatus*, *S. borellii,* and *S. maculatus* (Table 3). For *T. galeatus* (7 stomachs) and *S. maculatus* (5 stomachs) microplastics were observed only in RBSH 1. For *S. borelli* (3 stomachs) only in RBSH 2.

Trachelyopterus galeatus consumed mainly Coleoptera adult (29.24%), terrestrial insect (27.16%), and Hymenoptera (11.6%) being classified a terrestrial insectivore (Figure 2). *Hoplias malabaricus, S. maculatus,* and *S. marginatus* were classified as piscivores due to the high consumption of fish fragments (78.81%, 82.87%, and 72.9%, respectively) (Figure 2). *Schizodon borellii* mainly consumed aquatic plants (76.96%) and algae (1.84%), being classified as herbivores (Figure 2), and *P. squamosissimus* consumed 90.52% of decapods, so it was classified as a carcinophages (Figure 2).

4. Discussion

The fish community evaluated here consumed items of autochthonous and allochthonous origin, representing different trophic guilds. *Trachelyopterus*

Table 2. Species, origin, number of stomachs analyzed (n), standard length (Sl), and weight (Wt) of the individuals sampled in the Refúgio Biológico de Santa Helena, Itaipu Reservoir, Paraná III River Basin, Brazil. Mean (ME) and Standard deviation (SD), followed by the minimum and maximum values.

Order	Family	Species	Origin*	n	Sl – ME ± SD (min – max) cm	Wt – ME ± SD (min – max) g
Perciformes						
	Sciaenidae					
		Plagioscion squamosissimus (Heckel, 1840)	non-native	24	20.1 ± 4.8 (14.0 –31.5)	190.9 ± 99.5 (67.9 – 562.6)
Siluriformes						
	Auchenipteridae					
		Trachelyopterus cf. *galeatus* (Linnaeus, 1766)	native	37	14.7 ± 0.9 (13.5 –16.0)	104.0 ± 19.7 (32.1 – 162.5)
Characiformes						
	Anostomidae					
		Schizodon borellii (Boulenger, 1900)	non-native	38	19.6 ± 1.6 (16.5 –22.5)	208.4 ± 52.1 (126.5 – 317.4)
	Serrasalmidae					
		Serrasalmus maculatus (Kner, 1858)	native	17	14.8 ± 4.2 (10.0 – 24.5)	172.2 ± 194.6 (37.4 – 732.7)
		Serrasalmus marginatus (Valenciennes, 1837)	non-native	15	11.9 ± 3.1 (9.0 –18.5)	67.9 ± 53.6 (26.2 – 193.0)
	Erythrinidae					
		Hoplias malabaricus (Bloch, 1794)	native	7	25.8 ± 2.4 (22.0 –30.0)	322.6 ± 147.1 (214.7 – 643.0)

*According to Ota et al. (2018).

Table 3. Diet composition in the percentage of volume for *Plagioscion squamosissimus* (*Pss*), *Trachelyopterus galeatus* (*Tg*), *Schizodon borellii* (*Sb*), *Hoplias malabaricus* (*Hm*), *Serrasalmus maculatus* (*Sm*), *Serrasalmus marginatus* (*Smr*), in the Refúgio Biológico de Santa Helena, Itaipu Reservoir, Paraná III River Basin, Brazil.

Species	***Pss***	***Tg***	***Sb***	***Hm***	***Sm***	***Smr***
Trophic guild	**CARC**	**TINS**	**HERB**	**PISC**	**PISC**	**PISC**
Food item			Volume percentage (%)			
Autochthonous (83.44%)						
Aquatic insect	0.03	1.54		0.47	0.07	
Gastropoda			0.07			
Bivalvia		1.58	16.15	0.15	0.62	3.44
Microcrustacean			0.01			
Crustacea			0.24		0.02	
Decapoda	**90.52**	10.95		13.09	8.6	2.64
Fish	8.38	4.54	2.56	**78.81**	**82.87**	**72.9**
Aquatic plant	0.32	0.05	**76.96**	7.48	4.9	8.82
Algae		*	1.84			
Periphyton	0.01					
Allochthonous (14.6%)						
Terrestrial insect	0.41	**27.16**			1.2	1.15
Coleoptera (adult)		**29.24**				
Hymenoptera		**11.6**				
Hemiptera	0.13	4.35			1.3	
Terrestrial plant	0.09	9.02			0.4	1.55
Undetermined (2%)						
Detritus	0.11		2.17			9.52
Microplastic		*	*		0.02	

CARC = Carcinophage, TINS = Terrestrial insectivore, HERB = Herbivore, PISC = Piscivore. *Percentage <0.01.

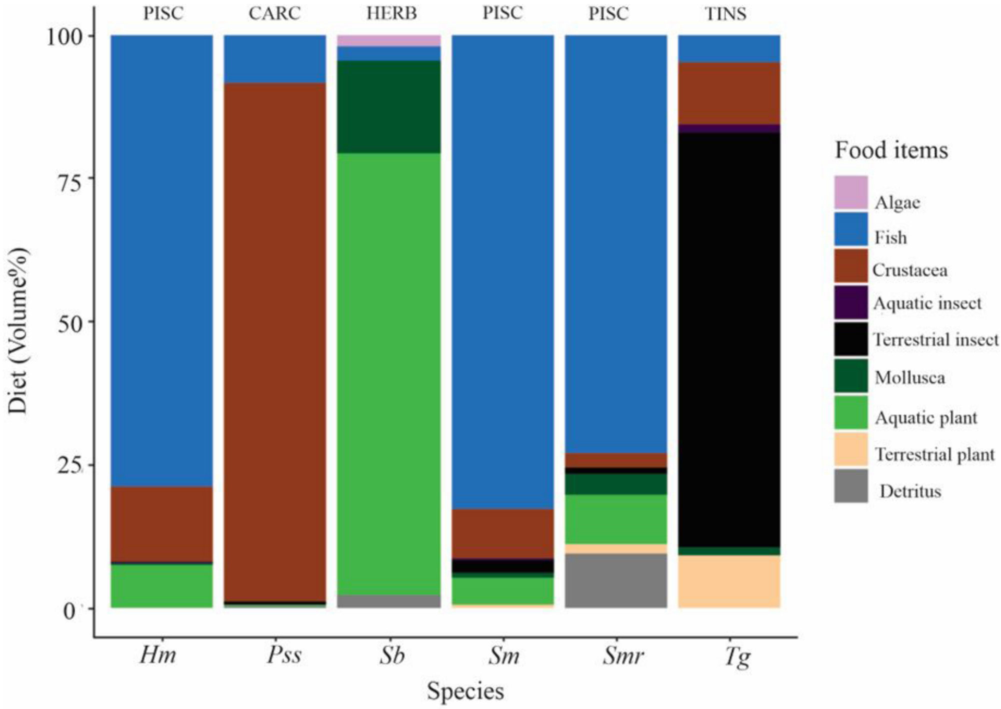

Figure 2. Volume percentage (%) of food items consumed by fish species and their trophic guild in the Refúgio Biológico de Santa Helena, Itaipu Reservoir, Paraná III River Basin, Brazil. *Hoplias malabaricus* (*Hm*), *Plagioscion squamosissimus* (*Pss*), *Schizodon borellii* (*Sb*), *Serrasalmus maculatus* (*Sm*), *Serrasalmus marginatus* (*Smr*), *Trachelyopterus galeatus* (*Tg*), PISC = Piscivore, CARC = Carcinofage, HERB = Herbivore, TINS = Terrestrial insectivore.

g*aleatus* consumed Coleoptera, Hymenoptera, and other insect fragments, as observed in other studies (Garcia et al., 2018; Peretti & Andrian, 2004, 2008; Tonella et al., 2018). Garcia et al. (2018) and Tonella et al. (2018), classified this species as omnivorous due to the consumed of plants and animals (fish and invertebrates), however, *Parauchenipterus galeatus* (Linnaeus, 1766) (=*T. galeatus*) consumed much more terrestrial insects than plants and other animals, so it was considered a terrestrial insectivore, corroborating our study. *S. borellii* showed a predominantly plant-based diet, corroborating other studies which classified it as a herbivore (Ferretti et al., 1996; Pereira & Resende, 2002). However, we also report the consumption of Bivalvia, Crustacea, and detritus such as Peretti & Andrian (2004). Thus, we observed that *T. galeatus* and *S. borellii* presented ample trophic plasticity, managing to take advantage of more available items when foraging (Gerking, 1994).

The three species of piscivores evaluated here (*H. malabaricus*, *S. marginatus*, and *S. maculatus*) mainly consumed fish fragments but also consumed plants, insects, decapods, and Bivalvia, as observed in other studies (Bozza & Hahn, 2010; Garcia et al., 2018; Peretti & Andrian, 2004, 2008; Santana-Porto & Andrian, 2009; Tonella et al., 2018). Considering that plants were found in small quantities, we inferred that the consumption of these items was an accidental act due to the predatory behavior of these species (Behr & Signor, 2008; Costa et al., 2005; Garcia et al., 2018; Moraes & Bárbola, 1995; Peretti & Andrian, 2008).

Plagioscion squamosissimus diet was composed mainly of shrimp and classified as carcinophages. According to Garcia, et al. (2018), *P. squamosissimus* was considered a specialist in shrimp in a lentic environment. In addition, there are reports of environmental and ontogenetic variation in the diet with the consumption of fish and other aquatic invertebrates (Bozza & Hahn, 2010; Neves et al., 2015), demonstrating that feeding can change according to energy requirements and availability resources (Bennemann et al., 2006; Cardoso et al., 2019). Opportunistic behavior is reported for some Neotropical fish that can take advantage of the most abundant food source in time and space, consuming resources according to their availability and preferences (Abelha et al., 2001; Dias et al., 2017; Gerking, 1994; Pereira et al., 2016).

The consumption of items from various sources and the trophic guilds classified in this work demonstrate that the fish community evaluated belongs to different trophic levels. Each trophic level plays a role in maintaining the ecosystem (Díaz & Cabido, 2001), essential for the maintenance of conservation units. The carnivorous fish that are represented here as terrestrial insectivores, piscivores, and carcinophages, have a functional role in the population control of invertebrates and vertebrates (Manna et al., 2013). Carnivorous fishes promote long-term selective pressure and helping to increase species diversity (Townsend et al., 2011). Herbivorous fish, represented here by *S. borellii*, contribute to the entry of nutrients into the food chain in that area, cycling nutrients from plants, such as nitrogen and phosphorus (Hulot et al., 2000; Manna et al., 2013).

Here, three non-native species were analyzed (*S. marginatus* (piscivores), *P. squamosissimus* (carcinophages), and *S. borellii* (herbivores)). Although trophic guilds contribute to population control of some species or nutrient cycling, non-native species can compete for food resources with native species (Gallardo et al., 2016). Soon after their introduction, non-native species have a competitive advantage over native species due to the absence of natural predators in the invaded environment, which can lead to their establishment and population increase (Britton, 2019; De La Torre Zavala et al., 2018) and decrease the population of native species (Gallardo et al., 2016; Levis et al., 2013). Agostinho et al. (2003), has shown this impact with an experiment between the non-native piranha (*S. marginatus*) and native (*Serrasalmus spilopleura* Kner, 1958 (= *S. maculatus*) species in the upper Paraná River, where there was competition. The authors noted that due the greater advantage of the non-native species, the population of native piranhas decreased (Agostinho et al., 2003).

Another relevant aspect was the occurrence of microplastics in the diet of some species in the RBSH 1 and RBSH 2. This record is worrying since the conservation unit is located close to the urban area, demonstrating the current problem of plastic contamination in freshwater biota, associated with urbanization (Azevedo-Santos et al., 2019; Pinheiro et al., 2017; Silva-Cavalcanti et al., 2017). The consumption of microplastics by fish promotes several negative physiological effects (Fu et al., 2020; Lönnstedt & Eklöv, 2016), and due to their characteristics, they can reach fish of all levels of development (Azevedo-Santos et al., 2019).

As it is a conservation unit, knowing the trophic guilds that make up the fish community can contribute to understanding the dynamics of food chains, the structure of the community, and the functioning of the ecosystem. In addition, the occurrence of microplastics reported here raises the issue about the contamination of conservation units in aquatic environments, since there is no way to contain the effluents coming from urban areas, for example. Finally, this work can contribute information about the biology of the species evaluated for future work and conservation programs.

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