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Food ecology and presence of microplastic in the stomach content of neotropical fish in an urban river of the upper Paraná River Basin

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ABSTRACT

Plastic is a useful material; but along with its benefits also come several disadvantages. One of these is the consequences of the improper discarding of plastic in the environment and its eventual fragmentation into microplastics. Plastic can reach rivers and affect their biota as microparticles from its degradation. The entry of plastic into the food chain occurs by the consumption of fish or other organisms. Furthermore, persistent organic pollutants can accumulate, and the consumption of materials containing plastic pollutants can cause several physiological problems in animals. Such pollutants can reach man when water or fish is consumed. There are numerous studies in the marine environment that show that microplastics significantly interfere with marine biota; but there are relatively few studies addressing this topic in freshwater environments. This work characterized the diet of the most abundant fish species in an urban river of the Upper Paraná Brazil Watershed, investigating the occurrence of microplastic. As much as the studied river suffers from urban river syndrome, a wide variety of food consumed by fish was found. The stomach content of 220 individuals belonging to fourteen species was analyzed. In the stomach content analysis, 16 types of food items were found, most of them autochthonous. The species analyzed were grouped into four trophic categories, with iliophage as dominant one. The presence of microplastics was found in 2% of the studied individuals, two individuals of the species *Rhamdia quelen*, one specimen of *Hoplosternum littorale* and one specimen of *Astyanax fasciatus*.

Keywords: benthic species, sediment, urbanization.

Ecologia alimentar e presença de microplástico no conteúdo estomacal de peixes neotropicais em um rio urbano da bacia do alto rio Paraná

RESUMO

O plástico é um material relevante, mas junto com seus benefícios também surgem diversos problemas, um deles são as consequências do seu descarte irregular no meio ambiente e sua eventual fragmentação em microplásticos. O plástico pode alcançar rios e afetar sua biota através da sua degradação em micropartículas. A entrada de plástico na cadeia alimentar ocorre pelo consumo dos peixes ou outros organismos. Além disso, poluentes orgânicos persistentes podem acumular nessas micropartículas, e ao serem ingeridos pela biota podem causar vários problemas fisiológicos. Tais poluentes podem atingir o homem ao consumir água ou peixe. Existem inúmeros estudos no ambiente marinho que mostram que os microplásticos interferem significativamente na biota marinha, mas estudos que abordam esse tópico em ambientes de água doce ainda são incipientes. O presente trabalho teve como objetivo caracterizar a dieta das espécies de peixes mais abundantes em um rio urbano da Bacia do Alto Paraná, investigando a ocorrência de microplástico. Por mais que o rio estudado sofra da síndrome do rio urbano, verificou-se uma grande variedade de alimentos consumidos pelos peixes. Foi analisado o conteúdo estomacal de 220 indivíduos pertencentes a 14 espécies. Na análise do conteúdo estomacal, foram encontrados 16 tipos de itens alimentares, a maioria autóctone. As espécies analisadas foram agrupadas em 4 categorias tróficas, sendo o iliófago dominante. A presença de microplásticos foi encontrada em 2% dos indivíduos estudados, dois indivíduos da espécie *Rhamdia quelen*, um espécime de *Hoplosternum littorale* e um espécime de *Astyanax fasciatus*.

Palavras-chave: espécies bentônicas, sedimento, urbanização.

1. INTRODUCTION

Analysis of the food habits of fish allows us to outline several processes of ecology involved in their diets (Ribeiro *et al.*, 2014). Thus, the investigation of diet based on stomach content analysis provides consistent information about the trophic organization of fish communities as well as species biology, and is an important asset in the evaluation of interactive processes in aquatic environments (Bennemann *et al.*, 2006). Studies regarding diet can also highlight and elucidate the impacts to which aquatic ecosystems are subjected, such as impoundment, effluent discharge, and channeling, among others. More recently, the study of fish diet has been used to detect the presence of microplastic and characterize more susceptible trophic groups.

Plastic is an important component of several materials and objects today, with numerous applications and a wide use (Andrady and Neal, 2009; Faggio *et al.*, 2018; Savoca *et al.*, 2019a; 2019b). It is, however, considered one of the main pollutants that act on aquatic ecosystems (Islam and Tanaka, 2004), accounting for 60% to 80% of the waste found in marine environments (Derraik, 2002; Moore, 2008). Regarding freshwater ecosystems, information is still sarce (Pinheiro *et al.*, 2017), a fact corroborated by Guzzetti *et al.* (2018), Blettler and Wantzen (2019), Prokić *et al.* (2019) and Pagano *et al.* (2019). These authors reinforce the need for research on this subject, although there is already a rush of researchers to develop studies on it. This is justified by the strong evidence that the ingestion of plastic by aquatic organisms occurs globally (Azevedo-Santos *et al.*, 2019). Studies have recently been conducted that highlight the contamination of freshwater ecosystems by microplastics (Wagner *et al.*, 2014); however, information is still limited, and little has been disseminated regarding these environments and their biota.

Microplastic consists of plastic particles measuring between one and five millimeters and it has been raising concerns as it shows a consistent and growing presence on both marine and freshwater environments. These environments have great potential to be exposure vectors and transfers of persistent and highly toxic organic compounds (Thompson *et al.*, 2004); this increases their ability to accumulate these compounds over time (Wurl and Obbard, 2004; Strungaru *et al.*, 2018; Alimba and Faggio, 2019). Microplastics can be generated by the breakdown of larger plastic particles, and may originate in personal care products such as exfoliation products and some toothpastes. Another possible origin is pellets, which are small plastic granules that correspond to the main shape of plastic resins and are used as raw material in industries to produce several objects (Manzano, 2009). The disposal of plastics in freshwater environments is practically the same as the oceans, such as direct dumping into rivers, or being carried into nearby bodies of water by rain or wind, or through the disposal of synthetic fibers from fishing or cosmetic equipment (Browne *et al.*, 2011).

There are few studies focused on microplastic intake by freshwater fish, and Wagner *et al.* (2014) shows only five studies have investigated its occurrence in freshwater fish. Among these, we can mention a study in China, where it was found that the fragments most ingested by freshwater fish are microplastic fibers (Jabeen, 2016). In Brazil, a study by Silva-Cavalcanti *et al.* (2017) in the Pajeú River in the city of Serra Talhada in northeastern Brazil, collected 48 individuals of *H. littorale*, and of this total, 75% of the samples contained microplastic in the stomach content. Therefore, our work investigated the diet of the main fish species of an urban stretch of a tropical river and verified the occurrence of microplastic in their stomach contents.

2. MATERIAL AND METHODS

2.1. Area of study

The present study was conducted on the Sorocaba River (Figure 1), in the urban area of Sorocaba, located in the state of São Paulo, Brazil. The river is 180 km long, and its source is located in the municipality of Ibiúna. Its mouth in the Tietê River, in the municipality of Laranjal Paulista (Smith, 2003).

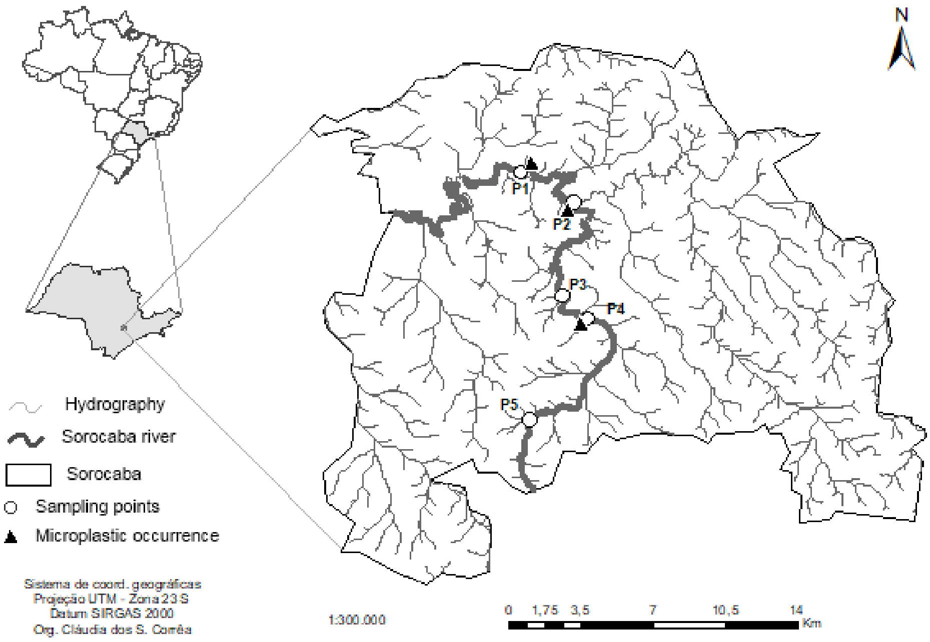

Figure 1. The municipality of Sorocaba, indicating the Sorocaba River and the sampling points analyzed.

2.2. Ichthyofauna sampling

The samples were gathered in June 2017 and January 2018, at five points along the river, located in the municipality of Sorocaba (Figure 1). Sampling sites were defined according to their distinct hydrological and ecological characteristics between the sampled points. Fish collections were made using standby nets divided into two batteries containing eight nets, ten meters long and 1.5 meters high, with mesh sizes varying (3.0 cm, 4.0 cm, 5.0 centimeters, 6.0 centimeters, 7.0 centimeters, 8.0 centimeters, 10.0 centimeters, 12.0 centimeters, between opposite nodes), remaining in place for 12 hours (Malabarba and Reis, 1987). The captured specimens were then weighed, measured (standard length) and separated into plastic bags containing information about the time of the year and location. The specimens were fixed using 10% formaldehyde and preserved in 70% alcohol, stored in plastic containers and deposited in the Laboratório de Ecologia Estrutural e Funcional de Ecossistemas of the Universidade

Paulista, on the Sorocaba campus, where they were screened and identified through identification keys and specialized literature. The collections were performed under the registration of SISBIO license (Authorization No. 6017122) and with a CEUA certificate of the Universidade Paulista (UNIP) No. 004/18.

2.3. Stomach Content and Data Analysis

The specimens were dissected to remove the stomachs, with the beginning of the incision in the anal opening and ending near the region of the pectoral fins (Santos *et al.*, 2009). The stomachs were measured (centimeters) and weighed (grams) and later stored in plastic containers with information of the species, location and date of collection, and were preserved in 70% alcohol (Carmo *et al.*, 2015). Some of the residues found in the analyzed stomachs were visually identified as microplastics, using a stereomicroscope, and it was not possible to analyze them through other types of identification. In order to be classified as microplastics, particles between 1 and 5 millimeters were considered, according to Arthur *et al.* (2008).

For the analysis of stomach content, adult individuals were used, so that the results were not altered by possible variations in the diet of juvenile individuals (Abelha *et al.*, 2001). Food items were analyzed by stereomicroscope and identified to the lowest taxonomic level possible by consulting the specialized literature, and were classified into autochthonous and allochthonous. For each of the analyzed species, the food index (Kawakami and Vazzoler, 1980) was determined by collection point and for each species, where $IAi = (FOi * Pi)/\sum(FOi * Pi) * 100$, where: IAi is the food index of item i, FOi is the frequency of occurrence of item i and Pi is the weight of item i. This method allowed the determination of the food preference of fish species and their trophic level, and could be applied even to smaller food items (Corrêa and Smith, 2019).

3. RESULTS

Two hundred and twenty individuals belonging to 14 species were analyzed, among which 90 in individuals collected in the dry period; the month of collection in that period was June. The month of collection in the rainy period was January, and in this period 130 individuals were collected. The most abundant species collected in the two periods were: *Prochilodus lineatus, Astyanax fasciatus*, *Hoplosternum littorale* and *Hoplias malabaricus*. The variation in the size of all individuals collected was between 3.9 cm and 40.7 cm (Table 1).

Table 1. Number of individuals collected during the dry and rainy season and variation in standard length.

Species	Abundance (Dry)	Abundance (Rain)	Dry Size Range (cm)	Rain Size Range (cm)
Astyanax fasciatus	4	32	6.7 – 10.6	7.1 – 9.2
Astyanax lacustris	8	2	3.9 – 9.1	8.6 – 8.9
Crenicichla lacustris	-	2	–	8.6 – 8.9
Geophagus brasiliensis	-	3	–	14.8 – 18.2
Hoplias malabaricus	20	4	16.1 – 40.8	23.8 – 35.6
Hoplosternum littorale	10	15	7.8 – 24.6	13.5 – 19.3
Hypostomus sp.	-	10	–	11.7 – 40.7
Hypostomus ancistroides	9	16	14.3 – 29.3	11.5 – 25.5
Hypostomus margaritifer	4	6	16.4 – 22.7	19.6 – 24.7
Prochilodus lineatus	24	26	20 – 38.3	15.6 – 31.8
Pterygoplichtys ambrosetti	5	11	6.9 – 31.2	10.1 – 28.7
Rhamdia quelen	4	1	18.2 – 34.6	12.5
Steindachnerina insculpta	2	2	9.9 – 22.7	9.4 – 9.5
Serrasalmus maculatus	-	1	-	8.5

3.1. Stomach content

Sixteen types of food items were identified, among which only cladoceran, crustacean and coleoptera were present only in the rainy season; all other items were present in the two seasons evaluated. The most abundant food items in the two periods were plant material and sandy and muddy sediment (Tables 1 and 2, supplementary material). Among the 16 types of food items, most were identified as being of autochthonous, totaling 10 items. While the other 6 items were characterized as items of allochthonous (Tables 1 and 2, supplementary material). Four trophic groups were identified (Omnivore, Insectivore, Piscivore and Iliophage), seven Iliophages in the rainy season and six species in the dry season, making this trophic group the most abundant among the animals analyzed (Table 2).

Table 2. Trophic group (established based on% IAi, Supplementary Tables 1 and 2) consumed by the ichthyofauna of the Sorocaba River. Number of individuals with microplastic in stomach content (NIWM).

Species	Trophic group (rain)	Trophic group (dry)	NIWM
Astyanax fasciatus	Insectivore / Omnivore	Omnivore	1
Astyanax lacustris	Insectivorous	Omnivore	-
Crenicichla lacustris	Insectivorous	-	-
Geophagus brasiliensis	Insectivorous	-	-
Hoplias malabaricus	Piscivorous	Piscivorous	-
Hoplosternum littorale	Iliophage	Iliophage	1
Hypostomus sp.	Iliophage	-	-
Hypostomus ancistroides	Iliophage	Iliophage	-
Hypostomus margaritifer	Iliophage	Iliophage	-
Prochilodus lineatus	Iliophage	Iliophage	-
Pterygoplichtys ambrosetti	Iliophage	Iliophage	-
Rhamdia quelen	-	Insectivore / Piscivore	2
Steindachnerina insculpta	Iliophage	Iliophage	-
Serrasalmus maculatus	Piscívoro	-	-

3.2. Plastic debris

Plastic fragments classifiable as "microplastics" were found in the stomach contents of four individuals: *Rhamdia quelen* (2 individuals), *Hoplosternum littorale* (1 individual) and *Astyanax fasciatus* (1 individual) (Table 2). These specimens were collected at three sampling points. Individuals of the species *Rhamdia quelen* had ingested the microplastic during the dry season, while individuals of *Hoplosternum littorale* and *Astyanax fasciatus* in the rainy season (Tables 1 and 2, supplementary material). The plastic particles found had different shapes, but the type of plastic material was possible to identify only visually, wherein the size of the microplastics found varied by approximately 1 and 3 millimeters. Among the debris, one included latex paint and the others degraded plastic fragments (Figure 2).

4. DISCUSSION

The stretch of the studied river presents typical conditions of the urban river syndrome, as described by Walsh (2005). Nevertheless, the fish showed a considerable variety of food items ingested. The urban character of the river determined the predominance of autochthonous items, which limits and drastically reduces the availability of allochthonous items coming from the riparian forest, which has been significantly altered in the studied stretch. The results show that among the six trophic categories commonly found in freshwater courses, most of the representative trophic groups are present in the studied river (four categories), suggesting environmental changes (Souto *et al.*, 2016), since the abundance trophic guilds is very dependent on environmental conditions (Vidotto-Magnoni and Carvalho, 2009).

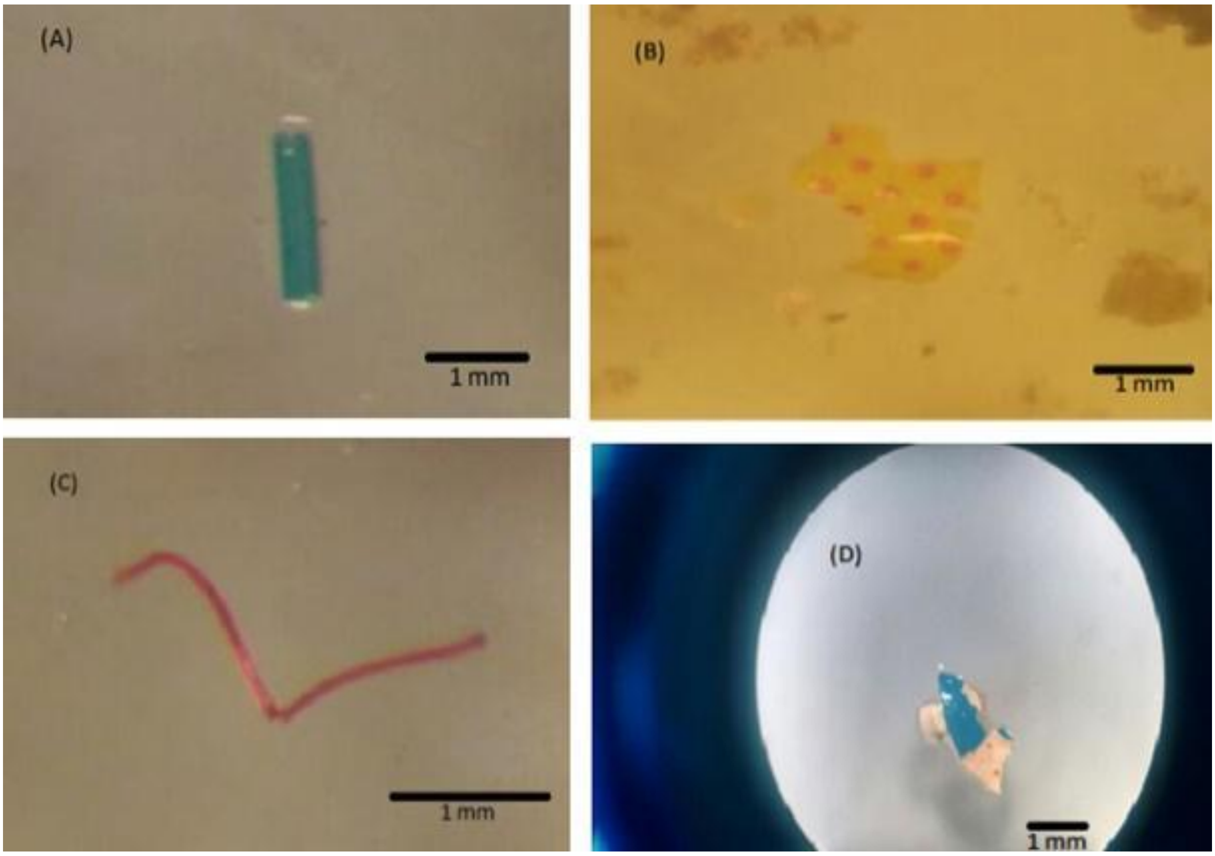

Figure 2. Stereoscopic microscope. (a) Small fiber found in *Astyanax fasciatus*. (b) Packaging remnant found in *Hoplosternum littorale*. (c) Small fiber found in *Rhamdia quelen*. (d) Probable trace of ink found in *Rhamdia quelen*.

The trophic change observed in the present study from the rainy season to the dry season was the result of the greater supply of autochthonous food in the rainy season, which favored greater specialization in the species diet (Junk and Wantzen, 2004) which emphasized that hydrological changes affect not only the quantity, but also the quality of the food. This result corroborates with the premise that most neotropical fish have sufficient capacity to adjust their diet (food plasticity) (Hahn and Fugi, 2007), and when a food becomes available many species are able to take advantage of this resource (Gerking, 1994), exploring items from these food categories that are in greater quantity (Marçal-Shimabuku and Peret, 2002).

The presence of microplastic in the food content of three species studied indicates how much the rivers are already affected by this debris, there are still few published works carefully studying these phenomena, mainly in Brazil. Of the three species in which microplastics were found, *R. quelen* and *H. littorale* have benthic habits, with a habit of foraging the bottom, whether in search of organisms or debris present in the sediment (Hahn *et al.*, 1997 and Ximenes *et al.*, 2011). The ingestion of microplastic by these species may have occurred accidentally, when these individuals ingested sediment or food present in the substrate, since the microplastic tends to be deposited in the beds of water bodies (Chubarenko *et al.*, 2016; Jabeen, 2016). Benthic species have a greater amount of microplastics than pelagic fish, noting that the feeding habits and habitats of freshwater fish play an important role in the ingestion of this debris (Sanchez *et al.*, 2014). The present study corroborates this statement, when it was found that *H. littorale* ingested plastic debris mixed with sediment when it foraged the bottom substrate, which was documented by Silva-Cavalcanti *et al.* (2017). When found in large quantities, microplastics can harm the health of fish, for example through the accumulation of heavy metals and the loss of satiety (Kuśmierek and Popiołek, 2020). However, despite having been found in low quantities in the present study, this result can already be considered an indication that the health of fish can be impaired over time due to the increase in plastic in aquatic ecosystems.

Our results showed that of the four fragments of microplastic found, the exact origin of the microplastic is uncertain; two of them resemble plastic fibers. The particles found (all between 1 and 3 millimeters in size) apparently come from larger plastic particles, being fragments, and reinforcing the fact that the microplastics ingested by the fish came from larger materials that were degraded until they reached the environment where they were ingested. Studies developed in other rivers have shown that plastic fibers are found in large quantities in freshwater fish (Sanchez, *et al.*, 2014; Peters and Bratton, 2016 and Jabeen, 2016; Silva-Cavalcanti *et al.*, 2017). In addition to the fibers, two other fragments resemble the plastic packaging that was degraded to the point of microplastic, which certainly contributed to its being consumed, in addition to another particle that, although not necessarily plastic, is a particle of anthropic origin that seems to have been generated by some type of latex-based paint and can accumulate pollutants and cause damage. In the present study, only visual analysis was performed, which makes it difficult to know the origin of this debris and its composition. More careful analysis and the use of equipment capable of identifying the type of plastic is of great relevance to assess the origin and impacts to be caused (Teuten, 2009; Gregory, 2009; Setala *et al.*, 2014).

The presence of microplastics in fish in the present study corroborates other studies that suggested that fish inhabiting freshwater environments in urbanized areas are more exposed to ingestion of microplastics (Jabeen, 2016; Silva-Cavalcanti *et al.*, 2017, Phillips and Bonner, 2015; Peters and Bratton 2016). Microplastics cause negative physical effects to fish by preventing the absorption of nutrients due to their accumulation in food appendages and damage to gastrointestinal tissues, and physiological effects causing behavioral changes (Pinheiro *et al.*, 2017; Derraik, 2002; Moore, 2008). Freshwater fish are extremely vulnerable to microplastic pollution and urbanized areas are the most susceptible to this situation. The investigation of microplastics in aquatic freshwater environments should be encouraged, as in recent years research is still very focused on the marine ecosystem, with very little research on freshwater ecosystems (Azevedo-Santos *et al.*, 2019). We hope that in the future the knowledge gaps that still exist in relation to freshwater ecosystems will be minimized with the increase in research on the theme and the management of urban solid waste will be expanded. This is a key issue to be solved, especially in developing countries like Brazil.

It is likely that future studies of microplastics in tropical rivers such as the Sorocaba River will reveal new evidence regarding pollution by these agents, and the results may lead to discussions of how to mitigate the effects, since this problem impacts the entire aquatic ecosystem and its biota, becoming a public health issue.

5. CONCLUSION

This study showed that the Sorocaba River is affected by microplastics and that fish consume these particles. The study also justified the concern raised at the beginning of the research, as microplastics were detected by the analysis of the stomach contents of the fish. The three species that ingested the microplastics are considered omnivorous and explore the bottom or the water column. Further studies involving not only the stomach contents of fish, but also the analysis of sediments found at different points of the river and studies involving invertebrates can help to clarify the magnitude of the impact caused by microplastics in the food chain, reinforcing the need for future research.

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