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First record of microplastics in two freshwater fish species (*Iheringhthys labrosus* and *Astyanax lacustris*) from the middle section of the Uruguay River, Brazil

Primeiro registro de microplástico em duas espécies de peixe (*Iheringhthys labrosus e Astyanax lacustris)* num trecho do Médio Rio Uruguai, Brasil

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Abstract: Aim: The aim was to analyze the incidence of microplastics in the diet of fish in the middle Uruguay River. **Methods:** The present work analyzed the gastrointestinal content of two species: *Astyanax lacustris* and *Iheringichthys labrosus*. **Results:** This study provides the first evidence of synthetic materials, such as fibres and plastics in the gastrointestinal tract of fish species in the Middle Uruguay River basin. A sample of sixty-one *A. lacustris* and twenty-nine *I. labrosus* were examined to highlight the ingestion of microplastics in the middle section of the Uruguay River in Brazil. In the *A. lacustris,* eleven fibres and two fragments were found in their gastrointestinal contents, corresponding to 18.1% of the specimens, while in the *I. labrosus,* twelve fibres and one fragment were found, corresponding to 34.5% of the specimens analyzed. Blue staining was also prevalent in the occurrence of microplastics. **Conclusions:** These data are the first record of microplastics in this zone and represent a baseline for this contamination for future studies.

Keywords: environmental contamination; freshwater fishes; Uruguay River; microplastic ingestion.

Resumo: Objetivo: O objectivo foi analisar a incidência de microplásticos na dieta dos peixes no meio do Rio Uruguai. **Métodos:** O presente trabalho analisou o conteúdo gastrointestinal de duas espécies: *Astyanax lacustris* e *Iheringichthys labrosus*. **Resultados:** Uma amostra de sessenta e um indivíduos de *A. lacustris* e vinte e nove indivíduos de *I. labrosus* foi examinada para destacar a ingestão de microplásticos na seção média do rio Uruguai no Brasil. Em *A. lacustris*, foram encontradas onze fibras e dois fragmentos em seu conteúdo gastrointestinal, correspondendo a 18,1% das amostras, enquanto no *I. labrosus* foram encontradas doze fibras e um fragmento, correspondentes a 34,5% das amostras analisadas. A coloração azul também foi prevalente na ocorrência de microplásticos. **Conclusões:** Esses dados são o primeiro registro de microplásticos nessa zona e representam uma linha de base para essa contaminação para estudos futuros.

Keywords: contaminação ambiental; peixes de água doce; Rio Uruguai; ingestão microplástica.

Global plastic production has increased exponentially since the 1950s, from 5 million tonnes/year to 300 million tonnes/year in 2013 (Rochman et al., 2013). Research efforts have also increased, which show that this is a global problem with local implications for biodiversity. Microplastics (MPs, < 5mm) can be found around the world, even in remote areas such as the Antarctic (Lacerda et al., 2019).

In the last few years, different studies have identified MPs in rivers, lakes, and estuaries in Africa, Asia, Europe, and North America and in these studies the MPs are from primary and secondary sources (Eerkes-Medrano et al., 2015; Wu et al., 2020). MPs can have long residence times in freshwater systems as they are transported (Browne et al., 2011). As the number of MPs in freshwater systems and the residence times increase, the risk of exposure and interaction with fauna also increases. The aim of this work was to analyze the incidence of microplastics in the diet of fish in the middle Uruguay River. For this purpose, we selected two species that are representative of the Uruguay River with different trophic habits in five different sites.

The studied species *Astyanax lacustris* (Lutken, 1875) is a small an omnivorous fish, widely distributed throughout the basin, it inhabits the water column and also usually feeds on macroinvertebrates in the bottom sediments (Hartz et al., 1996). On the other hand, *Iheringichthys labrosus* (Lütken, 1874) is a midsize catfish species also widely distributed throughout the basin, living in the bottom of the river and feeds mainly on macroinvertebrates and also plant remains (Abes et al., 2001; Masdeu et al., 2011; Silva et al., 2019). These two omnivorous species play an important role in the trophic web because they represent the connection between macroinvertebrates and basal resources with the piscivorous fish (Abes et al., 2001; Gomiero & Braga, 2003; Masdeu et al., 2011; D'Anatro et al., 2013). In addition to their flexible trophic habits, these species have great reproductive flexibility, both characteristics contribute to their widely distribution throughout the Uruguay River basin (Esteves, 1996; Andrian et al., 2001; D'Anatro et al., 2013; Silva et al., 2019; Vidal et al., 2020).

The Uruguay River course covers about 2,200 km^2 in length which is divided into the upper, middle and lower Uruguay River. The species were collected from five sampling stations (RS, Brazil) in the middle section of the Uruguay river between February 2018 and January 2019. Three of the stations were located in the main river channel in the municipalities of Alecrim (27°34'26"S-54°49'55"W), Porto Vera Cruz (27°42'01"S-54°53'47"W), and São Borja (28°34'15"S-56°09'51"W) and two were in the tributaries in the municipalities of São Paulo das Missões (Comandaí river, 27°59'26"S-54°53'04"W) and Roque Gonzales (Ijuí river, 28°02'05"S-55°11'401"W) (Figure 1).

For this study, the individuals were collected with multi-mesh gill nets (15 to 25 mm knot to knot) and seine nets. Immediately after the fish were captured, the standard body length and total weight of all the individuals were measured and the gastrointestinal tract was removed and fixed in 10% formaldehyde. To separate MP from the digestive tract of fish, the methodology recommended by Rochman et al. (2015) and Woodall et al. (2015) was used. A direct light microscope (Nikon Eclipse 50i) equipped with a polarized light system was used to reduce the number of fibres that might not be synthetic material. We classified and selected as plastic the particles that showed some kind of birefringence behavior (Sierra et al., 2020), we only included the MPs that were at least 1 mm in size. During the microscopic analysis, using direct light, the characteristics of the MPs were noted such as: type (fibre or fragment, Figure 2), color and size (mm).

Ninety gastrointestinal contents were analyzed, of which sixty-one belonged to *A. lacustris* and twenty-nine to *I. labrosus*. Of the total analyzed, 18.1% of *A. lacustris* and 34.5% of *I. labrosus* presented MPs in the gastrointestinal tract. A larger proportion of the *I. labrosus* species had MPs in their gastrointestinal tract in comparison with *A. lacustris*, this finding may be due to differences in the foraging feeding strategies of both species. The *I. labrosus,* unlike *A. lacustris,* feeds in the bottom sediments, so there could be a higher density of MPs in this environment. Also, the *I. labrosus* had the highest number of fibres in one individual (3 *versus* 2) and the maximum fibre size (10 mm *versus* 4 mm) probably related to the larger size of the mouth when compared to *A. lacustris* (Figure 3).

In this study, the most frequent synthetic materials were fibres, representing 88.5%. The predominant color was blue, 73.9% (white 17.5%, red 4.3% and black 4.3%), with an average size of 2.6±1.9 mm. Both analyzed species presented MPs in all the stations that were studied (Table 1).

Ingestion of synthetic material can occur directly in the water column or indirectly when

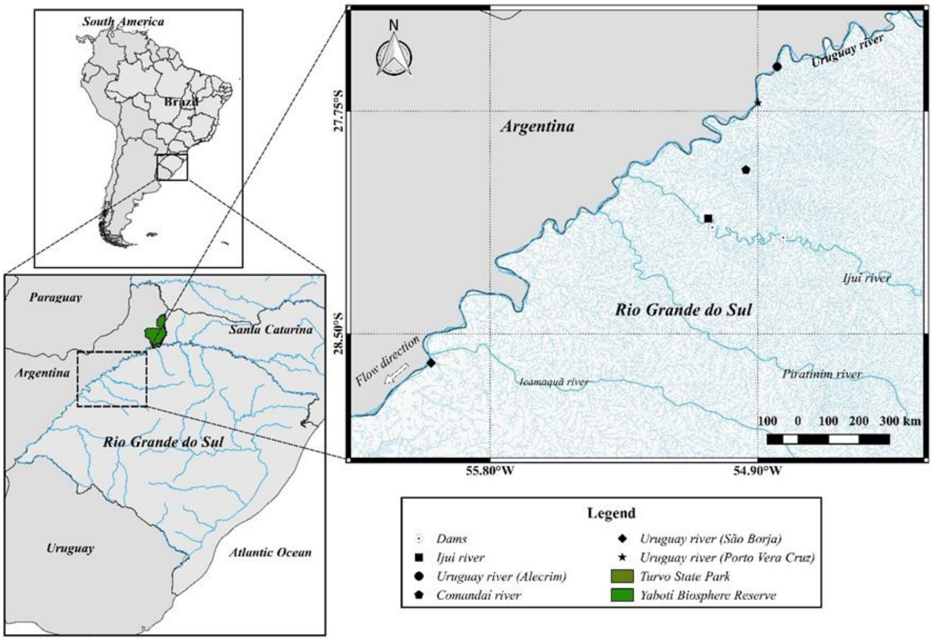

Figure 1. Study area, the location of the sampled sites can be observed.

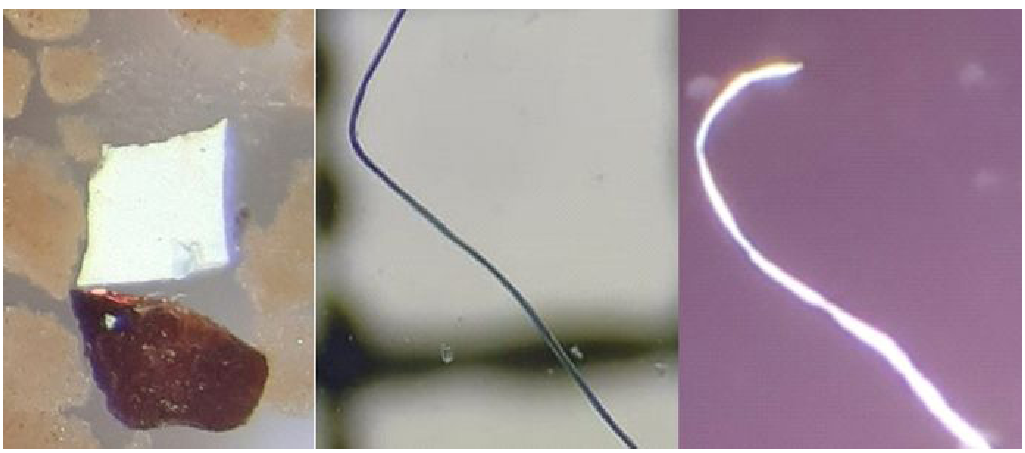

Figure 2. Microplastics found in stomach contents, left to right: white fragment under the microscope with direct light, blue fibre with direct light and the same fibre with polarized light showing birefringence behavior.

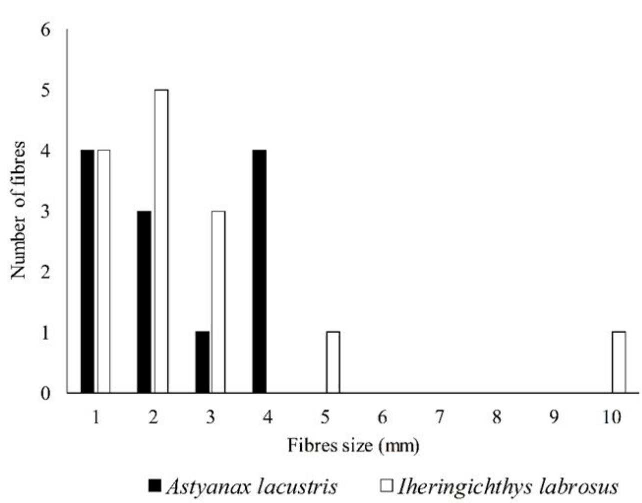

Figure 3. Fibre size frequency found in the gastrointestinal analyses of *Astyanax lacustris* and *Iheringichthys labrosus*.

Table 1. The number of individuals (n), standard length (mm), and percentage of individuals with microplastics (MPs) of both analyzed species in Uruguay River, Brazil.

Municipalities (Brazil)	*Astyanax lacustris*			*Iheringichthys labrosus*		
	n	(mm)	%MPs	n	(mm)	%MPs
Alecrim	6	111.5 ± 30.1	33.3	11	205.5 ± 39.9	27.3
Porto Vera Cruz	11	120.7 ± 16.3	10.0	9	238.6 ± 51.2	33.3
São Borja	13	132.4 ± 21.7	15.4	6	179.2 ± 31.8	33.3
São Paulo das Missões	13	121.4 ± 9.8	38.4	2	241.0 ± 21.1	50.0
Roque Gonzales	18	102.6 ± 14.3	16.7	1	101.0	100.0

fragmented particles mix with natural food sources, where color and buoyancy can be confused with natural food (Boerger et al., 2010; Davison & Asch, 2011). Among the plastic fragments, microplastics are considered very aggressive to the environment, because they are polymers that adsorbed hydrophobic organic pollutants (e.g. PCBs, organochlorine pesticides, PAHs) and heavy metals (e.g. Lozoya et al., 2016; Rodríguez et al., 2020). In the Uruguay River, the contamination by microplastics, probably from water and sediments, is added to the already existing contamination among others, oils, heavy metals and pesticides. (e.g. Costa et al., 2016; Soutullo et al., 2020). These contaminants can be adsorbed to plastics increasing their potential toxicity. Thus, when the fishes consume the particle voluntarily or even aggregated with another food, they can promote various toxic effects, including endocrine disturbance, oxidative and metabolic stress, enzyme activity, and cell necrosis (Oliveira et al., 2013; Rochman et al., 2013; Mazurais et al., 2015). The consequences can compromise the survival, growth, and reproduction (Mazurais et al., 2015; Nobre et al., 2015).

This is the first record of an analysis of MPs in the gastrointestinal tract of fish of the Middle Uruguay river basin. There is still a great lack of information to be able to estimate the real impact of microplastic consumption by fish in the Uruguay River. For this aim, further studies have to be done assessing the magnitude and frequency of consumption, as well as the possible toxicity at individual level, that can we extended to population and community levels. In addition, it is important to take into account the relative importance of microplastics sources associated with different productive activities as well as the effects of urbanization.

Finally, the use of species widely distributed in the basin, with different feeding habits and occupying different strata in the water column and sediments may be important to make comparative studies related to plastic pollution. Because a differential distribution of microplastics in the water column and in the sediments is to be expected in relation to their density (positive or negative buoyancy). In this sense some siluriforms, such as *I. labrosus* could be good indicators of microplastics contamination in bottom sediments.

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