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# Microplastic contamination in tropical fishes: An assessment of different feeding habits

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ABSTRACT

Marine ecosystems are reported to be contaminated by microplastics (MPs) (< 5 mm); however, the ecological mechanisms involved in the ingestion of debris by marine organisms are relatively unknown. By developing and optimising an appropriate protocol of gut digestion for fish species, this study explores a tropical estuarine environment to unriddle the processes responsible for the different ingestion rates of plastic debris. A total of 82 fishes with different feeding habits were analysed, *Centropomus undecimalis* (n = 30; Piscivore), *Bairdiella ronchus* (n = 21; Zoobenthivore) and *Gobionellus stomatus* (n = 31; Detritivore). The microplastic ingestion varied with the feeding strategy; *C. undecimalis*, the predator, was the most contaminated species. Overall, most MPs were fibres (47%), followed by pellets (40%) and fragments (13%), although these proportions varied among species. A high level of contamination was found in the Estuarine Complex of Santa Cruz Channel, Northeast of Brazil, with many potential input sources of MPs to the estuary, which likely accumulates in the sediment and water column, with unknown consequences for human health.

## 1. Introduction

Estuaries are well known for providing ecosystem services by supplying essential goods (raw materials and food) and offering an attractive environment for population growth and cultural activities (Atkins et al., 2011). In addition, they carry out important ecological functions since fishes use these environments for protection, feeding, reproduction, settlement, and nursery (Ferreira et al., 2019a; Krumme et al., 2008; Lima and Barletta, 2016; Potter et al., 2013; Ramos et al., 2016). Worldwide, estuaries are usually surrounded by large metropolises, and the expansion of the urban population is directly associated with impacts on the coastal ecosystems (Freeman et al., 2019). Plastic pollution is one of the most significant environmental problems of the 21st century since large quantities of these materials are being mismanaged and/or illegally disposed of in marine ecosystems (Dauvergne, 2018; Ostle et al., 2019). Once in the environment, these plastic materials are weakened by natural processes (*e.g.*, hydrodynamic forces, solar radiation, and biological actions) (Jambeck et al., 2015; Thompson et al., 2004) and fragmented into smaller parts known as microplastics (<5 mm).

Plastic debris poses several risks to marine biota (Galloway et al., 2017). Ingestion can be hazardous, causing digestive injuries, decreasing predatory efficiency, or inducing toxic effects (Barboza et al., 2018; de Sá et al., 2015; Moore, 2008; Teuten et al., 2007). Microplastics can adsorb pollutants available in the water column, such as persistent organic pollutants (POPs) (Frias et al., 2010; Oehlmann et al., 2009; Rochman et al., 2013) or heavy metals (Ashton et al., 2010; Holmes et al., 2012), which may be further bioaccumulated and biomagnified in the food web (Batel et al., 2016; Teuten et al., 2009). Furthermore, microplastics may be transferred in the trophic chain by predating contaminated prey (Chagnon et al., 2018; Ferreira et al., 2016, 2019a). The trophic transfer has already been pointed out as a relevant contamination mechanism for estuarine species (Athey et al., 2020).

Although the long-term effects of microplastic contamination are still unknown, the scientific community continually emphasises the importance of using reliable and replicable methods of investigations (Hermsen et al., 2018; Markic et al., 2020). A useful approach to obtain plastic in marine wildlife is based on chemical digestion protocols, which are efficient and low cost to work with (Karami et al., 2017; Kühn et al., 2017). Digestion protocols

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are a practical and secure way to extract and isolate microplastics in organisms, being widely used in the investigation of fish contamination by plastics (Bellas et al., 2016; Bessa et al., 2018; Foekema et al., 2013; Hermsen et al., 2017; Herrera et al., 2019; Pellini et al., 2018; Su et al., 2019; Tanaka and Takada, 2016). However, the lack of security procedures (*e.g.,* cleaned workroom and blanks procedures) during the implementation of digestion protocols may lead to the overestimation of contaminants since samples are more prone to airborne and cross-contamination (Hermsen et al., 2018, 2017; Torre et al., 2016). Moreover, the procedure must ensure data reliability through an effective and careful extraction of the microplastics and the implementation of a robust sample size with a minimum of 10 samples (Markic et al., 2020).

Several studies conducted in South Atlantic estuaries evaluated the ingestion of plastics by marine organisms, mammals (Attademo et al., 2015), mussels (Birnstiel et al., 2019; Santana et al., 2016), turtles (Guebert-Bartholo et al., 2011), and microplastics interactions with ichthyoplankton (Lima et al., 2016), and fish assemblages (Vendel et al., 2017). However, few studies investigated the ecological and biological dynamics associated with microplastic intakes on wild fishes (Amorim et al., 2020; Dantas et al., 2020; Ferreira et al., 2018, 2016). Furthermore, the bioaccumulation of microplastics in marine species is highly influenced by feeding strategies (Miller et al., 2020). However, the possible correlation between feeding habits and MPs ingestion is not yet well known.

Three estuarine species were chosen to test our hypothesis that microplastic ingestion varies according to the feeding strategies: (1) *Centropomus undecimalis* (Bloch, 1792); (2) *Bairdiella ronchus* (Cuvier, 1830); and (3) *Gobionellus stomatus* (Starks, 1913). *C. undecimalis* is an essential economic living resource for the commercial and subsistence fisheries in South America (Carpenter, 2002). While adults of *C. undecimalis* inhabit coastal areas and migrate towards the estuary, their juvenile stage uses the estuarine areas as a nursery ground (Ferreira et al., 2019a). *C. undecimalis* is classified as opportunistic predator, feeding on a large variety of available preys in the environment, with a piscivorous tendency (Ferreira et al., 2019a; Lira et al., 2017). *B. ronchus* is classified as zoobenthivore (Ferreira et al., 2019b), which preys on invertebrates associated with the sediment (Elliott et al., 2007). *G. stomatus* is a detritivore fish (Ferreira et al., 2019b) consuming detritus and microphytobenthos (Elliott et al., 2007). Although *B. ronchus* and *G. stomatus* were not of economic importance, they play a significant ecological role within the estuarine ecosystem and are the main energy source for the *C. undecimalis* (Gonzalez et al., 2019; Lira et al., 2018).

Understanding the role of microplastics as a component of anthropic pollution in this ecosystem is crucial to assess adverse impacts on regional biodiversity and the quality of fisheries resources that are being traded and consumed. Based on this information, the present study aims to (*i*) apply an adapted extraction protocol to assess MPs in the digestive tract of fishes, assuring the integrated quality control and appropriate sampling size, (*ii*) describe microplastics contamination in fishes in estuarine waters, and (*iii*) identify the main types of microplastics considering the different feeding strategies.

## 2. Materials and methods

### 2.1. Study area

The Estuarine Complex of the Santa Cruz Channel (ECSC) (Fig. 1) is located along the northeast Brazilian coast. The climate is classified as tropical, hot and humid, with an average of 26 °C (±2.8 °C) annual air temperature and two seasons defined according to the level of precipitation (rainy and dry seasons) (Medeiros et al., 2001).

The ECSC is a tidal channel that surrounds the Itamaracá Island, separating it from the mainland, with a total area of 22 km$^2$, a maximum width of 1.5 km and a depth between 4 and 5 m (Lira et al., 2017). The main tributaries are formed by the Arataca, Botafogo and Igarassu Rivers, and the predominant vegetation is the mangrove forest (Medeiros et al., 2001). The ECSC is surrounded by two cities (Itapissuma and Itamaracá), which have their economies mainly focused on the industrial and agricultural sectors (IBGE, 2011), whereas the local economy is primarily supported by artisanal fisheries, aquaculture, and tourism (de Moura et al., 2009).

### 2.2. Sampling and laboratory procedures

Three demersal species were selected for this study, given their commercial and or subsistence importance for riverine populations and their ecological interactions in the trophic chain (Lira et al., 2018, 2017): *Gobionellus stomatus*, *Bairdiella ronchus*, and *Centropomus undecimalis*, classified as Detritivores, Zoobenthivores and Piscivores respectively, according to Ferreira et al. (2019b) for the area. For the common snook *C. undecimalis*, individuals were collected in the juvenile stage (Total Length $<$ 26.3 cm) (Ferreira et al., 2019a), ensuring that the obtained specimens had not left the estuarine ecosystem. This procedure was carried out to ensure that contaminants' intake occurred within the estuarine boundary since adults of *C. undecimalis* perform migrations towards coastal areas. As *B. ronchus* and *G. stomatus* are not caught outside the estuary of ECSC (Ferreira et al., 2019b), we did not fix these species' ontogeny, and juveniles and adults were analysed. A total of 82 individuals were obtained by local fishermen. After each sampling, the specimens were labelled, and the individuals were frozen. In the laboratory, individuals were identified (Menezes and Figueiredo, 2000), measured and weighted. Each individual had their organs (stomach and intestine) carefully removed, weighed and stored again for the digestion analysis. The minimum sample size for each species was 20 individuals to avoid the bias of a low sample size, which is twice the number suggested by Markic et al. (2020).

### 2.3. Quality control and extraction protocol

Firstly, to guarantee quality control and avoid potential airborne contamination, several steps were implemented. The entire process of digestion, filtration, identification and storage of the microplastic samples was carried out in a cleaned and reserved room within the main laboratory, reserved only for microplastic analysis. The flow of people was limited; cotton lab coats and disposable latex gloves were worn during the entire process. Also, all used tools were previously cleaned with alcohol 70% and rinsed with filtered distilled water. The solutions utilised in the various procedures were filtered using a vacuum pump system (equipped with laboratory glassware) through a 47 mm GF/F 0.7 μm glass fibre filter (Whatman).

The digestive tracts were rinsed with distilled water before being placed in a beaker, submerged in NaOH (1 mol/L; PA 97%) solution, and covered by a glass lid (Fig. 2). The entire digestive tracts were submitted to NaOH without further dissection of those organs to avoid airborne contamination during handling. The proportion used was 1:100 w/v for 1 g of digestive tract weight, 100 ml of NaOH (1 mol/L) solution. The mixture was oven-dried at 60 °C for 24 h, mixed from time to time with a glass stick. The samples digested in the previous step, and the procedural blanks were filtered using a vacuum pump system through a 47 mm glass fibre filter (GF/F 0.7 μm Whatman).

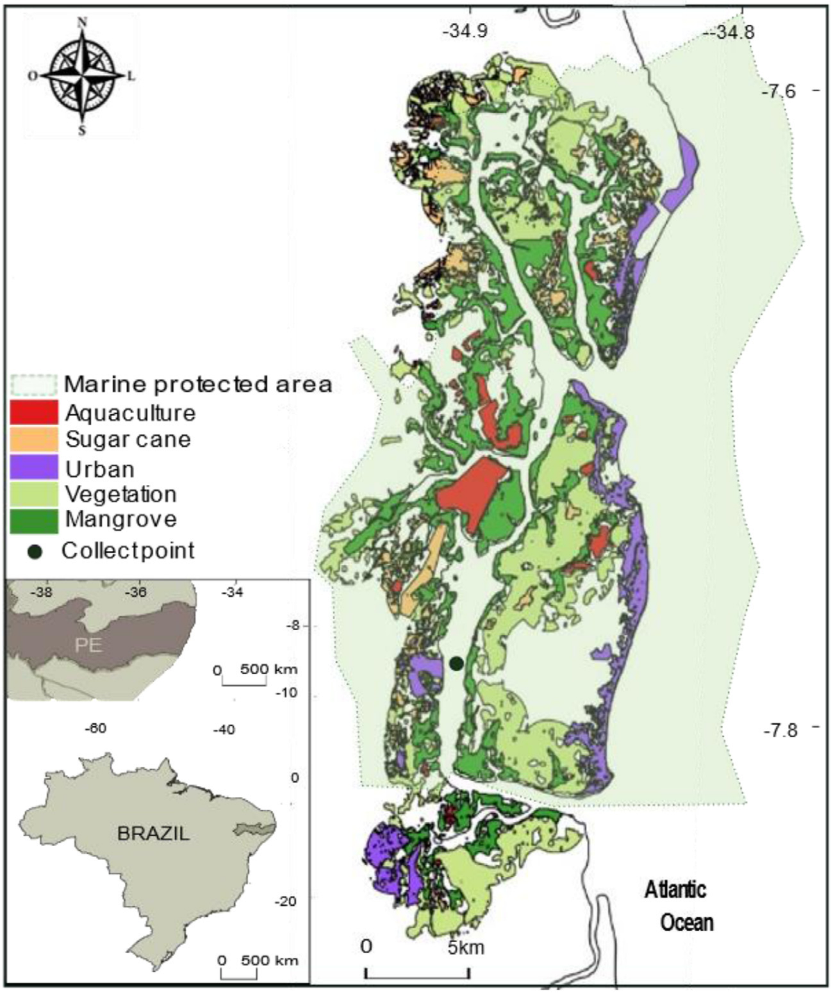

**Fig. 1.** Map of the Estuarine Complex of the Santa Cruz Channel (ECSC).
*Source:* Adapted from Pelage et al. (2019).

After filtration, filters were carefully set in a Petri dish (47 mm diameter) and covered. These filters were oven-dried at 60 °C for 24 h. The microplastics were identified using a stereomicroscope (Zeiss Stemi 508) with 6.3–50 times magnification with a detection limit of 20 µm, photographed (Axiocam 105 Color), measured (Zeiss Zen 3.2) from the filter and stored in covered Petri dishes (Fig. 2). They were then categorised by type: (*i*) fibres (filamentous shape), (*ii*) fragments (irregular shape) or (*iii*) pellets (spherical shape). The digestion protocol is a useful tool to separate the organic materials and facilitates visual identification, although it is not sufficient for identifying the polymers. Thereby, we also applied the method described by Ferreira et al. (2019a) to confirm plastic debris by drying the samples in an oven to verify whether their physical characteristics changed or not.

Procedural blanks were made for each day of analysis before beginning the sample digestion. For blanks, a beaker was filled with 50 ml of NaOH (1 mol/L) solution and covered with a glass lid, and these blanks were exposed to the same protocol applied to the samples. A total of 10 blanks were made; among them, four blanks were observed with eight tiny particles (<100 µm) considered as paint fragments. Thereby, all particles further identified with any resemblance to those observed in the blanks were excluded from posterior analyses.

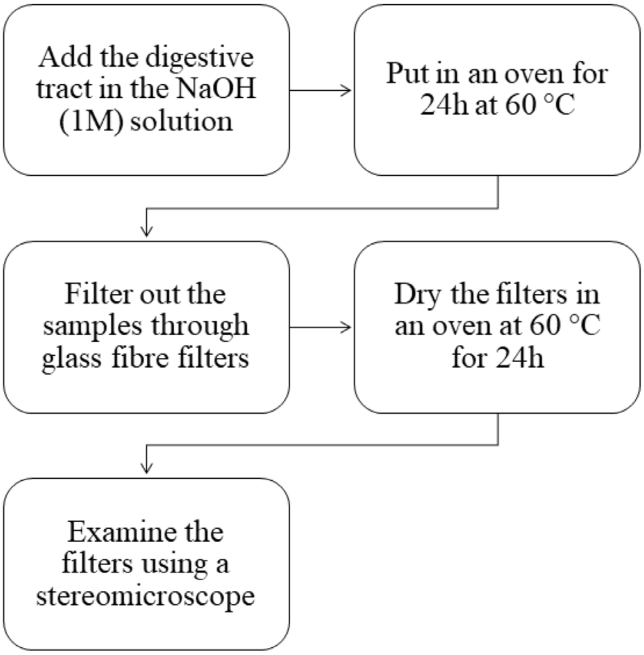

**Fig. 2.** Flowchart with the stepwise of the extraction protocol.

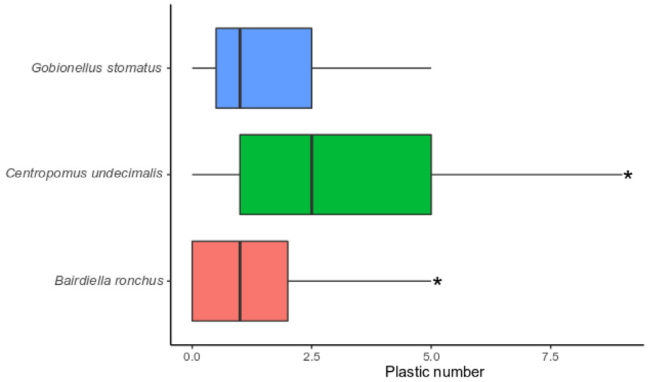

**Fig. 3.** Mean number of microplastics ingested by fishes collected in the Estuarine Complex of the Santa Cruz Channel. The asterisks represent the statistical differences with a significance of 0.05.

### 2.4. Data analysis

Kruskal–Wallis tests were used to verify if ingested microplastics (total MPs and different types) presented significant differences in number and length in relation to species (*C. undecimalis*, *B. ronchus* and *G. stomatus*). When the Kruskal–Wallis showed significant differences, the *post hoc* pairwise comparisons Dunn's test was performed to investigate the sources of variance (Dunn, 1964). All statistical analyses were carried out with the software R version 3.6.3 (R Core Team, 2020) and were conducted considering a level of significance of 5%.

### 2.5. Results

A total of 30 individuals of *Centropomus undecimalis*, 31 *Gobionellus stomatus*, 21 *Bairdiella ronchus* were analysed. Microplastics were present in 77% of *C. undecimalis*, 74% of *G. stomatus*, and 67% of *B. ronchus* individuals. A total of 176 particles of MPs were recovered from 82 fishes (Table 1).

According to the number of MPs, ingestion significantly differed between species (*p-value* $\leq$ 0.05), *C. undecimalis* being the most contaminated (3.3 ± 2.9 MPs fish$^{-1}$), followed by *G. stomatus* (1.7 ± 1.5 MPs fish$^{-1}$) and *B. ronchus* (1.2 ± 1.3 MPs fish$^{-1}$) (Fig. 3). Significant differences were recorded between *C. undecimalis* and *B. ronchus* (chi-squared = 7.873, df = 2, *p-value* $\leq$ 0.05). Concerning the length of ingested MPs, no significant differences among the same type of MPs were observed when comparing MPs' size between species.

Regarding the types of MPs ingested by fishes, most were fibres (47%), followed by pellets (40%) and fragments (13%), and proportions varied between the species. *C. undecimalis* registered 68% of pellets, 28% of fibres, and 4% of fragments, *B. ronchus* and *G. stomatus* registered 23% and 4% of pellets, 62% and 71% of fibres and 15% and 25% of fragments, respectively (Figs. 4 and 5).

## 3. Discussion

### 3.1. Quality assurance and quality control

In our study, we applied an analytical method to extract microplastics (MPs) in the digestive tract of fishes, involving a careful procedure of quality control, using sodium hydroxide (NaOH 1 M), and sample size with a minimum of 20 individuals for each species, following the recent recommendations of Markic et al. (2020) and Hermsen et al. (2018).

The application of digestion protocols to extract microplastics in marine biota has been growing worldwide (Lusher et al., 2017). Digestion protocol is a reliable method for isolating microplastics in the biota, facilitating the observation of non-organic materials. For fish, the most common method used is alkaline digestion. Indeed, for many authors, this is considered the most suitable method to remove organic material and isolate plastic debris (Karami et al., 2017; Kühn et al., 2017; Schirinzi et al., 2020). Although the efficiency of potassium hydroxide (KOH 10%) has been more often tested, the use of sodium hydroxide (NaOH 1M) has also been widely tested and demonstrated to be very useful (Baalkhuyur et al., 2018; Bellas et al., 2016; Budimir et al., 2018; Morgana et al., 2018; Su et al., 2019; Wieczorek et al., 2018), and ensures polymer integrity after chemical digestion (Budimir et al., 2018).

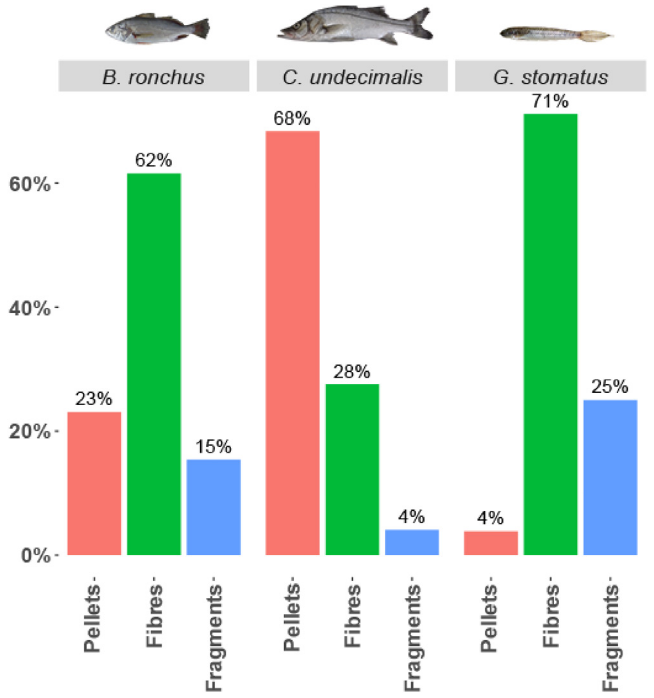

**Fig. 4.** Different types of microplastics (fibres, fragments, and pellets) ingested by fish species, expressed as a percentage.

Although adaptations of digestion protocol have been carried out around the world (Karami et al., 2017; Kühn et al., 2017), so far, only three studies in estuaries of South America have used a chemical digestion protocol for microplastics extraction in the digestive tract of fishes, with the implementation of adequate quality assurance and quality control (QA/QC) (Arias et al., 2019; Garcés-Ordóñez et al., 2020; Ribeiro-Brasil et al., 2020). Implementing QA/QC procedures is essential to avoid cross-contamination of microplastic samples (Hermsen et al., 2017; Lusher et al., 2017; O'Connor et al., 2020). This treatment is necessary to minimise over/underestimation of microplastics due to airborne contamination and or loss of particles during sample handling. Moreover, the sample size is also an essential factor in the analysis. Among the studies in South America, only Arias et al. (2019) have chosen a sample size n > 10. Such bias is unlikely to occur in our study, where the sample size was the highest in South America. Therefore, our results are expected to adequately reflect the contamination in the Estuarine Complex of Santa Cruz Channel (ECSC), and the protocol here used can safely be replicated in other studies.

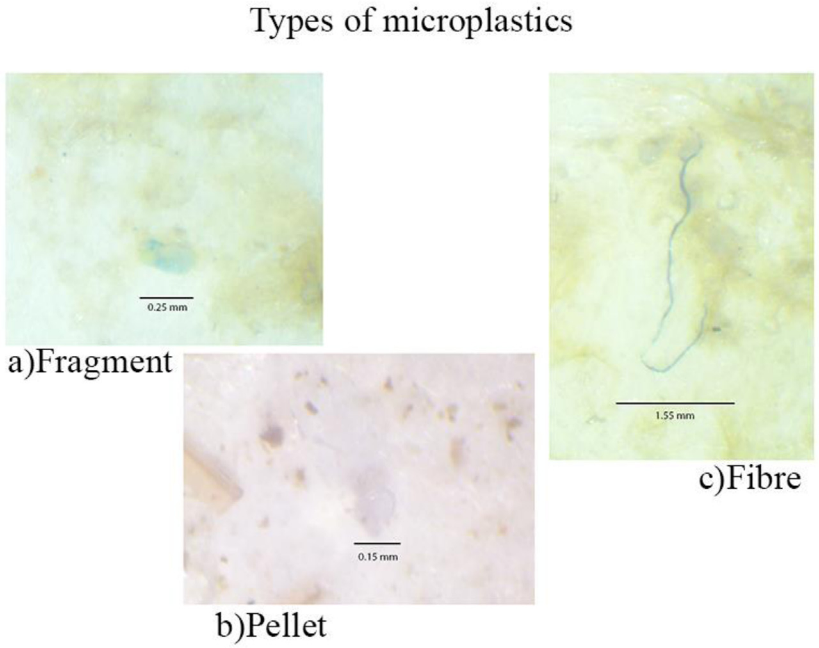

**Fig. 5.** Types of microplastics ingested by fishes of the estuarine complex of Santa Cruz Channel. (a) fragment (irregular shape); (b) pellet (spherical shape); and (c) fibre (filamentous shape).

**Table 1**
The ecological parameters and biological aspects of the analysed species. Feeding habit: DV (Detritivores), ZB (Zoobenthivores) and PV (Piscivores) obtained for the area by Ferreira et al. (2019b). TL (Total Length); SL (Standard Length); TW (Total Weight); FO% (Frequency of occurrence).

| Family/Species | Ecological parameters | | | Biometry | | | Microplastics occurrence | | |
|---|---|---|---|---|---|---|---|---|---|
| | N | Habitat use | Feeding habit | TL (cm) Min–Max | SL (cm) Min–Max | TW (g) Min–Max | MPs | FO% | TL (mm) Min–Max |
| **Gobiidae** | | | | | | | | | |
| *Gobionellus stomatus* (Starks, 1913) | 31 | Demersal | DV | 9.3–11.9 | 7.0–9.0 | 3.78–7.89 | 52 | 67 | 0.02–5.00 |
| **Sciaenidae** | | | | | | | | | |
| *Bairdiella ronchus* (Cuvier, 1830) | 21 | Demersal | ZB | 12.5–16.5 | 10.0–14.0 | 22.2–62.4 | 26 | 74 | 0.11–3.35 |
| **Centropomidae** | | | | | | | | | |
| *Centropomus undecimalis* (Bloch, 1792) | 30 | Demersal | PV | 8.9–14.0 | 7.3–11.0 | 5.8–16.78 | 98 | 77 | 0.06–3.79 |

### *3.2. Microplastic ingestion by fishes*

In our study, microplastic (MP) contamination rates were high (frequency of occurrence of 73%) for the three analysed demersal estuarine species. Overall, demersal estuarine species are frequently reported to have a high ingestion rate of MPs (Arias et al., 2019; Ferreira et al., 2016, 2019a). Demersal species inhabit and feed on the fauna associated with the substrate (Elliott et al., 2007), which might be in direct contact with contaminated sediment. Indeed, the estuarine sediment is a significant accumulation zone for MPs (Zhang, 2017). As estuaries are a transitional ecosystem, often surrounded by urban areas and exposed to domestic sewage discharge, they receive contaminants of both riverine and tidal inputs (Lebreton et al., 2017). Thereby, these ecosystems are more prone to be contaminated by MPs, especially fibres (Bessa et al., 2018; Browne et al., 2011). In addition to the sewage discharge, fibres can also be originated by the use, maintenance, discarding and loss of fisheries gear (Lima et al., 2014). MPs fibres occurred the most in the estuarine bottom, possibly due to the rapid sinking of these types of MPs (Lima et al., 2014).

Overall, the most ingested MP type were fibres, as observed in most of the studies worldwide (Bellas et al., 2016; Bessa et al., 2018; Foekema et al., 2013; Herrera et al., 2019; Wright et al., 2013). Although all types of plastics were ingested by the species analysed in this study, ingestion rates varied. Fibres were the most frequent type in *G. stomatus* (representing 71% of the ingested MPs) and *B. ronchus* (68%). *G. stomatus* and *B. ronchus* are detritivore and zoobenthivore species, respectively, depending on the organisms associated with the substrate or the organic matter available. Consequently, they are more vulnerable to the MPs fibres contaminating estuarine sediments. However, pellets were the most frequent type in *C. undecimalis* (68%), differently from the results observed by Ferreira et al. (2019a) for the same species, which registered mostly ingestion of filaments. Pellets also dominated the diet of fish along the coast of Salvador (Brazil) (Miranda and de Carvalho-Souza, 2016) and from the Amazon estuary (Pegado et al., 2018). Different from the other types of MP ingested, pellets are primary microplastics (Fendall and Sewell, 2009), which are manufactured as MPs mainly for the cosmetics industry (*e.g.*, microbeads). This type of MP can be accidentally discharged into the environment during the transport of this raw material (Ogata et al., 2009) or by the release of domestic sewage (Tanaka and Takada, 2016). The increase in urbanisation in the Santa Cruz Channel (Pelage et al., 2019) surely amplified the sewage discharge in this area. Pellets can be found floating in the water column, and they can even have fish eggs attached to them (Ivar Do Sul and Costa, 2014). Predatory fishes such as *C. undecimalis* can ingest the pellets directly by confusing them with their natural prey. Moreover, when feeding, opportunistic predators ingest a large amount of prey, which might increase the momentary build-up of MPs particles prior to egestion.

Predator species are more vulnerable to microplastic contamination due to the trophic transference, which occurs when they ingest contaminated prey (Chagnon et al., 2018; Eriksson and Burton, 2003; Ferreira et al., 2019a,b, 2016; Nelms et al., 2018). Consequently, as we observed among the three analysed demersal fish species, the predator, *C. undecimalis,* had the highest contamination rate (3.3 ± 2.9 MPs fish$^{-1}$) despite being in its early life stage (juvenile), followed by *G. stomatus* (1.7 ± 1.5 MPs fish$^{-1}$) and *B. ronchus* (1.2 ± 1.3 MPs fish$^{-1}$). Our study corroborated previous studies hypothesising that microplastic ingestion varies with the different feeding strategies (Ferreira et al., 2018, 2016; Mizraji et al., 2017). However, regardless of the diet preferences of the species, in our study area, there are several potential input sources of MPs contaminants, which probably accumulate in the sediment and water column, negatively affecting the life-strategies of fish species and mostly the juvenile stages which utilise estuaries as a nursery ground. Thus, the predators are more prone to be contaminated by microplastics through two main exposure routes: (1) the highly contaminated estuarine habitats and (2) the ingestion of contaminated prey.

Anthropogenic activities in the ECSC (urban areas, manufacturers, aquaculture plants, and sugarcane fields) are found surrounding the whole floodplain, and these activities might be an important source of microplastics and other contaminants (*e.g.,* pesticides and heavy metals). Indeed, the ECSC has registered many impacts such as habitat loss and mercury releases (Albuquerque et al., 2019; Araújo et al., 2019; Pelage et al., 2019), which likely affect the estuarine community. Besides, our study has identified high contamination by microplastics in fish species that are a relevant source of protein locally and regionally. Despite being a Marine Protected Area, which provides essential ecosystems services, there is a lack of awareness and public policies, highlighting the importance of monitoring and management policies to control and mitigate social and health problems.

Further studies regarding the microplastic impacts on marine fauna and whether they could transfer adsorbed pollutants such as persistent organic pollutants (POPs), heavy metals, and plastic additives to the food web are necessary, as microplastics particles can be transferred along the trophic chain, the chances to accumulate other pollutants in the food web increases. In fishery resources, this question is a public health matter because it is linked to human uptake of these pollutants. Our study also emphasises the importance of implementing protocols to extract microplastics in biological samples, which guarantee the quality of samples, avoid under or overestimation and airborne contamination, and which can be easily replicable.

## CRediT authorship contribution statement

**Anne K.S. Justino:** Conceptualization, Methodology, Validation, Investigation, Formal analysis, Writing - original draft. **Véronique Lenoble:** Supervision, Methodology, Writing - review & editing. **Latifa Pelage:** Formal analysis, Writing - review & editing. **Guilherme V.B. Ferreira:** Methodology, Validation, Writing - review & editing. **Rafaela Passarone:** Validation, Writing - review & editing. **Thierry Frédou:** Supervision, Writing - review & editing, Funding acquisition. **Flávia Lucena Frédou:** Project administration, Supervision, Resources, Writing - review & editing, Funding acquisition.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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