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# Ingestion of microplastics by *Hypanus guttatus* stingrays in the Western Atlantic Ocean (Brazilian Amazon Coast)

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## ARTICLE INFO

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Longnose stingray
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## ABSTRACT

The present study documents, for the first time, the ingestion of microplastics (MPs) by Longnose stingrays in the Western Atlantic Ocean. We examined 23 specimens of *Hypanus guttatus* from the Brazilian Amazon coast and found microplastic particles in the stomach contents of almost a third of the individuals. Fibers were the most frequent item (82%), blue was the most frequent color (47%) and Polyethylene Terephthalate (PET) was the most frequent polymer recorded (35%), as identified by 2D imaging - Fourier Transform Infrared (FTIR). The ingestion of microplastics by Longnose stingray has not been previously recorded. The findings of the present study thus provide an important baseline for future studies of microplastic ingestion by dasyatid rays and other batoid species in the Atlantic Ocean, and contribute to the broader understanding of the spatial and temporal dimensions of the growing problem of plastic pollution in aquatic ecosystems and organisms.

Microplastics (MPs) are now widely distributed in the environment, reaching even the remotest areas of the oceans, and infiltrating food webs worldwide (Germanov et al., 2019). These particles are potential carriers of persistent organic pollutants (POPs) and metals (Yu et al., 2019). Microplastics are normally defined as plastic particles with a maximum dimension of less than 5 mm (Arthur et al., 2009). These particles can be classified according to their origin as either primary or secondary MPs. Primary MPs are produced intentionally as micro-sized particles for use in cosmetics and a range of other industrial applications (Ogata et al., 2009), while secondary MPs are produced by the physical or chemical degradation of larger plastic waste by the environment (Cole et al., 2011; Godoy et al., 2019). Given their small size and abundance, MPs can be actively ingested by a wide range of organisms (Eriksen et al., 2014; Herrera et al., 2019), when the MPs are mistaken for prey, or passively, through the unintentional ingestion of the particles during normal feeding activities (Campbell et al., 2017; Desforges et al., 2015).

Despite the large number of studies that have focused on the ingestion of MPs by marine teleost fishes (e.g. Markic et al., 2018; Murphy et al., 2017; Pegado et al., 2018), few data are available on elasmobranchs, and most of which refer to sharks or pelagic rays (Alomar and Deudero, 2017; Anastasopoulou et al., 2013; Germanov et al., 2019; Valente et al., 2019). Up to now, only two reports have apparently been published on the ingestion of MPs by benthonic rays in marine environments; Neves et al. (2015) recorded MPs in specimens of *Raja asterias*, off the coast of Portugal and Pegado et al. (2018) that found MPs in an individual of *Narcine brasiliensis* from Amazon river estuary. However, both studies analyzed less than 10 individuals, which Markic et al. (2020) considered to be a suboptimal sample size for a reliable estimate of plastic ingestion rates.

Elasmobranchs are commercially important fishes, being consumed widely by some Latin American populations, from the Caribbean coast to northeastern Brazil (Feitosa et al., 2018; Rodrigues et al., 2020; Schmid et al., 2019). This suggests that the ingestion of microplastics by stingrays and sharks may eventually also affect human food safety and health (Van Cauwenberghe and Janssen, 2014). The Longnose stingray, *Hypanus guttatus* (Bloch and Schneider, 1801), a species of the family Dasyatidae, is an opportunistic, benthonic predator (Gianeti et al., 2019; Last et al., 2016), distributed from the southern Gulf of Mexico to southeastern Brazil (Bigelow and Schroeder, 1953; Rosa and Furtado, 2016). This species may reach up to 2 m in disc width and is very common as by-catch in the artisanal and industrial fisheries along the northern and northeastern coasts of Brazil (Rodrigues et al., 2020; Tagliafico et al., 2013). The present study investigated the presence of

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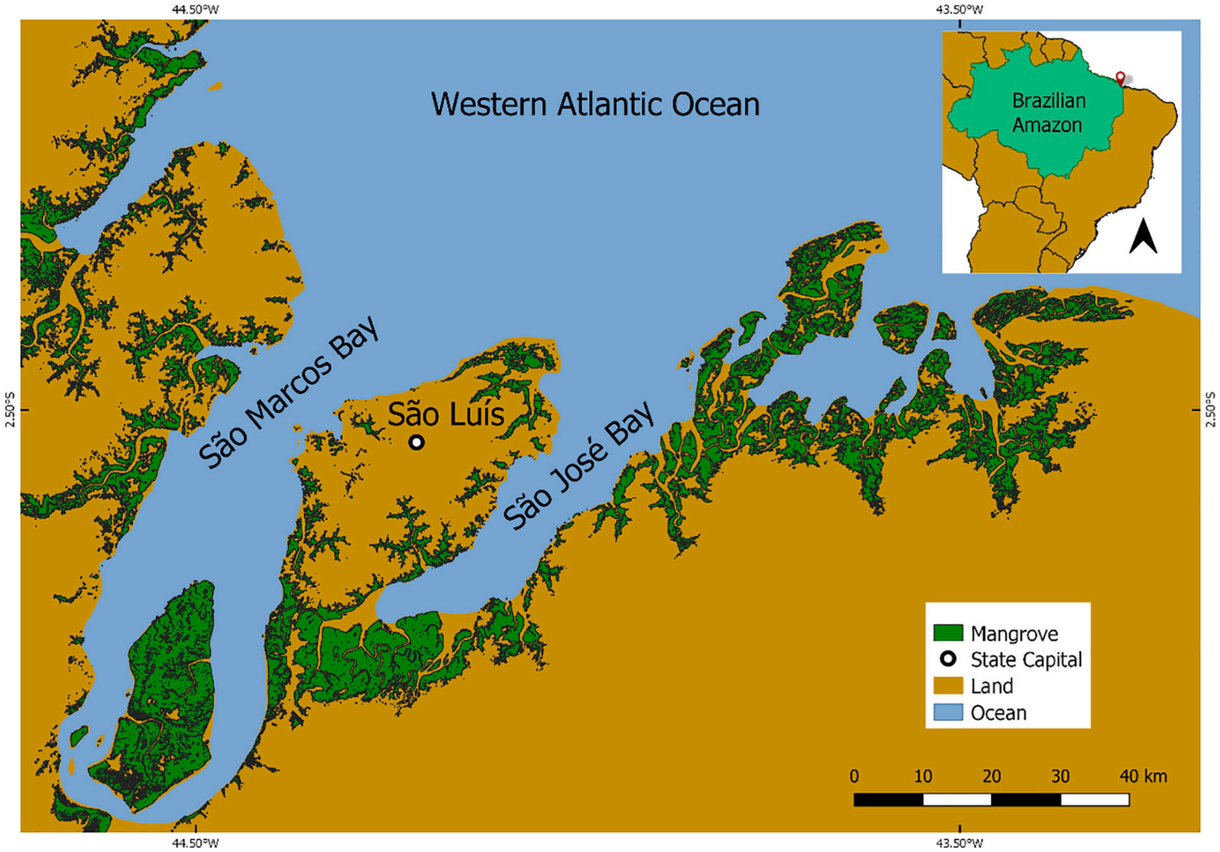

**Fig. 1.** Map of the Maranhão Gulf estuarine complex, located on southern extreme of the Brazilian Amazon coast in the Western Atlantic Ocean, where the Longnose stingray (*Hypanus guttatus*) individuals analyzed in this study were captured.

MPs in *H. guttatus* from the southern extreme of the Brazilian Amazonian coast. The study also provides an important baseline for future comparisons of the abundance, shape, and color of the microplastics found in the stomach contents of elasmobranch species.

The Maranhão Gulf is located at the southern extreme of the Brazilian Amazonian coast (Fig. 1), and is formed by the bay of São Marcos and São José, on either side of São Luís Island (Castro et al., 2018; Teixeira and Souza Filho, 2009). São Luís, the capital of Maranhão state, with its population of more than one million inhabitants, is located on this island (IBGE, 2010). This whole area forms an estuarine complex that covers an area of 5414 km$^2$ (Souza Filho, 2005) and has an extreme semidiurnal macrotidal regime, with mean tidal amplitude of 3–7 m (Castro et al., 2018; Teixeira and Souza Filho, 2009). The local climate is tropical humid, with an annual precipitation of approximately 2300 mm (Fisch et al., 1998) and a mean temperature of 26 °C (Castro et al., 2018; Teixeira and Souza Filho, 2009).

The 23 Longnose stingray specimens analyzed in the present study were obtained from local fishers, and were captured by longlines and gillnets between August 2018 and March 2019. All individuals were immediately transported to the laboratory on ice in portable coolers. The length and width of the disc of each specimen were measured, and they were then eviscerated through a longitudinal incision in the abdominal area, using surgical forceps and a scalpel. The stomachs were removed carefully, and their contents placed in Petri dishes for analysis under a stereomicroscope (ZEISS Stemi DV4) at a magnification of 8× to 32×. All the MPs identified during this analysis were placed in Petri dishes containing distilled water, dried at 35 °C for 48 h, and then separated according to shape and color. All the material and equipment used during the laboratory processing were cleaned constantly and protected from possible external contamination. Therefore, sample processing (extraction and stomachs contents analysis) was executed under a laboratory fume hood, by personnel using natural fiber clothing and maintaining doors and windows closed. To guarantee the accuracy of the readings, a clean Petri dish was placed beside the stereomicroscope during the analysis of the stomach contents and inspected after the processing of the sample, to identify possible external contamination by MPs existing in the laboratory environment.

The findings of this analysis are presented here through descriptive statistics, including the mean, minimum, and maximum numbers of microplastic items, the percentages of the different categories of shape and color, as well as the polymeric composition of the particles, and the frequency of occurrence (FO%) of the microplastics found in the stomach contents. The FO% was calculated by: FO% = (Ni / N) × 100, where Ni = the number of stomachs that contained microplastic particles, and N = total number of stomachs examined.

Samples of each category of microplastic particle found in the gastrointestinal tracts of the stingrays were separated for 2D imaging-Fourier transform infrared (FTIR) analysis. The FTIR analysis was conducted directly on the dry filters (with no further processing), using a Cary 620–670 FTIR microscope, equipped with a 128 × 128 FPA detector (Agilent Technologies). The spectra were recorded directly on the surface of the samples (or of the Au background) in reflectance mode, with an open aperture and a spectral resolution of 8 cm$^{-1}$, with 128 scans being acquired for each spectrum. A "single-tile" analysis resulted in a map of 700 × 700 μm$^2$ (128 × 128 pixels), with each imaging map having a spatial resolution of 5.5 μm (i.e., each pixel has an area of 5.5 × 5.5 μm$^2$).

The discs of the stingray specimens had a mean length of 52.3 (SD ± 8.68) cm, with a minimum of 32.4 cm and maximum of 72.0 cm, and a mean width of 54.6 (SD ± 10.0) cm, ranging from 34 cm to 83 cm (Table 1). Almost a third (FO% = 30.43%) of the samples contained microplastics, a value similar to that recorded in

**Table 1**
Biometrics of the Longnose stingray (*Hypanus guttatus*) specimens and the characteristics (shape, color, and type of polymer) of the microplastic particles (MPs) found in their stomach contents. The presence of MPs is expressed as the presence (1) or absence (0). The polymers are: ABS = Acrylonitrile Butadiene Styrene; PA = Polyamide; PE = Polyethylene; PET = Polyethylene Terephthalate; PP = Polypropylene; SBR = Styrene-Butadiene Rubber.

| Stingray | Disc length (cm) | Disc width (cm) | Presence of MPs | Shape of the MPs | Color of the MPs | Number of MPs | Polymer |
|---|---|---|---|---|---|---|---|
| 1 | 55 | 58 | 0 | – | – | 0 | – |
| 2 | 55 | 59 | 0 | – | – | 0 | – |
| 3 | 56.5 | 60 | 1 | Fiber | Transparent | 6 | PET, PP, PA |
| 4 | 56 | 57.5 | 1 | Fragment | Blue | 2 | ABS |
| 5 | 51 | 54 | 0 | – | – | 0 | – |
| 6 | 56.5 | 54.5 | 0 | – | – | 0 | – |
| 7 | 51 | 56 | 0 | – | – | 0 | – |
| 8 | 57 | 61 | 0 | – | – | 0 | – |
| 9 | 57.5 | 58.5 | 1 | Fiber | Red | 1 | Blend (PET + SBR) |
| 10 | 72 | 73.5 | 0 | – | – | 0 | – |
| 11 | 41.5 | 41 | 1 | Fiber | Blue | 3 | PET, PE |
| 12 | 51.5 | 55 | 0 | – | – | 0 | – |
| 13 | 72 | 83 | 1 | Fiber | Black | 2 | PA |
| 14 | 52 | 55.5 | 0 | – | – | 0 | – |
| 15 | 52.5 | 56 | 0 | – | – | 0 | – |
| 16 | 45.5 | 48 | 1 | Fragment | Blue | 1 | ABS |
| 17 | 48.3 | 52 | 0 | – | – | 0 | – |
| 18 | 43.3 | 45.5 | 0 | – | – | 0 | – |
| 19 | 44.8 | 48.5 | 0 | – | – | 0 | – |
| 20 | 49 | 53 | 0 | – | – | 0 | – |
| 21 | 43 | 45 | 0 | – | – | 0 | – |
| 22 | 46 | 49 | 1 | Fiber | Blue | 2 | PE |
| 23 | 32.4 | 34 | 0 | – | – | 0 | – |

benthonic rays (43%) from the Portuguese coast (Neves et al., 2015). This relatively high incidence of MP ingestion may be related to the foraging strategy of the species (Romeo et al., 2015). The stingray *H. guttatus* is an important predator of benthic and benthopelagic coastal organisms, feeding on a wide range of prey. As a generalist top predator when adult, it seems likely that these individuals were susceptible to bioaccumulated microplastic contamination through the food chain, by passive ingestion (Gianeti et al., 2019).

A total of 17 microplastic particles were found in the stomach contents of seven stingrays, with a mean of 2.4 (SD ± 1.7) particles per individual ($N = 7$ individuals), ranging from one to six particles in a given individual. The majority (82%) of the particles found in our study were classified as fibers and the other 18% as fragments, which were primarily blue (47%) or transparent (35.3%), with some black (11.8%) and red (5.9%) particles (Fig. 2).

Neves et al. (2015) recorded a mean of only 0.5 (SD ± 0.8) particles per individual in *Raja asterias*, and found only fibers in the stomach content of this ray. Many authors have found that fibers are the most abundant microplastic particles in marine environments (Alomar and Deudero, 2017; de Lucia et al., 2018, 2014; Neves et al., 2015; Rochman et al., 2015). Our findings further support that the marine biota, including benthic stingrays like *H. guttatus*, may be most exposed to microplastic fibers. The distribution of microplastics in the oceans may be influenced directly by anthropogenic processes (Barnes et al., 2009) and large amounts are found in aquatic environments near areas of urban development (Garcia et al., 2020). In Sao Luis, like many other largest cities in the Amazonian region, such as Manaus and Belém, due to the lack of environmental awareness and efficient waste management, more than 19% of the urban solid waste, including plastics, is not collected by municipalities and an unknown fraction of this mismanaged waste is washed into the Gulf of Maranhão (Giarrizzo et al., 2019).

Further, Maranhão is recognized as one of the most important states for artisanal fisheries in Brazil's northern and northeastern regions (Almeida and Isaac-Nahum, 2015). This potentially contributes to the high presence of filaments in the coastal and estuarine ecosystems, originated by the fragmentation of fishing gear (Soares et al., 2017). These particles are introduced into marine environments through ports and fisheries activity, wastewater treatment plants, urban runoff (Peters and Bratton, 2016), and river discharge (Woodall et al., 2014). Strong macro-tidal currents and other oceanographic phenomena (e.g. the permanent east-to-west prevailing winds) found in this region may contribute to the ample dispersal of microplastics through the known accumulating effects of enclosed or semi-enclosed bays within metropolitan urban areas (Auta et al., 2017).

Six types of polymer were identified in the microplastic particles analyzed by 2D FTIR Imaging in the present study (Fig. 3). The most frequent polymer was Polyethylene Terephthalate (PET; 35.3%), followed by Polyamide (PA), Acrylonitrile butadiene styrene (ABS), and Polyethylene (PE), each with a frequency of occurrence of 17.6%, and then Polypropylene (PP) and PET + SBR (Styrene Butadiene Rubber), both with a frequency of 5.9%. The predominance of PET is consistent with the fact that it is one of the polymers most produced by industries, worldwide, and thus more likely than others to be present in the marine environment (Andrady, 2011). This polymer is used in the production of textiles, including clothes, blankets, and fleeces, as well as bottles (Wang et al., 2017). Therefore, PET fibers are common in domestic wastewater, in particular from washing machines, which contaminates river basins and, eventually, oceans (Browne et al., 2011; Napper and Thompson, 2016). As a relatively dense polymer, PET is also more likely to sink to the bottom of aquatic environments, where it can be ingested by benthic organisms (GESAMP, 2015), including the Longnose stingray. The second most common polymers were PE and PA, which could come from the fishing gears, like nets and floats that are often have these polymers in their composition (GESAMP, 2016). Over time, however, lower-density polymers, such as PP and PE, may decompose and sink, and thus become available to a variety of benthic organisms (Long et al., 2015; Morét-Ferguson et al., 2010).

In the present study, microplastic particles were found in the stomach contents of almost one third of the analyzed *H. guttatus* specimens. This stingray species is an important target of the artisanal fisheries of Maranhão State, at the Latin America and in southern extreme of the Brazilian Amazon coast. Most of the particles were fibers, and the most frequent polymer was PET. With 23 specimens analyzed, the present study provides a more reliable estimate than the previous reports of microplastic ingestion by benthonic rays. Our study provides the first record of ingestion of MPs by *Hypanus guttatus* from the Western Atlantic Ocean, as well as an important database for further comparisons of the exposure of this elasmobranch group to plastic

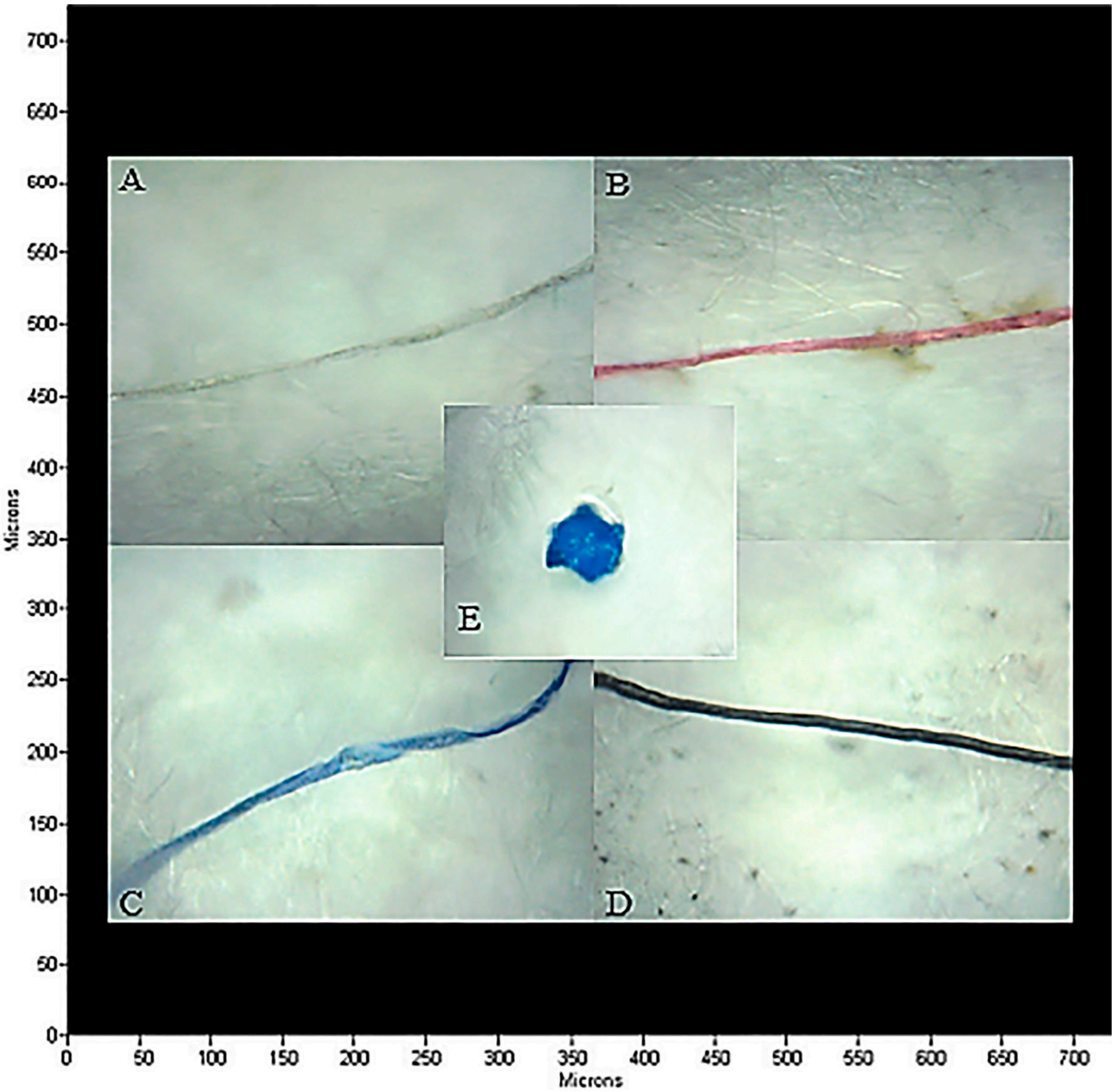

**Fig. 2.** Examples of the different categories of microplastic found in the stomach content of the Longnose stingray *Hypanus guttatus* specimens collected from the Gulf of Maranhão. A) Transparent Fiber; B) Red Fiber; C) Blue Fiber; D) Black Fiber; E) Blue Fragment. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

contaminants in the marine environment. Such investigations, specifically for understudied areas and species, are important contributions towards the understanding of spatial and temporal patterns of plastic pollution in aquatic ecosystems and organisms, as well as to support effective prevention and conservation efforts in response to this global problem.

## CRediT authorship contribution statement

**Tamyris Pegado:** Methodology, Formal analysis, Writing - original draft, Visualization, Writing - review & editing. **Lucio Brabo:** Formal analysis, Writing - original draft, Visualization, Writing - review & editing. **Kurt Schmid:** Writing - original draft, Visualization, Writing - review & editing. **Francesco Sarti:** Formal analysis, Resources, Writing - review & editing. **Thaís T. Gava:** Formal analysis, Investigation, Writing - review & editing. **Jorge Nunes:** Conceptualization, Resources, Writing - review & editing. **David Chelazzi:** Methodology, Formal analysis, Resources, Writing - review & editing. **Alessandra Cincinelli:** Resources, Writing - review & editing. **Tommaso Giarrizzo:** Conceptualization, Methodology, Formal analysis, Writing - review & editing, Writing - original draft, Resources, Visualization, Supervision.

## 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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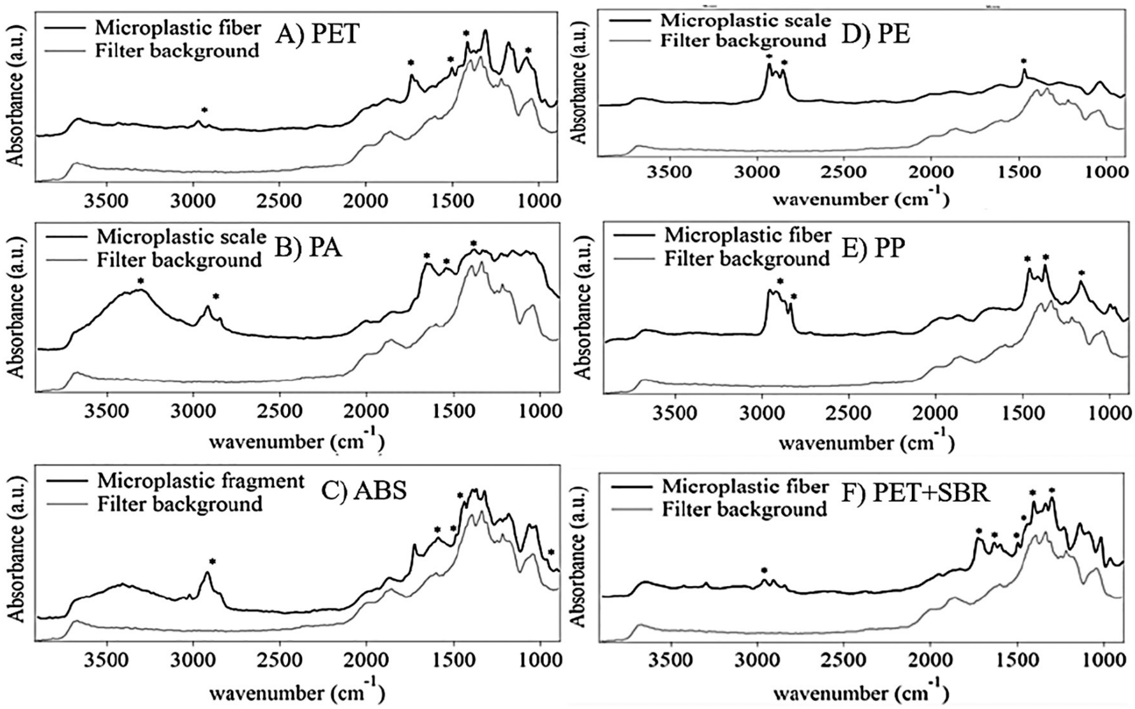

**Fig. 3.** Representative FTIR reflectance spectra acquired different microplastic polymers, collected from the stomach contents of the Longnose stingray *Hypanus guttatus* from the Maranhão Gulf, Brazil. A) PET: Polyethylene Terephthalate; B) PA: Polyamide; C) ABS: Acrylonitrile Butadiene Styrene; D) PE: Polyethylene; E) PP: Polypropylene; F) Blend of PET (Polyethylene Terephthalate), and SBR (Styrene-butadiene rubber).

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