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Plastic intake does not depend on fish eating habits: Identification of microplastics in the stomach contents of fish on an urban beach in Brazil

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ABSTRACT

This study aims to identify, classify, quantify the ingested microplastic by marine teleost fish, in order to analyze the relationship between microplastic and trophic guilds. Food items of 214 individuals of *Opisthonema oglinum*, *Bagre marinus*, *Cathorops spixii*, *Sciades herzbergii*, *Chloroscombrus chrysurus*, *Conodon nobilis*, *Haemulopsis corvinaeformis* were analyzed. The species were classified according to their trophic guilds (zoobenthivorous or opportunistic/omnivorous). All species ingested microplastic and contamination occurred independently of the trophic guild. Of the sampled fish, 55% were contaminated by microplastic. The most consumed categories were blue (28%) and transparent filaments (20%). Raman spectroscopy measurements detected that most sampled filament corresponds to blue synthetic fiber (polyester). This study can contribute by filling gaps in knowledge regarding sandy beach impacts, which are environments so highly threatened by human activities around the world and are neglected in terms of use and conservation plans.

1. Introduction

Marine litter is recognized as an environmental problem, and plastic is the most abundant waste and the most discussed and studied component lately (Galgani et al., 2015; Gesamp, 2016; Brate et al., 2017). The plastic material dispersed in the sea, when fragmented, due to weathering, aggression of ultraviolet rays and action of waves and currents, degrades into smaller size, becoming microplastic (ranging from 0.0001 to 5.0 mm) (Cole et al., 2011; Gesamp, 2016; UNE, 2018).

Ingestion of microplastics has been documented in a variety of marine taxa representing various trophic levels. This variety includes mammals (Denuncio et al., 2011; Besseling et al., 2015), turtles (Santos et al., 2015; Duncan et al., 2018), seabirds (Brandão et al., 2011; Kühn and Franeker, 2012; Roman et al., 2016) and fish (Boerger et al., 2010; Romeo et al., 2015; Baalkhuyur et al., 2018). Zoobenthic organisms are also suffering from contamination by microplastic particles, such as annelids (Cauwenberghe et al., 2015), bivalve mollusks (Cauwenberghe and Janssen, 2014; Li Jiana et al., 2016), sea cucumbers (Graham and Thompson, 2009), crustaceans (Murray and Cowie, 2011), amphipods and barnacles (Thompson et al., 2004; Hodgson et al., 2018) as well as the zooplankton as copepods, daphnia and fish larvae (Collignon et al., 2012; Cole et al., 2013; Desforges et al., 2015; Sun et al., 2017; Steer et al., 2017).

Several studies have been developed with the purpose of identifying the fish species containing microplastic particles in their stomachs. Microplastics were recorded in planktivorous fish caught in the North Pacific Gyre (Boerger et al., 2010), in pelagic and demersal species caught in the English Channel (Lusher et al., 2013), in piscivorous sampled in the North Pacific Ocean (Jantz et al., 2013), in the North Sea and the Baltic Sea (Foekema et al., 2013; Rummel et al., 2016) in pelagic and demersal fishes of the Mediterranean Sea (Romeo et al., 2015; Bellas et al., 2016), in the Portuguese Coast (Neves et al., 2015), in Atlantic cod on the coast of Norway (Brate et al., 2016), in coastal and freshwater fish in China (Jabeen et al., 2017) and along the coast of the Red Sea in Saudi Arabia (Baalkhuyur et al., 2018).

In Brazil, studies are concentrated in the Northeast region, where microplastic ingestion was recorded in three catfish species (*Cathorops spixii*, *Cathorops agassizii* e *Sciades herzbergii*) (Possatto et al., 2011) and

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the contamination by nylon fragments in two species of Sciaenidae (*Stellifer brasiliensis* and *Stellifer stellifer*) in estuary and coastal zone in northeastern Brazil (Dantas et al., 2012). Pellets were found in the stomach of two species of fish (*Scomberomorus cavala* and *Rhizoprionodon lalandii*) commercialized in the city of Salvador, State of Bahia (Miranda and Carvalho-Souza, 2016); there was record of microplastic ingestion in the fish assemblage of two estuaries subjected to different anthropogenic pressures in the state of Paraíba (Vendel et al., 2017). Contamination by microplastic was evaluated in the life cycle of yellow hake (*Cynoscion acoupa*) (Ferreira et al., 2016; Ferreira et al., 2018) and two haemulids (*Pomadasys ramosus* and *Haemulopsis corvinaeformis*) (Silva et al., 2018) in estuarine regions. The diet of two top predators (*Centropomus undecimalis* and *C. mexicanus*) from an estuary was analyzed and it was recorded the intake of filaments in different sites of the estuary and life cycle (Ferreira et al., 2019). Another study described the ingestion of plastic fragments and identified the possible effect of this contamination on the condition factor of the catfish *Genidens genidens*, in the Laguna estuarine system (Dantas et al., 2019). In this sense, it is important to report the problem of plastic pollution in fishery resources as an environmental monitoring tool (Brate et al., 2017).

Since microplastic contamination has been documented in marine food web, studies that focus on the relationship between microplastic contamination and trophic guilds are needed. Trophic guild is defined as a group of species that exploit the same kind of environmental resources in a similar way (Root, 1967). For this reason, guild classification synthesizes and reduces the complexity of dietary data and facilitates comparisons among food webs (Elliott and Quintino, 2007).

In this sense, it is not known exactly how the marine environment and its organisms will respond ecologically to the impacts of plastic pollution. For this purpose, studies focusing on microplastic ingestion are essential to understand and predict the consequences of this contamination. They are important for developing effective management plans for coastal and marine ecosystems threatened by human activities. In order to improve the knowledge to tackle these challenges created by plastic pollution, it was hypothesized that the amount of microplastic ingested varies according to the trophic guild of the species.

2. Material and methods

2.1. Study area

The study was carried out in Fortaleza coastal zone (03°43′01″ S e 038°32′35″ W), capital of Ceará state, on the Meireles urban beach (Fig. 1). The city has a tropical climate, with an annual average temperature and rainfall of 26.3 °C and 1448 mm per year, respectively. The rainy season occurs in summer, with a peak in April, registering an average of 329 mm and 13 mm of rainfall in the dry season, whose peak occurs in October (FUNCEME - Fundação Cearense de Meteorologia e Recursos Hídricos, 2018).

In Fortaleza city, the edge of Beira Mar Avenue is considered one of the main tourist attractions. Most hotels and tourist services are located on this avenue, representing a pole for national and international tourists. The beach is heavily used throughout the year as it has infrastructure available as several beach stalls and kiosks on the sand strip (Projeto Orla, 2006).

2.2. Sampling

Fish were sampled monthly from November 2015 to January 2017 in the low spring tide along two groins 1.4 km apart. A beach seine net (15 m long, 2 m high, 2 m deep and 3 cm mesh) was used to capture the specimens. The net was directed and extended by a wooden boat of 4.5 m of length, powered by a 5.5 HP outboard motor. After carrying out the siege of the net with the boat, the gear was pulled out by human traction and perpendicular to the beach line, so as to run the entire lateral extension of the groin.

Two trawls were performed at each groin, being one on the leeward side (E1S, E2S) and the other on the windward (E1B, E2B), totaling 60 trawls. Fish were sampled under the authorization for activities with scientific purpose of number 29819669 issued by ICMBio/SISBIO (Chico Mendes Institute for Biodiversity Conservation/Biodiversity Information and Authorization System).

2.3. Analysis of stomach content (food items and microplastic)

Fish were identified, counted and dissected, stomachs were removed and preserved in Eppendorf microtubes containing 70% ethanol. The species selected for the analysis of stomach contents were: *Opisthonema oglinum* (Lesueur, 1818), *Bagre marinus* (Mitchill, 1815), *Cathorops spixii*

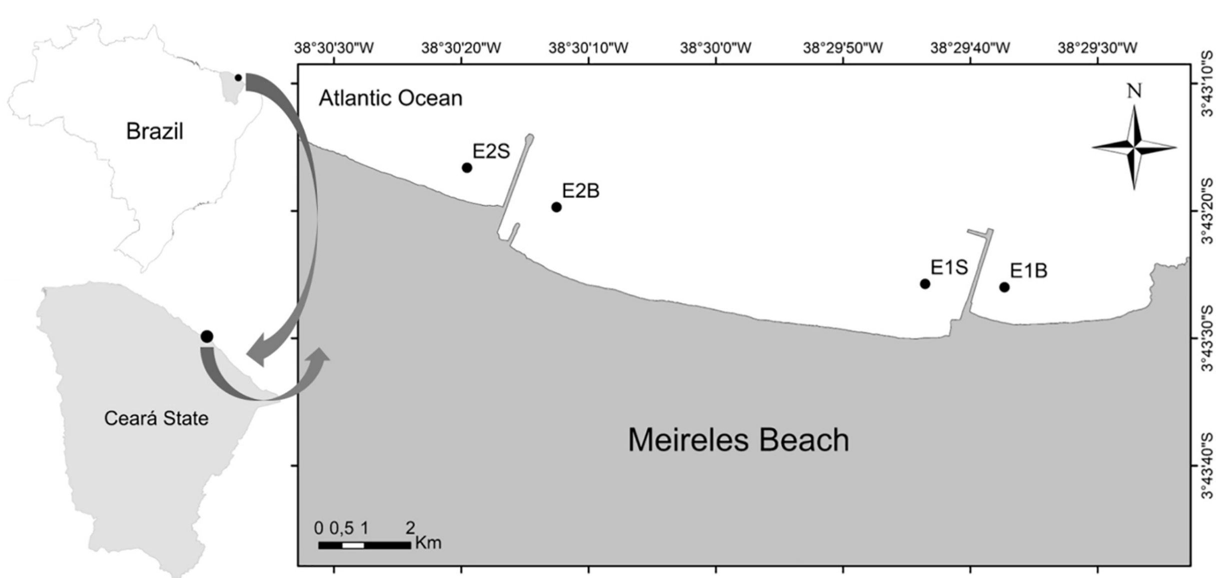

Fig. 1. Map of the study area showing the sampling sites (E1B, E1S, E2B, E2S) in Meireles Beach, Ceará State, Northeastern, Brazil.

Table 1
Classification of the trophic guilds, frequency and abundance of microplastic ingestion found in the stomachs of the fish species *Opisthonema oglinum, Sciades herzbergii, Cathorops spixii, Chloroscombrus chrysurus, Bagre marinus, Haemulopsis corvinaeformis, Conodon nobilis* caught at Meireles beach, Fortaleza, Brazil. Legend: Trophic guilds: ZB (zoobenthivorous), OP (opportunistic/omnivorous), N: Number of stomachs analyzed per species, Relative Frequency (%), Numerical Abundance, Med: Median abundance of ingestion per species and range of data.

Family/species	Guild	N	Relative frequency (%)	Numerical abundance	Med. max/min
Clupeidae					
Opisthonema oglinum (Lesueur, 1818)	OP	31	65	54	1.5 (0–6)
Ariidae					
Bagre marinus (Mitchill, 1815)	OP	27	37	25	1.0 (1–5)
Cathorops spixii (Agassiz, 1829)	ZB	33	75	103	2.5 (1–14)
Sciades herzbergii (Bloch, 1794)	ZB	31	42	48	1.0 (1 – 10)
Carangidae					
Chloroscombrus chrysurus (Linnaeus, 1766)	OP	31	61	42	1.0 (1–5)
Haemulidae					
Conodon nobilis (Linnaeus. 1758)	OP	34	56	34	1.0 (1–5)
Haemulopsis corvinaeformis (Steindachner. 1868)	ZB	28	46	21	1.0 (1–5)

Source: Prepared by the authors.

(Agassiz, 1829), *Sciades herzbergii* (Bloch, 1794), *Chloroscombrus chrysurus* (Linnaeus, 1766), *Conodon nobilis* (Linnaeus, 1758), *Haemulopsis corvinaeformis* (Steindachner, 1868).

The stomach contents were placed in a Petri dish, where it was possible to separate food items and microplastic. The volume of each food item was determined using a Petri dish with 1 mm of height. A graph paper was placed below the Petri dish in order to calculate the volume of the item by the ratio of width and height (Albrecht and Caramaschi, 2003). The microplastic was separated using tissue forceps and after separation, the microplastics were counted and classified according to the type (filament or fragment) and coloration (Herring et al., 2015). Filaments and fragments < 5 mm were classified as microplastics (Arthur et al., 2009). Microplastic were identified under a binocular stereo microscope.

To avoid contamination, Petri dishes, scissors and tweezers were cleaned with distilled water and then with 70% alcohol, disposable latex gloves were used during all analyses of the stomach contents. After the microplastic classification, they were treated for the removal of organic matter from their surfaces. They were immersed in 5 mL 35% PA Hydrogen Peroxide and heated on a TECNAL® hot plate at 75 °C for 30 min, according to the methodology adapted from NOAA - National Oceanic and Atmospheric Administration (Herring et al., 2015).

For each specimen the relative frequency (expressed by the percentage of microplastic intake per species and each microplastic category) and numerical abundance (amount of intake per species and of each microplastic category) were estimated.

In order to characterize the particles of the plastic polymers, the Raman spectroscopy technique was utilized. This method consists in a non-destructive chemical analysis technique that provides information about chemical structure, crystallinity, and molecular interactions. The Raman spectroscopy measurements were performed in a Horiba LabRaman spectrometer equipped with a liquid N_2-cooled CCD system. Adapted methodology (Gesamp, 2016). The spectrometer slits were set for a resolution of 2 cm^{-1}. The 785 nm line laser was used to excite the Raman signal. The microplastics samples were mounted on slides and covered with coverslips. Five slides were analyzed per species. For analysis with the Raman technique, 35 slides were prepared, but only 16 were possible to be analyzed. The difficulty in reading was related to a strong fluorescent background observed on some slides. This color pattern significantly overlapped the intensity of the Raman signal, making it impossible to accurately characterize the microplastics. The resolution and laser line adjustments that enabled the reading were not found. The plastic polymers were identified using Bio-Rad's knowltAll® QC Expert software data base.

2.4. Analysis of the trophic level and classification of the trophic guilds

The trophic position of fish was calculated using TrophLab software, available at Fishbase (http://www.fishbase.org/Download/) (Froese and Pauly, 2018). The estimated Troph values range from 2.0 to 5.0, with values close to 2.0 for herbivorous/detritivorous consumers and 5.0 for piscivorous/carnivorous animals (Pauly and Palomares, 2000).

Fish were classified into functional food groups (trophic guilds) based on their food preferences (predominant items in the diet) and Troph values. The functional groups were classified according to methodology proposed by Elliott and Quintino (2007).

2.5. Data analysis

To evaluate significant differences in microplastic composition ingestion among species, a Permutational Multivariate Analysis of Variance (PERMANOVA; Anderson, 2014), using Bray-Curtis dissimilarity matrices as a measure of distance, with log10-transformed abundance of microplastic ingested and trophic guilds data was applied. Fishes with empty stomach were exclude from analysis. Statistical analyses were conducted in R Programming Environment using the vegan package (www.r-project.org).

3. Results

A total of 214 stomach contents of the seven fish species from the urban beach of Meireles, Fortaleza, Ceará were analyzed (Table 1). Among the analyzed stomachs, 55% were contaminated by microplastic particles, containing 0 to 14 particles. The frequency of contamination varied among species, where *Cathorops spixii* (75%), *Opisthonema oglinum* (65%), *Chloroscombrus chrysurus* (61%), *Conodon nobilis* (56%), *Haemulopsis corvinaeformis* (46%) and *Bagre marinus* (37%) specimens were contaminated by microplastic (Table 1, Fig. 2). A total of 327 microplastics were counted, divided into 10 different types (Table 2, Fig. 3).

C. spixii presented alone an amount of ingestion of 48% of the total of all microplastic sampled (Table 1), however PERMANOVA result was not significant for the species ($F_{(4,130)}$ = 0.97; p = .46) or guilds ($F_{(1,130)}$ = 0,01; p = .06) (Fig. 4, Table 3), indicating that it was not possible to determine a trophic guild as more susceptible to microplastic ingestion, contrary to our initial hypothesis. In other words, the species consumed microplastics in similar amounts and this amount of ingestion is independent of feeding habits.

The Raman spectra of sixteen slides were measured on the LabRaman Spectrometer (Figs. 5 and 6). A total of three plastic polymers were identified using Bio-Rad's knowltAll® QC Expert software

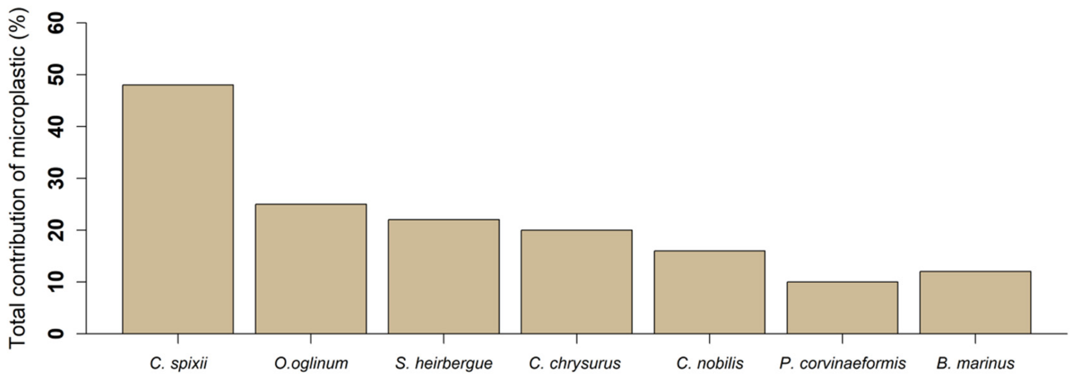

Fig. 2. Values of Relative Abundance (%) of microplastic ingestion by the fish species *Cathorops spixii, Opisthonema oglinum, Sciades herzbergii, Chloroscombrus chrysurus, Conodon nobilis, Haemulopsis corvinaeformis, Bagre marinus,* caught at Meireles beach, Fortaleza, State of Ceará, Brazil.

Table 2
Classification, frequency and abundance of the types of microplastic found in stomachs of the fish species *Opisthonema oglinum, Sciades herzbergii, Cathorops spixii, Chloroscombrus chrysurus, Bagre marinus, Haemulopsis corvinaeformis, Conodon nobilis* caught at Meireles beach, Fortaleza, Brazil. Microplastic: classification of microplastic as to type and color.

Microplastic	Relative frequency (%)	Numerical abundance
Blue filament	28	112
Transparent filament	20	85
Transparent fragment	14	35
Black filament	11	30
Red filament	7	19
Green filament	5	13
Blue fragment	4	9
Purple Filament	2	8
Pellets	2	14
Styrofoam Fragment	0	2

Source: Prepared by the authors.

data base: Pristine Polyester textile + impurity, being these impurities detected as TNT (Non-Woven Fabric), which represented 68.75% of the compounds identified (Fig. 7), Poly (*p*-phenylene terephthalamide) – Kevlar was 12.5% and Copper Phthalocyanine with 6.25%.

4. Discussion

It is likely that the ingestion should occur during normal fish feeding activities (Dantas et al., 2012). This is because there are particles of different sizes present in the sediment and in the water column (Cole et al., 2011; Chubarenko et al., 2016) and are available for fish mixed with their food items, being accidentally ingested (Possatto et al., 2011). For this reason, it was not possible to identify which species is most susceptible to microplastic ingestion based on their feeding habits.

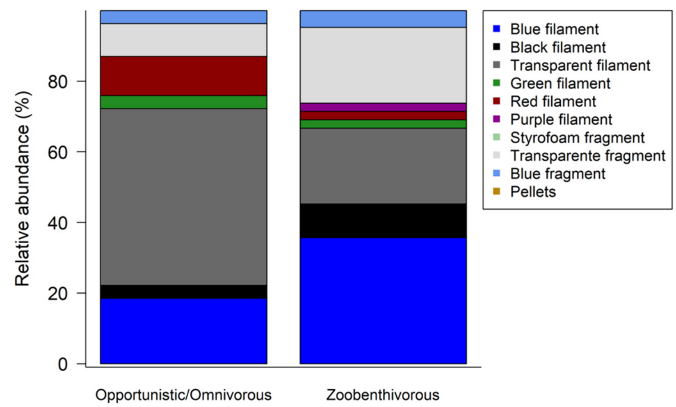

Fig. 4. Relative Abundance (%) of quantity ingestion of each microplastic category for trophic guild of the fish species Opisthonema oglinum, Sciades herzbergii, Cathorops spixii, Chloroscombrus chrysurus, Bagre marinus, Haemulopsis corvinaeformis, Conodon nobilis caught at Meireles beach, Fortaleza, State of Ceará, Brazil.

However, it is likely that organisms such as copepods and shrimp, which are selective foragers, may preferentially ingest microplastic particles that are involved by biofilm (community of microorganisms) by confounding it with a food of high nutritional value (Dahms et al., 2007). This can facilitate the transfer of these particles along the trophic chain (Ward and Kach, 2009; Rummel et al., 2017). In this case, fish can ingest microplastic directly when feeding in the water column, when foraging by stirring the substrate and indirectly when consuming

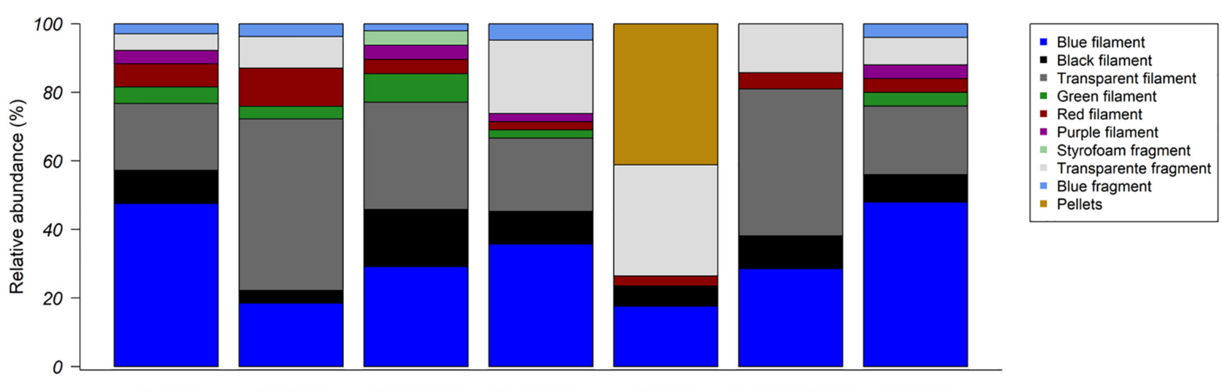

Fig. 3. Relative Abundance (%) of quantity ingestion of each microplastic category for *Cathorops spixii, Opisthonema oglinum, Sciades herzbergii, Chloroscombrus chrysurus, Conodon nobilis, Haemulopsis corvinaeformis, Bagre marinus,* caught at Meireles beach, Fortaleza, State of Ceará, Brazil.

Table 3
PERMANOVA results based on the matrix of abundance of microplastic ingested and species and trophic guilds. Highlighted *p* values in bold indicate significant variation for $p < .05$. Legend: Sum of sqrs - sum of squared deviations, df - degrees of freedom, F - PERMANOVA F statistic, p - statistical significance.

	Sum of sqrs	df	Mean square	F	p
Trophic guilds	0.75	1	0.75	2.16	0.06
Species	1.35	4	0.34	0.03	0.46
Residual	43.14	125	0.35		
Total	45.24	130			

Source: Prepared by the authors.

contaminated prey (Dantas et al., 2012; Lima et al., 2015; Rummel et al., 2017). In all cases, microplastic contamination occurs accidentally.

Three compounds were identified, which were classified in the filament category. Among the different types of microplastic ingested by fish, the fibers represented the majority (Lusher et al., 2013; Neves et al., 2015; Vendel et al., 2017). Poly (*p*-phenylene terephthalamide), whose brand name is Kevlar®, is a synthetic fiber widely used in construction and the textile industry (Dupont, 2018). Although it is formed by a combination of high strength fibers (Washer et al., 2009), the material can undergo degradation after water absorption and other environmental effects such as exposure to UV radiation, which can damage the fibers and change their chemical structure (Prasad and Grubb, 1990). Its easy degradation upon contact with water and UV rays can explain the ability of this fiber to become a microplastic.

Pristine Polyester textile is considered the synthetic textile fiber most commonly manufactured by the industry and used in the manufacture of different types of fabrics (Carmichael, 2015). The main source of this microplastic is the effluent from washing machines from wastewater treatment plants and domestic sewage discharged directly into the environment (Browne et al., 2011; Sillanpää and Sainio, 2017). Polyester fibers were found in the digestive tract of deep-sea benthic invertebrates (2200 m) (Courtene-Jones et al., 2017), which shows that this type of microplastic is one of the most widespread categories across the oceans and can easily contaminate coastal fish, such as the species studied here.

The third compound identified was Copper Phthalocyanine, a synthetic blue pigment used in the composition of paints and the coating of certain types of plastic, widely used in the packaging industry (Lewis, 2004). The presence of this pigment in the particles interferes with the identification of the type of plastic, but its presence validates that it is a particle of anthropogenic origin (Cauwenberghe et al., 2013). In terms of color, blue-colored microplastics are the most frequent in the stomachs of several fish species (Possatto et al., 2011; Lusher et al., 2016; Vendel et al., 2017; Ferreira et al., 2018), which may be a reflection of their high availability in the environment. Apparently, blue-colored fibers are the type of microplastic most present in the oceans and most commonly ingested by fish.

Among the species analyzed in this study, the Atlantic thread herring (*Opisthonema oglinum*) is considered an important fishing resource in the region, whose fishing practiced by the artisanal fleet occurs throughout the coast of the State of Ceará (Teixeira et al., 2014). This species is used as bait or food by fishermen (Teixeira et al., 2014). This microplastic contamination causes physical damage and/or inflammatory reactions in fish (Tourinho et al., 2010; Thompson, 2006), thus compromising survival (Cole et al., 2011) and consequently the sustainability of fishery resources.

Besides the physical damage, microplastic particles can adsorb persistent organic pollutants (POPs) and heavy metals that are present in the aquatic environment (Rios et al., 2007; Ogata et al., 2009; Van et al., 2012). In this case, there is a concern about food quality and safety and the effects of this contamination on human health, since the species *Opisthonema oglinum* and *Chloroscombrus chrysurus* are considered sources of animal protein for the fishing communities of the region (Cunha et al., 2000; Teixeira et al., 2014). In addition, these contaminants associated with microplastics (POPs) can be transferred along the trophic chain and reach humans more intensely (biomagnification process), since these small pelagic fish serve as prey for large pelagic fish such as Spanish mackerel (*Scomberomorus brasiliensis*) and King mackerel (*Scomberomorus cavalla*) (Sánchez-Ramírez, 2003), species of high commercial value in northern and northeastern Brazil (Nóbrega and Lessa, 2007; Maia et al., 2015).

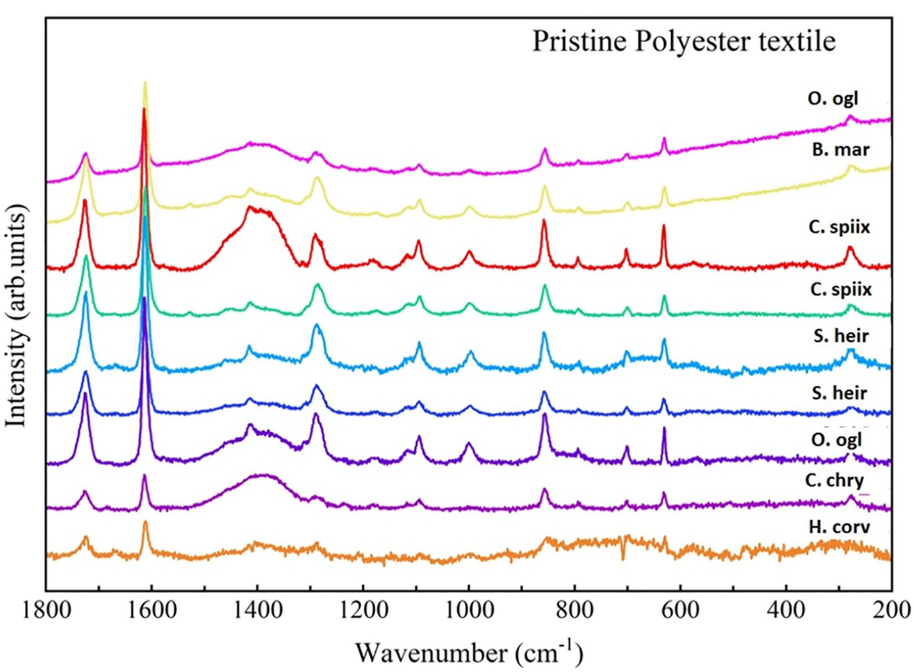

Fig. 5. Raman Spectra of the microplastic particles ingested by the fish species O. ogl: *Opisthonema oglinum,* B. mar: *Bagre marinus, C. spixii*: *Cathorops spixii,* S. heir: *Sciades herzbergii,* C. chry: *Chloroscombrus chrysuru,* H. cor: *Haemulopsis corvinaeformis* caught at Meireles beach, Fortaleza, State of Ceará, Brazil.

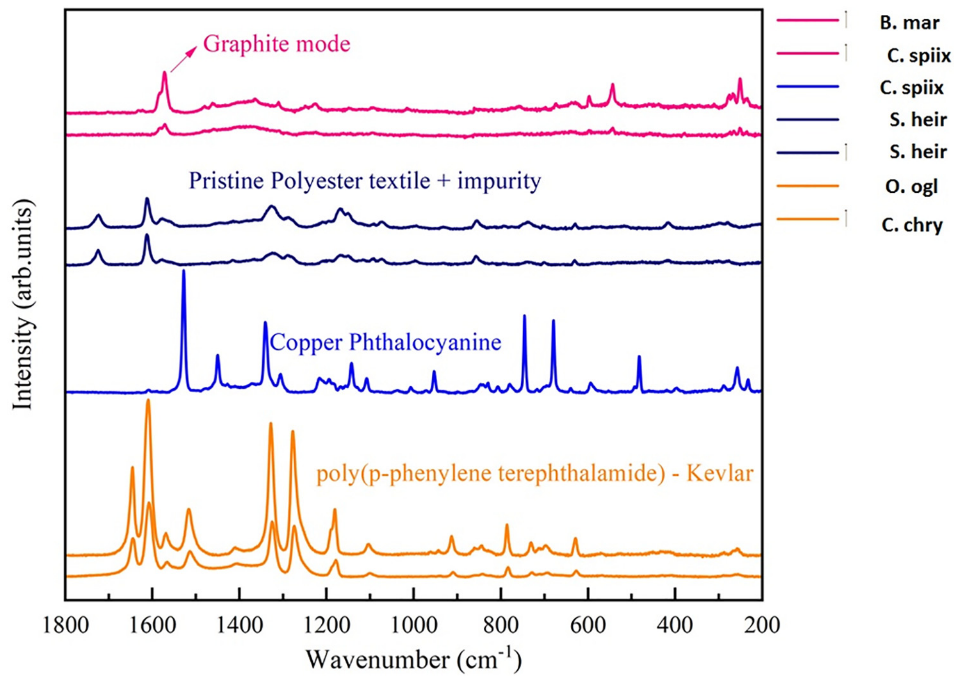

Fig. 6. Raman Spectra of the microplastic particles ingested by the fish species B. mar: *Bagre marinus, C. spixii*: *Cathorops spixii,* S. heir: *Sciades herzbergii,* O. ogl: *Opisthonema oglinum,* C. chry: *Chloroscombrus chrysurus,* caught at Meireles beach, Fortaleza, State of Ceará, Brazil.

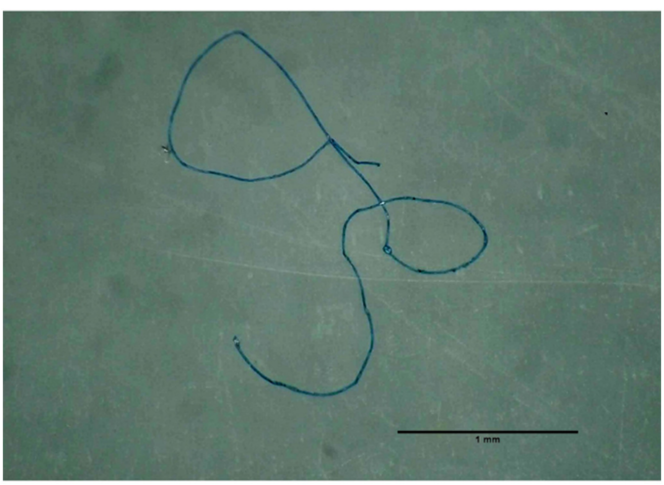

Fig. 7. Microplastic particles (polyester) ingested by the fish species caught at Meireles beach, Fortaleza, Ceará, Brazil.

5. Conclusion

The analyzed species present high amount and frequency of microplastic ingested. The amount of ingestion and the type of microplastic ingested does not depend on the feeding habit of the species. The most ingested microplastic was the blue fiber (Pristine Polyester Textile) which represented 68.75% of the compounds identified. It is expected that this study can contribute by filling in gaps that still exist regarding sandy beach, which are environments so highly threatened by human activities around the world, are largely neglected in terms of use and conservation plans.

CRediT authorship contribution statement

Natália C.F.M. Dantas: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Visualization. **Oscar S. Duarte:** Validation, Investigation. **Wellington C. Ferreira:** Validation. **Alejandro P. Ayala:** Validation. **Carla F. Rezende:** Resources, Validation, Writing - review & editing, Supervision. **Caroline V. Feitosa:** Resources, Writing - review & editing, Supervision.

Declaration of competing interest

The authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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