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Tamyris de Souza e Silva Pegado[1], Kurt Schmid[1], Kirk O. Winemiller[2], David Chelazzi[3], Alessandra Cincinelli[3,4], Luigi Dei[3,4], Tommaso Giarrizzo[1].

[1]Laboratório de Biologia Pesqueira e Manejo dos Recursos Aquáticos, Universidade Federal do Pará, Av. Perimetral 2651, Terra Firme, 66077830 Belém, Pará, Brazil.
[2]Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, TX, 77843-2258, USA.
[3]Consorzio Interuniversitario per lo Sviluppo dei Sistemi a Grande Interfase (CSGI), Via della Lastruccia, 3, 50019 Sesto Fiorentino, Florence, Italy.
[4]Department of Chemistry "Ugo Schiff", University of Florence, Via della Lastruccia, 3, 50019 Sesto Fiorentino, Florence, Italy.

Abstract

This study investigated occurrence of microplastic particles in digestive tracts of fishes from the Amazon River estuary. A total of 189 fish specimens representing 46 species from 22 families was sampled from bycatch of the shrimp fishery. Microplastic particles removed from fish gastrointestinal tracts were identified using Attenuated Total Reflectance – Fourier Transform Infrared (ATR-FTIR). In total, 228 microplastic particles were removed from gastrointestinal tracts of 26 specimens representing 14 species (30% of those examined). Microplastic particles were categorized as pellets (97.4%), sheets (1.3%), fragments (0.4%) and threads (0.9%), with size ranging from 0.38 to 4.16 mm. There was a positive correlation between fish standard length and number of particles found in gastrointestinal tracts. The main polymers identified by ATR-FTIR were polyamide, rayon and polyethylene. These findings provide the first evidence of microplastic contamination of biota from the Amazon estuary and northern coast of Brazil.

Keywords: Brazil, Bycatch, Pollution, Trophic level

Introduction

During recent decades, changes in manufacturing and consumer behavior together with insufficient waste management have resulted in accumulation of plastic debris in oceans throughout the world (e.g., Costa and Barletta, 2015; Jambeck et al., 2015), with plastic now composing between 60% and 80% of all marine debris (Barnes et al., 2009). It has been estimated that nearly half of all plastic products are discarded in less than 12 months after production (Hopewell et al., 2009). Once introduced into marine ecosystems, plastic waste becomes fragmented as it disperses via wind and oceanic currents (Barnes et al., 2009; Lebreton et al., 2012) and is distributed throughout the water column (Bellas et al., 2016). Plastic debris

accumulates not only in the open ocean, but also on beaches, mangrove forests and other coastal habitats (Ivar do Sul et al., 2007). Although some plastic debris is dumped directly into marine waters, rivers accumulate discarded material throughout their watersheds and transport it to the oceans (Lechner et al., 2014; Vendel et al., 2017). Unfortunately, rivers and estuaries have received relatively little attention with regard to the plastic pollution problem (Costa and Barletta, 2015), especially within the southern hemisphere (Cannon et al., 2016).

Reports of interactions between marine fauna and plastic debris have increased by 75% over the last two decades, including 267 species reported in 1997 (Laist, 1997) and 693 species reported in 2015 (Gall and Thompson, 2015). Plastic waste in the environment negatively impacts biota, including entanglement of animals within large items (macroplastics) and ingestion of microplastics (particles < 5 mm) by organisms, with subsequent transfer within the food web (Fossi et al., 2012; Cole et al., 2013; Ivar do Sul and Costa, 2014). Ingestion of plastic can affect organisms both physically and physiologically, including direct mortality from entanglement and choking as well as sub-lethal effects, such as compromised feeding, digestion, and reproduction activities (Gregory, 2009; Vendel et al., 2017). Exposure to chemical pollutants that bind to plastic particles has become a major concern, especially when chemicals bioaccumulate in fish destined for human consumption (Teuten et al., 2009). The effects of human consumption of organisms that contain microplastics are still poorly understood. Some evidence has been reported that plastic particles may cause immunotoxic responses, resulting either from chemical exposure or particle-induced mechanical stress (Seltenrich, 2015).

In aquatic and marine environments, plastics undergo a continuous process of disintegration from the action of water and wind causing abrasion from contact with solid particles, and through chemical decomposition by exposure to solar radiation (Moore, 2008; Barnes et al., 2009). Plastic debris is classified as macroplastics (particle diameter >25 mm), microplastics (diameter <5 mm) (GESAMP, 2015) or mesoplastics (5-25 mm) (Jabeen et al., 2017). Microplastics are further classified according to their origin. Primary microplastics are resin pellets and microbeads used in cleaning products, cosmetics, medicines and other products; secondary microplastics are formed from the fragmentation of larger meso- and macroplastics (Cole et al., 2011). Plastic pellets are used worldwide as a raw material in the production of plastic products (Ogata et al., 2009). With exposure to solar radiation, plastic pellets often lose or change their initial white or translucid coloration and many anthropogenic and biogenic chemicals can be adsorbed by their surface (Endo et al., 2005; Miranda et al., 2016).

Hydrophobic characteristics of plastics allow them to function as vectors for organic contaminants and heavy metals (Colabuono et al., 2010; Holmes et al., 2012).

Many fishes ingest tiny plastic particles either intentionally or accidentally while feeding in the water column or the benthos (Browne et al., 2010). Most investigations of microplastic ingestion by wild fish have been conducted in the northern hemisphere (e.g. Boerger et al., 2010; Phillips and Bonner, 2015), especially in Europe (e.g. Neves et al., 2015; Bellas et al., 2016; McGoran et al., 2017) and North America (e. g. Carson, 2013; Petters e Bratton, 2016). Microplastic ingestion by fishes in the Southern Hemisphere has been documented by studies performed in Africa (e.g. Biginagwa et al., 2016; Naidoo et al., 2016), Australia (e.g. Cannon et al., 2016), Easter Island (e.g. Ory et al., 2017), Indonesia (e.g. Rochman et al., 2015), and South America (Mizraji et al., 2017; Ory et al., 2017). Studies in Brazil have been conducted in the northeastern and southeastern regions (e.g. Possatto et al., 2011; Ferreira et al., 2016; Silva-Cavalcanti et al., 2017), with no investigations as yet for the northern region that includes the Amazon River estuary.

Brazil's northern coastline has low human population density and contains the world's second-longest, continuous area of largely undisturbed mangrove forest (ca. 7,000 km^2) (Giarrizzo and Krumme, 2008). In 2016, an extensive and biodiverse reef system (~9,500 km^2) was discovered offshore from the mouth of the Amazon River (Moura et al., 2016). This discovery, paired with the fact that 20% of Brazil's fisheries landings come from the northern coast (Krumme et al., 2015), lends urgency to the need to improve knowledge about plastic pollution in the region. Based on experiences in estuaries from northeastern Brazil, Costa and Barletta (2015) identified the Amazon River estuary as a priority area for future studies on marine plastic pollution. The goal of our study was to investigate the presence of microplastics ingestion by fishes from the Amazon River estuary on the coast of Brazil. We hypothesized that quantity and size of the ingested particles increases with fish body size, weight and vertical trophic position within the estuarine food web.

Material and Methods

Study area

Brazil's North Coast extends over 1,400 km along the states of Amapá and Pará, covering an area of approximately 488,000 km^2 and a variety of ecosystems including mesophotic reefs, islands, tidal flats, and estuaries with extensive mangrove forests (Marceniuk et al., 2013) (Fig.

1). The region's equatorial humid climate (Kottek et al., 2006) has annual rainfall up to 3,300 mm and average annual temperatures of 27.5 to 29.5 °C (Pereira et al., 2009).

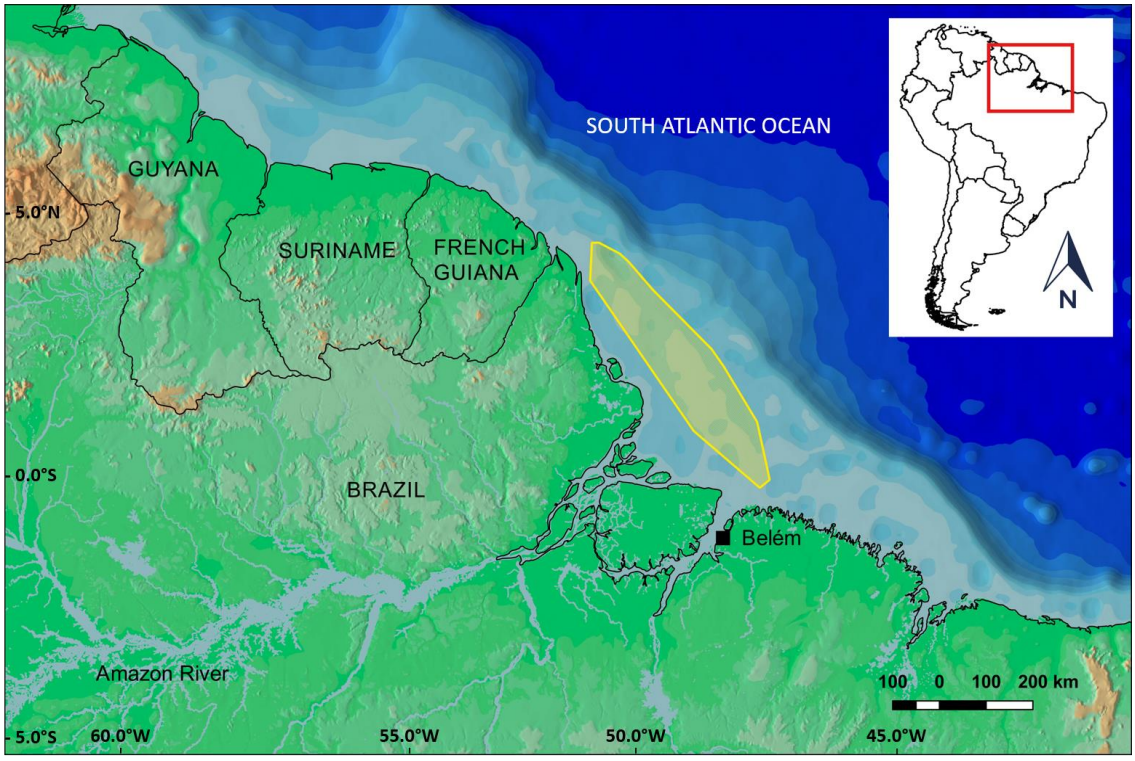

Fig.1. Location of the Amazon River estuary in northeastern Brazil (inset) showing the survey area (yellow shaded area).

The region includes the estuary of the world's largest river, the Amazon, with its mean annual discharge of 6.3 trillion m^3 of freshwater, 1.2 billion tons of sediments and 290 million tons of solutes that flow onto the continental shelf (Oltman, 1968; Meade et al., 1985; Nittrouer et al., 1995). The Amazon's freshwater plume can seasonally expand up to 120 km from the river mouth to the open ocean where salinities can close to zero.

The Amazon's massive freshwater discharge affects oceanographic processes, creating dynamic system of currents and tidal fluxes. The large sediment discharge contributes to high primary and secondary productivity, sustaining important artisanal and commercial fisheries (Neiva and Moura 1977, Wolff et al. 2000). Estuarine fishes and crustaceans have great economic importance and many of them interact with substrates, influencing physical and chemical processes, including nutrient dynamics (Lana et al., 1996).

Fish sampling

Fish specimens analyzed in this study were obtained from bycatch of the southern brown-shrimp fishery, which is monitored by the Center for Research and Management of Fishing Resources of Brazil's North Coast (CEPNOR) of the Brazilian Ministry of the Environment (MMA). Samples were obtained from 104 bottom trawls carried out between July 2015 and August 2016 at depths varying from 35 to 85 m. Due to ship's limited freezer space, a sample of the most abundant fish species were collected from each haul, frozen immediately, and stored for up to 2 weeks at -20 °C in the laboratory prior to processing.

Sample processing

In the laboratory, the fishes were identified to species level and each specimen was measured for standard length (SL) with a caliper (0.01 cm precision) and weighed with a digital scale (Marte BL3200H; 0.01 g precision). For each species trophic level was assigned according to values provided in FishBase (Froese and Pauly, 2017). To remove the digestive tract, a longitudinal incision was made in the abdominal area using surgical forceps and a scalpel. Each digestive tract (stomach and intestine) was cut longitudinally, and contents were washed into a Petri dish using 70% ethanol.

Stomach and intestine contents were examined under a stereo-microscope (Opton Tim-2b) at 6.5× to 50× magnification. Plastic particles were separated from other ingested particles, counted, classified according to shape and colour, measured (diameter in longest dimension to 0.001 mm precision), and photographed using a ZEISS SteREO Discovery V12 stereo microscope with the Zen software (blue edition, v2.0, Zeiss, Oberkochen, Germany).

Before and after each procedure for each specimen, all work surfaces and instruments were thoroughly cleaned with 70% ethanol and a new, clean pair of latex gloves was worn. To test for potential presence of airborne plastic fibers at the work station, a clean (using 70% ethanol) glass Petri dish that was placed at the work station at the start of each day was examined with the stereo-microscope at the end of the day.

A sample of each microplastic type recorded in the study was randomly selected for polymer identification. Recent studies showed that Fourier Transform Infrared (FTIR) spectroscopy is the most reliable method to identify the composition of maritime plastic debris, using either single-element or Focal Plane Array (FPA) detectors (Srinivasa Reddy et al., 2006; Mecozzi et al., 2016; Cincinelli et al., 2017). In this study, FTIR spectra were collected in

Attenuated Total Reflectance (ATR) mode, using a single-element MCT detector, which was deemed as the optimal set up, given the type and morphology of the plastic samples. The ATR-FTIR analysis of the samples was carried out using a Cary 620-670 FTIR microscope, equipped with a GeATR crystal (Agilent Technologies). The spectra were recorded directly on the samples with a spectral resolution of 8 cm^{-1}, acquiring 128 scans for each spectrum in the 4000-650 cm^{-1}spectral range.

Statistical analysis

The percentage of frequency of occurrence of microplastics within digestive tracts was calculated using the following formula: FO% = (Ni/N) x 100, where FO% = frequency of occurrence of microplastic particles; Ni = number of gastrointestinal tracts that contained microplastic particles; N = total number of gastrointestinal tracts examined.

Kendall's rank correlation was performed to assess the association between i) number of ingested microplastic particles and fish body size, ii) number of ingested microplastic particles and fish weight, iii) number of ingested microplastic particles and fish trophic level, and iv) the size of microplastic particles and fish body size. Whenever the correlation was significant, a linear regression was performed to derive an equation predicting the relationship between the response variable and independent variable. Statistical tests only included specimens that had microplastic particles in the gastrointestinal tract, and were performed using the R statistical package (R Core Team, 2017).

Results

Overall, 189 fish specimens representing 46 species from 22 families were analyzed (Table 1). Standard length (± SD) averaged 24.7 (± 13.3) cm, varying from 10.2 to 92 cm, and average body weight was 558.3 g (± 1429.2) ranging from 28.2 g to 10000g. All fishes are carnivorous and the species trophic level (TL), based on values reported in FishBase, ranged from ranged from 3.1 to 4.5.

Table 1. Summary information for fishes examined for microplastic particles.

Family	Species	Trophic level	Nº of fish	Nº of fish with microplastic	Frequency of occurrence (%)	Nº of microplastic particles in gastrointestinal tract	Average Nº of microplastic particles per specimen**	Nº of fish with microplastic in stomach	Nº of microplastic particles in stomach	Nº of fish with microplastic in intestine	Nº of microplastic particles in intestine
Ariidae	***Bagre bagre****	4	7	5	71.4	64	12.8	5	62	2	2
	Bagre marinus*	3.5	4	4	100	31	7.8	4	31	0	0
	Notarius grandicassis	4	4	0	0	0	0	0	0	0	0
Batrachoididae	***Batrachoides surinamensis***	3.7	1	0	0	0	0	0	0	0	0
Carangidae	***Caranx crysos***	4.1	3	0	0	0	0	0	0	0	0
	Caranx hippos*	3.6	3	3	100	92	30.7	3	92	0	0
	Selene setapinnis	3.7	1	0	0	0	0	0	0	0	0
	Selene vomer*	4.3	2	1	50	2	2.0	1	2	0	0
Ephippidae	***Chaetodipterus faber***	4.5	5	0	0	0	0	0	0	0	0
Haemulidae	***Anisotremus surinamensis***	3.6	1	0	0	0	0	0	0	0	0
	Anisotremus virginicus	3.6	1	0	0	0	0	0	0	0	0
	Conodon nobilis	3.6	8	0	0	0	0	0	0	0	0
	Genyatremus luteus	3.5	8	0	0	0	0	0	0	0	0
	Haemulon plumierii	3.8	13	0	0	0	0	0	0	0	0
	Haemulon steindachneri	3.7	5	0	0	0	0	0	0	0	0
	Orthopristis ruber	3.6	2	0	0	0	0	0	0	0	0
Lutjanidae	***Lutjanus analis****	3.9	3	1	33.3	1	1.0	0	0	1	1
	Lutjanus synagris*	3.8	2	1	50	1	1.0	0	0	2	1
Muraenesocidae	*Cynoponticus savanna*	3.5	1	0	0	0	0	0	0	0	0
Muraenidae	*Gymnothorax ocellatus*	4.1	1	0	0	0	0	0	0	0	0
Myliobatidae	***Rhinoptera bonasus***	3.2	1	0	0	0	0	0	0	0	0
Narcinidae	*Narcine brasiliensis**	3.2	6	1	16.7	3	3.0	0	0	1	3
Ophichthidae	*Ophichthus cylindroideus*	4	1	0	0	0	0	0	0	0	0
	Ophichthus ophis	4.5	1	0	0	0	0	0	0	0	0
Polynemidae	*Polydactylus oligodon**	3.7	1	1	100	3	3.0	1	3	0	0

	Polydactylus virginicus	3.7	13	0	0	0	0	0	0	0	0
Pomatomidae	***Pomatomus saltatrix***	4.5	2	0	0	0	0	0	0	0	0
Rachycentridae	***Rachycentron canadum***	4	1	0	0	0	0	0	0	0	0
Sciaenidae	***Bairdiella ronchus***	3.5	4	0	0	0	0	0	0	0	0
	Ctenosciaena gracilicirrhus	3.9	11	0	0	0	0	0	0	0	0
	Cynoscion jamaicensis	3.8	3	0	0	0	0	0	0	0	0
	Cynoscion leiarchus*	3.1	2	1	50	2	2.0	1	2	0	0
	Cynoscion microlepidotus*	4	16	3	18.7	4	1.3	3	3	1	1
	Cynoscion virescens*	4	7	1	14.3	3	3.0	1	3	0	0
	*Macrodon ancylodon**	3.9	13	1	7.7	2	2.0	1	2	0	0
	Menticirrhus americanus	3.5	1	0	0	0	0	0	0	0	0
	Micropogonias furnieri	3.1	6	0	0	0	0	0	0	0	0
	Paralonchurus brasiliensis	3.4	6	0	0	0	0	0	0	0	0
Scombridae	***Scomberomorus brasiliensis***	3.3	1	0	0	0	0	0	0	0	0
Serranidae	***Epinephelus itajara***	4.1	2	0	0	0	0	0	0	0	0
Sphyrnidae	***Sphyrna tiburo****	3.9	2	2	100	18	9.0	1	8	2	10
Stromateidae	*Peprilus paru*	4.5	2	0	0	0	0	0	0	0	0
Tetraodontidae	***Colomesus psittacus***	3.6	2	0	0	0	0	0	0	0	0
Triakidae	***Mustelus canis***	3.6	2	0	0	0	0	0	0	0	0
	Mustelus higmani	3.6	3	0	0	0	0	0	0	0	0
Trichiuridae	***Trichiurus lepturus****	4.4	5	1	20	2	2.0	1	2	0	0
TOTAL			**189**	**26**	**-**	**228**	**1.75**	**22**	**210**	**9**	**18**

* Fishes that ingested microplastic, ** Sample based only on specimens that ingested microplastic. Species in bold font support important commercial fisheries.

In total, 228 microplastic particles were recovered from gastrointestinal tracts of 26 specimens belonging to 14 species (Table 1), which represented 13.7% of the total abundance and 30.4% of the total species richness in samples. From the total number of microplastic particles, 210 (92.1%) were found in the stomachs of 21 specimens representing 11 species, and 18 particles (7.9%) were recovered from the intestines of 13 specimens representing six species (Table 1). On average, 1.2 (± 5.0) particles per fish were found. The standard length of fishes with ingested microplastics varied from 16.0 to 57.5 cm (mean SL = 32.7 cm ± 11.7), and weight varied between 72.7 and 10,000 g (mean = 1,531.9 g (± 3,132.7). Size, weight and number of microplastic particles found in gastrointestinal tract for each fish are provided as supplementary material (Appendix 1).

The greatest number of ingested microplastics per specimen (50 particles) was recorded for *C. hippos*. This species was responsible for 40.3% of all recovered microplastic particles. The frequency of occurrence (FO%) of microplastics per species, among those with three or more specimens examined, varied between 18.7% (*Cynoscion microlepidotus*) and 100% (*Bagre marinus*, *Caranx hippos*).

A positive correlation was found between fish SL and number of microplastic particles in the gastrointestinal tract (Kendall's Tau= 0.41, p= 0.003). Linear regression analysis indicated that each additional centimeter of SL was associated with an additional 0.58 ingested particles (r^2= 0.37; p= 0.0008; y= -10.374+0.586x) (Fig. 2). In contrast, no significant correlation (p= 0.43) was found between the quantity of ingested particles and trophic level, between the size microplastic particles and fish SL (p= 0.48), or between the quantity of ingested particles and fishes weight (p= 0.15).

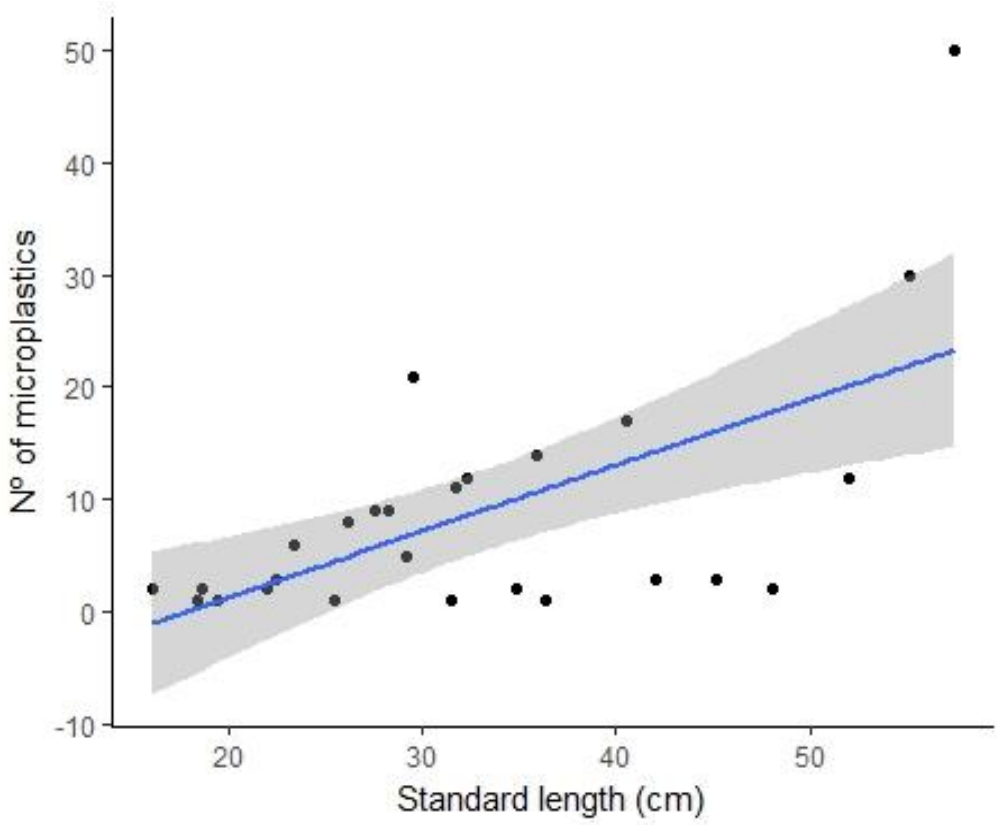

Fig. 2. Scatter plot showing the relationship between the number of ingested microplastic particles and SL among fishes that had microplastic particles in their gastrointestinal tracts (n=26). Shaded area indicates 95% confidence interval for the linear regression.

The size of plastic particles recovered from fish gastrointestinal tracts was always < 5 mm (range= 0.38–4.16 mm, average (± SD) = 1.82 (± 0.68)). Consequently, all particles are classified as microplastics (Arthur et al., 2009). Particles were recorded as four shape categories: pellets (97.4%), sheets (1.3%), fragments (0.4%) and threads (0.9%). Colours were either clear, yellow, orange or blue (Fig. 3).

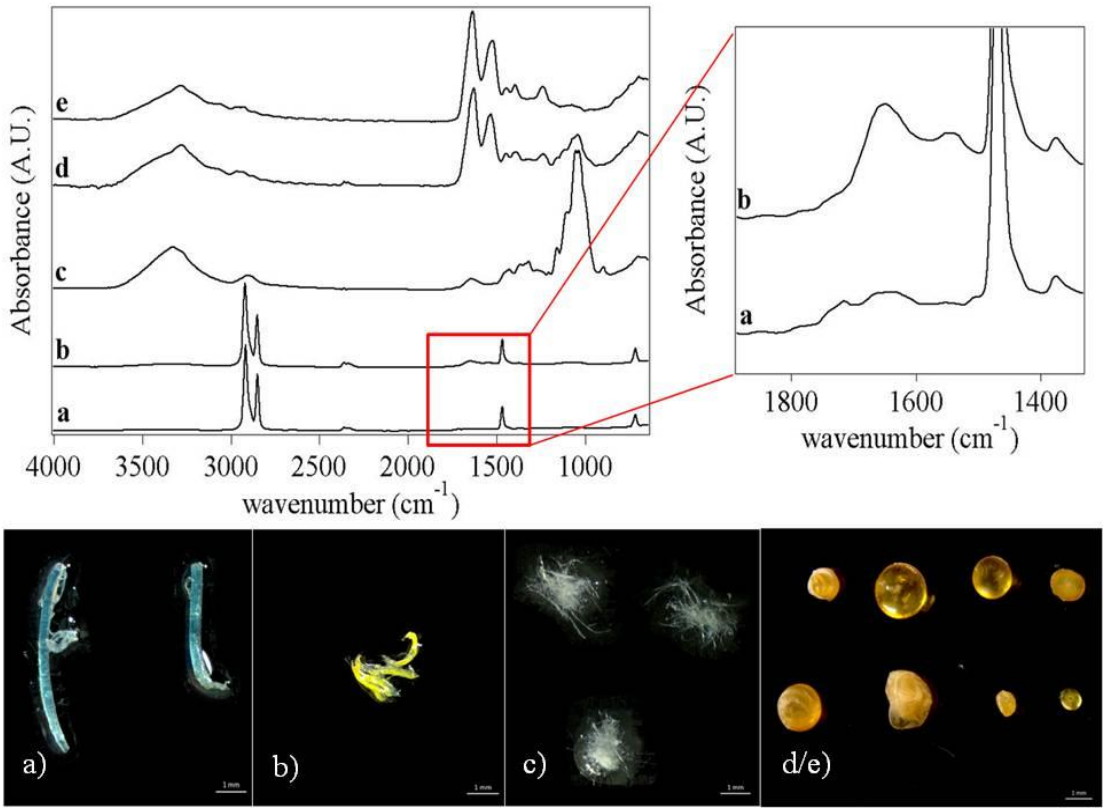

Fig. 3. ATR-FTIR spectra of the microplastic particles: (a) blue thread (polyethylene); (b) yellow fragment (polyethylene); (c) transparent sheet (rayon); (d) pellet I (polyamide); (e) pellet II (polyamide). Highlighted in red the FTIR inset showing the oxidation bands of polyethylene in spectra a and b.

Miroplastic polymers were identified using ATR-FTIR and comparison with reference spectra (Fig. 3). Samples classified as blue thread and yellow fragments had absorption spectra consistent with polyethylene (Fig. 03a,b): 2920 (ν_{as} CH_2), 2850 (ν_s CH_2), 1471 (bending deformation), 1373 ($\delta_s CH_3$) and 720 cm^{-1} (rocking deformation) (Gulmine et al., 2002). Among blue and yellow fragments, some spectral bands also could be consistent with presence of adsorbed algae: ca. 3350 (N-H, O-H stretching), 1650 (amide I) and 1540 cm^{-1} (amide II) (Kong and Yu, 2007). Among blue fragments, some weak bands and shoulders suggest the presence of oxygenated groups that form during the abiotic oxidation of polyethylene (Gewert et al., 2015): 1715 (C=O stretching ketones, carboxylic acids) and 1738 cm^{-1} (C=O stretching esters) (Gardette et al., 2013). Transparent sheets (Fig. 03c) showed spectral bands characteristic of Rayon at 3330 (O-H stretching), 2901(CH, CH_2 stretching), 1648 (HOH bending of water), 1445 (H-C-H and H-

O-C bending), 1373 and 1320 (H-C-C, H-C-O, and H-O-C bend), 1110 and 1045 (C-C and C-O-C stretching), and 901cm^{-1} (C-O-C in plane, symmetric) (Kaur et al., 2013; Li-Ling, 2007). Finally, samples classified as pellet I and pellet II (Fig. 4e,d) had spectra indicative of nylon at 3285 (N-H stretching), 3060 (C-H stretching, asymm., N-H band), 2953 (CH_2 asymm. stretching), 1640 (amide I), 1529 (amide II), 1447 and 1400 (CH_2 bending), and 1242 cm^{-1} (amide III) (Mahdi, 2011; Charles et al., 2009; Fayemi et al., 2016). For the pellet I sample, the broad band around 1045 cm^{-1} (C-O-C stretching) suggests the presence of adsorbed cellulosic fibers.

Discussion

This study provides the first evidence of ingestion of microplastic particles by fishes from the Amazon River estuary. In recent studies conducted in the northern Atlantic Ocean, 19.80% (Neves et al., 2015), 17.50% (Bellas et al., 2016) and 2.98% (Brate et al., 2016) of fishes had microplastics in their gastrointestinal tracts. Microplastics were reported from guts of 50% of marine fishes near the city of Salvador (Miranda et al., 2016) and in 23% of fishes from the Goiana estuary (Possatto et al., 2011), both in Northeast Brazil. Another study of fishes from the Goiana estuary (Ramos et al., 2012) found levels of microplastic ingestion (13.4%) similar to the level we found for fishes the Amazon estuary (13.7%).

The greatest number of ingested microplastics (50 particles) was encountered in a Crevalle jack (*Caranx hippos*) a predatory fish that can attain 124 cm SL and 32 kg (Meyer et al., 2001; Froese and Pauly, 2017). Considered generalists, Crevalle jack feed on abundant prey, such as schooling fish and crustaceans (Kwei, 1978; Sancho, 2000). A previous study had confirmed the relationship between the ingestion of microplastics and the feeding strategies of demersal fishes in the Eastern Mediterranean (Anastasopoulou et al., 2013). Romeo et al. (2015) proposed that microplastics are ingested most frequently by generalist foragers that target abundant small prey - a characteristic consistent with Crevalle jack feeding habits. The authors further proposed that microplastics also are ingested secondarily when their prey already contain microplastics. Given that microplastics tend to accumulate in the benthos (Bellas et al., 2016), biomagnification may be accelerated in predators that target bottom-feeding fishes and crustaceans. Ingestion of microplastics by the Crevalle jack is cause for concern, given the species' ecological importance in many tropical and subtropical marine ecosystems. Additionally, fishes of the Carangidae family are among the most important in tropical fisheries (Reuben et al., 1992, Crabtree et al.,

2002). Our finding of a significant correlation between fish length and the quantity of ingested microplastic particles corroborates those of Alomar et al. (2017) for goatfish, *Mullus surmuletus*, in the Western Mediterranean. Our failure to find a relationship between the number of ingested microplastics and trophic level may have been influenced by the limited range of trophic levels (3.1 to 4.5) among the species surveyed.

Several investigations of marine vertebrates and invertebrates have found that microfibers are the most commonly ingested microplastic particles (Cole et al., 2013; Bellas et al., 2016; Mizraji et al., 2017). In our study, plastic pellets comprised more than 97% of the microplastics recovered from fish gastrointestinal tracts. This corroborates the findings of Miranda et al. (2016) that fishes from the Brazil's northeastern coast near the city of Salvador had exclusively ingested pellets. This region is home to the largest petrochemical complex in Latin America, which could contribute to plastic pollution of rivers and coastal habitats (Ogata et al., 2009).

The color of the recovered pellets (predominately shades of yellow) was similar to that observed by Miranda et al. (2016) and indicative of persistent organic pollutants (POPs) attached to the surface of plastics (Endo et al., 2005). Furthermore, the yellowish color suggests that the pellets experienced oxidation and photo-oxidation while adrift in the sea (Ogata et al., 2009; Gewert et al., 2015), implying the original introduction into the environment might have occurred far from our study area. Yellow colour also can be caused by interaction with digestive enzymes (Miranda et al., 2016).

As estimated by ATR-FTIR analysis, polyamide (Nylon) represented 97.4% of all microplastics ingested by fishes in our sample from the Amazon estuary. We conclude that the high density (1.13–1.15 g/m^3) of polyamide (GESAMP, 2015), paired with the round shape of pellets, contributed to their accessibility to fishes in the study area. Most fishing gear is manufactured from nylon (Timmers et al., 2005), and the fishing industry has been estimated to contribute approximately 18% of all plastic debris found in the oceans (Andrady, 2011).

Apart from the nylon pellets, blue thread, yellow fragments and transparent sheets each contributed < 2% to the total of plastic particles recovered from fish gastrointestinal tracts. Blue thread and yellow fragments were identified as polyethylene (PE), a commonly produced polymer (Jambeck et al. 2015) used to make plastic bags and storage containers (GESAMP, 2015). Globally, PE is the most abundant polymer found in the environment (Andrady and Neal, 2009; Hidalgo-Ruz et al., 2012). Relative to nylon pellets, PE microplastics were uncommon in fish gastrointestinal tracts, and this could be related to their relatively low density (0.91–0.96

g/cm^3) (Coutinho et al., 2003; GESAMP, 2015). Floating PE particles, therefore, would be less available to demersal fishes. Transparent sheets were made of rayon, a man-made, semi-synthetic polymer that is used as an artificial textile material (Kauffman, 1993; Woodall et al., 2014). Possible sources are clothing, furniture, and personal hygiene products in sewage effluents (Lusher et al., 2012). In the Amazon estuary, this rayon contributed insignificantly, probably due to this material's rapid rate of degradation (Park, et al., 2004).

Some marine systems, such as enclosed bodies (e.g., Mediterranean Sea) and zones of gyre convergence, appear to accumulate large amounts of microplastics (Barnes et al. 2009; Ryan et al. 2009), and rivers are considered major contributors to pollution in these regions (Jambeck et al., 2015). Schmidt et al. (2017) demonstrated that rivers transport microplastics more efficiently than macroplastics, and that the concentration of microplastic particles is positively correlated to river size. Along with mismanagement of solid waste disposal, wastewater discharge, inland navigation and industrial pollution all contribute to entry of microplastics into fluvial ecosystems (Lechner et al., 2014). The Amazon River discharges the world's greatest volume of freshwater. Lebreton et al. (2017) estimated that 38,900 tons of plastic wastes are transported from the Amazon River into the Atlantic Ocean annually, suggesting that most of the microplastics found in our study could have originated in the river.

Microplastics, more than macroplastics, are influenced by advection and circulation patterns, which contributed to the accumulation of particles in deep-sea environments (Woodall et al., 2014; Van Cauwenberghe et al., 2015). Offshore convection, saline subduction, and other oceanographic processes along the coast near the Amazon River Mouth could contribute to accumulate microplastics in marine sediments (Talley, 2002; Stabholz et al., 2013).

The United Nations Environment Program (UNEP) listed plastic pollution as a critical problem, comparable to climate change (UNEP, 2014). Research that identifies and quantifies microplastics within the environment and bodies of organisms will help us to understand the magnitude and scope of microplastic pollution, and pave the way to manage this problem (Boerguer et al., 2010; Alomar and Deudero, 2017). Our findings indicate significant ingestion of microplastics by diverse fishes inhabiting the Amazon estuary and half of the species, including many that support important fisheries (Table 1). This raises human health concerns, because ingestion of fish that consume plastics has the potential to increase the body burden of hazardous chemicals that adsorb to plastics in the environment (Rochman et al., 2015) and subsequently

bioaccumulate (Oehlmann et al., 2009; Rochman et al., 2013). The degree to which microplastics and associated compounds biomagnify in food chains is poorly understood at present.

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