Marine Pollution Bulletin 192 (2023) 115087

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Baseline

Exploring microplastic contamination in reef-associated fishes of the Tropical Atlantic

Anne K.S. Justino[a,b,*], Guilherme V.B. Ferreira[a], Vincent Fauvelle[c], Natascha Schmidt[d], Véronique Lenoble[b], Latifa Pelage[a], Flávia Lucena-Frédou[a]

[a] Universidade Federal Rural de Pernambuco (UFRPE), Departamento de Pesca e Aquicultura (DEPAQ), Rua Dom Manuel de Medeiros, s/n, 52171-900 Recife, Brazil
[b] Université de Toulon, Aix Marseille Univ., CNRS, IRD, MIO, Toulon, France
[c] Université de Toulouse, LEGOS (CNES/CNRS/IRD/UPS), Toulouse, France
[d] NILU - Norwegian Institute for Air Research, Fram Centre, Tromsø, Norway

ARTICLE INFO

Keywords:
Plastic pollution
Tropical Atlantic
Reef fish
Demersal fish

ABSTRACT

Microplastics (MPs) are ubiquitous in marine compartments, and their transboundary distribution favours the dispersion and accumulation of particles in ecosystems. This study investigated MP contamination in four coastal fish species (*Haemulon squamipinna*, *Chaetodon ocellatus*, *Syacium micrurum*, and *Alphestes afer*) from the southwestern Tropical Atlantic. An alkaline treatment was applied to extract MPs from the digestive tracts, and a Laser Direct Infrared (LDIR) system was used to identify polymers. All species analysed were contaminated with MPs, with *Alphestes afer* being the most contaminated (1.45 ± 1.09 MPs individual^{-1}; frequency of occurrence 80 %). No significant differences were found in the number and size of detected particles among species. The most common shapes were fibres and films, and polyethylene was the most abundant polymer. This study provides important baseline data on MP contamination in coastal fish species inhabiting complex habitat areas relevant for conserving marine biodiversity.

1. Introduction

Plastic is a versatile, resistant and cheap material, and society is becoming increasingly dependent on this synthetic material (Geyer, 2020). However, the incorrect disposal of plastic waste makes its presence in ecosystems progressively hazardous. About 80 % of plastics from continental sources enter the oceans, mainly through riverine discharges (Andrady, 2011; Meijer et al., 2021). Once in the marine environment, these anthropogenic materials are easily distributed through ocean currents and degraded by physicochemical and biological weathering (*e. g.* solar radiation and interaction with marine organisms; (Jambeck et al., 2015; Thompson et al., 2004). The breakdown of larger plastics results in particles of different sizes, the ones smaller than 5 mm being called microplastics (MPs; Arthur et al., 2009).

MPs are widely available in marine ecosystems and can be mistaken for natural prey by marine species or swallowed by accident while breathing (Boerger et al., 2010; Li et al., 2021). MPs can pose various threats to marine biota (Galloway et al., 2017); their intake is associated with damage to the digestive system and decreased predation efficiency (de Sá et al., 2015; Moore, 2008). In addition, MPs can release additive burdens into the environment and adsorb other available pollutants (Fauvelle et al., 2021; Rochman et al., 2013; Teuten et al., 2007). Furthermore, MPs can serve as habitats for microorganisms such as viruses and bacteria (Amaral-Zettler et al., 2020; Pinheiro et al., 2021), carrying these organisms as well as their potential pathogenicity.

MPs have been detected in estuaries, coastal subsurface waters, and oceanic islands in the western equatorial Atlantic region (Lima et al., 2016; Lins-Silva et al., 2021; Monteiro et al., 2020). Ingestion of MPs by marine species is widely documented (Savoca et al., 2021) and has also been reported for species in the equatorial Atlantic (Justino et al., 2023; Bruzaca et al., 2022; Ferreira et al., 2016; Morais et al., 2020; Vendel et al., 2017). However, globally, few information regarding the ingestion of MP by reef fish species is available (Baalkhuyur et al., 2018; Garnier et al., 2019; Huang et al., 2023; Macieira et al., 2021; Nie et al., 2019). Reefs are extremely important habitats for marine biodiversity and for maintaining the important ecosystem services they provide. Thus, monitoring contamination in reef-associated fishes contributes to a better understanding of the ecosystem's health.

* Corresponding author at: Universidade Federal Rural de Pernambuco (UFRPE), Departamento de Pesca e Aquicultura (DEPAQ), Rua Dom Manuel de Medeiros, s/n, 52171-900 Recife, Brazil.
E-mail address: anne.justino@ufrpe.br (A.K.S. Justino).

https://doi.org/10.1016/j.marpolbul.2023.115087
Received 1 April 2023; Received in revised form 17 May 2023; Accepted 19 May 2023
Available online 30 May 2023

Along the Southwestern Tropical Atlantic (SWTA), the coastal region of northeast Brazil is inhabited by nearly 30 million people and is a major tourist region on a global and regional scale, which receive tourists throughout the year. In addition to tourism, the primary economic sectors are located in or near the coastal region, represented by industries, agriculture and commercial and subsistence fisheries (IBGE, 2011). Artisanal fishing in this area involves >200,000 people and is responsible for the highest sea fishes volume landed in the country (Nóbrega et al., 2009). This large volume of fish landings is due to the high fish diversity and abundance found on the continental shelf of northeast Brazil (MMA, 2006), a region included in Ecologically or Biologically Significant Marine Areas (EBSA; CBD, 2014). The incredible biodiversity is explained by the presence of complex habitats in this region, such as mangroves, seagrass meadows and reefs (Eduardo et al., 2018).

Given the relevance of this ecosystem, both in terms of biodiversity and importance to fisheries, and considering that one-quarter of marine fish species are coral reef-associated (Spalding et al., 2001), but they represent <10 % of marine species evaluated for MP contamination (Wootton et al., 2021), our study aims to identify and characterise MP contamination in coastal demersal fish species from the Northeast Brazilian coast, along with verifying the differences in MP ingestion rates among species.

2. Materials and methods

2.1. Study area

The studied coastal area is located on the continental shelf along the Northeast Brazilian coast in the Southwestern Tropical Atlantic and comprise the states of Rio Grande do Norte, Paraíba, and Pernambuco (Fig. 1). The tropical climate offers air temperature between 20 and 25 °C throughout the year, with a seasonality defined according to the rainfall patterns, comprised of a dry and a rainy season (Macêdo et al., 2004). In proximity, several Marine Protected Areas (MPA) have been established (*e.g.*, "APA dos Recifes de Corais" and "APA Costa dos Corais") (Ferreira and Maida, 2006; Prates et al., 2007).

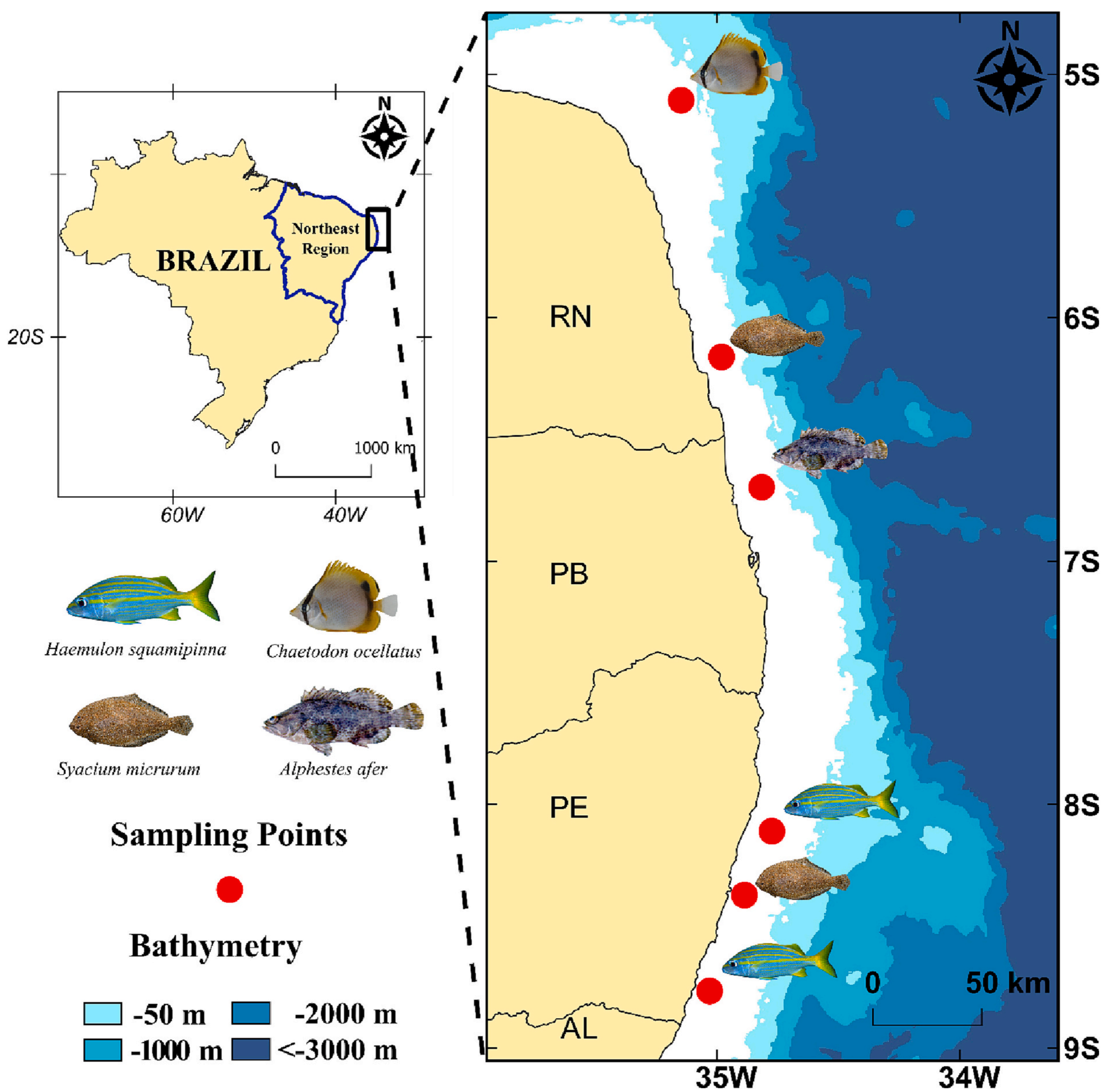

Fig. 1. Map of the continental shelf on Northeast Brazil in the Southwestern Tropical Atlantic. The red dots represent the stations where fishes were sampled. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.2. Sample collection and laboratory procedures

Two scientific expeditions were performed on the R/V ANTEA in the years 2015 (August and September) and 2017 (April and May), whereby 6 stations were selected for the study along the continental shelf through the Acoustics Along Brazilian Coast (ABRACOS) campaign (Eduardo et al., 2018). The coastal fish samples were collected using a bottom trawl (mesh: 40 mm; bag mesh: 25 mm; mouth dimensions: 28 × 10 m), between 15 and 65 m depth, and the haul was carried out for about 5 min at 3.2 kt. The SCANMAR system estimated the net's height, depth, and width. During the second cruise, bobbins were added to the ground rope to reduce the impact on the benthic habitat caused by trawling and avoid damage to the net.

A total of 47 individuals from four species were selected in this study due to their ecological and economic importance in the region. The coastal demersal species collected were: *Haemulon squamipinna* Rocha & Rosa, 1999; *Chaetodon ocellatus* Bloch, 1787; *Syacium micrurum* Ranzani, 1842 and *Alphestes afer* (Bloch, 1793). Individuals were measured for standard and total length in cm and weighed for total weight in g. Afterwards, they were frozen at −18 °C in the laboratory, and each individual had their organs (stomach and intestine) carefully removed, weighed and stored again for MP analysis.

2.3. Contamination control and microplastic identification

Several steps were carried out to ensure quality assurance and quality control to avoid cross-contamination, following the protocol proposed by Justino et al. (2021). This includes using cotton lab coats and the filtration of all solutions through a glass fibre filter (47 mm GF/F, 0.7 μm pore size, ©Whatman) mounted on a vacuum pump system equipped with laboratory glassware. Blank procedures were done for each set of 10 samples and received the same treatment as the samples. Particles found in the samples with similarities to the particles in the blank samples were excluded from the study. Only two black fragments were found in the blank samples and were identified as chitin. Additionally, the particles classified as biopolymers were excluded from further analysis.

To extract MPs from the digestive tract (stomach and intestine) of fishes, we used an alkaline digestion protocol with sodium hydroxide ($NaOH$, 1 mol L^{-1}; PA 97 %), which removed the organic matter around the particles (Justino et al., 2021). The digestive tracts were placed in a beaker and submerged in the NaOH solution (the proportion used was 1:100 *w/v*), covered by a glass lid and oven-dried at 60 °C for 24 h. Later, samples were filtered through a 47 mm GF/F 0.7 μm pore size glass fibre filter (©Whatman) using a vacuum pump system. After filtration, samples were oven-dried again at 60 °C for 24 h and proceeded to visualise MPs using a stereomicroscope (©Zeiss Stemi 508; size detection limits 0.04–5 mm).

All the particles suspected to be MPs were counted, photographed (©Zeiss Zen 3.2; Axiocam 105 Color), and measured in the longest axis (mm). Particles were first categorised according to their shape as fibres (filamentous shape), fragments (irregular shape), films (flat shape), foams (soft with an irregular shape), or pellets (spherical shape) (Justino et al., 2021). Thereafter, a subset of suspected MP particles (52 % of detected MPs) was analysed with a Laser Direct Infrared (LDIR) analyser Agilent 8700 Chemical Imaging System with the Microplastic Starter 1.0 library (Ourgaud et al., 2022). The LDIR analyser scans the particles (size range 20–5000 μm) to obtain a spectral curve using a wavelength range of 1800–975 cm^{-1}. The information is collected with the Clarity image software (©Agilent version 1.3.9) and compared with the polymer spectrum library (~400 reference spectra). The polymer nature was confirmed when the identification match was >70 % (Ferreira et al., 2022; Justino et al., 2022, 2023).

2.4. Data analysis

The frequency of occurrence (percentage of individuals in a given species in which MPs were recorded; FO%) was calculated to assess the general contamination status of the species. Since the data did not meet the parametric premises, the Kruskal-Wallis test was used to verify if the MP detected in the digestive tract presented significant differences among the species according to mean number and size. Additionally, Spearman's correlation test was used to evaluate the relationship between MPs and the biometry (length and weight) of fishes. All statistical analyses were performed with the software R version 3.6.3 (R Core Team, 2020) and were conducted considering a significance level of 5 %.

3. Results

From the 47 individuals of four coastal fish species analysed in this work, 52 MP particles were recovered (FO = 70 %, Table 1). The mean number and size of ingested MPs did not vary between the species ($p > 0.05$). However, *A. afer* was the most contaminated species in terms of the number of MPs ingested (1.45 ± 1.09 particles indindividual^{-1}; FO = 80 %), followed by *H. squamipinna* (1.00 ± 0.81 part. Ind.$^{-1}$; FO = 71 %), *S. micrurum* (0.90 ± 0.73 part. Ind.$^{-1}$; FO = 70 %), and *C. ocellatus* (0.70 ± 0.82 part. Ind.$^{-1}$; FO = 50 %) (Table 1, Fig. 2a and b). *Alphestes afer* showed the longest particle size ingested (0.63 ± 0.92 mm ind.$^{-1}$), followed by *C. ocellatus* (0.28 ± 0.72 mm ind.$^{-1}$), *S. micrurum* (0.24 ± 0.45 mm ind.$^{-1}$), and *H. squamipinna* (0.07 ± 0.10 mm ind.$^{-1}$). There was no relationship between the number and size of MPs and the biological parameters of fishes (Spearman's rank correlation, $p > 0.05$).

Regarding the shapes of the ingested MPs (Fig. 2d), *A. afer* registered 41 % of fibres, 37 % fragments, 31 % films, and 10 % of pellets, while *S. micrurum* registered 44 % fragments, 22 % fibres and films each and 11 % of pellets. *Chaetodon ocellatus* and *H. squamipinna* reported 57 % and 28 % fibres and 42 % and 71 % of films, respectively. The colours blue, black, and white were predominant (Table 1).

Overall, plastic polymers were successfully identified in 33 % of particles from the subset of samples analysed by the LDIR (Fig. 2e and c). Partially identified particles (60–69.9 % of similarity to the plastic polymers from the reference spectrum) and comprised 45 % of the samples. Biopolymers identified as cellulose, natural polyamide, and polylactic acid were observed in 22 % of all particles. The most common polymers were identified as polyethylene (PE) at 16 % abundance, polyamide (PA), polyvinyl alcohol (PVA), polycarbonate (PC), polypropylene (PP) at 11 % abundance, and alkyd varnish and styrene-butadiene rubber (SBR) with a similar abundance at 10 %. The other polymers, such as chlorinated polyethylene (CPE), polyurethane (PU), ethylene vinyl acetate (EVA) and polyvinyl chloride (PVC), contribute to a similar abundance of 5 %.

4. Discussion

This study provides new insights into the contamination of coastal fishes in Northeast Brazil, SWTA. Despite many studies evaluating MP contamination in fish species, there are limited reports on reef species (Baalkhuyur et al., 2018; Garnier et al., 2019; Huang et al., 2023; Macieira et al., 2021; Nie et al., 2019). Our results confirmed widespread contamination in reef species inhabiting the area, with a frequency of occurrence of 70 %.

While MPs were detected in all the fish species, no statistical difference was observed in the contamination rate and particle size among the four species, which varied between 0.70 ± 0.82 part. ind.$^{-1}$ (mean number) for *C. ocellatus* and 1.45 ± 1.09 part. ind.$^{-1}$ for *A. afer*. The number of MP found is similar to that in reef fish species at Guarapari Islands in Southeast Brazil (Macieira et al., 2021) and from Moorea Island in French Polynesia (Garnier et al., 2019).

The four species analysed here are demersal and share similarities, such as their feeding habits and habitat use. They are classified as mobile

Table 1
Summary of results regarding the mean (±standard deviation) number (particles individual^{-1}), size (mm), and FO% (frequency of occurrence) of microplastics detected in fishes, according to shape and colours. SL = Standard length; TW = Total weight.

Species/sample size		*Haemulon squamipinna* 7	*Chaetodon ocellatus* 10	*Syacium micrurum* 10	*Alphestes afer* 20
SLmin-max (mm)		10.7–14.4	8.5–10	8.6–20.5	11.6–16.4
TWmin-ma (g)		30.3–75.8	33.3–56.4	10.1–149.3	32.5–118.2
MP FO%		71 %	50 %	70 %	80 %
MP size (mm)		0.07 ± 0.10	0.28 ± 0.72	0.24 ± 0.45	0.63 ± 0.92
MP number		1 ± 0.81	0.70 ± 0.82	0.90 ± 0.73	1.45 ± 1.09
Shape	Fibre	0.28 ± 0.48 (29 %)	0.40 ± 0.69 (30 %)	0.20 ± 0.42 (20 %)	0.60 ± 0.68 (50 %)
	Fragment	0 %	0 %	0.40 ± 0.69 (30 %)	0.25 ± 0.44 (25 %)
	Film	0.71 ± 0.75 (57 %)	0.30 ± 0.48 (30 %)	0.20 ± 0.42 (20 %)	0.45 ± 0.82 (30 %)
	Foam	0 %	0 %	0 %	0 %
	Pellet	0 %	0 %	0.10 ± 0.31 (10 %)	0.15 ± 0.48 (10 %)
Colour	Blue	0.71 ± 0.75 (57 %)	0.50 ± 0.52 (50 %)	0.30 ± 0.48 (30 %)	0.45 ± 0.51 (45 %)
	White	0.14 ± 0.37 (14 %)	0 %	0.10 ± 0.31 (10 %)	0.35 ± 0.58 (30 %)
	Black	0.14 ± 0.37 (14 %)	0.20 ± 0.42 (20 %)	0.50 ± 0.70 (40 %)	0.65 ± 0.81 (45 %)
	Green	0 %	0 %	0 %	0.05 ± 0.22 (5 %)
	Red	0 %	0 %	0 %	0 %

invertebrate feeders, primarily feeding on benthic invertebrates, catching prey on the bottom or in the water column (Pereira et al., 2015; Pinheiro et al., 2018). Despite having active swimming ability, they spend most of their time in association with the substrate. In fact, species which rely on substrate resources may be more susceptible to MPs available in the sediment (Justino et al., 2021) since they may accidentally ingest these particles by mistaking them for prey or foraging on the substrate. Besides the accidental ingestion of MPs mistaken for prey, small particles can be passively captured while fish breathe (Li et al., 2021). Overall, the MPs detected in this study were, on average, smaller than 1 mm.

Fibres and films were the most frequent MP shapes in this study. Fibre is the MP shape most frequently associated with ingestion by fish in different marine ecosystems (Ferreira et al., 2016; Foekema et al., 2013), as was also observed in reef species (Baalkhuyur et al., 2018; Huang et al., 2023; Macieira et al., 2021). Possible sources of fibres in these regions can be either the fragmentation of fishing gear or the textile industry, which releases fibres through the flow of domestic sewage from the continent, reaching the continental shelf (Ferreira et al., 2016). However, not only fisheries but also touristic activities in reef areas may be impacting such habitats. Films and fragments can originate from maintenance or fragmentation by abrasion between the boats sailing in these regions that release paint particles (Lima et al., 2014).

The most abundant colours were blue, black and white, as observed in other studies, and are similar to the typical colour used in fishing nets (Baalkhuyur et al., 2018; Ferreira et al., 2016). The types of polymers identified in our study suggest that the primary source of MPs in this region is from fishing activities. Among the encountered polymers, the most abundant types were PE, PA and PP, which are used in fishing nets and fabrics and are frequently reported in fishes (Lima et al., 2021; Nie et al., 2019). In addition, we identified alkyd varnish, which is widely used in paints of boats, varnishes and synthetic enamels and as thermosetting plastics that can be moulded (Polymer DataBase, 2022). Finally, we identified other thermoplastics, such as PVA and PC; and SBR, used in manufacturing car tires and as a substitute for natural rubber (Polymer DataBase, 2022).

MP particles can adsorb environmental pollutants and release toxic additives, posing significant risks to organisms (Fauvelle et al., 2021; Teuten et al., 2009). The ingestion of MP particles can lead to various detrimental effects, including digestive damage, reduced predation efficiency, and the induction of toxic responses (Barboza et al., 2018; de Sá et al., 2015; Moore, 2008). Moreover, the uptake of progressively smaller particles can be particularly concerning as they have the potential to accumulate in tissues, exacerbating the risks for organisms (Dong et al., 2023). In addition to physical damage, MPs can disrupt the behavioural and neurological functions of fish, impact their metabolism, and alter the composition of their intestinal microbiome (Jacob et al., 2020).

In the environment, MPs can host a wide diversity of microorganisms, such as viruses and bacteria (Pinheiro et al., 2021), which may increase disease proneness on coral reefs (Lamb et al., 2018). In addition, some polymers, such as polypropylene, can cause adverse effects on coral reefs and act as a stressor (Isa et al., 2023). The wide-ranging impacts of microplastic pollution underscore the pressing necessity to tackle this issue and mitigate its harmful consequences on aquatic organisms.

Reef ecosystems on the continental shelf play a vital role in species conservation (Eduardo et al., 2018). However, a wide range of MP polymers in these ecosystems adds significant pressure, considering their crucial role in maintaining biodiversity. The fish species examined in this study, particularly those belonging to the *Haemulon* genus, hold substantial ecological importance and are extensively exploited by traditional communities in the Northeastern region of Brazil (Cardoso de Melo et al., 2020; Frédou et al., 2006). The contamination of *H. squamipinna* observed in this study reflects a global trend of plastic ingestion by marine resources (Savoca et al., 2021), raising concerns about the future sustainability of fish stocks and ecosystem functioning. Additionally, MPs in fishery stocks, which are essential for traditional communities, can pose potential risks to human health. Furthermore, by analysing the characteristics of MP particles (such as shape, colour, and polymer composition) identified in this study, we provide evidence suggesting that fishery and tourism activities in the region could be potential sources of contamination for the studied species. These findings significantly contribute to the expanding knowledge on MP contamination in the Southwestern Tropical Atlantic (SWTA) and offer valuable insights for developing effective mitigation strategies to safeguard these vulnerable ecosystems.

CRediT authorship contribution statement

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

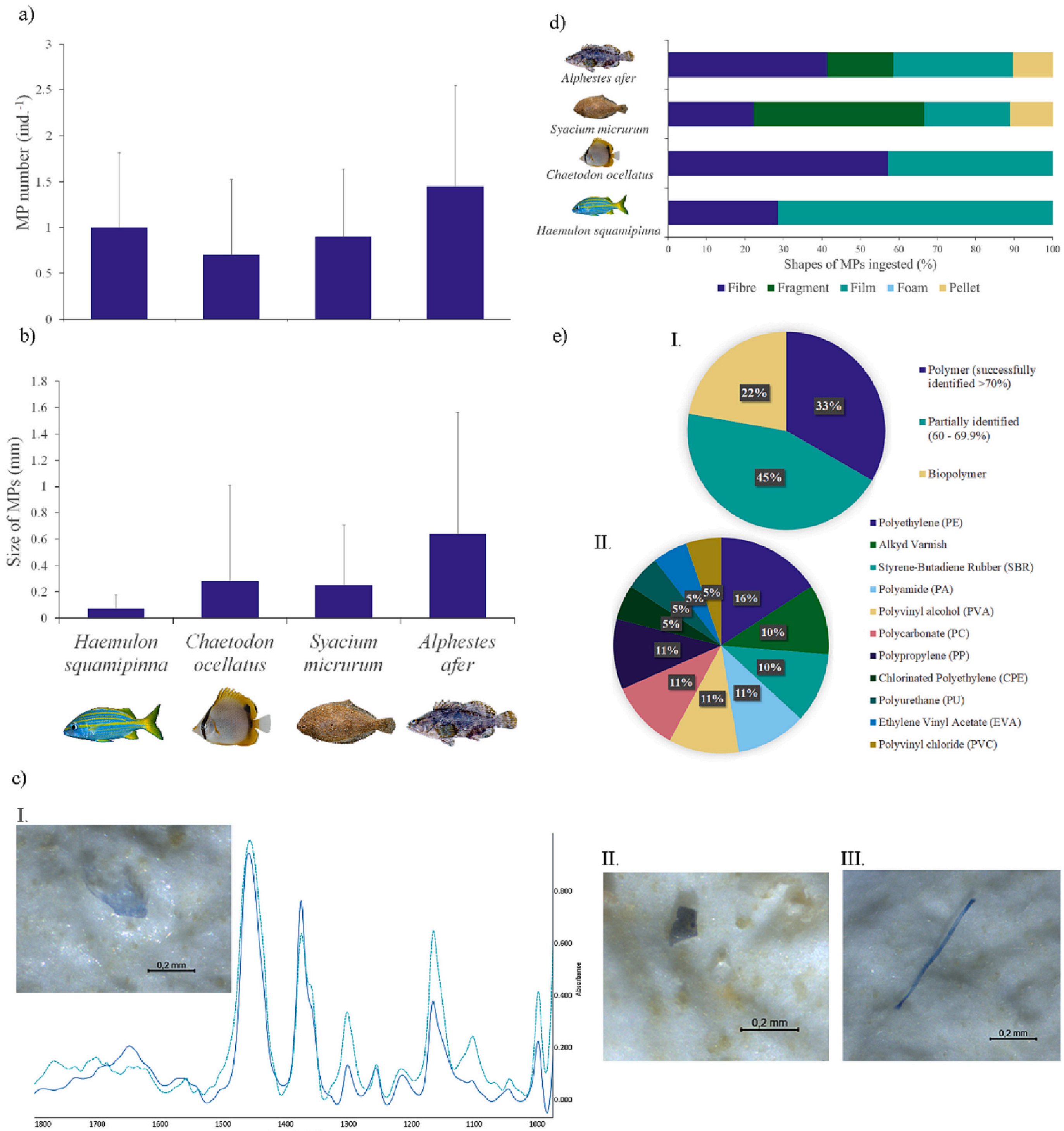

Fig. 2. a) Mean number (± standard deviation), b) mean size (mm) of microplastics ingested per fish species, c) Polymers and shapes detected in the analysed sample: I. blue fragment - polypropylene (PP) with the reference spectrum, II. black fragment - styrene-butadiene rubber (SBR), III. blue fibre - polyethylene (PE), d) shapes of microplastics found in the coastal fishes, expressed as a percentage (%), and e) polymers identified: I. particle composition in the detected samples, and II. the abundance of the polymers found. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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.

Data availability

Data will be made available on request.

Acknowledgements

Our research was supported by the LMI TAPIOCA program CAPES/

COFECUB (88881.142689/2017-01); FUNBIO and HUMANIZE under the grant “Programa Bolsas Funbio - Conservando o Futuro 2019 (02/2019)”; FACEPE (Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco); and MicroplastiX Project/JPI-Oceans/CONFAP/FACEPE (n°APQ-0035-1.08/19). We thank the CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), which provided research grants to F. Lucena-Frédou (CNPq n° 308554/2019-1); FACEPE for granting a scholarship to G. Ferreira (BFP-0107-5.06/21) and CAPES for granting a scholarship at doctoral level for the first author. We thank Richard Sempéré and Christos Panagiotopoulos for access to the LDIR system. All the authors thank the members of the BIOIMPACT laboratory (UFRPE) for their constant support.

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