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From prey to predators: evidence of microplastic trophic transfer in tuna and large pelagic species in the Southwestern Tropical Atlantic

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## Abstract

Plastic pollution is present in most marine environments; however, contamination in pelagic predators, including species of economic interest, is still poorly understood. This study aims to access the macro- and microplastic contamination in tuna and large pelagic species and verify whether a trophic transfer occurs from prey to tunas captured by two fleets in the Southwestern Tropical Atlantic (SWTA). We combined different methodological approaches to analyse the intake of macro- and microplastics. In addition to examining the plastics in the fish' stomachs, we investigated the contamination in the prey retrieved from the guts of predators. A low frequency of occurrence (3%) of macroplastic was detected in the tuna and large pelagic species; conversely, we observed a high frequency of microplastic in the tuna's stomachs (100%) and prey analysed (70%). We evinced the trophic transfer of microplastics by analysing the ingestion rate of particles in prey retrieved from the tuna stomachs. In the 34 analysed prey, we detected 355 microplastic particles. The most contaminated prey were cephalopods and fishes of the Bramidae family. The most frequent microplastic shapes in both prey and tuna stomachs were foams, pellets and fibres (< 1 mm). A variety of polymers were identified; the most frequent were styrene-butadiene rubber (SBR), polyamide (PA), polyethylene terephthalate (PET) and polyethylene (PE). Our findings enhance scientific knowledge of how the ecological behaviour of marine species can affect microplastics intake.

**Keywords:** Tropical fishery, Plastic Pollution, Predators, Microplastic, South Atlantic.

## Introduction

Plastic pollution is one of the most preoccupying environmental issues of the 21st century, with production that has drastically increased and is expected to hit 1100 million tons (Mt) by 2050 (Geyer, 2020). Considerable amounts of continental plastic materials are improperly managed and transported by riverine discharges into marine ecosystems (Koelmans et al., 2017; Meijer et al., 2021). To tackle the issue of plastic pollution on an international level, representatives at the fifth session of the United Nations Environment Assembly (UNEA-5.2) recently endorsed a landmark resolution to forge an international legally binding agreement by 2024 (UNEP, 2022). However, even if all available solutions to minimise the impact of plastic on the environment were to be applied, annual emissions of plastic into the environment could only be reduced by 79% by 2040 (Lau et al., 2020).

Plastic particles are commonly categorised by size into macroplastics (> 20 mm), mesoplastics (5 – 20 mm), and microplastics (< 5 mm) (Barnes et al., 2009). Microplastics can also be classified according to their sources: “primary” microplastics are produced for direct use or as precursors to other products, such as plastic pellets and exfoliants (Arthur et al., 2009), while “secondary” microplastics are particles formed from the breakdown of larger plastics, such as marine debris (Tanaka and Takada, 2016). Moreover, once present in the environment, plastic waste is weathered by natural processes, such as solar radiation, hydrodynamics and interaction with biota (Jambeck et al., 2015; Thompson et al., 2004).

Microplastics are present in marine ecosystems globally, spanning from coastal to polar regions, and are distributed throughout the water column vertically (Choy et al., 2019; Lins-Silva et al., 2021; Waller et al., 2017). Due to their small size and abundance, fish can inadvertently ingest microplastics through the respiratory process and be mistaken as prey (Boerger et al., 2010; Li et al., 2021). The ingestion of microplastics can pose several threats to marine biota (Galloway et al., 2017), such as damage to the digestive system, reduction in predation efficiency, and induction of toxic effects (Barboza et al., 2018; de Sá et al., 2015; Moore, 2008; Teuten et al., 2007). Additionally, species’ ecological behaviour is an important factor influencing microplastic intake (Savoca et al., 2021; Justino et al., 2022; Ferreira et al., 2023).

Microplastics are likely to be transferred from prey to predators through trophic transfer (Eriksson and Burton, 2003). Laboratory studies have confirmed the trophic transfer of plastics from mussels to crabs (Farrell and Nelson, 2013) and among planktonic organisms

with different trophic levels (mesozooplankton to macrozooplankton) (Setälä et al., 2014). In a study carried out in the South Pacific, it was observed that the prey ingested by tuna was contaminated with microplastic, probably associated with the trophic transfer between predator-prey (flying fish) in the natural environment (Chagnon et al., 2018). Indeed, predators are hypothesised to ingest more plastics than other species due to the large prey intake and a momentary build-up of particles in the stomachs (Ferreira et al., 2019).

Currently, although a large amount of information on microplastic contamination in marine fish is available (Savoca et al., 2021), our understanding of the extent of this problem in tuna and large pelagic species, which are crucial fisheries stocks (FAO, 2020), is still limited. This is compounded by the fact that only a small fraction of studies have used appropriate microplastic extraction methods, such as digestion and quality assurance/quality control (QA/QC) protocols, which are recommended by the scientific community to ensure reliable and reproducible research (Markic et al., 2020; Müller, 2021). Adhering to these protocols is critical in preventing biases arising from cross-contamination and sample loss, which can lead to overestimation or underestimation of microplastic concentrations. Nevertheless, microplastic contamination has been detected in several large pelagic species, including the common dolphinfish (*Coryphaena hippurus*) in the Mediterranean Sea (Schirinzi et al., 2020), skipjack tuna (*Euthynnus affinis* and *Katsuwonus pelamis*) in Indonesia (Andreas et al., 2021; Lessy and Sabar, 2021), and dolphinfish in the eastern Pacific Ocean (Li et al., 2022).

In the Southwestern Tropical Atlantic (SWTA), tropical tuna and large pelagic fisheries contribute significantly to the regional economy and provide an essential source of income and livelihood for fishers (FAO, 2020; Silva et al., 2018). The industrial fleets, which represent most of the catches, target mainly the yellowfin tuna (*Thunnus albacares* Bonnaterre, 1788) and the bigeye tuna (*Thunnus obesus* Lowe, 1839) and occur in offshore areas (Silva et al., 2016). Meanwhile, artisanal and recreational fisheries occur close to ocean islands, such as in the Fernando de Noronha Archipelago (FNA), mainly targeting the barracuda (*Sphyraena barracuda* Walbaum, 1792), the wahoo (*Acanthocybium solandri* Cuvier, 1832) and the yellowfin tuna (Martins et al., 2021).

In the South Atlantic, some data regarding the ingestion of plastic debris by pelagic predators are available: in the southeast-south of Brazil (Neto et al., 2020), on the coast of

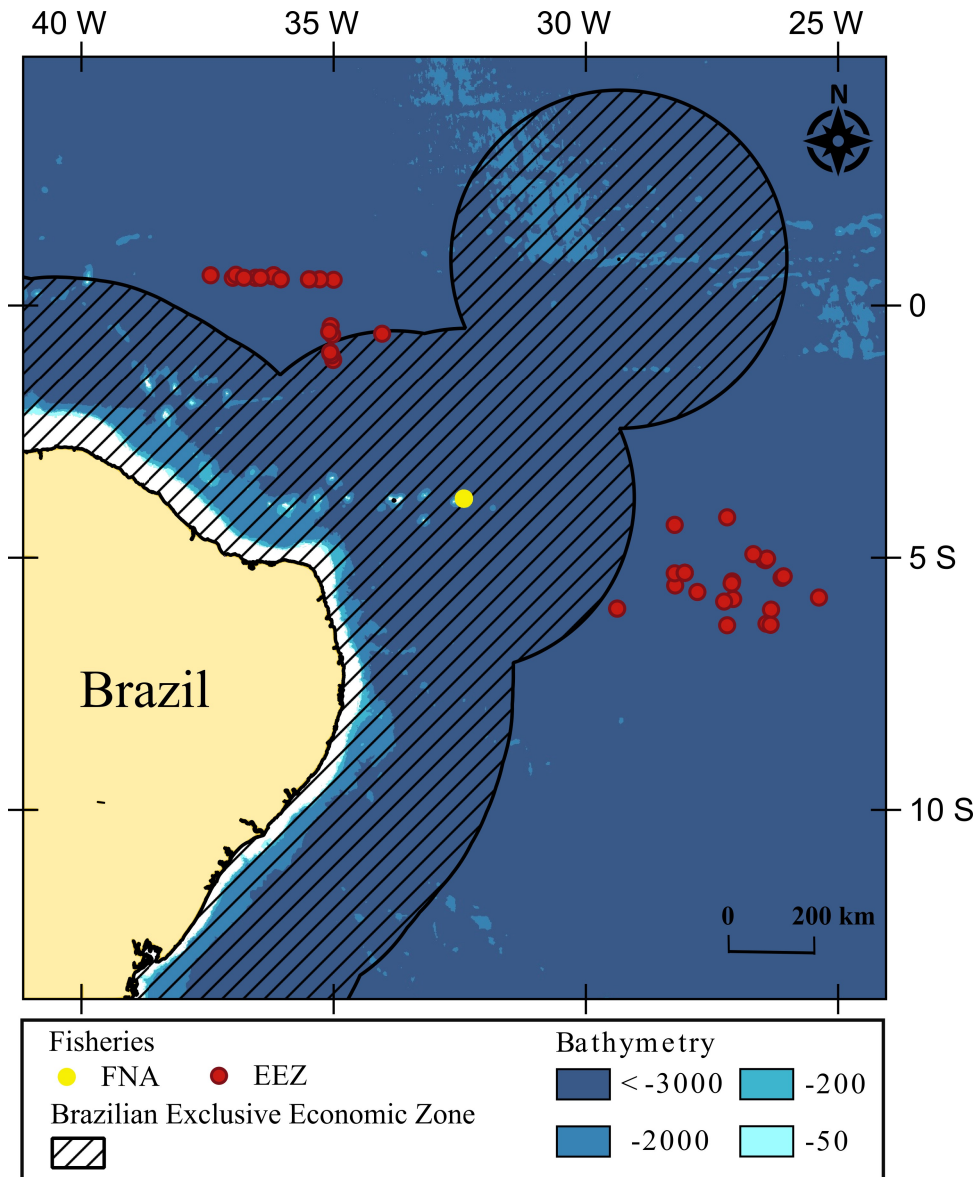
Salvador, in northeast Brazil (Miranda and de Carvalho-Souza, 2016), and reports for the Western Equatorial Atlantic (de Mesquita et al., 2021; Menezes et al., 2019; Vaske-Júnior and Lessa, 2004). However, despite their vast importance as a source of wealth and food security worldwide, to our best knowledge, there is no information regarding the microplastic contamination in tuna and large pelagic species in the SWTA.

Analysing microplastics in larger predatory fishes is a great challenge, mainly due to laboratory procedures. The use of standard techniques for the digestion of stomachs to separate the organic matter and the plastic items (*e.g.*, alkaline and acid digestion) is very time-consuming and must be done with caution to avoid cross-contamination and over/underestimation (Justino et al., 2021). Our study used combined methodologies to analyse the contamination of macro- and microplastics in tuna and large pelagic species targeted by industrial, recreational and artisanal fisheries operating in the SWTA. In addition to examining the microplastics in the stomachs, we investigated the contamination of the prey found inside the guts (Chagnon et al., 2018; Ferreira et al., 2019) to verify if there might be a trophic transfer of microplastics in the pelagic predators, also considering potential differences concerning contamination rates (number of microplastic extracted in the digestive tract) among the target species, fleets and their analysed prey (prey groups and species).

## **Materials and Methods**

### *Study area and sampling*

The study area is located along the Southwestern Tropical Atlantic (SWTA) (Fig. 1). The climate there is tropical, with well-defined rainy (March to July) and dry (August to February) seasons, and the warm and oligotrophic waters are influenced by the South Equatorial Current (SEC) and South Equatorial Undercurrent (SEUC) (Almeida, 2006; Assunção et al., 2020). The Fernando de Noronha Archipelago (FNA), registered on the UNESCO world heritage, is located in this area at ~360 km from the Northeastern Brazilian coast and inserted in a Marine Protected Area (MPA) with a National Marine Park (PARNAMAR) and an Environmental Protection Area (EPA).



**Figure 1.** Map with the fishing fleets' collection points along the Southwestern Tropical Atlantic (SWTA). Red dots show that the fishery operates outside the Brazilian Exclusive Economic Zone (EEZ), and the yellow dot indicates the captures in the Fernando de Noronha Archipelago (FNA).

Tuna and large pelagic species were collected by the industrial, recreational, and artisanal fleets that operated along the SWTA in 2018 and 2019. The industrial fleet operates with longlines mainly outside the Brazilian Exclusive Economic Zone (EEZ). The main target species are *T. albacares* and *T. obesus*, usually destined for exportation and the important

centres of fishery trades. On the other hand, artisanal and recreational fleets based in the FNA operate using rods and reels, and the main species caught are *S. barracuda*, *A. solandri*, and *T. albacares*. In FNA, the catches usually supply the island solely. We relied on onboard observers who recorded information about the fisheries. The specimens were labelled, measured (nearest 0.1 cm of fork length), weighed (kg of total weight), and dissected onboard. The stomachs were carefully removed and frozen at -18°C and kept in freezers until laboratory analysis.

In both fisheries (EEZ and FNA), a total of 350 samples of tuna and large pelagic species were collected: *T. albacares* (yellowfin tuna, YFT; n = 102); *T. obesus* (bigeye tuna, BET; n = 63); *S. barracuda* (barracuda, BAR; n = 136); and *A. solandri* (wahoo, WAH; n = 49).

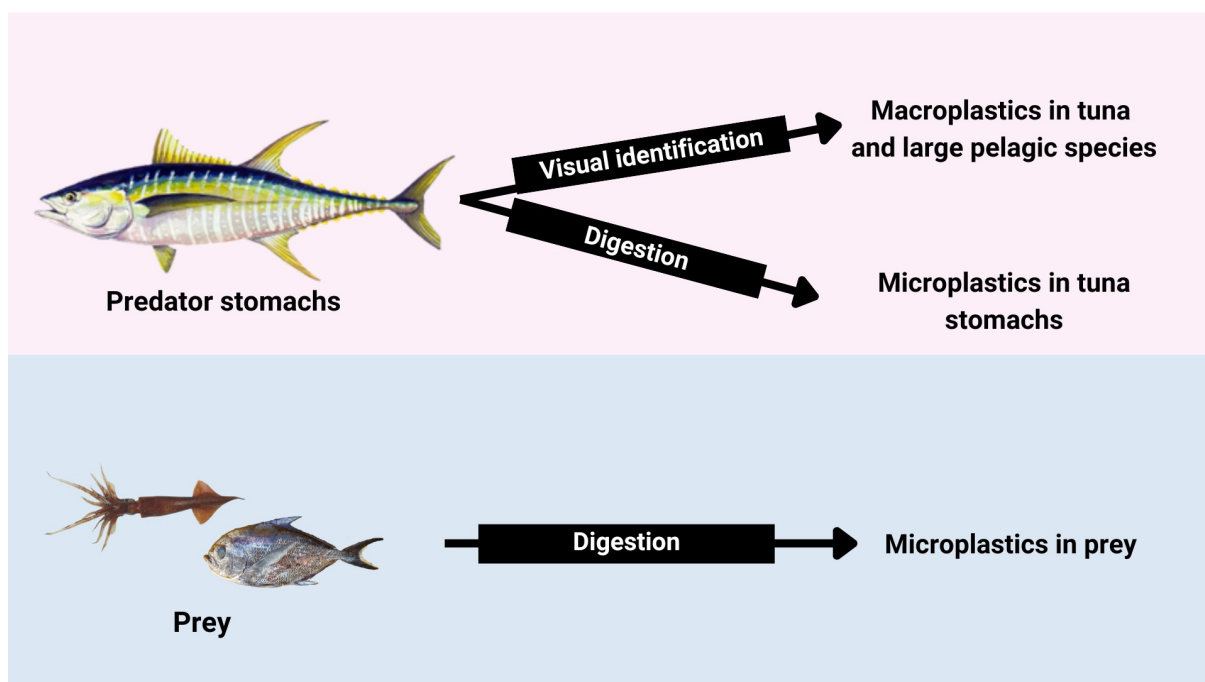
#### *Contamination control*

Several steps were carried out before the extractions (macro- & microplastics) to ensure quality assurance and quality control (QA/QC) and to avoid cross-contamination, following the protocol proposed by Justino et al. (2021). The protocol includes using 100% cotton lab coats and disposable latex gloves in a dedicated workspace with a limited flow of people. Moreover, all the utilised solutions were filtered through a glass fibre filter (47 mm GF/F 0.7 µm pore size, © Whatman) using a vacuum pump system equipped with laboratory glassware. To ensure a sterile working environment, all work surfaces were meticulously cleaned using 70% filtered ethanol, and all handling equipment was made exclusively of metal and glass. Before use, all equipment, including beakers and Petri dishes, were thoroughly rinsed with filtered distilled water and examined for any attached particles under a stereomicroscope. Reagent preparation and sample handling were performed in a fume hood cabinet to prevent airborne contamination.

For the macroplastic analysis, visual identification was applied. However, since visual inspection is not a safe method for analysing smaller particles (microplastics), only anthropogenic items that could be identified with the “human eye” were identified (see next section). This was done to avoid airborne contamination, as the stomachs were large, and due to the time taken to separate the food items, there would be a greater risk of cross-contamination. For the microplastic analysis, we implemented procedural blanks for each set

of 10 samples. The blanks received the same treatment as the samples. We excluded the particles found in the samples with similarity (colour and shape) to those observed in the blanks from the study. A total of three particles were detected in all observed blanks, two blue fibres and one black fibre. All particles were identified as cellulosic and excluded from further analysis.

### *Laboratory procedures and plastic extraction*



**Figure 2.** Flowchart illustrating the experimental strategy applied to detect macro- and microplastics in tuna and large pelagic species from the SWTA. Macroplastics from 341 stomachs were detected by visual identification. Microplastics from 9 *Thunnus albacares* stomachs and 34 preys were identified by Laser Directed InfraRed after an alkaline digestion protocol.

### *Macroplastic and prey identification in tuna and large pelagic species*

We visually inspected a total of 341 stomachs to identify plastic items. Additionally, we separated the prey in good condition found in the stomachs of tuna and large pelagic species with an intact digestive tract for further analysis (Fig. 2). Suspected plastic items were oven dried at 60°C for 24 hours to confirm their identification. To confirm its anthropogenic



nature, we considered certain characteristics, such as shape and physical consistency, which are not easily cut or broken. Finally, after confirming identification, particles were measured, counted, and photographed.

Tuna prey was measured, weighed, and stored for microplastic (< 5 mm) analysis (see next section). A total of 34 prey items were identified at the lowest taxonomic level possible and classified into large groups (mainly fishes and cephalopods) (Humann and DeLoach, 2002; Vaske-Júnior, 2006). In the case of prey identified as fish, only the digestive tract (stomach and intestine) was used for digestion, whereas for cephalopods, beaks and pen were removed, and then the whole animal was digested (Ferreira et al., 2022).

#### Microplastic detection in tuna stomachs and prey

Nine *T. albacares* stomachs from EEZ were separated for microplastic analysis regarding the predator. The stomachs were carefully eviscerated, and only the gut content was placed in a beaker for the microplastic extraction. The material found inside the stomachs analysed was in an advanced degree of digestion and could not be taxonomically identified.

Microplastic extraction from prey and *T. albacares* stomachs was performed with the help of an alkaline digestion protocol using sodium hydroxide (NaOH, 1 mol L<sup>-1</sup>; PA 97%) (Justino et al., 2021). The prey samples (digestive tract of fish and the whole cephalopod) and the gut content of *T. albacares* stomachs were carefully washed before analysis with filtered distilled water to remove any particles attached to the external tissue. Then, samples 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. After that, 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 carefully set in a Petri dish and covered; these filters were oven-dried again at 60 °C for 24 h. Finally, the filters were visually inspected for microplastics using a stereomicroscope (© Zeiss Stemi 508, with a size detection limit of 0.04 mm). All the particles suspected to be microplastics were counted, photographed (© Zeiss Zen 3.2; AxioCam 105 Color), and measured in length (mm). Microplastics 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).

## Polymer analysis

A random subset (15% of total microplastic extracted; 67 particles) of samples was selected to identify the main polymers using the Laser Direct Infrared (LDIR) analyser Agilent 8700 Chemical Imaging System with the Microplastic Starter 1.0 library. The LDIR analyser scans the particles (size range 20–5000  $\mu\text{m}$ ) within a wavelength range of 1800–975  $\text{cm}^{-1}$  (Ourgaud et al., 2022). The information is collected with the Clarity image software (© Agilent version 1.3.9) and compared with the polymer spectrum library (~400 references spectra). We confirmed the polymer type of a particle when the identification match was >70% (Ferreira et al., 2022; Eo et al., 2021). It is important to remark that since the polymer composition was only accessed in a sub-sample of the detected particles, there is a possibility that non-plastic particles may have been accounted as microplastics to some extent.

## Data analysis

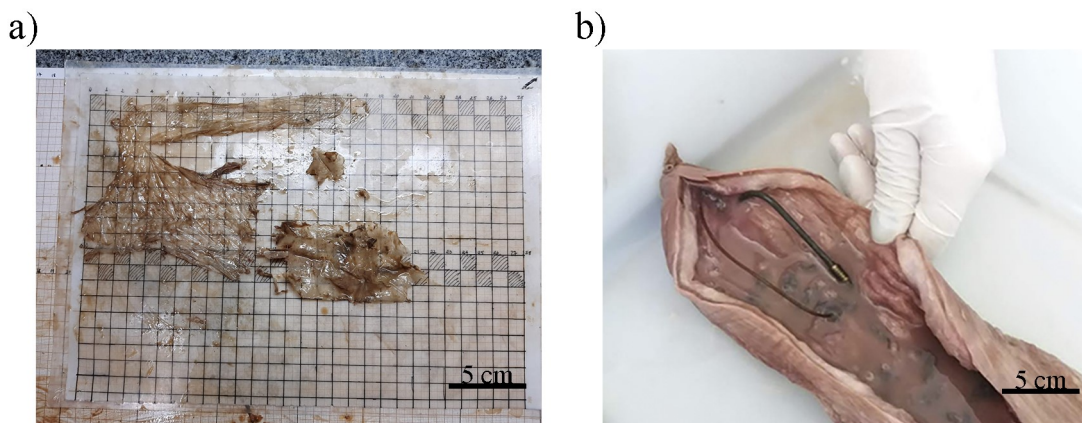
As the data on microplastic particles did not meet parametric assumptions, a Kruskal-Wallis test was used to verify whether detected particles in tuna prey presented significant differences among the fleets, predator species, prey groups, and prey species according to microplastic mean number and size. When significant differences were detected, a *post hoc* pairwise comparison, Dunn's test, was used to investigate the sources of variance (Dunn, 1964). A Spearman's correlation test was used to verify the relationship between microplastics and the predator's biometry. 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%.

## Results

### *Macroplastics in tuna and large pelagic species*

Macroplastics (> 5 mm) were found in 10 of the 341 examined stomachs, presenting, regardless of species, a low frequency of occurrence (FO= 3%) in both FNA (FO= 4%) and EEZ (FO= 1%). Among the species, *A. solandri* (FO= 8%, FNA) individuals were the most contaminated, followed by *S. barracuda* (FO= 3%, FNA) and *T. albacares* (FO= 2%, EEZ; FO= 3%, FNA). No plastics were found in *T. obesus* samples. A single particle resembling a

plastic bag was observed in a *T. albacares* caught in the EEZ, whereas for the FNA catches, we observed mostly fibres, plastic tape, synthetic fishhook, and artificial bait (Fig. 3).



**Figure 3.** Macroplastics detected in the tuna and large pelagic species from the Southwestern Tropical Atlantic: a) plastic bag and b) synthetic fishhook.

#### *Microplastics in the stomachs of *T. albacares**

A total of 93 microplastic particles were recovered from the nine stomachs of *T. albacares* (FO= 100%) captured outside the EEZ, with an average of  $10.33 \pm$  standard deviation of 14.06 particles per individual<sup>-1</sup> and a mean size of  $0.77 \pm 0.92$  mm ind.<sup>-1</sup>. The analysed *T. albacares* ranged from 40 to 145 cm in fork length and weighed from 1 to 47.8 kg. However, there was no relationship between the number and size of detected particles and the size of tuna (Spearman's rank correlation,  $p > 0.05$ ). Overall, regarding the shapes of the microplastics, the most abundant were foams (61%), followed by fibres (22%), films (10%), pellets (6%) and fragments (1%). The colours white and blue were the most predominant (Table 1).

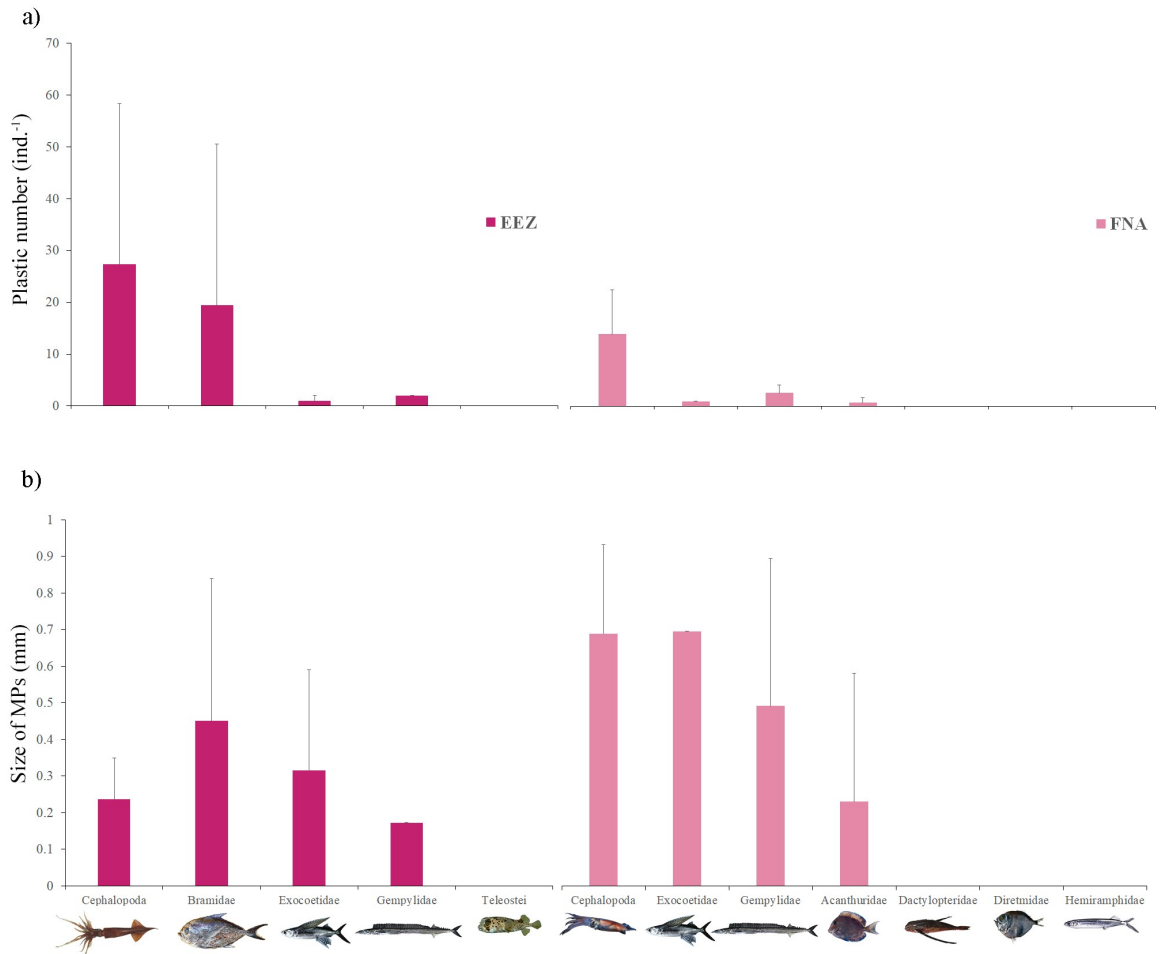
**Table 1.** Summary of results regarding the mean ( $\pm$ standard deviation) number (particles individuals<sup>-1</sup>), size (mm), and FO% (frequency of occurrence) of microplastics extracted from *Thunnus albacares* stomachs, according to shape and colours.

		Number	Size	FO%
	MPs	10.33 ( $\pm$ 14.06)	0.77 ( $\pm$ 0.92)	100
	Fibre	2.22 ( $\pm$ 3.11)	0.89 ( $\pm$ 0.98)	66
	Fragment	0.11 ( $\pm$ 0.33)	0.06 ( $\pm$ 0.19)	11
Shape	Film	1 ( $\pm$ 0.86)	0.10 ( $\pm$ 0.19)	66
	Foam	6.33 ( $\pm$ 10.92)	0.09 ( $\pm$ 0.13)	44
	Pellet	0.66 ( $\pm$ 1.32)	0.04 ( $\pm$ 0.07)	33
Colour	White	7.44 ( $\pm$ 10.52)	0.94 ( $\pm$ 1.15)	77
	Black	0.22 ( $\pm$ 0.44)	0.45 ( $\pm$ 0.44)	22
	Blue	1.66 ( $\pm$ 2.39)	0.20 ( $\pm$ 0.21)	55
	Yellow	0.11 ( $\pm$ 0.33)	0.61 ( $\pm$ 0)	11
	Red	0.66 ( $\pm$ 1.65)	0.96 ( $\pm$ 0.71)	22

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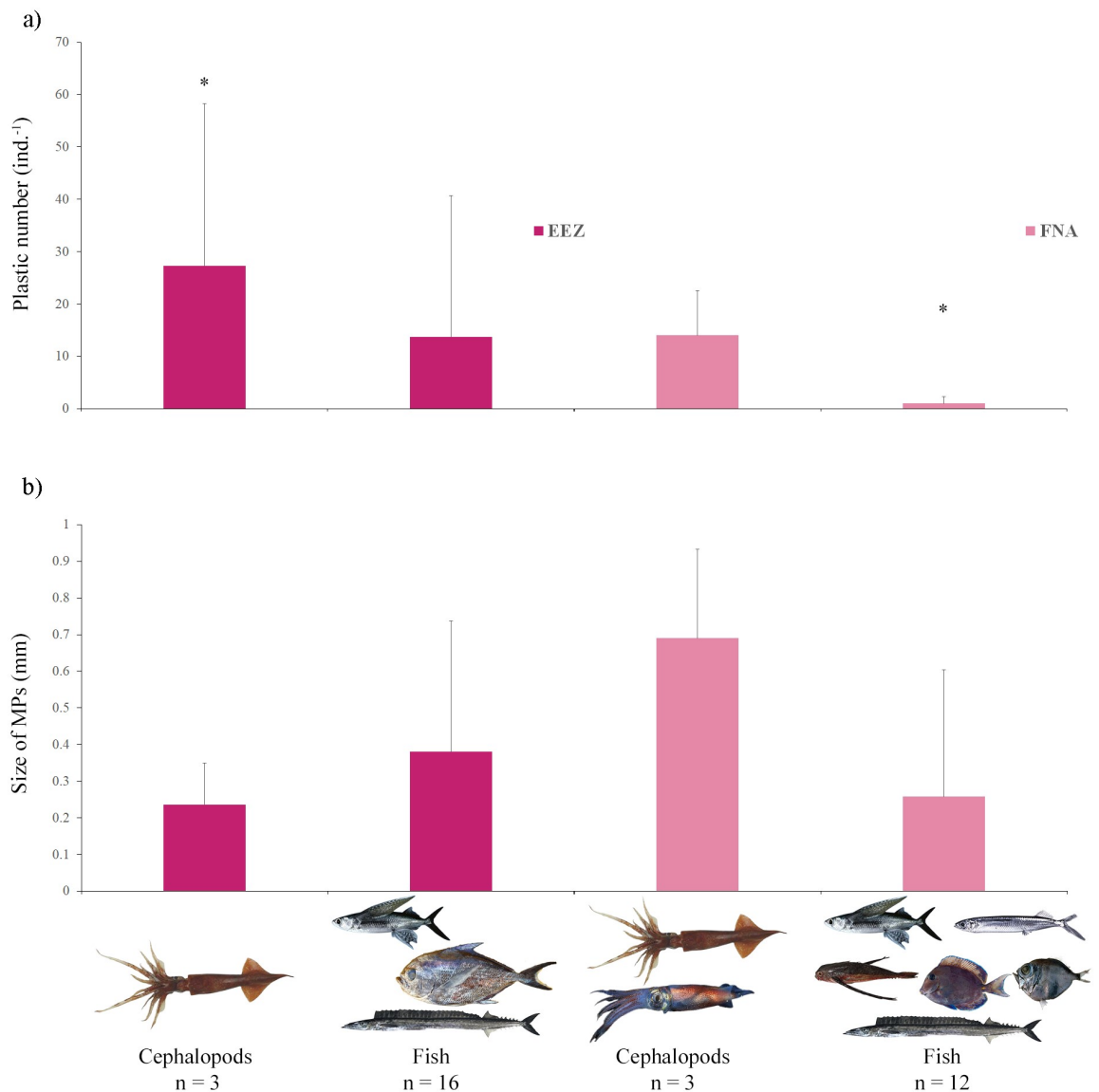
## 262 *Microplastics in tuna prey*

263 Among all the analysed stomachs, we recovered 34 tuna prey items with their organs  
264 intact. The main prey items found in tuna caught outside the EEZ were identified as  
265 Cephalopoda, Bramidae, Exocoetidae, Gempylidae, and Teleostei (unidentified fish). The  
266 prey found in the FNA catches were identified as Cephalopoda and the fish families  
267 Exocoetidae, Gempylidae, Acanthuridae, Dactylopteridae, Diretmidae, and Hemiramphidae  
268 (Table 2). In the recovered samples, 355 microplastics were detected in tuna prey. According  
269 to the mean number of microplastics, ingestion significantly differed between tuna prey  
270 species (chi-squared = 20.636, df = 11,  $p < 0.05$ ), the Cephalopoda from the EEZ was the  
271 most contaminated prey with an average of  $27.33 \pm 30.98$  part. ind.<sup>-1</sup>, followed by Bramidae  
272 from the EEZ ( $19.45 \pm 31.15$  part. ind.<sup>-1</sup>). Overall, the prey of tuna and large pelagic species  
273 caught in the FNA were less contaminated than EEZ prey (Fig. 4). However, the number and  
274 size of microplastics found in the prey did not vary statistically significantly between the  
275 areas (chi-squared = 3.2216, df = 1; chi-squared = 0.11138, df = 1,  $p > 0.05$ ).



**Figure 4.** a) mean number ( $\pm$ standard deviation) and b) mean size (length mm) of microplastics found in the prey species.

Nevertheless, when we grouped prey into larger groups, fish and cephalopods, we also observed significant differences in contamination rates (chi-squared = 13.226, df = 3,  $p < 0.05$ ). Fish predated in FNA were less contaminated ( $1 \pm 1.34$  part. ind.<sup>-1</sup>) than cephalopods predated outside of the EEZ ( $27.33 \pm 30.99$  part. ind.<sup>-1</sup>), and the cephalopods from the FNA ( $14 \pm 8.54$  part. ind.<sup>-1</sup>), and also fish from the outside of the EEZ ( $13.75 \pm 26.96$  part. ind.<sup>-1</sup>) (Fig. 5). Dunn's *post hoc* test showed that the number of microplastics detected in fish predated on FNA differed from that found in the cephalopods of EEZ and FNA.

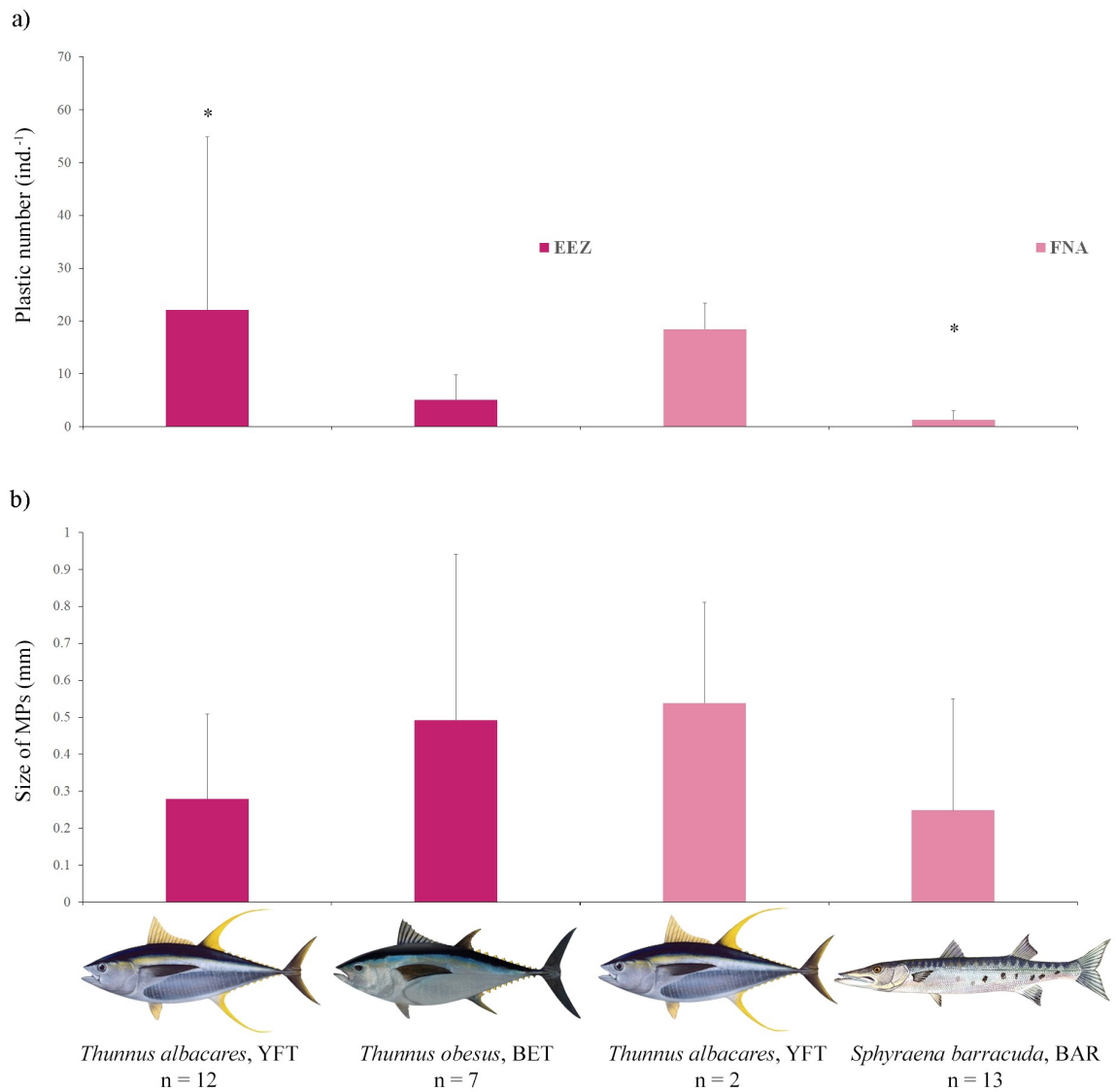


**Figure 5.** a) mean number ( $\pm$ standard deviation) and b) mean size (length mm) of microplastics detected in grouped prey. The asterisks represent the statistical differences with a significance of 0.05.

#### *Prey as a data proxy for tuna contamination*

We used the prey contamination data as a proxy to access the contamination rate (microplastics extracted in the prey) of tuna and large pelagic species. The data on microplastic number detected in prey significantly differed between the tuna species (chi-

squared = 9.3041, df = 3,  $p < 0.05$ ). The *T. albacares* captured outside the EEZ were the most contaminated ( $22.08 \pm 32.85$  part. ind.<sup>-1</sup>, 75%), followed by the specimens of this species captured in the FNA ( $18.5 \pm 4.94$  part. ind.<sup>-1</sup>, 100%) and *T. obesus* captured outside of the EEZ ( $5.14 \pm 4.70$  part. ind.<sup>-1</sup>, 85%). The least contaminated species was *S. barracuda* caught in the FNA ( $1.30 \pm 1.70$  part. ind.<sup>-1</sup>, 53%). However, the mean size of microplastics did not vary significantly between species (chi-squared = 3.3636, df = 3,  $p > 0.05$ ), and in general, particles were small ( $< 1$  mm) (Fig. 6, Table 2).



**Figure 6.** a) mean number ( $\pm$ standard deviation) and b) mean size (length mm) of microplastics detected per tuna and large pelagic species captured in the SWTA. The asterisks represent the statistical differences with a significance of 0.05.

**Table 2.** Summary of results regarding the mean ( $\pm$ standard deviation) number (particles individuals<sup>-1</sup>), size (mm), and FO% (frequency of occurrence) of microplastics (MPs) extracted from tuna prey.  $\Sigma$  = sum of MPs found in prey. The ecological importance of prey was obtained in the literature for the area and is expressed as frequencies in number (%N), weight (%W) and FO%. Reference set as:  $\alpha$  = Silva et al., 2019;  $\beta$  = Martins et al., 2021. n/d = unidentified.



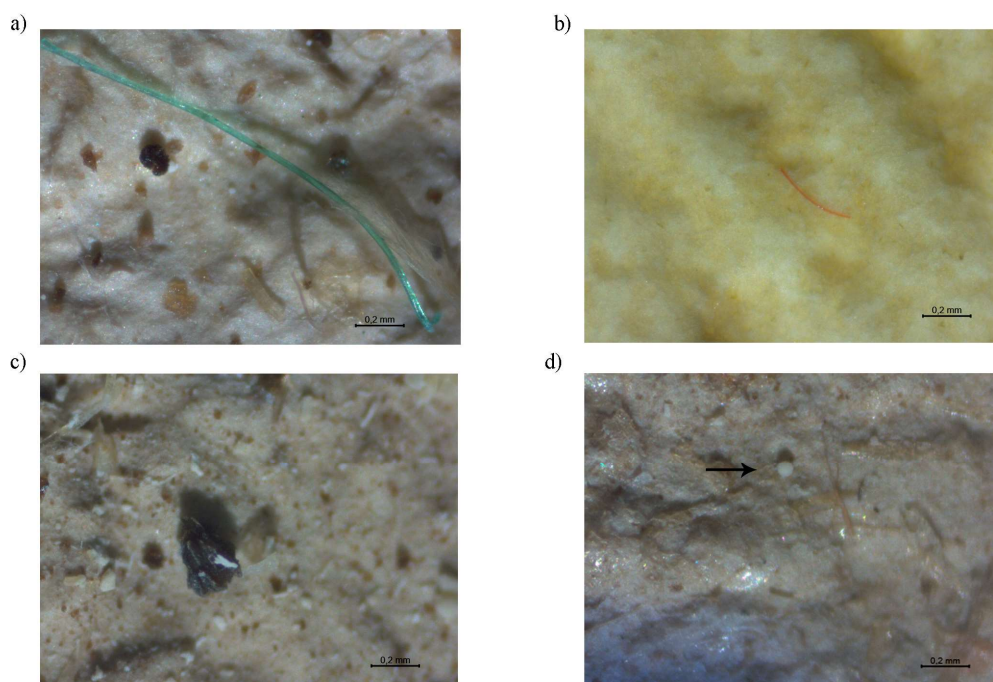
Predator	Prey (group/taxa)	Sampling		Microplastics (MPs) occurrence in prey				Ecological importance of prey			
		Number of prey	Fishery	$\Sigma$	FO%	MPs mean $\pm$ SD	Length (mm) mean $\pm$ SD	%N	%W	%FO	Reference
<i>Thunnus obesus</i> Bigeye tuna (BET)	Fish; Bramidae	4	EEZ	22	100	5.50 ( $\pm$ 4.04)	0.78 ( $\pm$ 0.34)	6.17	14.24	20	$\alpha$
	Fish; Gempylidae	1	EEZ	2	100	2	0.17 ( $\pm$ 0.04)	0.11	0.08	0.95	$\alpha$
	Fish; Teleostei n/d	1	EEZ	-	-	-	-	9.31	9.69	23.81	$\alpha$
	Cephalopod; Cephalopoda	1	EEZ	12	100	12	0.11 ( $\pm$ 0.05)	8.58	14.92	26.6	$\alpha$
<i>Thunnus albacares</i> Yellowfin tuna (YFT)	Fish; Bramidae	7	EEZ	192	71	27.4 ( $\pm$ 37.4)	0.25 ( $\pm$ 0.26)	6.31	6.41	11.36	$\alpha$
	Fish; Exocoetidae	3	EEZ	3	67	1 ( $\pm$ 1)	0.31 ( $\pm$ 0.27)	33.18	82.97	47.16	$\alpha$
	Cephalopod; Cephalopoda n/d	2	EEZ	70	100	35 ( $\pm$ 39.5)	0.29 ( $\pm$ 0.04)	10.05	2.61	23.29	$\alpha$
	Cephalopod; <i>Abralia veranyi</i>	1	FNA	22	100	22	0.41 ( $\pm$ 0.25)	0.26	0.02	2.94	$\beta$
	Cephalopod; <i>Ornitotheuthis antillarum</i>	1	FNA	15	100	15	0.87 ( $\pm$ 0.85)	8.85	3.19	26.47	$\beta$
<i>Sphyraena barracuda</i> Barracuda (BAR)	Fish; Exocoetidae <i>Exocoetus volitans</i>	1	FNA	1	100	1	0.69	18.18	46.27	11.67	$\beta$
	Fish; Gempylidae <i>Gempylus serpens</i>	3	FNA	8	100	2.66 ( $\pm$ 1.52)	0.49 ( $\pm$ 0.40)	2.39	1.11	0.83	$\beta$
	Fish; Achanturidae <i>Acanthurus</i> sp.	4	FNA	3	50	0.75 ( $\pm$ 0.95)	0.23 ( $\pm$ 0.35)	4.78	12.49	2.5	$\beta$
	Fish; Dactylopteridae <i>Dactylopterus volitans</i>	2	FNA	-	-	-	-	3.83	1.48	2.5	$\beta$
	Fish; Diretmidae <i>Diretmus argenteus</i>	1	FNA	-	-	-	-	0.48	0.49	0.83	$\beta$
	Fish; Hemiramphidae <i>Oxyporhamphus</i>	1	FNA	-	-	-	-	0.96	5.27	0.83	$\beta$

	<i>micropterus</i>											
	Cephalopod;											
	<i>Ornitotheuthis antillarum</i>	1	FNA	5	100	5	0.77 ( $\pm$ 0.73)	1.91	0.25	0.83	$\beta$	
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Regarding the shapes of microplastics identified in the prey and used as a proxy for tuna contamination, we observed that *T. albacares* captured outside the EEZ ingested mainly pellets (61%), fragments (24%), foams (12%), fibres (2%) and films (1%) (Fig. S1). The *T. obesus* from the EEZ ingested mostly foams (75%), followed by fibres (14%), pellets and fragments (6%) (Fig. S1). Meanwhile, in *T. albacares* caught in the FNA, ingested mostly pellets (51%) and fibres (43%), whereas films represented only 5% of the total (Fig. S1). For the *S. barracuda*, the main shapes observed were fibres (76%), followed by fragments (12%), pellets and foams (6%) (Fig. S1).

### *Identified polymers*

Overall, plastic polymers were successfully identified in 35% of particles from the subset of samples analysed by LDIR. Particles that were identified between 60-69.9% similarity to the reference spectrum were considered partially identified and comprise 34% of the samples (Fig. S2). The lower similarity between partially identified particle spectra to reference spectra might be explained by the advanced weathering of the particles. LDIR is still a novel technique for identifying plastic polymers in environmental samples; the number of reference spectra of weathered polymers will increase in the future, thereby diminishing the percentage of partially identified particles. Biopolymers identified as cellulose and natural polyamide were observed in 19% of all particles, and 12% were unidentified. A wide range of polymers was identified, but the most commons were Styrene-Butadiene Rubber (SBR) with 17% abundance, followed by Polyamide (PA) with 15%, Polyethylene Terephthalate (PET) and Polyethylene (PE) with a similar abundance of 12%, and Polyurethane (PU) with 10%. The other polymers, such as Low-density polyethylene (LDPE), Polyvinyl chloride (PVC), Acrylonitrile Butadiene Styrene (ABS), Alkyd Varnish, Polypropylene (PP), Polystyrene (PS), Polymethylmethacrylate (PMMA), Polytetrafluoroethylene (PTFE), and Chlorinated Polyisoprene contributed with a similar abundance of 2-5% (Fig. S2).



**Figure 7.** Microplastics identify by the LDIR analysis. a) green fibre - polypropylene (PP), b) red fibre - polyurethane (PU), c) foam - styrene-butadiene rubber (SBR), and d) pellet - polyethylene (PE), the arrow indicates the pellet shape in the image.

## Discussion

### Macroplastic in tuna

In general, we found a low frequency of macroplastic contamination (FO= 3%) in tuna and large pelagic species caught in the Southwestern Tropical Atlantic (SWTA). This low frequency of occurrence has also been observed in other studies analysing the ingestion of marine debris in *T. albacares*, *T. obesus* and *Katsuwonus pelamis* (FO= 0, 0 and 0.75%, respectively) from the Western Atlantic (de Mesquita et al., 2021), in *T. albacares* from the South Pacific subtropical gyre (2%) (Chagnon et al., 2018), and in *K. pelamis*, *T. albacares*, *C. hippurus*, and *T. obesus* (FO= 0, 0, 2 and 9%, respectively) from the North Pacific subtropical gyre (Choy and Drazen, 2013). Researchers often suggest that the lower intake of large plastics by pelagic fishes can be explained due to geographical location (de Mesquita et al., 2021) since plastics tend to accumulate in oceanic gyres (Cózar et al., 2014; Jiang et al., 2020) and areas that do not have a convergence zone would accumulate fewer plastics.

Conversely, even in the most polluted regions of the ocean (e.g., North Pacific subtropical gyre), some species may exhibit a low frequency of ingested macroplastics (Choy and Drazen, 2013). Therefore, the contamination of marine species does not seem to be solely linked to the availability of plastic debris since plastic debris is ubiquitous, and the interaction of marine organisms with plastic waste might be associated with the ecological behaviour of species (Justino et al., 2022).

In the present study, among tuna caught by industrial fleets outside the EEZ, only *T. albacares* presented a single plastic bag. However, concerning the tuna and large pelagic species caught in the FNA, *A. solandri* was the most contaminated species (FO= 8%), followed by *S. barracuda* (FO= 3%) and *T. albacares* (FO= 3%) captured in the same area. The items extracted from the fishes were filaments, synthetic fishhooks, artificial bait, and plastic tape. The availability of macroplastics in the vicinity of the Archipelago is mainly due to the intense tourist activity and fishery in the region and the island's topography. Islands can accumulate plastic material on the water's surface due to the island effect (Lima et al., 2016). Besides that, these predator species are generalists-opportunists, feeding mainly on fishes, cephalopods and crustaceans (Martins et al., 2021). Plastic fragments are more abundant in shallow waters due to their buoyancy, and tuna's feeding behaviour, which involves rounding up and chasing prey schools into shallow water, increases the chances of ingesting plastic (Romeo et al., 2015). Thus, their foraging habits in the islands are closely associated with coastal regions, which may be subjected to higher amounts of plastic waste due to their proximity to urban centres.

In addition, some of these plastic items may serve as a habitat for microorganisms due to biofouling (Pinheiro et al., 2021) and could be attractive to fishes, which may accidentally ingest them by confusion with prey items. In the tropical and oligotrophic waters away from the islands, tuna species need to forage over large areas in search of feeding resources; as a result, they might be even more exposed to plastic pollution (Roch et al., 2020). However, the low presence of macroplastics in individuals caught by the industrial fisheries outside the EEZ may be related to the fast evacuation rate (~10-12h) of these tuna (Magnuson, 1969; Olson and Boggs, 1986) and strategies of regurgitation (Li et al., 2021). Ingestion of plastics can lead to several sub-lethal effects for the individual, such as digestive damage, gut

blockage, decreased predatory efficiency and starvation (de Sá et al., 2015; Menezes et al., 2019; Moore, 2008).

#### Microplastics in tuna stomachs

Microplastics were detected in the stomachs of *T. albacares* captured outside the EEZ in a high number (mean of  $10.33 \pm 14.06$  particles per ind.<sup>-1</sup>; FO= 100%). However, we did not find any correlation between the number and size of particles detected and tuna size. This high number may be related to the fact that, as opportunistic predators, due to the intake of large amounts of contaminated prey, the momentary build-up of microplastics increases before egestion (Ferreira et al., 2019; Justino et al., 2021). In addition, for tropical tuna, it is vital to have the ability to process large quantities of food in a brief period when food is available (Olson and Boggs, 1986). Hence, it may lead to numerous microplastics accumulating through trophic transfer, as was observed in our study (see next section). The tiny sizes and shapes of microplastics found in the stomachs of yellowfin tuna observed in our study strongly suggest that this accumulation is probably due to particles ingested and transferred by the prey. The length of microplastics found in the stomachs was generally smaller (mean size of  $0.77 \pm 0.92$  mm ind.<sup>-1</sup>) than previously observed for other oceanic predator species, such as the *C. hippurus* captured in the Eastern Pacific Ocean (Li et al., 2022). Besides accidental ingestion, these particles of such small size can be unintentionally swallowed when fish breathe (Li et al., 2021). However, to analyse the relevance of microplastic uptake through breathing, particles need to be detected in both gills and stomachs.

#### Trophic transfer of microplastics

The presence of microplastic in ingested prey of tuna and large pelagic species suggests that trophic transfer of microplastics might occur in fishes from the SWTA. From the analysed prey items found in fishes, we detected 355 microplastic particles. Differences in the contamination rate were observed between the ingested prey. Cephalopoda outside the EEZ was the most contaminated prey ( $27.33 \pm 30.98$  part. ind.<sup>-1</sup>), followed by Bramidae fishes ( $19.45 \pm 31.15$  part. ind.<sup>-1</sup>), also predated in the EEZ. Compared with prey that was preyed upon outside the EEZ, in FNA, they were less contaminated (microplastic number). For example, when grouping the data, fishes predated in the FNA had an average of  $1 \pm 1.34$  MPs

(part. ind.<sup>-1</sup>). In the FNA, previous studies have reported the ubiquitous presence of microplastics in the water column, mainly fibres (Ivar Do Sul et al., 2014), and also extracted in the digestive tract of deep-sea fish (Justino et al., 2022). In fact, in our study, fish that were predated in the FNA had more fibres in their stomachs when compared with fishes predated outside the EEZ. Islands can retain these particles near shore due to the action of waves and winds, which might explain these findings (Gove et al., 2019). However, when we compare the shapes of microplastics in the prey, we observe a clear difference between the areas. Prey predated outside the EEZ had most foams and pellets in their digestive tract.

Tuna that prey in areas far from islands may be feeding in deeper layers. Observations of the behaviour of *T. obesus* in the Pacific Ocean have recorded that this species performs daily vertical migration to forage, diving at night at 100 m depth and between 400-500 m during daytime (Dagorn et al., 2000). On the other hand, *T. albacares* spends most of its time in shallow waters (75 m); however, they can dive deeper than 500 m in some cases (Dagorn et al., 2006). We have observed some preys in our samples that are commonly found in deeper areas like Cephalopods (*e.g.*, *A. veranyi*), Bramidae and Gempylidae (Ferreira et al., 2022; Klautau et al., 2020; Roper et al., 1984). These prey items had already been reported for the tuna species analysed in the study area, emphasising their importance as a resource for the tuna (Silva et al., 2019). It is the first time that microplastic contamination has been registered for the preys Bramidae and Gempylidae, highlighting the lack of information for some important groups that serve as a source of energy for fish stocks. The high contamination rate in individuals caught outside the EEZ and the different shapes of particles found may be due to differences in the feeding habits of the prey. Indeed, the fish prey groups outside the EEZ (Gempylidae and Bramidae) are also opportunistic predators, like tunas, and feed mainly on cephalopods and other fish species (Froese and Pauly, 2022).

In our study, using prey as bioindicators to verify the presence of microplastics, we observed that the planktivorous fishes (*e.g.*, Exocoetidae), which feed mainly in the epipelagic zone, were less exposed to microplastics than deep-sea fishes and organisms that feed on marine aggregates (*e.g.*, Cephalopods; Hoving and Robison, 2012). Fishes of the family Exocoetidae are among the essential energy sources for tunas (Martins et al., 2021; Silva et al., 2019) and were already reported to be contaminated with plastics (Chagnon et al., 2018; Gove et al., 2019). Here, we observed a contamination rate of one particle per individual,

similar to the one observed in the Pacific Ocean for a species of the family Exocoetidae (1.5 particles per fish; Chagnon et al., 2018). Cephalopods were the prey that registered the highest contamination rate in our study. In recent research conducted in the SWTA, Ferreira et al. (2022) reported a high contamination rate in deep-sea cephalopods, which was attributed to the feeding strategy of the species, which usually feed on fish, zooplankton and marine snow. Marine snow is an organic matter aggregate that can be originated from the release of substances by decomposed organisms or other organic matter in marine environments (Tansel, 2018). Additionally, marine aggregates serve as an important energy source for various organisms, including midwater zooplankton (Steinberg et al., 1994). Incorporating microplastics into marine snow is hypothesised to be an important microplastic sinking mechanism (Kvale et al., 2020). Furthermore, most marine organisms can egest microplastics, a possible route for their incorporation into marine aggregates (Wright et al., 2013).

For the Cephalopods, Bramidae and tuna, we observed plastics of sizes  $< 1$  mm (suggesting that these particles had already been quite degraded through weathering) and were mostly foams and pellets (shapes mainly associated with aggregates). Therefore, we assume that these microplastics may have been accidentally ingested by the prey when foraging in deeper waters and transferred along the trophic chain to predators. However, we emphasise the importance of studying marine aggregates and verifying the association of microplastics and the main types of polymers present.

#### Characterisation of microplastic polymers

Among the polymers identified in our study, we found various polymer types in tuna stomachs and prey items. Nevertheless, the main polymers were identified as SBR, PA, PET and PE. The SBR polymer is often used in manufacturing car tires and as a substitute for natural rubber due to its resistance to abrasion (Polymer DataBase, 2022). Tiny particles generated from the abrasion of car tires against the road surface are widely available in the environment but still rarely reported as microplastic contaminants in environmental studies (Arias et al., 2022; Knight et al., 2020; Kreider et al., 2010). Tire wear particles (TWP) could be one of the likely sources of SBR particles in the marine environment. While it is still unclear how these particles reach the oceans, possible pathways could be atmospheric fallout, wastewater effluent, rivers, and oceanic currents (Knight et al., 2020; Luo et al., 2021). SBR was also found in the gastrointestinal tract of tuna-like species (*C. hippurus*) in the



Mediterranean Sea (Schirinzi et al., 2020), in mesopelagic fishes from the SWTA (Justino et al., 2022), and mussels *Mytilus* spp. from the Norwegian sea (Bråte et al., 2018). Moreover, the other polymers (PA, PET and PE) are primarily used in the textile industry and fishing activities (Lima et al., 2021) and are frequently reported in marine species (Justino et al., 2022; Li et al., 2022; Schirinzi et al., 2020).

In addition to the polymers themselves, there is a significant concern about the additives released from these particles, and the associated hazards are still poorly understood. For example, researchers found a compound (6PPD) derived from tire wear particles, which induced acute mortality in coho salmon in the Pacific Northwest (Tian et al., 2021). Moreover, the leachate of TWP can be toxic to organisms (Yang et al., 2022). In addition to additives, microplastics can adsorb and concentrate other pollutants (*e.g.*, heavy metals and persistent organic pollutants), which are widely available in the ocean (Ashton et al., 2010; Rochman et al., 2013) and can be bioaccumulated and biomagnified in the food web (Batel et al., 2016; Teuten et al., 2009). Furthermore, in an experimental study, trophic transfer of microplastics was reported as an important route to accumulating plastic additives in fish tissues (Hasegawa et al., 2022).

Moreover, the exposure of marine organisms to microplastic contaminants is of major concern for human health, which depends on fishery resources. For example, microplastics have already been detected in canned tuna (Akhbarizadeh et al., 2020; Diaz-Basantes et al., 2022). The fact that the tuna is ingesting such small particles serves as an additional warning to society, which is already exposed to these particles through various pathways, such as the atmosphere, water, salt and seafood (Bruzaca et al., 2022; Karami et al., 2017; Pratesi et al., 2021; Wang et al., 2020). The degradation of microplastics into progressively smaller particles, such as nano plastics, can increase health risks due to their ability to accumulate in tissues such as the brain and cause oxidative DNA damage in the regions where they bioaccumulate (Sökmen et al., 2020). Furthermore, plastic particles have recently been detected in human blood (Leslie et al., 2022). In our study, it was impossible to quantify nanoparticles due to the methodology used, so we merely reported microplastics here (0.04–5 mm). However, due to the potential risks of bioaccumulation of nanoparticles, and their associated risks with other pollutants available in the environment, we emphasise the importance of further investigations of the degradation of polymers and their impact on

marine organisms, and we reaffirm the urge for a debate on measures to establish appropriate limit values for safe consumption.

## Conclusions

This is the first study to assess microplastics contaminating tuna and large pelagic species and to observe strong evidence of microplastic trophic transfer in tunas from the South Atlantic Ocean. We found a low frequency of occurrence of macroplastic in the four species analysed. Conversely, we observed a high abundance of microplastics in the stomachs of *T. albacares*, including ingested prey.

The low occurrence of macroplastics is probably due to the species' rapid egestion and the regurgitation of items. On the other hand, the high contamination rate by microplastic may be due to the opportunistic behaviour of predatory species and its potential to accumulate these particles through trophic transfer. This study verified the possibility of trophic transfer of microplastics by analysing the ingestion rate of particles in prey found in tuna. Ingestion rates differed significantly between the prey species, and the most contaminated prey were the Cephalopods and fishes of the Bramidae family caught outside the EEZ. The ecological habits of organisms can explain the high number of microplastics found in the prey. Cephalopods from deeper waters usually feed on marine snow that may contain aggregated microplastics. On the other hand, the Bramidae are opportunistic predators such as the tunas. Additionally, predators generally feed on a large amount of available prey, accumulating the particles prior to egestion and thus transferring them into the trophic chain. The most frequent shapes of microplastics found in both prey and tuna stomachs were foams and pellets with sizes < 1 mm. A variety of polymers were identified; the most frequent were SBR, PA, PET and PE.

Our findings enhance scientific knowledge of how the ecological behaviour of marine species can affect the intake of microplastics. Moreover, it alerts the current contamination level of apex predators, such as tunas, which can pose severe risks to human health, given their worldwide high socio-economic value stocks. The information provided here may be used to monitor microplastic contamination in fish stocks and help decision-makers establish future mitigation strategies.

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