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

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13

14 **Abstract**

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

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

33 **Introduction**

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

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

52 Microplastics are present in marine ecosystems globally, spanning from coastal to  
53 polar regions, and are distributed throughout the water column vertically (Choy et al., 2019;  
54 Lins-Silva et al., 2021; Waller et al., 2017). Due to their small size and abundance, fish can  
55 inadvertently ingest microplastics through the respiratory process and be mistaken as prey  
56 (Boerger et al., 2010; Li et al., 2021). The ingestion of microplastics can pose several threats  
57 to marine biota (Galloway et al., 2017), such as damage to the digestive system, reduction in  
58 predation efficiency, and induction of toxic effects (Barboza et al., 2018; de Sá et al., 2015;  
59 Moore, 2008; Teuten et al., 2007). Additionally, species’ ecological behaviour is an important  
60 factor influencing microplastic intake (Savoca et al., 2021; Justino et al., 2022; Ferreira et al.,  
61 2023).

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

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

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

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

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

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

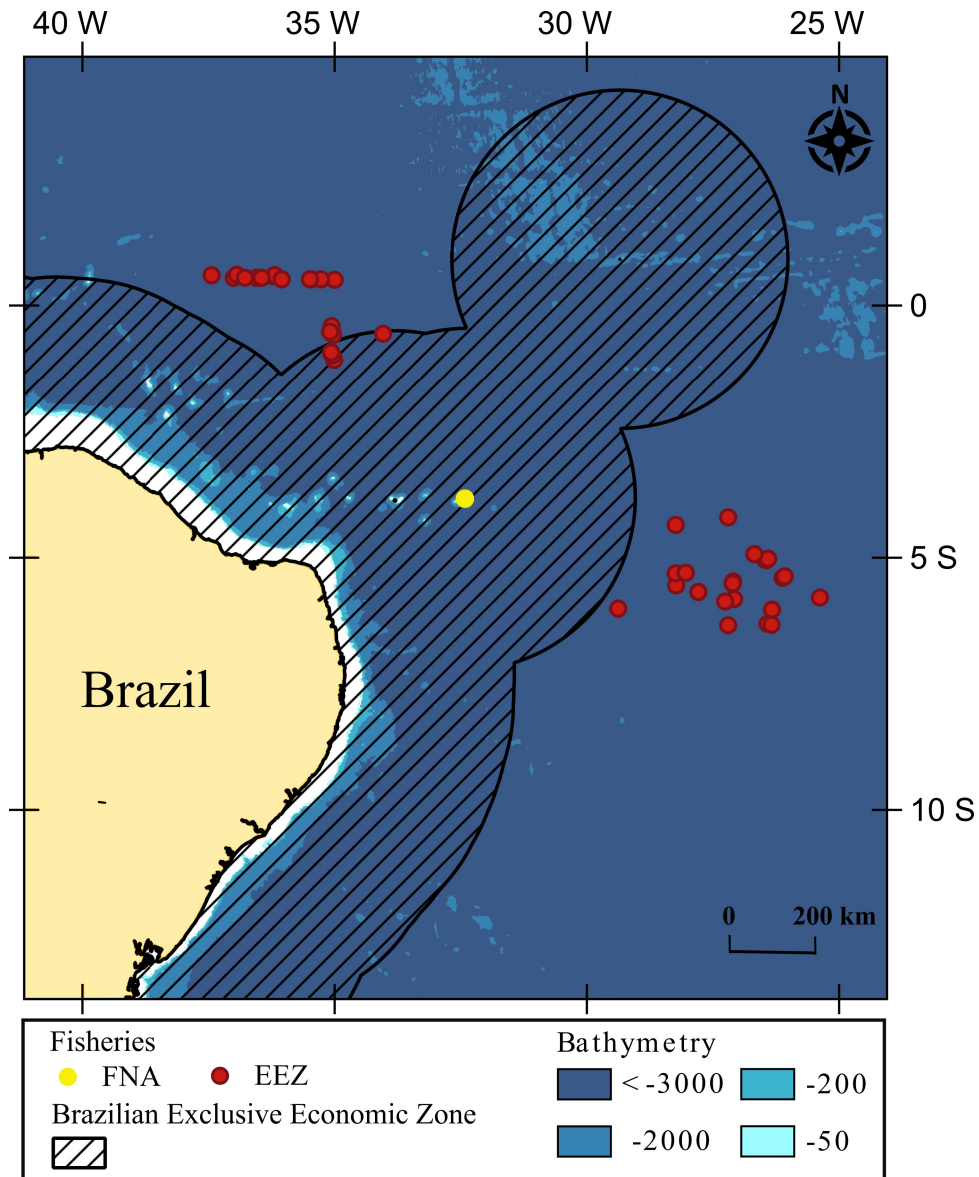
101         Analysing microplastics in larger predatory fishes is a great challenge, mainly due to  
102 laboratory procedures. The use of standard techniques for the digestion of stomachs to  
103 separate the organic matter and the plastic items (*e.g.*, alkaline and acid digestion) is very  
104 time-consuming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

## 113         **Materials and Methods**

### 114         *Study area and sampling*

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

123



124

125

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

129

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

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

141 In both fisheries (EEZ and FNA), a total of 350 samples of tuna and large pelagic  
142 species were collected: *T. albacares* (yellowfin tuna, YFT; n = 102); *T. obesus* (bigeye tuna,  
143 BET; n = 63); *S. barracuda* (barracuda, BAR; n = 136); and *A. solandri* (wahoo, WAH; n =  
144 49).

#### 145 *Contamination control*

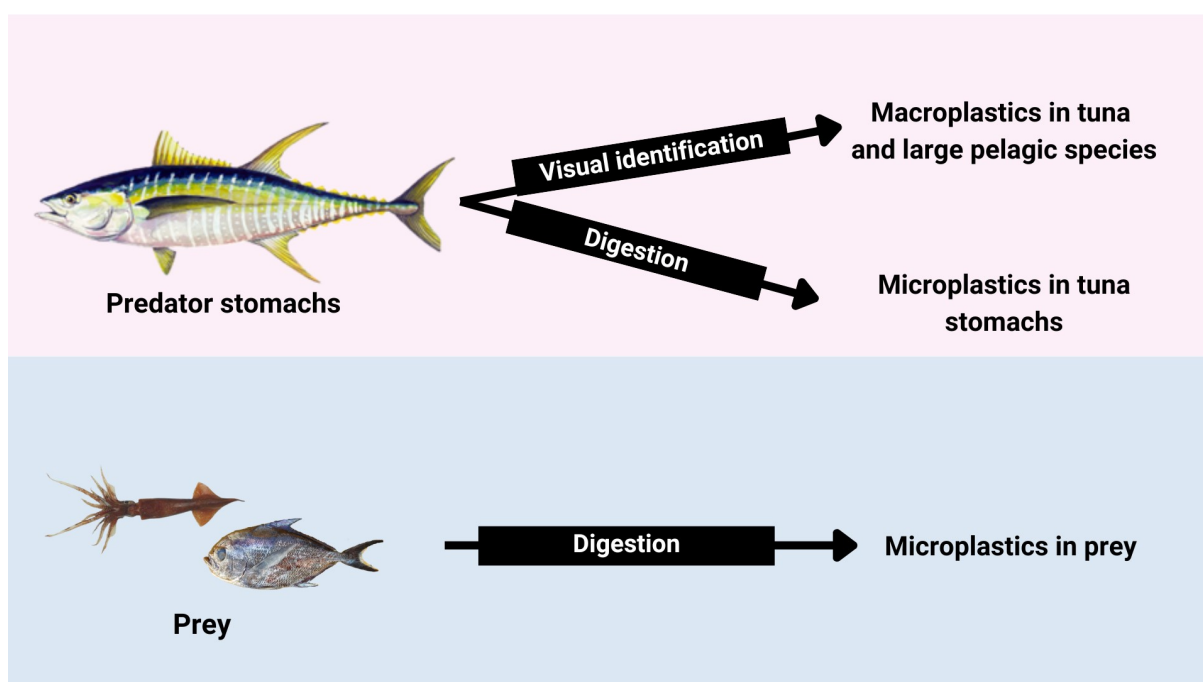
146 Several steps were carried out before the extractions (macro- & microplastics) to  
147 ensure quality assurance and quality control (QA/QC) and to avoid cross-contamination,  
148 following the protocol proposed by Justino et al. (2021). The protocol includes using 100%  
149 cotton lab coats and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

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

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

169 *Laboratory procedures and plastic extraction*

170



171

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

176

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

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



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

185 Tuna prey was measured, weighed, and stored for microplastic (< 5 mm) analysis (see  
186 next section). A total of 34 prey items were identified at the lowest taxonomic level possible  
187 and classified into large groups (mainly fishes and cephalopods) (Humann and DeLoach,  
188 2002; Vaske-Júnior, 2006). In the case of prey identified as fish, only the digestive tract  
189 (stomach and intestine) was used for digestion, whereas for cephalopods, beaks and pen were  
190 removed, and then the whole animal was digested (Ferreira et al., 2022).

#### 191 Microplastic detection in tuna stomachs and prey

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

196 Microplastic extraction from prey and *T. albacares* stomachs was performed with the  
197 help of an alkaline digestion protocol using sodium hydroxide (NaOH, 1 mol L<sup>-1</sup>; PA 97%)  
198 (Justino et al., 2021). The prey samples (digestive tract of fish and the whole cephalopod) and  
199 the gut content of *T. albacares* stomachs were carefully washed before analysis with filtered  
200 distilled water to remove any particles attached to the external tissue. Then, samples were  
201 placed in a beaker and submerged in the NaOH solution (the proportion used was 1:100 w/v),  
202 covered by a glass lid and oven-dried at 60 °C for 24 h. After that, samples were filtered  
203 through a 47 mm GF/F 0.7 µm pore size glass fibre filter (© Whatman) using a vacuum pump  
204 system. After filtration, samples were carefully set in a Petri dish and covered; these filters  
205 were oven-dried again at 60 °C for 24 h. Finally, the filters were visually inspected for  
206 microplastics using a stereomicroscope (© Zeiss Stemi 508, with a size detection limit of 0.04  
207 mm). All the particles suspected to be microplastics were counted, photographed (© Zeiss  
208 Zen 3.2; Axiocam 105 Color), and measured in length (mm). Microplastics were first  
209 categorised according to their shape as fibres (filamentous shape), fragments (irregular shape),  
210 films (flat shape), foams (soft with an irregular shape), or pellets (spherical shape) (Justino et  
211 al., 2021).

212 *Polymer analysis*

213 A random subset (15% of total microplastic extracted; 67 particles) of samples was  
214 selected to identify the main polymers using the Laser Direct Infrared (LDIR) analyser  
215 Agilent 8700 Chemical Imaging System with the Microplastic Starter 1.0 library. The LDIR  
216 analyser scans the particles (size range 20–5000  $\mu\text{m}$ ) within a wavelength range of 1800–975  
217  $\text{cm}^{-1}$  (Ourgaud et al., 2022). The information is collected with the Clarity image software (©  
218 Agilent version 1.3.9) and compared with the polymer spectrum library (~400 references  
219 spectra). We confirmed the polymer type of a particle when the identification match was  
220 >70% (Ferreira et al., 2022; Eo et al., 2021). It is important to remark that since the polymer  
221 composition was only accessed in a sub-sample of the detected particles, there is a possibility  
222 that non-plastic particles may have been accounted as microplastics to some extent.

223 *Data analysis*

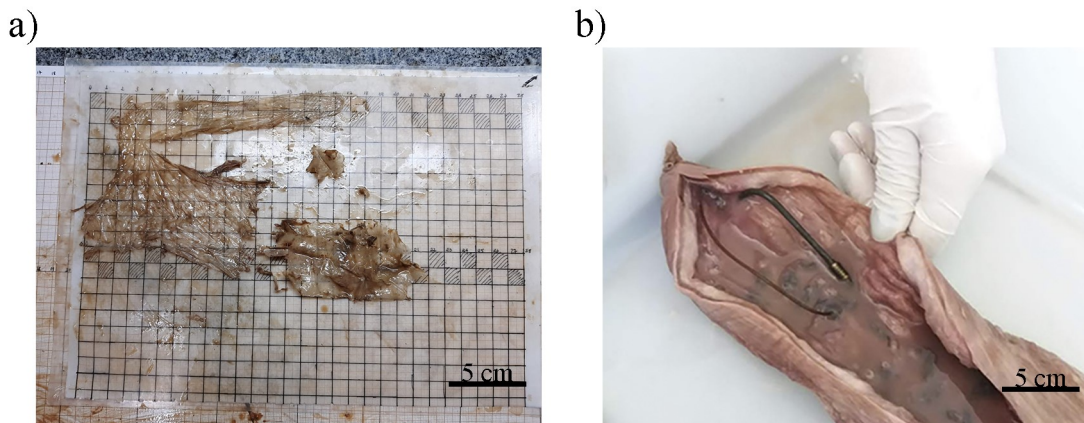
224 As the data on microplastic particles did not meet parametric assumptions, a Kruskal-  
225 Wallis test was used to verify whether detected particles in tuna prey presented significant  
226 differences among the fleets, predator species, prey groups, and prey species according to  
227 microplastic mean number and size. When significant differences were detected, a *post hoc*  
228 pairwise comparison, Dunn's test, was used to investigate the sources of variance (Dunn,  
229 1964). A Spearman's correlation test was used to verify the relationship between  
230 microplastics and the predator's biometry. All statistical analyses were performed with the  
231 software R version 3.6.3 (R Core Team, 2020) and were conducted considering a significance  
232 level of 5%.

233 **Results**

234 *Macroplastics in tuna and large pelagic species*

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

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



242  
243 **Figure 3.** Macroplastics detected in the tuna and large pelagic species from the Southwestern Tropical  
244 Atlantic: a) plastic bag and b) synthetic fishhook.

245 *Microplastics in the stomachs of T. albacares*

246 A total of 93 microplastic particles were recovered from the nine stomachs of *T.*  
247 *albacares* (FO= 100%) captured outside the EEZ, with an average of  $10.33 \pm$  standard  
248 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  
249 analysed *T. albacares* ranged from 40 to 145 cm in fork length and weighed from 1 to 47.8  
250 kg. However, there was no relationship between the number and size of detected particles and  
251 the size of tuna (Spearman's rank correlation,  $p > 0.05$ ). Overall, regarding the shapes of the  
252 microplastics, the most abundant were foams (61%), followed by fibres (22%), films (10%),  
253 pellets (6%) and fragments (1%). The colours white and blue were the most predominant  
254 (Table 1).

255

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

259

260

		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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### *Microplastics in tuna prey*

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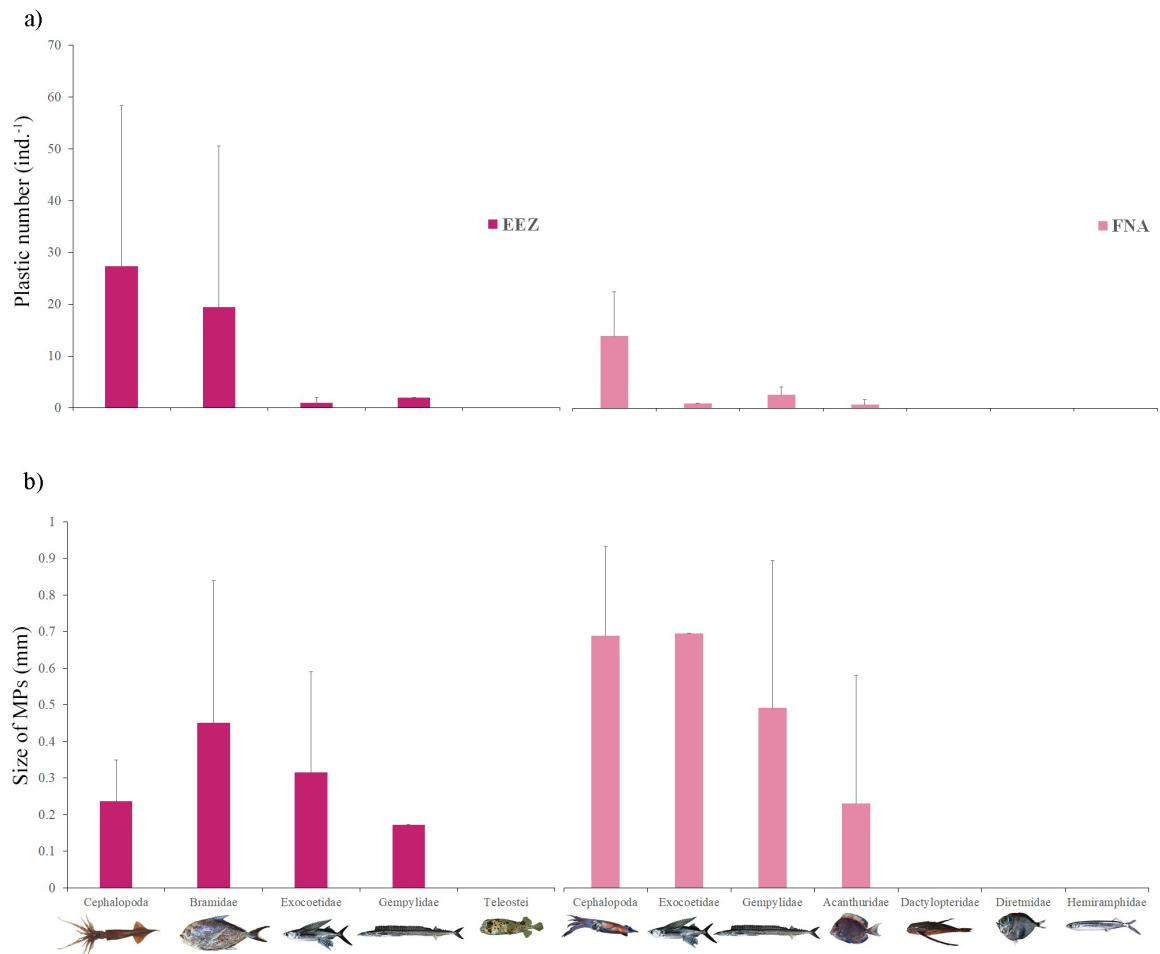
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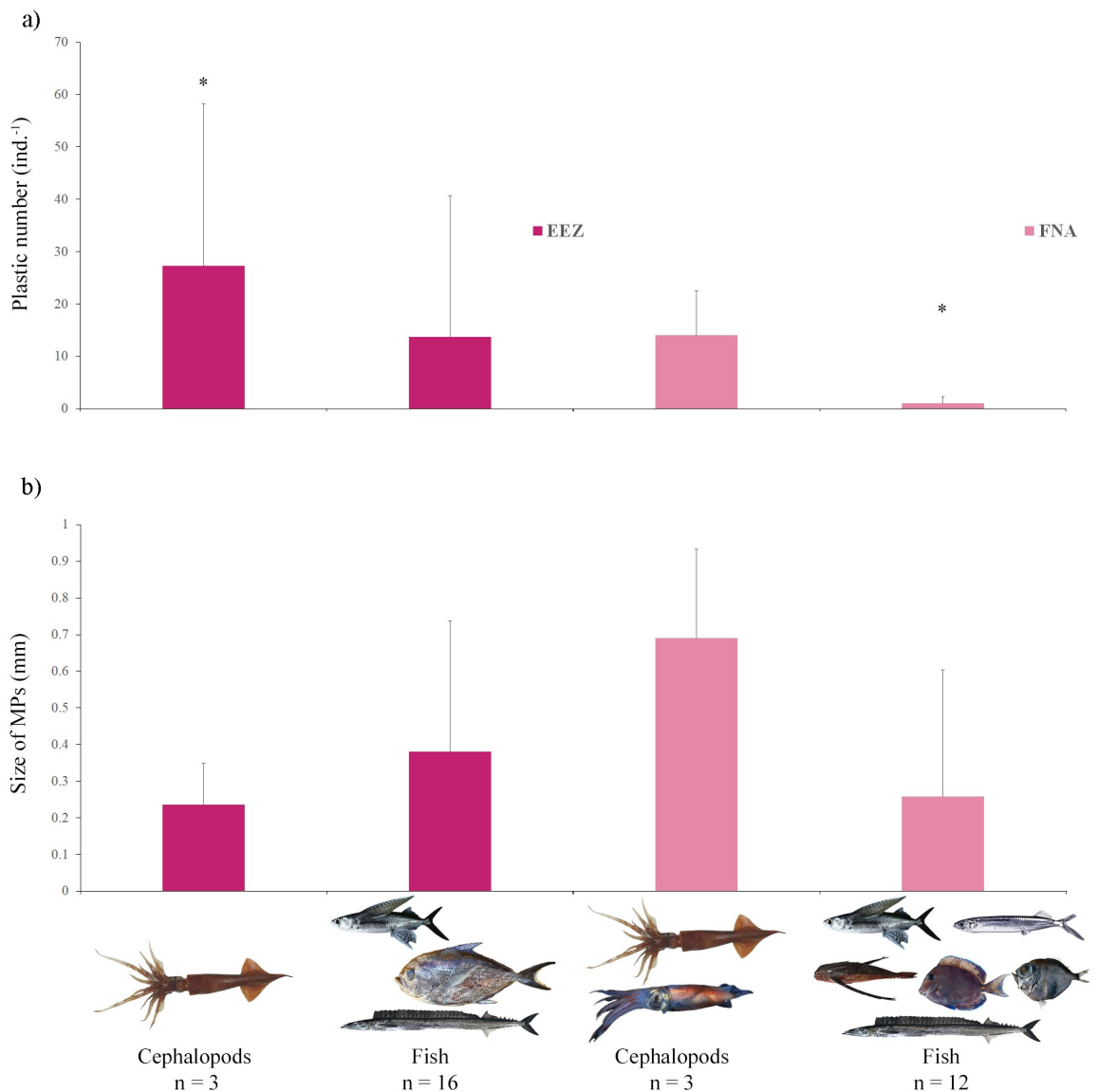
Among all the analysed stomachs, we recovered 34 tuna prey items with their organs intact. The main prey items found in tuna caught outside the EEZ were identified as Cephalopoda, Bramidae, Exocoetidae, Gempylidae, and Teleostei (unidentified fish). The prey found in the FNA catches were identified as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



276

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

279 Nevertheless, when we grouped prey into larger groups, fish and cephalopods, we also  
 280 observed significant differences in contamination rates (chi-squared = 13.226, df = 3,  $p <$   
 281 0.05). Fish predated in FNA were less contaminated ( $1 \pm 1.34$  part. ind.<sup>-1</sup>) than cephalopods  
 282 predated outside of the EEZ ( $27.33 \pm 30.99$  part. ind.<sup>-1</sup>), and the cephalopods from the FNA  
 283 ( $14 \pm 8.54$  part. ind.<sup>-1</sup>), and also fish from the outside of the EEZ ( $13.75 \pm 26.96$  part. ind.<sup>-1</sup>)  
 284 (Fig. 5). Dunn's *post hoc* test showed that the number of microplastics detected in fish  
 285 predated on FNA differed from that found in the cephalopods of EEZ and FNA.



286

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

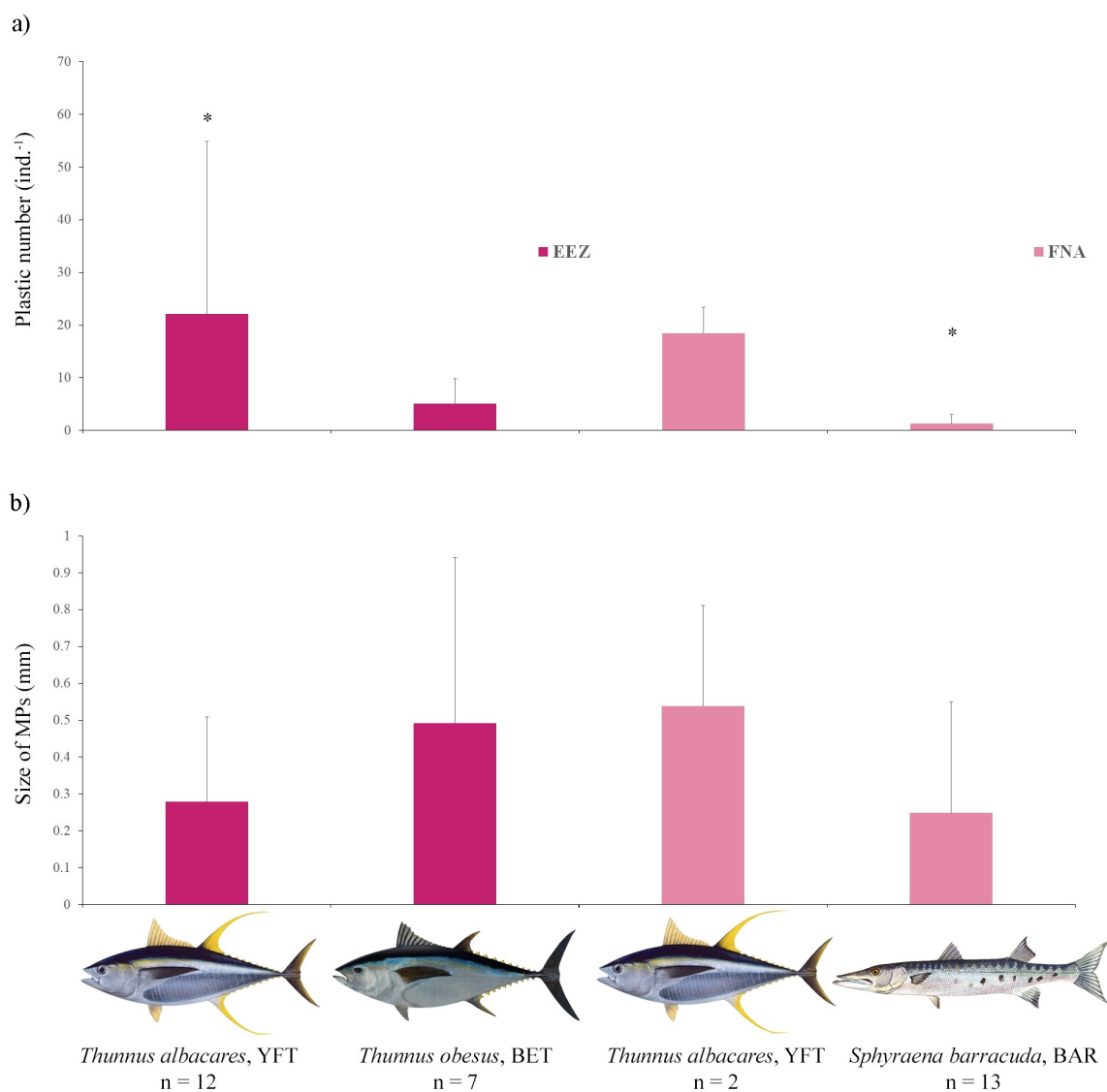
289

290 *Prey as a data proxy for tuna contamination*

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

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

301



302

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

306

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

312



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

*micropterus*

Cephalopod;

*Ornitotheuthis antillarum*

1

FNA

5

100

5

0.77 ( $\pm 0.73$ )

1.91

0.25

0.83

$\beta$

314

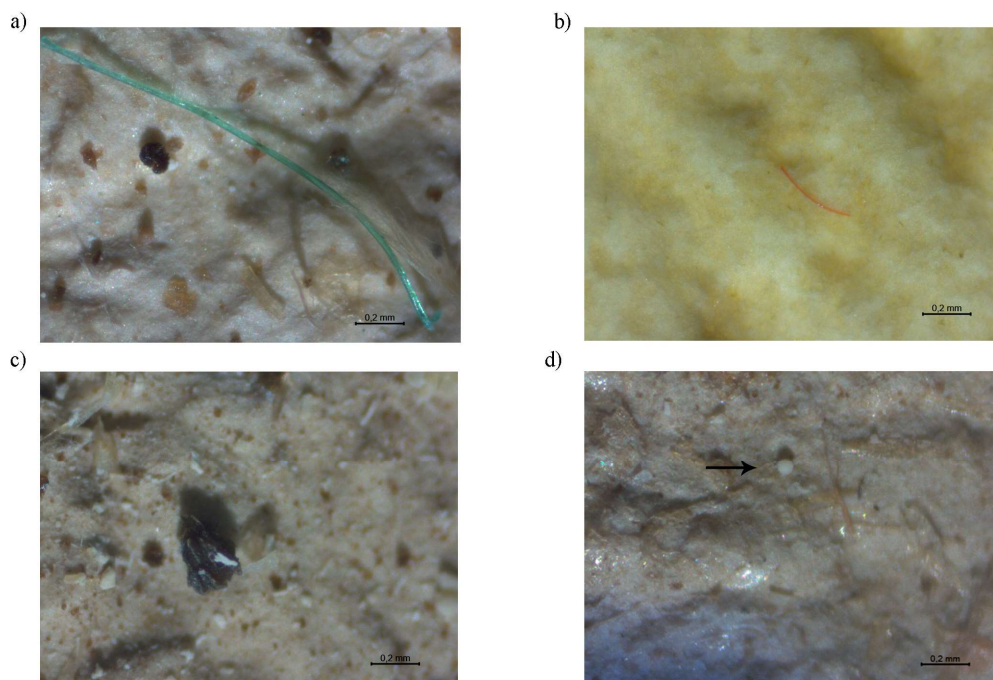
315

316

317           Regarding the shapes of microplastics identified in the prey and used as a proxy for  
318 tuna contamination, we observed that *T. albacares* captured outside the EEZ ingested mainly  
319 pellets (61%), fragments (24%), foams (12%), fibres (2%) and films (1%) (Fig. S1). The *T.*  
320 *obesus* from the EEZ ingested mostly foams (75%), followed by fibres (14%), pellets and  
321 fragments (6%) (Fig. S1). Meanwhile, in *T. albacares* caught in the FNA, ingested mostly  
322 pellets (51%) and fibres (43%), whereas films represented only 5% of the total (Fig. S1). For  
323 the *S. barracuda*, the main shapes observed were fibres (76%), followed by fragments (12%),  
324 pellets and foams (6%) (Fig. S1).

### 325           *Identified polymers*

326           Overall, plastic polymers were successfully identified in 35% of particles from the  
327 subset of samples analysed by LDIR. Particles that were identified between 60-69.9%  
328 similarity to the reference spectrum were considered partially identified and comprise 34% of  
329 the samples (Fig. S2). The lower similarity between partially identified particle spectra to  
330 reference spectra might be explained by the advanced weathering of the particles. LDIR is  
331 still a novel technique for identifying plastic polymers in environmental samples; the number  
332 of reference spectra of weathered polymers will increase in the future, thereby diminishing the  
333 percentage of partially identified particles. Biopolymers identified as cellulose and natural  
334 polyamide were observed in 19% of all particles, and 12% were unidentified. A wide range of  
335 polymers was identified, but the most commons were Styrene-Butadiene Rubber (SBR) with  
336 17% abundance, followed by Polyamide (PA) with 15%, Polyethylene Terephthalate (PET)  
337 and Polyethylene (PE) with a similar abundance of 12%, and Polyurethane (PU) with 10%.  
338 The other polymers, such as Low-density polyethylene (LDPE), Polyvinyl chloride (PVC),  
339 Acrylonitrile Butadiene Styrene (ABS), Alkyd Varnish, Polypropylene (PP), Polystyrene  
340 (PS), Polymethylmethacrylate (PMMA), Polytetrafluoroethylene (PTFE), and Chlorinated  
341 Polyisoprene contributed with a similar abundance of 2-5% (Fig. S2).



342

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

346

347 **Discussion**

348 Macroplastic in tuna

349 In general, we found a low frequency of macroplastic contamination (FO= 3%) in tuna  
 350 and large pelagic species caught in the Southwestern Tropical Atlantic (SWTA). This low  
 351 frequency of occurrence has also been observed in other studies analysing the ingestion of  
 352 marine debris in *T. albacares*, *T. obesus* and *Katsuwonus pelamis* (FO= 0, 0 and 0.75%,  
 353 respectively) from the Western Atlantic (de Mesquita et al., 2021), in *T. albacares* from the  
 354 South Pacific subtropical gyre (2%) (Chagnon et al., 2018), and in *K. pelamis*, *T. albacares*,  
 355 *C. hippurus*, and *T. obesus* (FO= 0, 0, 2 and 9%, respectively) from the North Pacific  
 356 subtropical gyre (Choy and Drazen, 2013). Researchers often suggest that the lower intake of  
 357 large plastics by pelagic fishes can be explained due to geographical location (de Mesquita et  
 358 al., 2021) since plastics tend to accumulate in oceanic gyres (Cózar et al., 2014; Jiang et al.,  
 359 2020) and areas that do not have a convergence zone would accumulate fewer plastics.

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

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

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

390 blockage, decreased predatory efficiency and starvation (de Sá et al., 2015; Menezes et al.,  
391 2019; Moore, 2008).

### 392 Microplastics in tuna stomachs

393 Microplastics were detected in the stomachs of *T. albacares* captured outside the EEZ  
394 in a high number (mean of  $10.33 \pm 14.06$  particles per ind.<sup>-1</sup>; FO= 100%). However, we did  
395 not find any correlation between the number and size of particles detected and tuna size. This  
396 high number may be related to the fact that, as opportunistic predators, due to the intake of  
397 large amounts of contaminated prey, the momentary build-up of microplastics increases  
398 before egestion (Ferreira et al., 2019; Justino et al., 2021). In addition, for tropical tuna, it is  
399 vital to have the ability to process large quantities of food in a brief period when food is  
400 available (Olson and Boggs, 1986). Hence, it may lead to numerous microplastics  
401 accumulating through trophic transfer, as was observed in our study (see next section). The  
402 tiny sizes and shapes of microplastics found in the stomachs of yellowfin tuna observed in our  
403 study strongly suggest that this accumulation is probably due to particles ingested and  
404 transferred by the prey. The length of microplastics found in the stomachs was generally  
405 smaller (mean size of  $0.77 \pm 0.92$  mm ind.<sup>-1</sup>) than previously observed for other oceanic  
406 predator species, such as the *C. hippurus* captured in the Eastern Pacific Ocean (Li et al.,  
407 2022). Besides accidental ingestion, these particles of such small size can be unintentionally  
408 swallowed when fish breathe (Li et al., 2021). However, to analyse the relevance of  
409 microplastic uptake through breathing, particles need to be detected in both gills and  
410 stomachs.

### 411 Trophic transfer of microplastics

412 The presence of microplastic in ingested prey of tuna and large pelagic species  
413 suggests that trophic transfer of microplastics might occur in fishes from the SWTA. From the  
414 analysed prey items found in fishes, we detected 355 microplastic particles. Differences in the  
415 contamination rate were observed between the ingested prey. Cephalopoda outside the EEZ  
416 was the most contaminated prey ( $27.33 \pm 30.98$  part. ind.<sup>-1</sup>), followed by Bramidae fishes  
417 ( $19.45 \pm 31.15$  part. ind.<sup>-1</sup>), also predated in the EEZ. Compared with prey that was preyed  
418 upon outside the EEZ, in FNA, they were less contaminated (microplastic number). For  
419 example, when grouping the data, fishes predated in the FNA had an average of  $1 \pm 1.34$  MPs

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

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

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

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

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

#### 470 Characterisation of microplastic polymers

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



482 Mediterranean Sea (Schirinzi et al., 2020), in mesopelagic fishes from the SWTA (Justino et  
483 al., 2022), and mussels *Mytilus* spp. from the Norwegian sea (Bråte et al., 2018). Moreover,  
484 the other polymers (PA, PET and PE) are primarily used in the textile industry and fishing  
485 activities (Lima et al., 2021) and are frequently reported in marine species (Justino et al.,  
486 2022; Li et al., 2022; Schirinzi et al., 2020).

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

498 Moreover, the exposure of marine organisms to microplastic contaminants is of major  
499 concern for human health, which depends on fishery resources. For example, microplastics  
500 have already been detected in canned tuna (Akhbarizadeh et al., 2020; Diaz-Basantes et al.,  
501 2022). The fact that the tuna is ingesting such small particles serves as an additional warning  
502 to society, which is already exposed to these particles through various pathways, such as the  
503 atmosphere, water, salt and seafood (Bruzaca et al., 2022; Karami et al., 2017; Pratesi et al.,  
504 2021; Wang et al., 2020). The degradation of microplastics into progressively smaller  
505 particles, such as nano plastics, can increase health risks due to their ability to accumulate in  
506 tissues such as the brain and cause oxidative DNA damage in the regions where they  
507 bioaccumulate (Sökmen et al., 2020). Furthermore, plastic particles have recently been  
508 detected in human blood (Leslie et al., 2022). In our study, it was impossible to quantify  
509 nanoparticles due to the methodology used, so we merely reported microplastics here (0.04–5  
510 mm). However, due to the potential risks of bioaccumulation of nanoparticles, and their  
511 associated risks with other pollutants available in the environment, we emphasise the  
512 importance of further investigations of the degradation of polymers and their impact on

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

515

## 516 **Conclusions**

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

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

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

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557

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