
The role of mesopelagic fishes as microplastics vectors across the deep-sea layers from the Southwestern Tropical Atlantic

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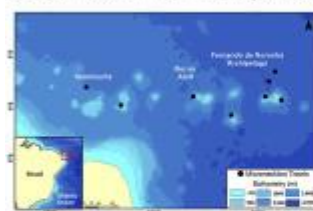
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Abstract :

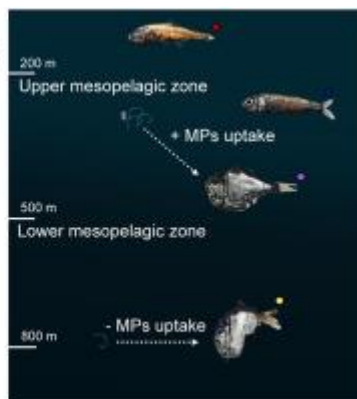
Microplastics (MPs; <5 mm) are a macro issue recognised worldwide as a threat to biodiversity and ecosystems. Widely distributed in marine ecosystems, MPs have already been found in the deep-sea environment. However, there is little information on ecological mechanisms driving MP uptake by deep-sea species. For the first time, this study generates data on MP contamination in mesopelagic fishes from the Southwestern Tropical Atlantic (SWTA) to help understand the deep-sea contamination patterns. An alkaline digestion protocol was applied to extract MPs from the digestive tract of four mesopelagic fish species: *Argyropelecus sladeni*, *Sternoptyx diaphana* (Sternoptychidae), *Diaphus brachycephalus*, and *Hygophum taaningi* (Myctophidae). A total of 213 particles were recovered from 170 specimens, and MPs were found in 67% of the specimens. Fibres were the most common shape found in all species, whereas polyamide, polyethylene, and polyethylene terephthalate were the most frequent polymers. The most contaminated species was *A. sladeni* (93%), and the least contaminated was *S. diaphana* (45%). Interestingly, individuals caught in the lower mesopelagic zone (500–1000 m depth) were less contaminated with MPs than those captured in the upper mesopelagic layer (200–500 m). Our results highlight significant contamination levels and reveal the influence of mesopelagic fishes on MPs transport in the deep waters of the SWTA.

Graphical abstract

67% of the fishes analysed were contaminated with microplastics



Species	MPs detected (mean \pm SD)
<i>Hypophum teaningsi</i>	1.07 \pm 1.20
<i>Diaphus brachycephalus</i>	1.63 \pm 1.41
<i>Argyroplectus alaberti</i>	1.66 \pm 1.23
<i>Sternopyx diaphana</i>	0.54 \pm 0.71



Highlights

► Microplastics were found in deep-sea fishes from the Southwestern Tropical Atlantic. ► The most frequent polymers identified were PA, PE, and PET. ► Ingestion rates of microplastics varied between species and depth. ► Fishes ingested more microplastics in the upper mesopelagic layer.

Keywords : Marine Pollution, Plastic ingestion, Myctophidae, Sternoptychidae, Oceanic islands

38 Since its invention, plastic production has risen considerably, reaching up to 348 million
39 tons (Mt) in 2017 (PlasticsEurope, 2018), with a prognosis to hit 1100 Mt by 2050 (Geyer,
40 2020). Vast quantities of plastic materials are mismanaged or illegally discarded in marine
41 ecosystems (Koelmans et al., 2017; Ostle et al., 2019). Land-based sources contribute to about
42 80% of plastics entering the oceans (Andrady, 2011) via riverine discharges (Meijer et al.,
43 2021). In marine ecosystems, plastic debris is weathered by natural processes (*e.g.*,
44 hydrodynamics, solar radiation and interaction with biota (Jambeck et al., 2015; Thompson et
45 al., 2004) and eventually fragmented into microplastics (MPs, < 5 mm; Arthur et al., 2009).

46 MPs are widely distributed all over the marine environment, from urban coastal areas
47 (Lins-Silva et al., 2021) to remote regions such as the Arctic and Antarctic polar seas (Lusher
48 et al., 2015; Waller et al., 2017). MPs accumulate in the ocean gyres (Jiang et al., 2020) due to
49 the interaction of winds and rotatory ocean currents. In the Atlantic Ocean, remote islands are
50 known to be contaminated with MPs, as is the case of Falklands and Ascension Islands (Green
51 et al., 2018); the Canary Islands (Álvarez-Hernández et al., 2019); Abrolhos Archipelago,
52 Fernando de Noronha Archipelago, and Trindade Island (Ivar do Sul et al., 2013, 2014). In the
53 short term, these islands might retain MPs in the nearshore due to the actions of winds, waves,
54 vortices, and eddies surrounding the islands (Lima et al., 2016). Nevertheless, not only the sea
55 surface is impacted by MPs, but also the deep-sea, which has been pointed out as a major MPs
56 reservoir (Woodall et al., 2014). Indeed, MPs have already been observed in the subsurface
57 waters, sediments, and fauna of the deep sea (Lusher et al., 2016; Courtene-Jones et al., 2017;
58 Choy et al., 2019; Jamieson et al., 2019; Kane et al., 2020). However, processes involved in the
59 dispersion and fate of MPs into deeper ocean layers are still poorly understood.

60 MPs can be transported from the surface to deep waters through interaction with marine
61 communities. For example, giant larvaceans can pack MPs filtered in the surface into faecal
62 pellets that quickly sink to the seafloor (Katija et al., 2017; Choy et al., 2019). MPs
63 incorporation into marine snow is hypothesised to be the main sinking mechanism for buoyant
64 polymers (Kvale et al., 2020). Additionally, many deep-sea species undertake epipelagic

65 vertical migrations to feed (Eduardo et al., 2020a) and may act as biological plastic transporter
66 whenever contaminated with MPs (Ferreira et al., 2022). Although the role of mesopelagic
67 fishes in the vertical movement of MPs in the water column has been proposed, it is still not
68 well understood (Lusher et al., 2016; Savoca et al., 2021). Thus, widespread MPs pose several
69 threats to marine biota (Galloway et al., 2017), as they can easily be mistaken with prey and
70 ingested by marine species (Boerger et al., 2010). Furthermore, they might be transferred from
71 prey to predator through trophic interactions (Ferreira et al., 2016, 2019; Nelms et al., 2018).
72 Once ingested, MPs can cause digestive damage, decrease predatory efficiency, and induce
73 toxic effects (Teuten et al., 2007; Moore, 2008; de Sá et al., 2015; Barboza et al., 2018).
74 Moreover, MPs can adsorb and concentrate pollutants available in the ocean (*e.g.*, persistent
75 organic pollutants and heavy metals; Oehlmann et al., 2009; Ashton et al., 2010; Rochman et
76 al., 2013c; Jamieson et al., 2017) or release their additive burden (Paluselli et al., 2019; Fauvelle
77 et al., 2021), and may be bioaccumulated and biomagnified in the food web (Teuten et al., 2009;
78 Batel et al., 2016).

79 The mesopelagic layer (200–1000 m) hosts remarkable marine biodiversity that plays a
80 pivotal role in sequestering carbon, recycling nutrients, and acting as a key trophic link between
81 primary consumers and higher trophic levels (*e.g.*, larger fishes, mammals, and seabirds;
82 Drazen and Sutton, 2017; Eduardo et al., 2020a). Additionally, many mesopelagic species
83 migrate vertically to the upper ocean layers to feed at night and return to deep waters during
84 daylight, contributing to the connection between shallow and deep-sea ecosystems (Davison et
85 al., 2013; St. John et al., 2016; Eduardo et al., 2020b).

86 MP ingestion by mesopelagic fishes has been already reported all over the world, as
87 observed in the North Pacific Central Gyre (Boerger et al., 2010), North Pacific Subtropical
88 Gyre (Davison and Asch, 2011), North Atlantic (Lusher et al., 2016; Wieczorek et al., 2018),
89 Mediterranean Sea (Romeo et al., 2016), South China Sea (Zhu et al., 2019), and in the South
90 Atlantic, around the Tristan da Cunha and St. Helena islands (McGoran et al., 2021). However,
91 this group is still poorly investigated in deep waters due to sampling difficulties (*e.g.*, high
92 sampling cost and operational complexity), especially in the least developed countries (Howell
93 et al., 2020). To date, no study has investigated MP contamination in fishes inhabiting the
94 mesopelagic zone of the Southwestern Tropical Atlantic (SWTA). Located in the SWTA, the
95 Fernando de Noronha Archipelago (FNA) is essential for the conservation of the marine

96 biodiversity in the tropical oceanic region, as it serves as a shelter, reproduction and nursery
97 area for several species, including the mesopelagic fishes (Lima et al., 2016; Eduardo et al.,
98 2020a; Martins et al., 2021).

99 Hatchetfishes (Sternoptychidae) and lanternfishes (Myctophidae) are among the most
100 abundant and widespread mesopelagic fish groups in the world (Gjøsaeter and Kawaguchi,
101 1980; Eduardo et al., 2020a, 2021). These groups present an essential linkage between the
102 epipelagic producers and deep-sea predators since they represent a key energy source in the
103 mesopelagic zone (Eduardo et al., 2020b, 2020a, 2021).

104 Within the SWTA, four species in the mesopelagic compartment are outstanding in
105 terms of abundance and/or vertical migration: the sternoptychids *Argyropelecus sladeni* Regan,
106 1908 and *Sternoptyx diaphana* Hermann, 1781; and the myctophids *Diaphus brachycephalus*
107 (Tåning, 1928) and *Hygophum taaningi* Becker, 1965. These species are zooplanktivorous,
108 feeding primarily on fish larvae, amphipods, gelatinous, and euphausiids (Drazen and Sutton,
109 2017; Eduardo et al., 2020a; Eduardo et al., 2021). Furthermore, they all perform diel vertical
110 migration, ascending to the epipelagic zone at night mainly to forage and avoid predators
111 (Eduardo et al., 2020a; Eduardo et al., 2021). However, these species present strong niche
112 segregation, belonging to functional groups with different diet preferences, isotopic
113 composition, and vertical distribution (Eduardo et al., 2020a; Eduardo et al., 2021). These
114 ecological differences, therefore, might also influence MP uptake.

115 In this study, we identify the patterns of MP contamination in mesopelagic fishes from
116 the SWTA and their relationship with different ecological habits. Specifically, this study aims
117 (i) to describe the occurrence of MP contamination in four mesopelagic species from the
118 SWTA, (ii) to identify the main shapes and polymer nature of the ingested particles, and (iii) to
119 investigate whether there are differences in MP ingestion rates according to depth and period
120 (day or night).

121 **Materials and Methods**

122 *Study area*

123 The study area is located along the Fernando de Noronha Ridge, SWTA, with
124 oligotrophic and warm waters influenced by the South Equatorial Current (SEC) and South

125 Equatorial Undercurrent (SEUC) (Assunção et al., 2020), specifically the Fernando de Noronha
 126 Archipelago (FNA), Rocas Atoll (RA), and adjacent seamounts (Figure 1). These areas are
 127 important for marine biodiversity and are recognised as an EBSA “Ecologically and
 128 Biologically Significant Marine Area” (CBD, 2014). Furthermore, FNA is inserted in a Marine
 129 Protected Area (MPA), with a National Marine Park (PARNAMAR) and an Environmental
 130 Protection Area (EPA), which is classified as a UNESCO natural heritage. The RA is also
 131 inserted in an MPA, and it is situated at the top of a submarine mountain chain, with its base at
 132 4000 m depth, located 148 km west of the Fernando de Noronha Archipelago (Soares et al.,
 133 2010).

134 *Sample collection and laboratory procedures*

135 Mesopelagic fishes were collected using a micronekton trawl (body mesh: 40 mm, cod-
 136 end mesh: 10 mm) during the day and at night, from 90 to 800 m depth for 30 min at 2–3 kt
 137 (Eduardo et al., 2020b). Samples were collected along the Fernando de Noronha Ridge during
 138 the scientific survey ABRACOS 2 (Acoustics along the BRAzilian COaSt), carried out from
 139 9th April to 6th May 2017, onboard the French RV *Antea* (Bertrand, 2017). After each sampling,
 140 the specimens were labelled, frozen, and subsequently identified.

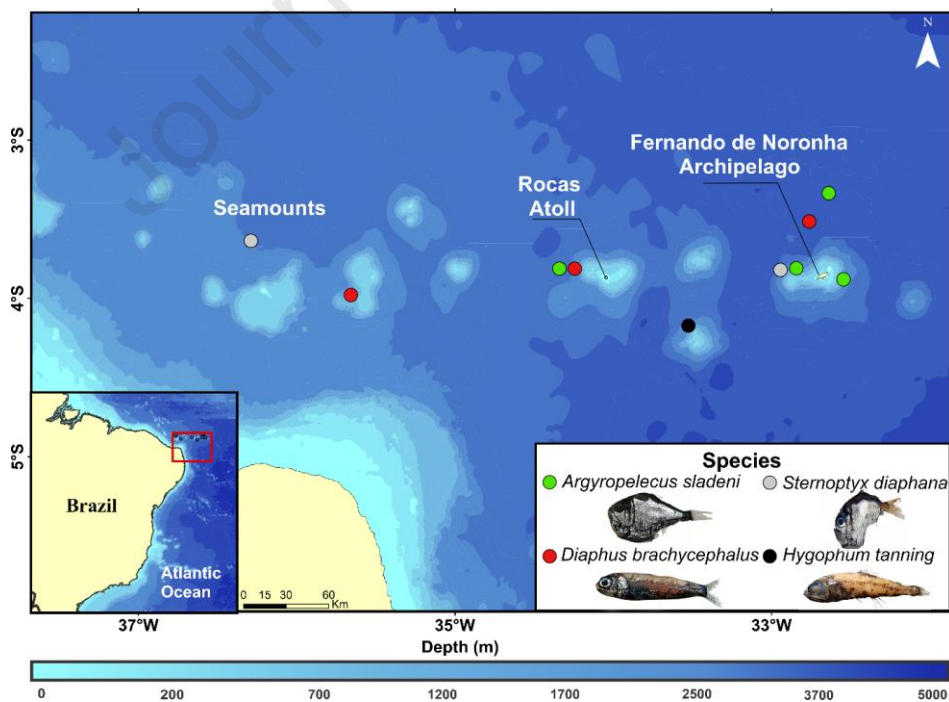


Figure 1. Fernando de Noronha Ridge, off northeastern Brazil (STWA). Sampling stations for each species are indicated by coloured circles.

141 Four mesopelagic species were selected for this study: *Argyropelecus sladeni* ($n = 15$);
142 *Sternoptyx diaphana* ($n = 33$); *Diaphus brachycephalus* ($n = 69$); and *Hygophum taaningi* ($n =$
143 53). Specimens were measured (nearest 0.1 cm of total length and standard length), weighed
144 (nearest 0.01 g of total weight), and dissected (Table I). The digestive tracts (stomach and
145 intestine) were carefully removed, weighed, and frozen again for the digestion analysis.

146 *Contamination control*

147 Before the extraction procedures, several steps were carefully carried out to ensure
148 quality assurance/quality control (QA/QC) and avoid possible airborne and cross-
149 contamination, following the protocol described by Justino et al. (2021). This QA/QC includes
150 using 100% cotton lab coats, face masks, and disposable gloves in a cleaned and reserved room,
151 with a limited flow of people during the whole process. Additionally, all solutions were filtered
152 using a vacuum pump system (equipped with laboratory glassware) through a 47 mm GF/F 0.7
153 μm pore size glass fibre filter (Whatman). Extraction tools were cleaned with ethanol 70%,
154 rinsed with filtered distilled water and checked for contamination.

155 Before starting the chemical digestion, blank procedures were done for each set of 10
156 samples. For the blanks, a beaker was filled with 50 mL of NaOH (1 mol L^{-1}) solution, covered
157 with a glass lid, and then treated with the same protocol applied to the samples (see next
158 section). A total of 4 particles were observed in the blank procedures, of which two were
159 filaments (one red and one white), and two resembled paint chips (blue). The red filament was
160 further identified as polylactic acid (PLA), and the blue particle resembled a paint chip as
161 styrene-butadiene rubber (SBR). Particles identified in the samples with any similarity to those
162 observed in the blanks were excluded from further analysis.

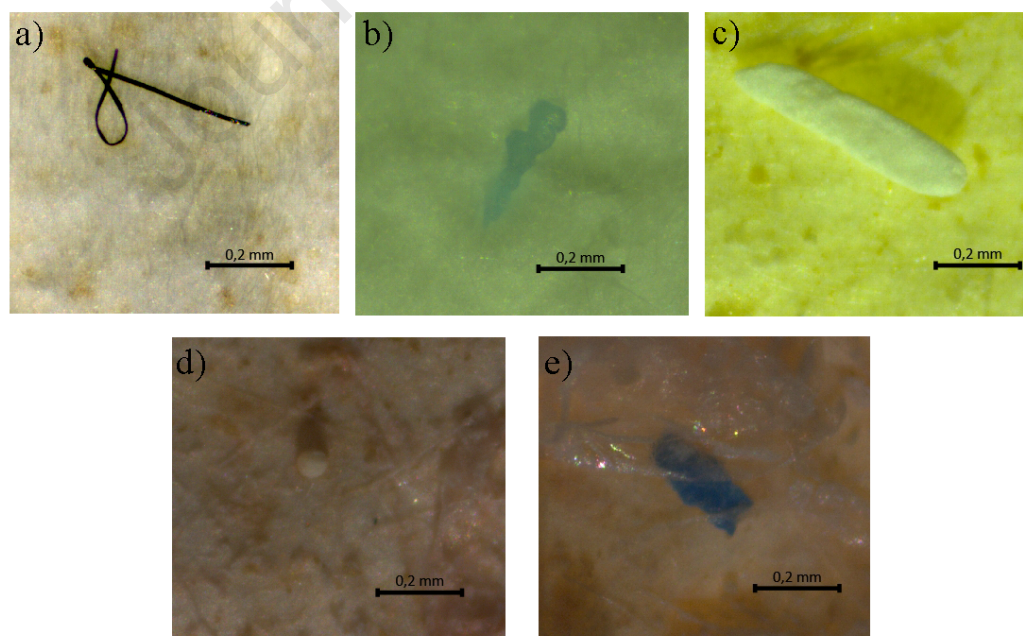
163 *Microplastic extraction protocol*

164 An alkaline digestion protocol using sodium hydroxide (NaOH) was used for extracting
165 MPs from the digestive tract of fish (Justino et al., 2021). Digestive tract samples were rinsed
166 with filtered distilled water to remove any particles adhering to the external tissue before being
167 placed in a beaker and submerged in NaOH (1 mol L^{-1} ; PA 97%) solution (the proportion used
168 was 1:100 (w/v), i.e. 1 g of digestive tract weight for 100 mL NaOH solution), covered by a
169 glass lid and oven-dried at $60 \text{ }^{\circ}\text{C}$ for 24 h. After that, samples were filtered using a vacuum
170 pump system through a 47 mm GF/F. After filtration, samples were carefully set in a Petri dish

171 and covered. These filters were oven-dried again at 60 °C for 24 h. Then, filters were visually
172 examined for MPs identification using a stereomicroscope (Zeiss Stemi 508, with 40–50 times
173 magnification with a size detection limit of 0.07–5 mm). The particles suspected to be MPs
174 were photographed (Axiocam 105 Color), counted, and measured in length (mm) (Zeiss Zen
175 3.2). MPs were categorised according to their shape (Figure 2; Justino et al., 2021) as fibres
176 (filamentous shape), fragments (irregular shape), films (flat shape), foams (soft with an
177 irregular shape), or pellets (spherical shape).

178 *Laser Direct Infrared (LDIR) Analysis of MPs polymers*

179 A subset (10% of the total particles extracted) of samples was selected to identify the
180 main types of MPs polymers using the LDIR analyser Agilent 8700 Chemical Imaging System
181 using the Microplastic Starter 1.0 library. The LDIR analyser scans the particles (size range 20–
182 5000 μm) in an automatic mode and obtains a spectral curve using a wavelength range of 1800–
183 975 cm^{-1} . The information is collected with the Clarity image software (© Agilent version 1.3.9)
184 and compared with the polymer spectrum library (~400 references spectra). A particle was
185 considered as identified if the accordance of its spectrum with the reference spectrum was \geq
186 70% (Ourgaud et al., In prep).



187
188 Figure 2. Shapes of microplastics identified in the mesopelagic fishes: a) fibre; b) fragment; c) foam; d) pellet; e) film.

189 *Data analysis*

190 Kruskal-Wallis test was used to verify whether ingested MPs presented significant
191 differences among species (*A. sladeni*, *S. diaphana*, *D. brachycephalus*, and *H. taaningi*)
192 considering the number and size of MPs. We also used Kruskal-Wallis to test whether the total
193 number of MPs ingested varied according to depth. When the Kruskal-Wallis test presented
194 significant differences, *post hoc* pairwise comparisons, Dunn's test was used to investigate the
195 sources of variance (Dunn, 1964). Mann-Whitney tests were applied to determine differences
196 in the MPs ingested according to the period (day or night). A Spearman's correlation test was
197 used to evaluate the relationship between MPs ingestion and biological parameters of fishes
198 (standard length and total weight). All statistical analyses were performed with the software R
199 version 3.6.3 (R Core Team, 2020) and were conducted considering a level of significance of
200 5%.

201 **Results**

202 A total of 213 microplastic (MPs) particles were recovered from the 170 analysed
203 specimens (frequency of occurrence 67%). MPs were presented in 93% of *Argyropelecus*
204 *sladeni*, 75% of *Diaphus brachycephalus*, 62% of *Hygophum taaningi*, and 45% of *Sternoptyx*
205 *diaphana* specimens (Table I). According to the number of MPs, ingestion significantly differed
206 between species (chi-squared = 20.437, df = 3, $p < 0.05$), with *A. sladeni* being the most
207 contaminated (1.66 ± 1.23 MPs ind.⁻¹), followed by *D. brachycephalus* (1.63 ± 1.41 MPs ind.⁻¹)
208 ¹), *H. taaningi* (1.07 ± 1.20 MPs ind.⁻¹), and *S. diaphana* (0.54 ± 0.71 MPs ind.⁻¹) (Table I).
209 Dunn's *post hoc* test showed that *S. diaphana* differed from *A. sladeni* and *D. brachycephalus*.
210 Additionally, there was no relationship between the MPs ingested by fish species and the
211 biological parameters (standard length and the total weight) (Spearman's rank correlation, $p >$
212 0.05).

213 In general, the mean size of ingested MPs also varied according to the species (chi-
214 squared = 12.247, df = 3, $p < 0.05$). *Argyropelecus sladeni* (0.74 ± 0.53 mm ind.⁻¹) showed the
215 longest size of MPs ingested, followed by *H. taaningi* (0.49 ± 0.80 mm ind.⁻¹), *D.*
216 *brachycephalus* (0.44 ± 0.53 mm ind.⁻¹), and *S. diaphana* (0.36 ± 0.82 mm ind.⁻¹), with
217 significant differences observed between *A. sladeni* and *S. diaphana* (Table I). Overall, fish MP
218 contamination levels were not significantly different between day or night sampling, regardless
219 of species (chi-squared = 1.4024, df = 1, $p > 0.05$), and by species individually ($p > 0.05$).
220 However, ingestion differed among the sampling depths (chi-squared = 18.80, df = 6, $p < 0.05$).

221 Fishes were generally most contaminated at 230 m (1.73 ± 1.25 MPs ind.⁻¹), followed by 430
 222 m (1.66 ± 0.57 MPs ind.⁻¹), and 610 m (1.62 ± 1.44 MPs ind.⁻¹), and less contaminated at 800
 223 m (0.57 ± 0.75 MPs ind.⁻¹) (Figure 3). Statistically significant differences were observed
 224 between depths of 800 and 230 m and between depths of 800 and 610 m ($p < 0.05$). Regarding
 225 the shape of MPs ingested by fishes, most were fibres (64%), followed by fragments (19%),
 226 pellets (6%), films and foams (4%). However, the shape of ingested MPs did not vary between
 227 the species (chi-squared = 3.1683, df = 4, $p > 0.05$). Fibres were mainly observed in *S. diaphana*
 228 (83%), *A. sladeni* (76%), *H. taaningi* (63%), and *D. brachycephalus* (58%), followed by
 229 fragments in *D. brachycephalus* (23%), *H. taaningi* (21%), *A. sladeni* (12%) and *S. diaphana*
 230 (11%). Pellets were found in *H. taaningi* (12%), *S. diaphana* and *D. brachycephalus* (5%), and
 231 films were found in *A. sladeni* (12%), *D. brachycephalus* (5%), *H. taaningi* (1%). Foams were
 232 only found in *D. brachycephalus* (7%) and *H. taaningi* (1%) (Figure 4**Error! Reference source**
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234 Overall, plastic polymers were identified in 80% of particles from the subset of samples.
 235 Natural particles identified as cellulose were observed in 15% of all particles, and 5% were
 236 unidentified. The most common polymers found were polyamide (PA) at 25% abundance,
 237 followed by polyethylene (PE) and polyethylene terephthalate (PET), with a similar abundance
 238 at 19%. The other polymers contributed to a similar percentage of 6-7% and included the
 239 ethylene-vinyl acetate (EVA), polyvinylchloride (PVC), styrene-butadiene rubber (SBR),
 240 polylactic acid (PLA), alkyd varnish and chlorinated polyisoprene (Figure 5).

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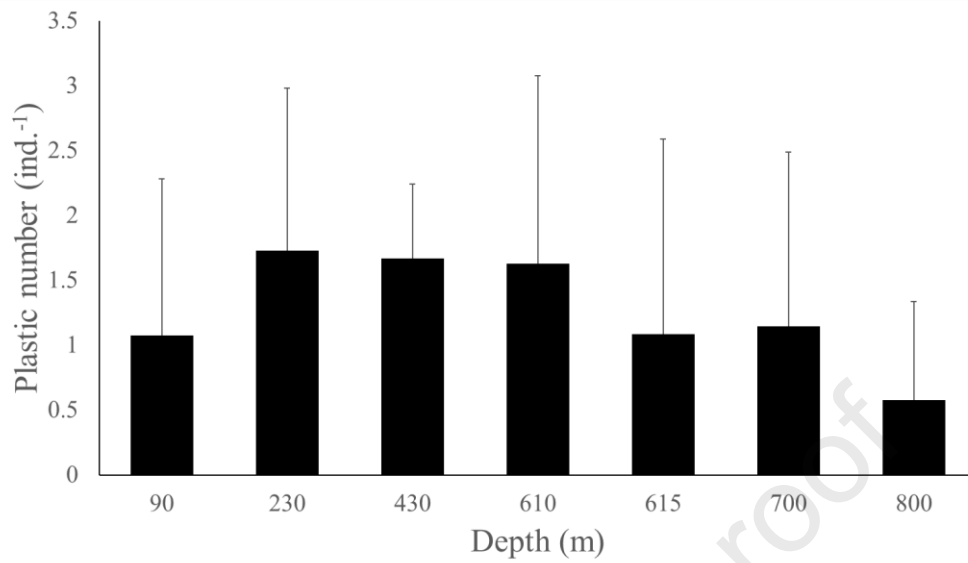
242 **Table I.** Biological aspects and sampling data of the species analysed. Abbreviations: SL, standard length; TW, total weight;
 243 FO%, frequency of occurrence; SD, standard deviation.

Family/Species	Sampling		Biometry		Microplastics occurrence		
	<i>n</i>	Depth (m)	SL (cm) range	TW (g) range	FO%	MPs mean \pm SD	Length (mm) mean \pm SD
Sternoptychidae							
<i>Argyropelecus sladeni</i>	15	430; 610; 615; 800	3.00–5.85	0.70–3.18	93	1.66 ± 1.23	0.74 ± 0.53
<i>Sternoptyx diaphana</i>	33	615; 800	1.92–3.06	0.18–0.97	45	0.54 ± 0.71	0.36 ± 0.82
Myctophidae							
<i>Diaphus brachycephalus</i>	69	230; 610; 700	2.51–4.98	0.34–2.15	75	1.63 ± 1.41	0.40 ± 0.55
<i>Hygophum taaningi</i>	53	90	4.13–5.99	1.14–2.68	62	1.07 ± 1.20	0.49 ± 0.80

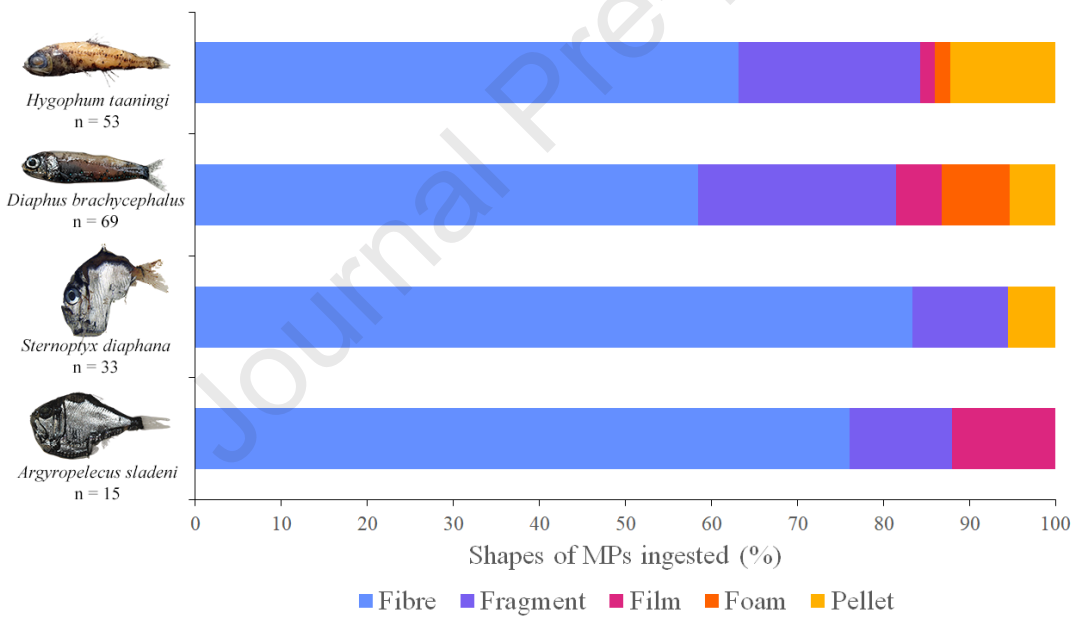
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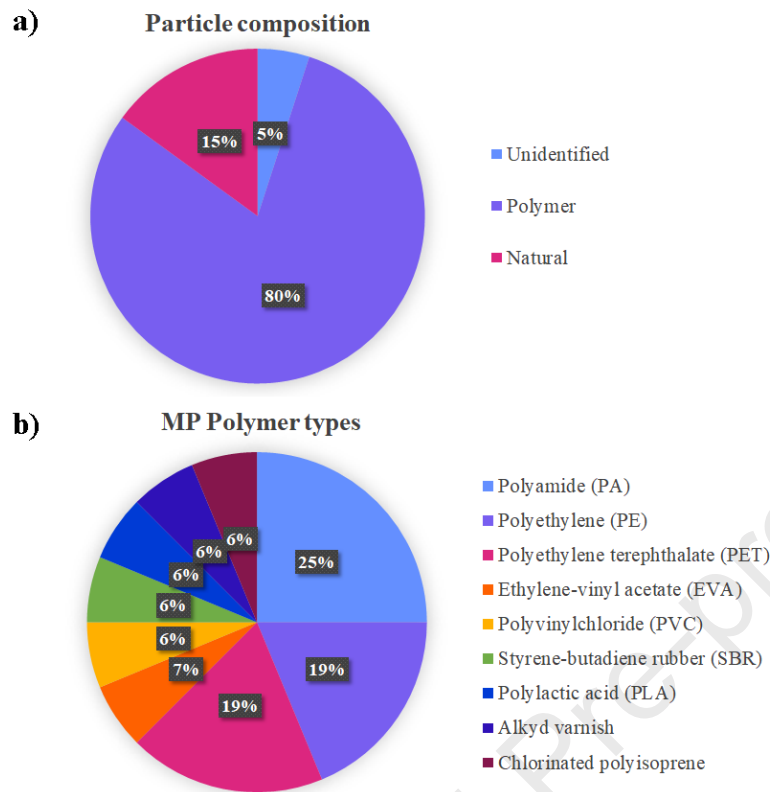


247 Figure 3. Mean number (\pm standard deviation) of MPs ingested per depth strata.



248

249 Figure 4. Relative abundances (%) of MP shapes ingested per fish species.



250

251 Figure 5. Polymers identified using the LDIR analyser. a) Particle composition in the samples analysed, and b) Percentage of
 252 microplastic polymers found in the samples.

253

254 Discussion

255 This study confirmed that the mesopelagic fishes from the SWTA are contaminated with
 256 MPs. The four species analysed here exhibited a high MP detection frequency in their digestive
 257 tract (67%). These findings bring new information into the contamination of the deep sea and
 258 shed light on the potential role of marine organisms in MPs sinking.

259 Worldwide, few studies have documented plastic ingestion by mesopelagic fishes. For
 260 example, in the North Pacific Gyre, Davison and Asch (2011) reported an MP detection
 261 frequency of 9.2% of the fishes sampled, whereas Boerger et al. (2010) found 35% in the same
 262 area. In the Mediterranean Sea, Romeo et al. (2016) found MPs in 2.7% of sampled
 263 lanternfishes, whereas Zhu et al. (2019) reported the presence of MPs in more than 90% of the
 264 deep-sea fishes sampled in the South China Sea. In the Islands of Tristan da Cunha and St.
 265 Helena, McGoran et al. (2021) found 73.3% of species contaminated with MPs; and in the

266 North Atlantic, Lusher et al. (2016) found 11% of individuals contaminated, in contrast with
267 Wieczorek et al. (2018) which detected MPs in 73% of the mesopelagic fish specimens from
268 the same area. The substantial divergence in the frequency of occurrence of MPs recovered in
269 mesopelagic fishes may be due to several factors such as ecological behaviour, site-specific
270 oceanographic differences, laboratory procedures, and sampling methods. However,
271 differences in the extraction methods, an issue previously addressed by Wieczorek et al. (2018),
272 might also influence the contamination rate. A lack of standardisation of the protocols for MPs
273 extraction in organisms is the main issue for comparing studies on plastic contamination. The
274 scientific community emphasises the importance of employing reliable and replicable research
275 methods (Hermesen et al., 2018; Markic et al., 2020; Müller, 2021), not only concerning the
276 choice of a suitable extraction method for MPs (*e.g.*, digestion and QA/QC protocols), but also
277 an adequate sample size (> 10 ; Justino et al., 2021) and size detection threshold of the particles,
278 which is determinant in the number of plastics recovered (Savoca et al., 2021). Such decisions
279 are important to avoid the bias of over/underestimation due to cross-contamination and loss of
280 samples and were carefully considered in the present study.

281 The wide availability of MPs is expected to threaten biodiversity throughout the marine
282 environment. Plastic debris is found all along the coastal zone, continental slope, around
283 oceanic islands, seamounts, and even in the deepest parts of the ocean (Cai et al., 2018;
284 Monteiro et al., 2018; Lins-Silva et al., 2021; Pinheiro et al., 2021). Differences in the
285 ecological habits, such as feeding strategy and migration, might influence the MP uptake by
286 marine species. A clear distinction was observed in our study between the number of MPs
287 ingested by species. For example, *A. sladeni* exhibited the highest number of particles (mean
288 of 1.66 ± 1.23 MPs ind.⁻¹; FO=93%), while *S. diaphana* exhibited the lowest number ($0.54 \pm$
289 0.71 MPs ind.⁻¹; FO=45%). A distinct pattern from that recorded in previous studies on
290 mesopelagic fishes, where two of the most up-to-date references did not observe any differences
291 between species and depths (Lusher et al., 2016; Wieczorek et al., 2018).

292 The difference observed in MPs ingestion might be explained by the species vertical
293 migration behaviour. For example, in our study area, *A. sladeni* is mostly distributed at 400–
294 500 m during the daytime, mainly feeding on fish larvae and ostracods (Eduardo et al., 2020a).
295 On the other hand, *S. diaphana* is found chiefly in deeper waters (700–900 m), primarily feeding
296 on amphipods (Eduardo et al., 2020a). Likewise, in the daytime, *D. brachycephalus* is mainly

297 distributed in the upper mesopelagic layer at 200–500 m, while *H. taaningi* was predominantly
298 found in deeper waters (700–1000 m) (Eduardo et al., 2020a, 2021). However, the *H. taaningi*
299 analysed in this study were only caught in the epipelagic zone, probably captured during
300 migration towards superficial areas. Even though all species analysed in this study performed
301 diel vertical migration (DVM), we did not observe any significant differences in the MP
302 concentration in specimens sampled day or night. However, differences in MP number were
303 observed depending on the depth strata.

304 Indeed, the most contaminated species (*A. sladeni* and *D. brachycephalus*) were mainly
305 caught in the upper mesopelagic layer (230–430 m), and *S. diaphana*, which ingested a lower
306 number of MPs particles, was captured in the lower mesopelagic layer (800 m). Therefore, we
307 suggest that when migrating to the upper layers, these species interact with MPs and, when
308 returning, they probably act as vectors of MPs to the deeper ocean layers (Figure 6). For
309 instance, in the study area, myctophids constitute 85% of the viperfish diet, the most abundant
310 mesopelagic micronektivore fish species (Eduardo et al., 2020b). To our best knowledge, there
311 is no information on MP in sediment and bottom organisms for the SWTA region, making the
312 real impact of MP and their transportation into the deep sea speculative. However, coupling the
313 data gathered in the present study with the widely acknowledged fact that mesopelagic species
314 transport carbon to deep waters (Davison et al., 2013; Drazen and Sutton, 2017; Eduardo et al.,
315 2020a), it seems that these species may also be transporting MPs to the deep sea.

316 Furthermore, our data support previous hypotheses that the deeper layers are less
317 contaminated (Kvale et al., 2020; Zobkov et al., 2019). In Monterey Bay, California, Choy et
318 al. (2019) also observed a similar pattern: a peak concentration of MPs in the mesopelagic zone
319 at a range of 200–600 m depth. Additionally, the size of MPs ingested was also influenced by
320 the depth in which species were caught (Ferreira et al., 2022). *Argyrolepecus sladeni* ingested
321 the longest MPs, whereas *S. diaphana* ingested significantly smaller MPs, coinciding with
322 surveys investigating MP size in the water column (Dai et al., 2018; Zobkov et al., 2019). The
323 ingestion of smaller size plastics was also observed in deep-water species in the North-East
324 Atlantic (Pereira et al., 2020). The sinking of MPs is associated with biological activities such
325 as biofouling, marine snow, faecal pellets, and plastic pump, contributing to the dispersion of
326 smaller particles in the deeper layers (Van Sebille et al., 2020). We corroborate previous

327 findings by linking MP size to depth since we found the smallest particles in species inhabiting
 328 the deepest layers.

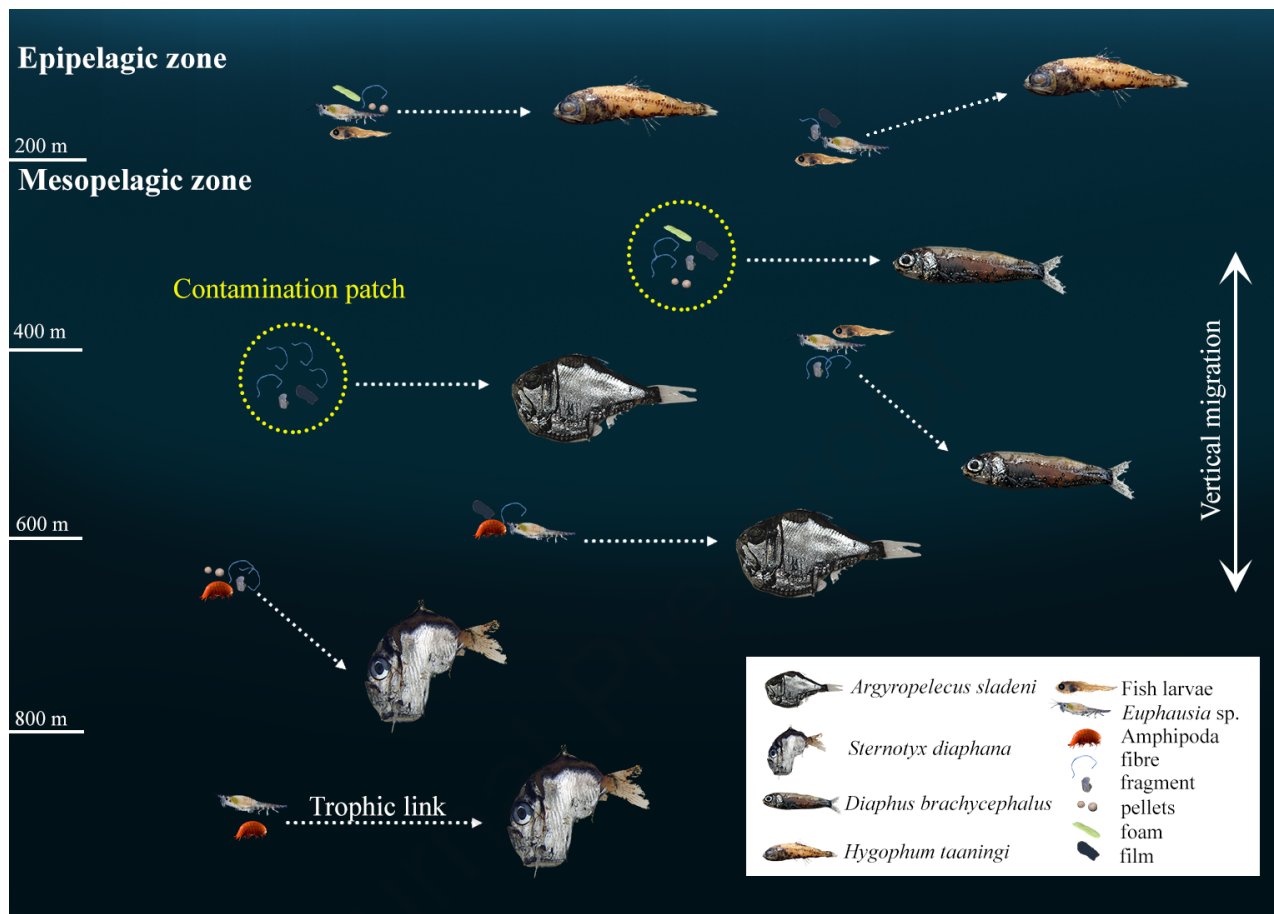


Figure 6. Schematic representation of the microplastic ingestion by mesopelagic fishes in the Southwestern Tropical Atlantic. White dotted arrows indicate the ingestion by trophic link, and yellow dotted circles the probable microplastic accumulation zone.

329 In our study, fibres were the common MP shape for all species (64%), and polyamide
 330 (PA), polyethylene (PE), and polyethylene terephthalate (PET) were the most common
 331 polymers identified, which are mainly used in the fishery and the textile industry (Lima et al.,
 332 2021). Previous research has already found lower density polymers as polyethylene in
 333 mesopelagic fishes (Wieczorek et al., 2018); these buoyant microplastics can be ingested by
 334 fish when they migrate towards epipelagic areas, thereby transporting these particles to deeper
 335 areas. Sources of fibres are related to the release of untreated water from the washing machine
 336 into aquatic environments (De Falco et al., 2019) and extensive fishery activities (Chen et al.,
 337 2018; Xue et al., 2020). Despite FNA including MPAs, this archipelago has a high influx of
 338 tourists and extensive subsistence and recreational fishing activities (Lopes et al., 2017). Nets
 339 and fishing lines are known to degrade and fragment in the environment by physical factors,
 340 such as solar radiation (Andrady, 2011). Indeed, microfibrils are the most common type

341 observed in marine ecosystems (Kanhai et al., 2018; Lima et al., 2021) and recorded in the FNA
342 and nearby islands (Ivar do Sul et al., 2014; Lima et al., 2016). Additionally, the Equatorial
343 Atlantic is not perceived as an accumulation zone of fibres in surface water masses, decreasing
344 the sinking of this type of MPs to deeper layers where fishes were captured (Lima et al., 2021).
345 However, in the short-term, these islands might retain MPs in the nearshore due to the actions
346 of winds, waves, vortices, and eddies surrounding the islands (Lima et al., 2016; Gove et al.,
347 2019). The most contaminated species were captured around the FNA, suggesting that
348 proximity to the MPs sources also influences ingestion rates.

349 Fibres are reported as the most ingested shape by mesopelagic fishes (Wieczorek et al.,
350 2018; McGoran et al., 2021) and were also found in deep-sea amphipods in the Mariana trench
351 (Jamieson et al., 2019); these tiny zooplankton act as energy sources in the oceanic trophic web.
352 All fish species analysed here are zooplanktivorous, and amphipods are one of their main prey
353 (Eduardo et al., 2020a, 2021). In the Mediterranean Sea, Romeo et al. (2016) observed
354 similarities in the size of MPs and the size of the copepods, prey of lanternfishes, suggesting
355 active and selective ingestion of MPs. We observed a similar pattern, as the dimensions of the
356 MPs found in the SWTA were similar to those of common prey of the species (< 2 mm), *e.g.*,
357 amphipods and fish larvae in this region (Figueiredo et al., 2020). Through experiments, Li et
358 al. (2021) demonstrated that fish could capture MPs passively by breathing but that some of
359 them are also ingested inadvertently due to the similarity between their prey or the tiny sizes,
360 which are hard to distinguish. Thus, MPs in mesopelagic fishes analysed here might be
361 accidentally consumed when confused as prey or by trophic transfer through ingestion of
362 contaminated prey. However, due to methodological limitations in our study, we cannot state
363 that these species interacted with MP by ingestion through food or swallowed by accident.

364 Regardless of the uptake routes (ingestion or breathing) of MPs in the mesopelagic
365 fishes, the contamination rates (MP extracted from the digestive tract) observed in this study
366 can be used as an indicator for the levels of MP available in the environment. The less
367 contaminated species, *S. diaphana* captured in the deepest region, is evidence of the lower
368 availability of MP particles in these areas. Additionally, this fact is corroborated by the smaller
369 dimensions of MP extracted from *S. diaphana*, as expected for greater depths.

370 MPs' wide availability in the deep ocean layers may be harmful to the marine
371 community, which is poorly investigated, but already interacts with these anthropogenic

372 particles. In addition to organic additives (phthalates, OPEs, bisphenols) contained in plastics
373 (Paluselli et al., 2019; Fauvelle et al., 2021), the surface of MPs can adsorb organic pollutants
374 such as polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs;
375 Rochman et al., 2013a), the latter process being enhanced by a longer transit time of MPs in
376 meso- and bathypelagic waters (Rochman et al., 2013b; Jamieson et al., 2017). All of these
377 compounds may very likely migrate into their surrounding environment, such as the digestive
378 tract of biological species. Besides, MPs ingestion can cause adverse effects in fishes, such as
379 physical injuries and blockage of the digestive tract, or even developmental, reproductive and
380 locomotor toxicity (Teuten et al., 2009; Bhagat et al., 2020). Additionally, smaller MPs can
381 bioaccumulate in tissues (Lee et al., 2019; Sökmen et al., 2020).

382 **Conclusions**

383 This study was the first to assess microplastic (MP) contamination in mesopelagic fishes
384 in the Southwestern Tropical Atlantic (SWTA). The four species analysed here were
385 contaminated with MPs in their digestive tract. The primary polymer types identified were
386 polyamide (PA), polyethylene (PE), and polyethylene terephthalate (PET). Ingestion rates of
387 MPs varied between species and depth. However, no difference between day or night sampling
388 was observed. Thus, even though all species interact at some level with MPs, individuals caught
389 at the lower mesopelagic zone seem to be less exposed to MPs than those captured in the upper
390 mesopelagic layer.

391 Mesopelagic fishes may act as a vector of MP to the deep sea as they perform vertical
392 migrations, presenting an important link between epipelagic and lower mesopelagic layers
393 (Lusher et al., 2016; Savoca et al., 2021). They also play an essential role in the energy transfer
394 in the ecosystem, transferring the energy of primary and secondary consumers to the top oceanic
395 predators, which are valuable for the fishery stocks. So, the presence of MPs in the SWTA
396 mesopelagic ecosystem will likely pose several risks to marine ecosystems if high
397 contamination is confirmed in the near future.

398 Further research on MP contamination is needed, especially concerning the deep-sea
399 community, whose crucial role in the marine ecosystem functioning has been proven.
400 Additionally, including the effects of oceanographic parameters (*e.g.*, oceanic currents,
401 microturbulence, salinity) and ecological interactions (*e.g.*, prey-predator interaction) into the

402 evaluation of MPs uptake is also needed since there are many factors involved in the transport,
403 sinking, and uptake of MPs in the deep ocean. Finally, the pressure of anthropogenic impacts
404 is rapidly increasing in the SWTA, so there is an urgent need to comprehend how
405 contaminations occur and affect the ecosystem to establish mitigation measures.

406

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1 **Research Highlights**

2 Microplastics were found in deep-sea fishes from the Southwestern Tropical
3 Atlantic.

4 The most frequent polymers identified were PA, PE, and PET.

5 Ingestion rates of microplastics varied between species and depth.

6 Fishes ingested more microplastics in the upper mesopelagic layer.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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