Marine Pollution Bulletin 167 (2021) 112371

Contents lists available at ScienceDirect

Marine Pollution Bulletin

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

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Microplastic and artificial cellulose microfibers ingestion by reef fishes in the Guarapari Islands, southwestern Atlantic

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ARTICLE INFO

Keywords:
Litter
Plastic
Marine fishes
Polymers
Cellulose-based fibers
Brazil

ABSTRACT

This study investigated the ingestion of microplastics and artificial cellulose particles by 103 specimens belonging to 21 reef fish species from the southwestern Atlantic. Specimens of six species had ingested microplastics and artificial cellulose particles, while those of another three species had ingested only one type of material. In our samples, man-made cellulose fibers were more common than microplastics. The tomtate grunt, *Haemulon aurolineatum*, ingested more particles than any of the other species. Overall, transparent particles were predominant, and polyamide was the most common plastic material. Household sewage, fishery activity, and navigation appear to be the principal sources of the artificial particles ingested by the reef fishes. Our results provide an important database on oceanic contamination by microplastics and artificial cellulose particles. Understanding this impact on tropical reef fish will contribute to the development of strategies to mitigate pollution by anthropogenic debris in reef systems.

Marine ecosystems are threatened globally by human activities, which have resulted in a decline in biodiversity, the disruption of ecosystem functions and services, and the loss of resilience (Barlow et al., 2018; Bellwood et al., 2004; Naeem et al., 2012). In particular, coral reefs are responsible for the provision of a variety of ecological goods and services to human society (Hughes et al., 2017a; Moberg and Folke, 1999), but are susceptible to a range of anthropogenic impacts worldwide (Bellwood et al., 2004; Hughes et al., 2017a; Hughes et al., 2018; Lamb et al., 2018). For instance, global warming resulting from human activities is responsible for the recurrent mass bleaching of corals (Hughes et al., 2017b), which results in deleterious changes (*e.g.*, loss diversity, functions, and resilience) to the affected reefs (Bellwood et al., 2004; Hughes et al., 2017a; Hughes et al., 2018). The combined effects of the anthropogenic impacts with mass bleaching represent a serious threat to the future for coral reefs.

In this context, pollution by plastics has become a prominent and progressive impact on aquatic ecosystems (Coleman and Wehle, 1984; Derraik, 2002; Jambeck et al., 2015; Ostle et al., 2019), including coral reefs (see Carvalho-Souza et al., 2018; Lamb et al., 2018). Pollution by plastics affects coral reef systems in a number of different ways, ranging from the entanglement of animals (Nunes et al., 2018; Sazima et al., 2002) to the ingestion of macro- and microplastic particles by sea turtles (Santos et al., 2015), fish (Cardozo et al., 2018), and the sessile fauna, such as corals (Rotjan et al., 2019).

Microplastic particles can be a source of eco-toxins for the reef fauna, which include polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), and polybrominated diphenyl ethers, or PBDEs (Rochman et al., 2019). One other emergent pollutant in marine ecosystems is the artificial cellulose microfibers produced primarily by the washing of textiles (Suaria et al., 2020), which are known to be ingested by fish (Savoca et al., 2019), although this phenomenon is still poorly-studied, and the potential impacts on the biota of coral reefs are still unclear. The principal differences between microplastics and artificial cellulose particles

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https://doi.org/10.1016/j.marpolbul.2021.112371
Received 19 October 2020; Received in revised form 6 April 2021; Accepted 12 April 2021

are in their (i) composition - microplastics are derived from petroleum-based polymers, while artificial cellulose (biomaterial) is obtained from plant material, which is transformed into a pulp and then extruded for use as a raw material; (ii) origin - while both types of particle are introduced into aquatic systems by runoff, microplastics are also produced by the weathering and breakdown of larger plastic debris, while fibers are released primarily by domestic washing and the textile industry, and (iii) persistence - in general, artificial cellulose fibers are decomposed more rapidly in the environment than microplastics (Andrady, 2011; Cesa et al., 2017; Liu et al., 2019; Mohanty et al., 2000; Singh et al., 2020).

Fish are an important component of aquatic ecosystems, in which they play key roles including herbivory, top-down and bottom-up control, and the energy uptake among trophic levels and between systems. They also constitute an important fishery resource for humans, to be exploited as a source of either subsistence or income (Cinner, 2014; Teh et al., 2013). Notwithstanding, fish are affected by manifold anthropogenic impacts such as overfishing (Barlow et al., 2018), the loss of habitat quality (Bellwood et al., 2004), and pollution (Tebbett et al., 2017). Although a reasonable amount of data is available on the impacts of pollutants, such as metals, on reef fishes, there is a substantial gap in our knowledge on the impact of microplastics and man-made cellulose fibers on reef fish species (Cardozo et al., 2018; Garnier et al., 2019). This includes the number of species affected and the susceptibility of specific trophic guilds. Here, 103 specimens representing 21 reef fish species were collected off the coast of southeastern Brazil. The gut content analysis of these specimens revealed that individuals of nine species had ingested microplastics and/or artificial cellulose microfibers.

The study was conducted in the Guarapari Islands (20°41′S, 40°23′W), 10 km off the Brazilian coast, in the southwestern Atlantic (Fig. 1). The local seascape is composed by a mosaic of habitats, including the Três Ilhas Archipelago, and Escalvada and Rasas islands. The substrate is formed primarily by rocky granite, in addition to biogenic reefs, formed mainly by encrusting coralline algae, bryozoans, and stony corals. Two artificial reefs, the shipwrecks of the Bellucia and Victory 8B, are also part of the consolidated bottom. The benthic cover is dominated by coralline algae, non-coralline algae, bryozoans, gorgonians, sponges, and corals (Simon et al., 2013). The unconsolidated bottoms surrounding both the natural and the artificial reefs are formed by rhodolith beds and gravel or sand. The principal source of anthropogenic impact (*e.g.*, sewage, litter, and fisheries) in the study area is the Metropolitan Region of Grande Vitória, which has approximately two million inhabitants (Andrades et al., 2020; IBGE, 2019).

Fish specimens were collected during autonomous dives using SCUBA (Self Contained Underwater Breathing Apparatus) in the austral summer (when oceanographic conditions are most adequate for diving) of 2015, 2016, 2017, and 2018. The specimens were stored in an ice chest on board the diving support vessel, and frozen at −20 °C in the laboratory.

The whole digestive tract (*i.e.*, from the esophagus to the anus) of each specimen was then removed through a longitudinal incision in the abdomen, and preserved in 10% formalin buffered with sodium tetraborate. Each digestive tract was dissected in a Petri dish under a compact stereo-microscope (Carl Zeiss - Stemi 305) at 16× magnification, and the contents were washed using 70% ethanol.

All the solutions used in this procedure were filtered through a 45-μm mesh stainless steel filter prior to use, to avoid the introduction of foreign particles. The wall of the digestive tract and its contents were examined meticulously at a magnification of between 16× and 80×. Suspected particles of plastic or cellulose microfiber were separated from the other ingested items, counted, measured (diameter in the longest dimension with precision of 0.001 mm), and classified according to their shape and color (see Rochman et al., 2019). Potential contamination of the samples by airborne plastics or fibers during handling at the work station was controlled by the analysis of samples following Pegado et al. (2018).

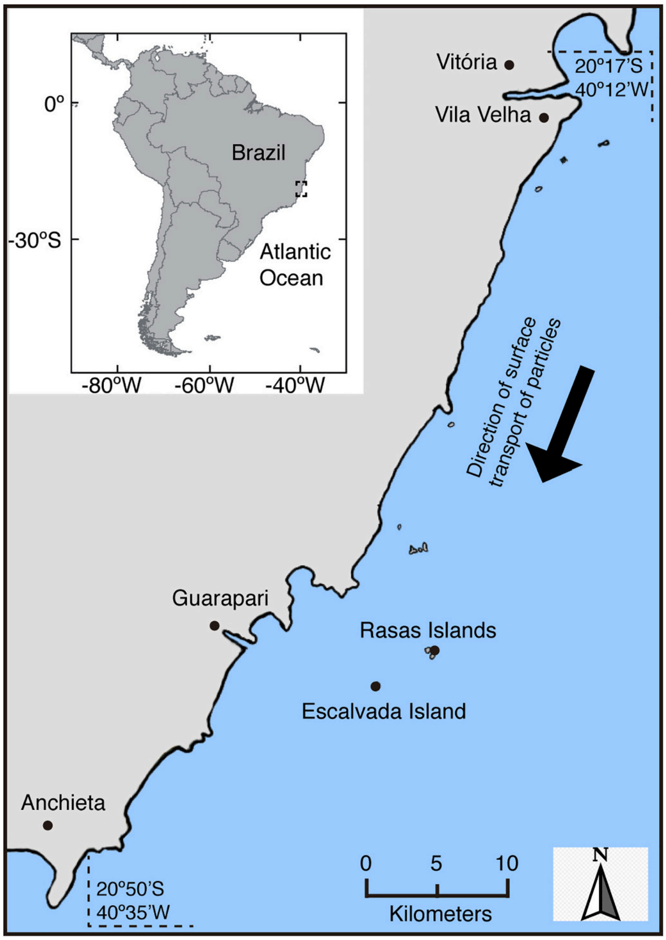

Fig. 1. Geographic location of the Guarapari Islands in the southwestern Atlantic (area delimited by the rectangle of coordinates), and details of the position of the islands off the shore of the Metropolitan Region of Grande Vitória. The arrow indicates the predominant direction of the surface transport of particles.

The fish species were assigned to trophic guilds, following Pinheiro et al. (2018) as Herbivore (HERB) - fish that consume a rich mass of macroalgae, turf algae and detritus; Macrocarnivore (MCAR) – fish that consume large mobile organisms, including invertebrates and fishes; Mobile Invertebrate Feeders (MIF) – fish that feed primarily on small benthic mobile invertebrates; Omnivores (OMN) – fish that consume a variety of organisms, including both animal and plant; Planktivores (PLK) – fish that feed primarily on plankton, and Sessile Invertebrate Feeder (SIF). The ingestion of microplastic particles and artificial cellulose microfibers by the reef fish was described by the percentage of frequency of occurrence (FO%).

The particles were analyzed using 2D imaging - Fourier transform infrared (FTIR) - applied directly to the dry filters (with no further preparation of the samples) using a Cary 620-670 FTIR microscope, equipped with an FPA 128 × 128 detector (Agilent Technologies). The spectra were recorded directly from the surface of the samples (or of the Au background) in reflectance mode, with an open aperture and a spectral resolution of 8 cm^{-1}, with 128 scans being acquired for each spectrum. The "single-tile" analysis resulted in a map of 700 × 700 μm^2 (128 × 128 pixels), with the spatial resolution of each Imaging map being 5.5 μm (*i.e.*, each pixel is 5.5 × 5.5 μm^2).

The ingestion of microplastics and microfibers was analyzed in a total of 103 fish specimens representing 21 species in 12 families (Table 1). Two of the species, *Sparisoma frondosum* and *Sparisoma* *tuiupiranga*, are endemic to Brazil. The parrotfish *S. frondosum* is classified as Vulnerable (VU-A3cd) in the Brazilian national red list (ICMBio, 2018). Most (14) of the fish species are mobile invertebrate feeders (68 specimens), followed by herbivores (two species; 8 individuals), macrocarnivores (2; 2), a single omnivore, one planktivore species (22 individuals), and a sessile invertebrate feeder (two individuals). Overall, individuals of nine fish species had ingested microplastics (12 specimens) and microfibers, found in 26 specimens (Fig. 2). Additional information on the microplastic particles and artificial cellulose microfibers ingested by the fish (*i.e.*, the size, color, and number of particles per fish) are provided in the Supplementary Material (Appendix 1).

Twelve specimens of seven species (five families) ingested microplastic particles, representing 12% of the total fish abundance and 33% of the species sampled. The majority of the microplastic particles (18 of 20; 90%) were found in the stomachs of the fish (11 specimens in six species), while the other two particles (10%) were recovered from the intestine of one specimen (Fig. 2 and Table 1). These particles ranged in size from 0.40 mm to 3.10 mm. The mean number of particles per fish ($n = 12$) was 1.67 (± 1.23 SD). The particles were ingested primarily by fishes of the MIF guild (80% of microplastic ingested), followed by the PLK (15%) and SIF guilds (5%). No particles were recorded in fish of the HERB, MCAR or OMN guilds. *Haemulon aurolineatum* and *Bodianus pulchellus* had the highest incidence of particles of all the fish species sampled, with five particles (25% of the total) each. Microplastic particles were predominantly transparent ($n = 5$) and yellow ($n = 5$) in color, followed by black ($n = 4$) and other colors, $n = 6$ (Fig. 2).

A total of 26 specimens ingested artificial cellulose microfibers, representing 25% of the total abundance of fish and 38% of the species sampled (Fig. 2 and Table 1). Overall, 55 (83%) of the 67 microfibers were found in stomachs, while the other 12 microfibers (17%) were recovered from the intestines of specimens of five species (Table 1). The mean number of particles collected per fish was 2.58 (± 2.04). Forty-five artificial cellulose microfibers (67.2% of all microfibers) were ingested by fish of the MIF guild, followed by the PLK (13; 19.4%) and SIF guilds (9; 13%). No microfibers were found in the HERB, MCAR or OMN guilds. *Haemulon aurolineatum* ingested the most fibers (26; 39% of total), followed by *Chromis multilineata*, with 13 microfibers (19%). Microfibers were the only type of debris found in *Anisotremus virginicus* and *Haemulon parra*. Most (44) artificial cellulose microfibers were transparent, followed by blue (13), and orange (3) fibers.

Overall, 87 microparticles (*i.e.*, particles or fibers) were found in the specimens analyzed. Most (68% of the total) items were composed of cellulose, eleven (12.6% the total) of polyamide, four (4.6%) of polyethylene terephthalate (PETE), three (3.4%) of polypropylene, one (1.1%) of acrylonitrile and one (1.1%) was polyethylene. The cellulose fibers were identified by the presence of bands at 3500–3050 (O—H stretching, hydroxyl groups of anhydroglucose unit), 3000–2800, centered on 2900 (stretching of methyl and methylene C—H bonds), and 1630 cm^{-1} (OH bending, adsorbed water) (Canché-Escamilla et al., 2006; Garside and Wyeth, 2003). Polyamide was identified by the bands at 3445–3265 (hydrogen bonded N—H stretching), 3000–2800 region (CH stretching), 1634 (C═O stretching) and 1538 cm^{-1} (NH bending, C—N stretching) (Noda et al., 2007; Rotter and Ishida, 1992; Verleye et al., 2001). Polyethylene microfragments and fibers were identified by the bands at 2919 and 2850 (CH stretching), and 1466 cm^{-1} (CH_2 bending deformation) (Chércoles Asensio et al., 2009; Coates, 2000; Gulmine et al., 2002; Nishikida and Coates, 2003; Noda et al., 2007).

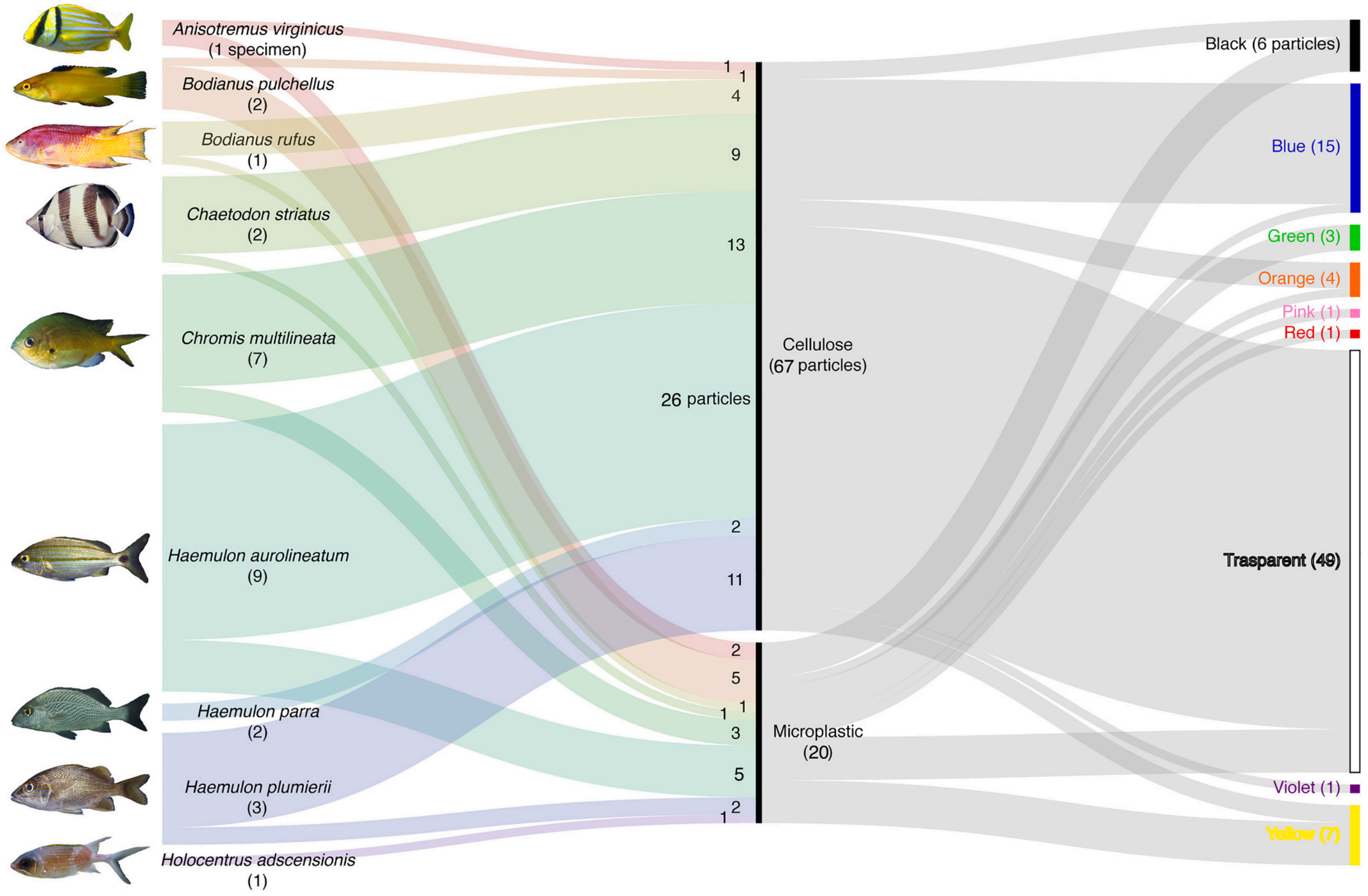

Fig. 2. Alluvial diagram (see Mauri et al., 2017) linking the reef fish species contaminated in the Guarapari islands to the type and color of the particles ingested. The numbers in parentheses below each species denote the number of specimens that had ingested particles. The numbers in the central column indicate the number of particles found in the gastrointestinal tract per type, and those in the right column, the number of particles per color.

Table 1
Taxonomic list and details of microplastic particles and artificial cellulose microfibers found in the reef fish specimens collected from the southwestern Atlantic, Brazil. The order of the families follows Nelson et al. (2016). Epinephelidae follows Craig and Hastings (2007) and Craig et al. (2011). Labridae follows Westneat and Alfaro (2005). * Endemic to Brazil. † Endangered species according to the Brazilian red list (ICMBio, 2018). Trophic guilds: Herbivore (HERB), Macrocarnivore (MCAR), Mobile Invertebrate Feeders (MIF), Omnivores (OMN), Planktivores (PLK), and Sessile Invertebrate Feeders (SIF). The values are formatted as "microplastic data/cellulose data".

Family/species	Trophic guild	Number of fish	Number of fish with particles	Frequency of occurrence (%)	N° of particles in gastrointestinal tract	Mean N° of particles per specimen	N° of fish with particles in stomach	N° of particles in stomach	N° of fish with particles in intestine	N° of particles in intestine
Holocentridae										
Holocentrus adscensionis (Osbeck 1765)	MIF	1	1/0	100/0	1/0	1.0/0.0	1/0	1/0	0/0	0/0
Myripristis jacobus Cuvier 1829	MIF	2	0/0	0/0	0/0	0.0/0.0	0/0	0/0	0/0	0/0
Plectrypops retrospinis (Guichenot 1853)	MIF	1	0/0	0/0	0/0	0.0/0.0	0/0	0/0	0/0	0/0
Pomacentridae										
Chromis multilineata (Guichenot 1853)	PLK	22	1/7	5/32	3/13	3.0/1.9	1/5	3/9	0/2	0/4
Dactylopteridae										
Dactylopterus volitans (Linnaeus 1758)	MIF	1	0/0	0/0	0/0	0.0/0.0	0/0	0/0	0/0	0/0
Labridae										
Bodianus pulchellus (Poey 1860)	MIF	4	1/1	25/25	5/1	5.0/1.0	1/1	5/1	0/0	0/0
Bodianus rufus (Linnaeus 1758)	MIF	6	1/1	17/17	2/3	2.0/3.0	0/1	0/2	1/1	2/1
Halichoeres poeyi (Steindachner 1867)	MIF	5	0/0	0/0	0/0	0.0/0.0	0/0	0/0	0/0	0/0
Sparisoma frondosum (Agassiz 1831)*†	HERB	2	0/0	0/0	0/0	0.0/0.0	0/0	0/0	0/0	0/0
Sparisoma tuiupiranga Gasparini, Joyeux & Floeter 2003*	HERB	6	0/0	0/0	0/0	0.0/0.0	0/0	0/0	0/0	0/0
Mullidae										
Pseudupeneus maculatus (Bloch 1793)	MIF	4	0/0	0/0	0/0	0.0/0.0	0/0	0/0	0/0	0/0
Epinephelidae										
Cephalopholis fulva (Linnaeus 1758)	MCAR	1	0/0	0/0	0/0	0.0/0.0	0/0	0/0	0/0	0/0
Priacanthidae										
Priacanthus arenatus Cuvier 1829	MIF	6	0/0	0/0	0/0	0.0/0.0	0/0	0/0	0/0	0/0
Chaetodontidae										
Chaetodon striatus Linnaeus 1758	SIF	2	1/2	50/100	1/9	1.0/4.5	1/2	1/9	0/0	0/0
Haemulidae										
Anisotremus virginicus (Linnaeus 1758)	MIF	4	0/1	0/25	0/3	0.0/1.3	0/0	0/0	0/1	0/3
Haemulon aurolineatum Cuvier 1830	MIF	29	5/9	17/31	5/26	1.0/2.8	5/8	5/25	0/1	0/1
Haemulon parra (Desmarest 1823)	MIF	2	0/2	0/100	0/2	0.0/1.0	0/2	0/2	0/0	0/0
Haemulon plumierii (Lacepède 1801)	MIF	4	2/3	50/75	3/10	1.5/3.3	2/3	2/8	0/1	0/2
Lutjanidae										
Ocyurus chrysurus (Bloch 1791)	MCAR	1	0/0	0/0	0/0	0.0/0.0	0/0	0/0	0/0	0/0
Ostraciidae										
Acanthostracion quadricornis (Linnaeus 1758)	OMN	1	0/0	0/0	0/0	0.0/0.0	0/0	0/0	0/0	0/0

(*continued on next page*)

Table 1 (*continued*)

Family/species	Trophic guild	Number of fish	Number of fish with particles	Frequency of occurrence (%)	N° of particles in gastrointestinal tract	Mean N° of particles per specimen	N° of fish with particles in stomach	N° of particles in stomach	N° of fish with particles in intestine	N° of particles in intestine
Sparidae										
Calamus pennatula Guichenot 1868	MIF	1	0/0	0/0	0/0	0.0/0.0	0/0	0/0	0/0	0/0

The polypropylene fragments were identified by the bands at 3000–2800 (CH stretching region), 1458 (δa_s CH_3, δ_s CH_2), 1373 (CH_3 bending), and 1166 cm^{-1} (νCC backbone, rocking CH_3, δ CH) (Andreassen, 1999). Polyethylene terephthalate (PETE) filaments were identified by the bands at 3000–2800 (CH stretching region), 1732 ($C{=}O$ stretching), 1407 (CH_2 bending ethylene glycol segment), 1236 and 1145 cm^{-1} (C—O stretching, terephthalate group $OOCC_6H_4$-COO), and 1022 cm^{-1} (vibration of ester C—O bond) (Chércoles Asensio et al., 2009; Noda et al., 2007; Pereira et al., 2017; Verleye et al., 2001). The 1678, 1571, and 1307 cm^{-1} bands may indicate the presence of polyurethane blended with PETE which is used in the form of a monomer for the synthesis of polyurethanes (Jankauskaitė et al., 2008). Acrylonitrile was identified by bands at 3000–2800 cm^{-1} region (C—H stretching), 2237 ($C{\equiv}N$ stretching of nitrile), 1732 ($C{=}O$ stretching), and 1440 cm^{-1} (δ_{as} CH_2). The carbonyl stretching band is sharp and has an intensity comparable to the δ_{as} CH_2 band, which may indicate the presence of acrylate fibers (Jung et al., 2018). The band at 1238 cm^{-1} could be assigned to the C-O-C stretching of acrylate copolymers (Duan et al., 2008) (Fig. 3).

The present study describes the contamination of reef fish in the southwestern Atlantic with anthropogenic microparticles. A total of 733 fish species are found in the reef ecosystems of the Brazilian Province (Pinheiro et al., 2018), of which, 21 (3% of the total richness) were analyzed here to evaluate the ingestion of microplastics and artificial cellulose microfibers. Man-made particles were found in nine (1%) species. Previous studies of reef fish (see Baalkhuyur et al., 2018; Nie et al., 2019) reported microplastic ingestion rates of approximately 50% and 60%. Despite the fact that a third (7) of the species analyzed in the present study were represented by only one specimen, man-made particles were found in almost half (43%) of the species, which implies that other species that occur in the study area are likely to be contaminated. Even so, no endemic Brazilian reef fish (based on Pinheiro et al., 2018) nor any endangered species (in the Brazilian red list; ICMBio, 2018) had ingested man-made microparticles in the present study, although more data (on both specimens and species) will be essential to confirm the contamination levels in all these *taxa*.

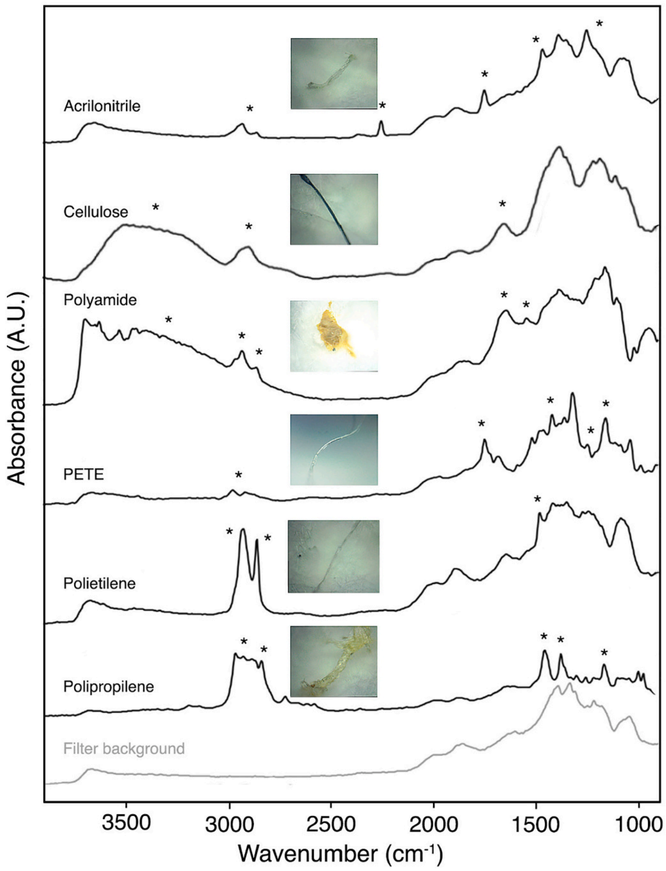

Fig. 3. ATR-FTIR spectra of the microplastic and cellulose particles recovered from reef fishes in the southwestern Atlantic, Brazil.

The large number of ingested particles recorded in *H. aurolineatum* is likely an effect of sample size, given that more particles were recovered from the more abundant species represented by more specimens. In fact, *C. multilineata*, which was the species represented by the second largest number of specimens, was also the species with the second largest number of particles ingested. Similarly, our data on many of the trophic guilds were derived from a small number of specimens, and thus need to be considered with caution. While more samples are needed to confirm the levels of contamination in both the species and the guilds, concerns on the threat of man-made particles for the conservation of these reef fish species is clearly urgent.

Anthropogenic microparticles may be ingested by fishes, intentionally or otherwise, during the ingestion of food. These particles may often match the characteristics of the food items exploited by the fish or they may be ingested involuntarily during feeding, physiological activities (*e.g.*, respiration, or drinking water) or even by being transferred through the food web (Coverton et al., 2021; Macali et al., 2018; Ory et al., 2017; Roch et al., 2020; Welden et al., 2018). In this context, the functional traits of a fish species may also contribute to its susceptibility to the ingestion of man-made particles (Anastasopoulou et al., 2013; Coverton et al., 2021). Using a Bayesian analysis Coverton et al. (2021), for example, found evidence of the susceptibility of one functional group, small planktivorous fish, to the ingestion microplastics related to its feeding mode (*i.e.*, acquisition of food by filtration in the water column). Both *C. multilineata* and *H. aurolineatum* feed opportunistically on zooplankton (Robertson and Van Tassell, 2019). The former species is planktivorous, and its food acquisition mode is based on the picking of items from the water column, while the latter species forages preferentially on benthos, but uses the same food acquisition mode. It is important to note here that these fish employ a bite-feeding strategy to select plankton, which is partially in agreement with the selective and semi-selective feeding mode of the fishes (Clupeidae) most susceptible to the ingestion of plastic (Coverton et al., 2021), although some features of the morphology of the gill rakers (see Collard et al., 2017) and the feeding mode (mid-water feeders) of the clupeids probably enhance their vulnerability to plastic pollution in comparison with other species.

The prevalence of the ingestion of transparent particles in the present study may be related to detection error in predatory fish (Ory et al., 2017), given that the transparency of plankton is an anti-predator trait (Cronin et al., 2014). However, there are at least two other reasonable interpretations here: (i) a greater abundance of transparent particles in the environment, and (ii) the involuntary ingestion of these particles by fish preying on plankton. The second most common particle color was blue, which may be related to the misidentification of bioluminescent marine organisms, such as copepods, amphipods, and mysids, which emit light in the blue wavelength (Cronin et al., 2014; Ory et al., 2017). It is nevertheless difficult to identify the factors that determine ingestion based only on the items found in the gastrointestinal tract of the fish. In fact, as transparent or semi-transparent and blue microparticles are prevalent in the marine environment (Andrades et al., 2018; Santos et al., 2016; Shaw and Day, 1994), the high rates of ingestion of particles of these colors recorded in the present study may simply reflect their availability in the environment, rather than any selective behavior. Most reef fishes have well-developed color vision (Cronin et al., 2014; Marshall, 2017; Siebeck et al., 2008), and more studies based on controlled experiments are needed to better evaluate the factors that determine the ingestion rates of particles of different colors.

Polyethylene and polyamide are the principal plastic polymers found in marine environments and in the gastrointestinal contents of fish in Brazil (Andrades et al., 2018; Pegado et al., 2018). However, microfibers were the most common type of particle recorded in the present study, with artificial cellulose fibers constituting 77% of the particles ingested by the fish examined, based on the μ-FTIR analysis. A similar prevalence of microfiber contamination has been reported in a number of previous studies (see Li et al., 2018; Savoca et al., 2019). These particles are shed from textiles, typically during the washing of clothing, upholstery, carpets, and other textiles (Rochman et al., 2019; Savoca et al., 2019; Suaria et al., 2020). In a global characterization of fibers found in oceanic environments, Suaria et al. (2020) identified fibers composed primarily of natural polymers, most of which (80%) were cellulose, followed by fibers of animal origin (12%), and 8% synthetic material. In the specific case of the Guarapari reefs, the principal source of these particles is likely the domestic effluents produced by the two million inhabitants of Grande Vitória, which are released into its coastal waters. Others common activities in the region, such as fisheries and shipping, together with the runoff of pluvial drainage systems, are the typical sources of microplastics in the coastal zone (Liu et al., 2019; Rochman et al., 2019; Singh et al., 2020; see Suaria et al., 2020). These findings emphasize the importance of identifying the chemical profile of microfibers before classifying them as microplastics (Suaria et al., 2020), and the need for further research to evaluate the impact of man-made cellulose particles on coral reef ecosystems.

Coral reefs are threatened by a wide range of impacts from human activities (Bellwood et al., 2004; Hughes et al., 2017a; Lamb et al., 2018). In a review of the experimental studies of the effects of microplastics in fishes Jacob et al. (2020) found evidence of significant deleterious effects (*e.g.*, on the sensory, behavioral, and immune systems, and metabolism). In a global meta-analysis, Salerno et al. (2021) identified a negative effect of the ingestion of microplastics on the functional traits of fish. In part, the health of coral reefs depends on their associated fish fauna and ecosystem functions to ensure that they play a key role in ecosystem dynamics. These combined impacts will likely worsen further the coral reef crisis (see Bellwood et al., 2004). Understanding the effects of these anthropogenic impacts is essential for the development of effective measures to avoid, mitigate or compensate for their long-term consequences for coral reefs. The present study provides a preliminary overview of the impact of microplastic particles and artificial cellulose fibers on the coral reefs of the southwestern Atlantic. We hope that our findings will contribute to the proposal of new public policies in Brazil that may help to stem the advance of this serious and growing threat to Brazilian reefs.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2021.112371.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. Animal handling was conducted in accordance with the Ethics Committee (CEUA–UVV). All specimen collection was licensed by ICMBio/MMA – Brazilian Ministry of the Environment, through the SISBIO system.

Funding

This study was funded by Fundação de Amparo à Pesquisa e Inovação do Espírito Santo - FAPES (grant FAPES/CNPq N° 04/2017 – PPP/119/2017). L.A. da S. de O. acknowledges financial support from Nossa Bolsa (FAPES), and R.A., G.C.C.-F., C.R.P. and J.L.G are grateful to the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) - Finance Code 001. C.R.P. also thanks the FAPES for a PhD scholarship. T.G. and L.C.G. received a research productivity grant from CNPq (#311078/2019-2 / #312431/2018-0). D.C. and A.C. gratefully acknowledge CSGI (Consorzio Interuniversitario per lo Sviluppo dei Sistemi a Grande Interfase), Florence (Italy) for financial support.

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Declaration of competing interest

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

Acknowledgments

We thank Jean-Christophe Joyeux, Raiane Prates de Jesus, Alexandre Villela, Kássia Rodnitzky, Lohaynne Zambon Dir, Micael Portugal, Larissa Benevides, Thayane Calazans de Lima, Fernando Campagnoli Junior (Cia do Mergulho), and Daniel Filgueiras for their cooperation and assistance during the field sampling.

References

Anastasopoulou, A., Mytilineou, C., Smith, C.J., Papadopoulou, K.N., 2013. Plastic debris ingested by deep-water fish of the Ionian Sea (Eastern Mediterranean). Deep-Sea Res. I Oceanogr. Res. Pap. 74, 11–13.

Andrades, R., Santos, R.G., Joyeux, J.-C., Chelazzi, D., Cincinelli, A., Giarrizzo, T., 2018. Marine debris in Trindade Island, a remote island of the South Atlantic. Mar. Pollut. Bull. 137, 180–184.

Andrades, R., Pegado, T., Godoy, B.S., Reis-Filho, J.A., Nunes, J.L.S., Grillo, A.C., Machado, R.C., Santos, R.G., Dalcin, R.H., Freitas, M.O., Kuhnen, V.V., Barbosa, N. D., Adelir-Alves, J., Albuquerque, T., Bentes, B., Giarrizzo, T., 2020. Anthropogenic litter on Brazilian beaches: baseline, trends and recommendations for future approaches. Mar. Pollut. Bull. 151, 110842.

Andrady, A.L., 2011. Microplastics in the marine environment. Mar. Pollut. Bull. 62, 1596–1605.

Andreassen, E., 1999. Infrared and Raman spectroscopy of polypropylene. In: Karger-Kocsis, J. (Ed.), Polypropylene: An A-Z Reference. Springer Netherlands, Dordrecht, pp. 320–328.

Baalkhuyur, F.M., Bin Dohaish, E.-J.A., Elhalwagy, M.E.A., Alikunhi, N.M., AlSuwailem, A.M., Røstad, A., Coker, D.J., Berumen, M.L., Duarte, C.M., 2018. Microplastic in the gastrointestinal tract of fishes along the Saudi Arabian Red Sea coast. Mar. Pollut. Bull. 131, 407–415.

Barlow, J., França, F., Gardner, T.A., Hicks, C.C., Lennox, G.D., Berenguer, E., Castello, L., Economo, E.P., Ferreira, J., Guénard, B., Gontijo Leal, C., Isaac, V., Lees, A.C., Parr, C.L., Wilson, S.K., Young, P.J., Graham, N.A.J., 2018. The future of hyperdiverse tropical ecosystems. Nature 559, 517–526.

Bellwood, D.R., Hughes, T.P., Folke, C., Nyström, M., 2004. Confronting the coral reef crisis. Nature 429, 827–833.

Canché-Escamilla, G., Pacheco-Catalán, D.E., Andrade-Canto, S.B., 2006. Modification of properties of rayon fibre by graft copolymerization with acrylic monomers. J. Mater. Sci. 41, 7296–7301.

Cardozo, A.L.P., Farias, E.G.G., Rodrigues-Filho, J.L., Moteiro, I.B., Scandolo, T.M., Dantas, D.V., 2018. Feeding ecology and ingestion of plastic fragments by *Priacanthus arenatus*: what's the fisheries contribution to the problem? Mar. Pollut. Bull. 130, 19–27.

Carvalho-Souza, G.F.d., Llope, M., Tinôco, M.S., Medeiros, D.V., Maia-Nogueira, R., Sampaio, C.L.S., 2018. Marine litter disrupts ecological processes in reef systems. Mar. Pollut. Bull. 133, 464–471.

Cesa, F.S., Turra, A., Baruque-Ramos, J., 2017. Synthetic fibers as microplastics in the marine environment: a review from textile perspective with a focus on domestic washings. Sci. Total Environ. 598, 1116–1129.

Chércoles Asensio, R., San Andrés Moya, M., de la Roja, J.M., Gómez, M., 2009. Analytical characterization of polymers used in conservation and restoration by ATR-FTIR spectroscopy. Anal. Bioanal. Chem. 395, 2081–2096.

Cinner, J., 2014. Coral reef livelihoods. Curr. Opin. Environ. Sustain. 7, 65–71.

Coates, J., 2000. Interpretation of infrared spectra, a practical approach. In: Meyers, R.A. (Ed.), Encyclopedia of Analytical Chemistry. John Wiley & Sons, Ltd., Chichester, pp. 10815–10837.

Coleman, F., Wehle, D.H.S., 1984. Plastic pollution: a worldwide oceanic problem. PARKS 9, 1–12.

Collard, F., Gilbert, B., Eppe, G., Roos, L., Compère, P., Das, K., Parmentier, E., 2017. Morphology of the filtration apparatus of three planktivorous fishes and relation with ingested anthropogenic particles. Mar. Pollut. Bull. 116, 182–191.

Covernton, G.A., Davies, H.L., Cox, K.D., El-Sabaawi, R., Juanes, F., Dudas, S.E., Dower, J.F., 2021. A Bayesian analysis of the factors determining microplastics ingestion in fishes. J. Hazard. Mater. 413, 125405.

Craig, M.T., Hastings, P.A., 2007. A molecular phylogeny of the groupers of the subfamily Epinephelinae (Serranidae) with a revised classification of the Epinephelini. Ichthyol. Res. 54, 1–17.

Craig, M.T., Mitcheson, Y.J.S.D., Heemstra, P.C., 2011. Groupers of the World: A Field and Market Guide. NISC, Grahamstown.

Cronin, T.W., Johnsen, S., Marshall, N.J., Warrant, E.J., 2014. Visual Ecology. Princeton University Press, Princeton, NJ.

Derraik, J.G.B., 2002. The pollution of the marine environment by plastic debris: a review. Mar. Pollut. Bull. 44, 842–852.

Duan, G., Zhang, C., Li, A., Yang, X., Lu, L., Wang, X., 2008. Preparation and characterization of Mesoporous zirconia made by using a poly (methyl methacrylate) template. Nanoscale Res. Lett. 3, 118.

Garnier, Y., Jacob, H., Guerra, A.S., Bertucci, F., Lecchini, D., 2019. Evaluation of microplastic ingestion by tropical fish from Moorea Island, French Polynesia. Mar. Pollut. Bull. 140, 165–170.

Garside, P., Wyeth, P., 2003. Identification of cellulosic fibres by FTIR spectroscopy - thread and single fibre analysis by attenuated total reflectance. Stud. Conserv. 48, 269–275.

Gulmine, J.V., Janissek, P.R., Heise, H.M., Akcelrud, L., 2002. Polyethylene characterization by FTIR. Polym. Test. 21, 557–563.

Hughes, T.P., Barnes, M.L., Bellwood, D.R., Cinner, J.E., Cumming, G.S., Jackson, J.B.C., Kleypas, J., van de Leemput, I.A., Lough, J.M., Morrison, T.H., Palumbi, S.R., van Nes, E.H., Scheffer, M., 2017a. Coral reefs in the anthropocene. Nature 546, 82–90.

Hughes, T.P., Kerry, J.T., Álvarez-Noriega, M., Álvarez-Romero, J.G., Anderson, K.D., Baird, A.H., Babcock, R.C., Beger, M., Bellwood, D.R., Berkelmans, R., Bridge, T.C., Butler, I.R., Byrne, M., Cantin, N.E., Comeau, S., Connolly, S.R., Cumming, G.S., Dalton, S.J., Diaz-Pulido, G., Eakin, C.M., Figueira, W.F., Gilmour, J.P., Harrison, H. B., Heron, S.F., Hoey, A.S., Hobbs, J.-P.A., Hoogenboom, M.O., Kennedy, E.V., Kuo, C.-y., Lough, J.M., Lowe, R.J., Liu, G., McCulloch, M.T., Malcolm, H.A., McWilliam, M.J., Pandolfi, J.M., Pears, R.J., Pratchett, M.S., Schoepf, V., Simpson, T., Skirving, W.J., Sommer, B., Torda, G., Wachenfeld, D.R., Willis, B.L., Wilson, S.K., 2017b. Global warming and recurrent mass bleaching of corals. Nature 543, 373–377.

Hughes, T.P., Kerry, J.T., Baird, A.H., Connolly, S.R., Dietzel, A., Eakin, C.M., Heron, S. F., Hoey, A.S., Hoogenboom, M.O., Liu, G., McWilliam, M.J., Pears, R.J., Pratchett, M.S., Skirving, W.J., Stella, J.S., Torda, G., 2018. Global warming transforms coral reef assemblages. Nature 556, 492–496.

IBGE, 2019. Estimativas da população residente no Brasil e unidades da federação com data de referência em 1° de julho de 2019. Instituto Brasileiro de Geografia e Estatística, Rio de Janeiro.

ICMBio, 2018. Livro Vermelho da Fauna Brasileira Ameaçada de Extinção: Volume VI - Peixes. ICMBio, Brasília.

Jacob, H., Besson, M., Swarzenski, P.W., Lecchini, D., Metian, M., 2020. Effects of virgin micro- and nanoplastics on fish: trends, meta-analysis, and perspectives. Environ. Sci. Technol. 54, 4733–4745.

Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. Science 347, 768–771.

Jankauskaitė, V., Macijauskas, G., Lygaitis, R., 2008. Polyethylene Terephthalate waste recycling and application possibilities: a review. Mater. Sci. (Medžiagotyra) 14, 119–127.

Jung, M.R., Horgen, F.D., Orski, S.V., Rodriguez .V., C, Beers, K.L., Balazs, G.H., Jones, T. T., Work, T.M., Brignac, K.C., Royer, S.-J., Hyrenbach, K.D., Jensen, B.A., Lynch, J. M., 2018. Validation of ATR FT-IR to identify polymers of plastic marine debris, including those ingested by marine organisms. Mar. Pollut. Bull. 127, 704–716.

Lamb, J.B., Willis, B.L., Fiorenza, E.A., Couch, C.S., Howard, R., Rader, D.N., True, J.D., Kelly, L.A., Ahmad, A., Jompa, J., Harvell, C.D., 2018. Plastic waste associated with disease on coral reefs. Science 359, 460–462.

Li, J., Green, C., Reynolds, A., Shi, H., Rotchell, J.M., 2018. Microplastics in mussels sampled from coastal waters and supermarkets in the United Kingdom. Environ. Pollut. 241, 35–44.

Liu, J., Yang, Y., Ding, J., Zhu, B., Gao, W., 2019. Microfibers: a preliminary discussion on their definition and sources. Environ. Sci. Pollut. Res. 26, 29497–29501.

Macali, A., Semenov, A., Venuti, V., Crupi, V., D'Amico, F., Rossi, B., Corsi, I., Bergami, E., 2018. Episodic records of jellyfish ingestion of plastic items reveal a novel pathway for trophic transference of marine litter. Sci. Rep. 8, 6105.

Marshall, J., 2017. Vision and lack of vision in the ocean. Curr. Biol. 27, R494–R502.

Mauri, M., Elli, T., Caviglia, G., Uboldi, G., Azzi, M., 2017. RAWGraphs: a visualisation platform to create open outputs. In: Proceedings of the 12th Biannual Conference on Italian SIGCHI Chapter ACM, New York, NY, USA, pp. 28:21–28:25.

Moberg, F., Folke, C., 1999. Ecological goods and services of coral reef ecosystems. Ecol. Econ. 29, 215–233.

Mohanty, A.K., Misra, M., Hinrichsen, G., 2000. Biofibres, biodegradable polymers and biocomposites: an overview. Macromol. Mater. Eng. 276-277, 1–24.

Naeem, S., Duffy, J.E., Zavaleta, E., 2012. The functions of biological diversity in an age of extinction. Science 336, 1401–1406.

Nelson, J.S., Grande, T.C., Wilson, M.V.H., 2016. Fishes of the World, 5th ed. John Wiley & Sons, Inc., Hoboken, New Jersey.

Nie, H., Wang, J., Xu, K., Huang, Y., Yan, M., 2019. Microplastic pollution in water and fish samples around Nanxun Reef in Nansha Islands, South China Sea. Sci. Total Environ. 696, 134022.

Nishikida, K., Coates, J., 2003. Infrared and Raman analysis of polymers. In: Lobo, H., Bonilla, J.V. (Eds.), Handbook of Plastics Analysis. Marcel Dekker, Inc, New York, pp. 186–316.

Noda, I., Dowrey, A.E., Haynes, J.L., Marcott, C., 2007. Group frequency assignments for major infrared bands observed in common synthetic polymers. In: Mark, J.E. (Ed.), Physical Properties of Polymers Handbook. Springer Science +Business Media, LLC, New York, pp. 395–406.

Nunes, J.A.C.C., Sampaio, C.L.S., Barros, F., Leduc, A.O.H.C., 2018. Plastic debris collars: an underreported stressor in tropical reef fishes. Mar. Pollut. Bull. 129, 802–805.

Ory, N.C., Sobral, P., Ferreira, J.L., Thiel, M., 2017. Amberstripe scad *Decapterus muroadsi* (Carangidae) fish ingest blue microplastics resembling their copepod prey along the coast of Rapa Nui (Easter Island) in the South Pacific subtropical gyre. Sci. Total Environ. 586, 430–437.

Ostle, C., Thompson, R.C., Broughton, D., Gregory, L., Wootton, M., Johns, D.G., 2019. The rise in ocean plastics evidenced from a 60-year time series. Nat. Commun. 10, 1622.

Pegado, T.d.S.e.S., Schmid, K., Winemiller, K.O., Chelazzi, D., Cincinelli, A., Dei, L., Giarrizzo, T., 2018. First evidence of microplastic ingestion by fishes from the Amazon River estuary. Mar. Pollut. Bull. 133, 814–821.

Pereira, A.P.D.S., Silva, M.H.P.D., Lima Júnior, É.P., Paula, A.D.S., Tommasini, F.J., 2017. Processing and characterization of PET composites reinforced with geopolymer concrete waste. Mater. Res. 20, 411–420.

Pinheiro, H.T., Rocha, L.A., Macieira, R.M., Carvalho-Filho, A., Anderson, A.B., Bender, M.G., Di Dario, F., Ferreira, C.E.L., Figueiredo-Filho, J., Francini-Filho, R., Gasparini, J.L., Joyeux, J.-C., Luiz, O.J., Mincarone, M.M., Moura, R.L., Nunes, J.d.A. C.C., Quimbayo, J.P., Rosa, R.S., Sampaio, C.L.S., Sazima, I., Simon, T., Vila-Nova, D. A., Floeter, S.R., 2018. South-western Atlantic reef fishes: zoogeographical patterns and ecological drivers reveal a secondary biodiversity centre in the Atlantic Ocean. Divers. Distrib. 24, 951–965.

Robertson, D.R., Van Tassell, J.L., 2019. Shorefishes of the Greater Caribbean: Online Information System. Version 2.0. Smithsonian Tropical Research Institute, Balboa, Panamá.

Roch, S., Friedrich, C., Brinker, A., 2020. Uptake routes of microplastics in fishes: practical and theoretical approaches to test existing theories. Sci. Rep. 10, 3896.

Rochman, C.M., Brookson, C., Bikker, J., Djuric, N., Earn, A., Bucci, K., Athey, S., Huntington, A., McIlwraith, H., Munno, K., De Frond, H., Kolomijeca, A., Erdle, L., Grbic, J., Bayoumi, M., Borrelle, S.B., Wu, T., Santoro, S., Werbowski, L.M., Zhu, X., Giles, R.K., Hamilton, B.M., Thaysen, C., Kaura, A., Klasios, N., Ead, L., Kim, J., Sherlock, C., Ho, A., Hung, C., 2019. Rethinking microplastics as a diverse contaminant suite. Environ. Toxicol. Chem. 38, 703–711.

Rotjan, R.D., Sharp, K.H., Gauthier, A.E., Yelton, R., Lopez, E.M.B., Carilli, J., Kagan, J. C., Urban-Rich, J., 2019. Patterns, dynamics and consequences of microplastic ingestion by the temperate coral, *Astrangia poculata*. Proc. R. Soc. B 286, 20190726.

Rotter, G., Ishida, H., 1992. FTIR separation of nylon-6 chain conformations: clarification of the mesomorphous and γ-crystalline phases. J. Polym. Sci. B Polym. Phys. 30, 489–495.

Salerno, M., Berlino, M., Mangano, M.C., Sarà, G., 2021. Microplastics and the functional traits of fishes: a global meta-analysis. Glob. Chang. Biol. n/a.

Santos, R.G., Andrades, R., Boldrini, M.A., Martins, A.S., 2015. Debris ingestion by juvenile marine turtles: an underestimated problem. Mar. Pollut. Bull. 93, 37–43.

Santos, R.G., Andrades, R., Fardim, L.M., Martins, A.S., 2016. Marine debris ingestion and Thayer's law – the importance of plastic color. Environ. Pollut. 214, 585–588.

Savoca, S., Capillo, G., Mancuso, M., Faggio, C., Panarello, G., Crupi, R., Bonsignore, M., D'Urso, L., Compagnini, G., Neri, F., Fazio, E., Romeo, T., Bottari, T., Spanò, N., 2019. Detection of artificial cellulose microfibers in *Boops boops* from the northern coasts of Sicily (Central Mediterranean). Sci. Total Environ. 691, 455–465.

Sazima, I., Gadig, O.B.F., Namora, R.C., Motta, F.S., 2002. Plastic debris collars on juvenile carcharhinid sharks (*Rhizoprionodon lalandii*) in southwest Atlantic. Mar. Pollut. Bull. 44, 1147–1149.

Shaw, D.G., Day, R.H., 1994. Colour- and form-dependent loss of plastic micro-debris from the North Pacific Ocean. Mar. Pollut. Bull. 28, 39–43.

Siebeck, U.E., Wallis, G.M., Litherland, L., 2008. Colour vision in coral reef fish. J. Exp. Biol. 211, 354–360.

Simon, T., Joyeux, J.-C., Pinheiro, H.T., 2013. Fish assemblages on shipwrecks and natural rocky reefs strongly differ in trophic structure. Mar. Environ. Res. 90, 55–65.

Singh, R.P., Mishra, S., Das, A.P., 2020. Synthetic microfibers: pollution toxicity and remediation. Chemosphere 257, 127199.

Suaria, G., Achtypi, A., Perold, V., Lee, J.R., Pierucci, A., Bornman, T.G., Aliani, S., Ryan, P.G., 2020. Microfibers in oceanic surface waters: a global characterization. Sci. Adv. 6, eaay8493.

Tebbett, S.B., Goatley, C.H.R., Bellwood, D.R., 2017. Fine sediments suppress detritivory on coral reefs. Mar. Pollut. Bull. 114, 934–940.

Teh, L.S.L., Teh, L.C.L., Sumaila, U.R., 2013. A global estimate of the number of coral reef fishers. PLoS One 8, e65397.

Verleye, G.A.L., Roeges, N.P.G., Moor, M.O.D., 2001. Easy Identification of Plastics and Rubbers. Rapra Technology Limited, Shropshire.

Welden, N.A., Abylkhani, B., Howarth, L.M., 2018. The effects of trophic transfer and environmental factors on microplastic uptake by plaice, *Pleuronectes plastessa*, and spider crab, *Maja squinado*. Environ. Pollut. 239, 351–358.

Westneat, M.W., Alfaro, M.E., 2005. Phylogenetic relationships and evolutionary history of the reef fish family Labridae. Mol. Phylogenet. Evol. 36, 370–390.