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The occurrence of microplastics in the gastrointestinal tract of demersal fish species

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Abstract

The study aimed to quantify and qualify the microplastics in the gastrointestinal tract contents of the six (6) dominant demersal fish species, determining the percentage frequency of occurrence and the condition factor. Out of 180 fish individuals examined, 21 samples (11.67 % of the total samples) have microplastics in their gastrointestinal tracts. The microplastics observed were fibers which occur the most (count = 22; %FO = 78.57 %), followed by microbeads (count = 5; %FO = 17.86 %) and rubber (count = 1; %FO = 3.57 %). The Spangled Emperor, *L. nebulosus,* was the highest consumption of 13 (46.43 %) fiber and 1 (3.57 %) microbeads in the stomach. No microplastic in the stomach of the Brown-stripe Snapper, *L. vitta*. The fish samples without microplastics ingestion differ significantly from fish with microplastics in the stomach. The fish samples without microplastics ingestion displayed a good and well-proportioned fish, while fish with microplastics is as a poor fish, long and thin.

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Introduction

Microplastics generally refer to plastic particles between 0.33 mm and 5 mm in size (Driedger *et al.*, 2015), which can be detrimental to the ocean and marine life. These come from a variety of outlets into natural ecosystems, including cosmetics, clothes, industrial processes *(Collignon et al., 2014) and careless resorts, operators of the boat, and tourists (Abir, 2019).*

Microplastics pose a danger to the health of marine environments as a result of different species eating them. Nearly all individuals possess plastic for certain species; for example, 96% of North Sea fulmars (birds that feed on fish and other aquatic animals) contained at least one piece of plastics (Jeftic *et al.,* 2009). Several species of fish include microplastic particles in their digestive system (Boerger *et al.*, 2010). Also, other aquatic animals such as polychaete worms, barnacles, amphipods, and marine cucumbers (Thompson *et al.*, 2004; Graham and Thompson, 2009). Microplastic ingestion is associated with several adverse health effects on aquatic organisms, including decreased immune response (Von Moos *et al.,* 2012). It reduced food intake, loss of weight and energy reduction (Besseling *et al.,* 2012; Wright *et al.,* 2013), reduced growth rate (Huerta-Lwanga *et al.,* 2016), lowered fecundity and adverse effects on subsequent generations (Sussarellu *et al.*, 2016). Many toxic effects have been documented by Avio *et al.* (2015) in mollusks related to immune response, oxidative stress, and genotoxicity.

The United Nations Environment Programme (UNEP) estimates that tourism activities alone can contribute 4.8 million tonnes, 14% of all solid waste. Littering is an issue in the tourist areas, especially those on the coast since plastics can be extremely harmful to both the local landscape and the marine environment (McDowall, 2016). Some studies claim that microplastics are being confused as natural prey for fishes and other aquatic organisms (Boerger *et al.*, 2010; Ramos *et al.*, 2012; Rummel *et al.*, 2016). Britania Group of Islands is one of the most visited tourist spots in Surigao del Sur, Philippines. The annual report of the Tourism Department of Municipality in San Agustin shown the highest number of annual visitors for the past four years recorded in 2018 with 125, 214 individuals (both local and foreign tourists). A study related to the extent of plastic pollution in this area is required, particularly for the microplastics not known to most people. Thus, the present study determined the presence of microplastics (MPs) in the gastrointestinal tracts of the demersal fishes. It includes the frequency of occurrence and the condition of the fish species with and without microplastics ingestions.

Materials and methods

Study area

The collection of the sample was conducted in Barangay Britania, San Agustin, Surigao del Sur, Philippines located at 8° 41' 52.4" N, 126° 12' 18.4" E where the majority of the fish catch of the fishermen was landed. The Municipality of San Agustin has a population of 22,779 people (PSA, 2015). Barangay Britania is blessed with 24 separate islands and islets in its coastal area called the Britania Group of Islands. The islands become one of the tourist destinations in the province (Wikipedia contributors, 2019).

Sampling procedures

Fish samples were collected every Wednesday and Saturday from November 2019 to January 2020 from fishers engaged in fishing activities within the municipal waters of San Agustin, Surigao del Sur. The fish samples were transported to the laboratory of Surigao del Sur State University (SDSSU) - Lianga Campus Biology Laboratory using an icebox with adequate ice. They were kept in a freezer until further analysis was carried out. The total length (TL) was measured using an ordinary ruler and weighed with Digital Weighing Scale (0.01× 500 g) in grams. Each species was photographed and identified based on Fishbase.org.

Laboratory analysis

Gastrointestinal tracts of fish have been dissected, and microplastics removed, identified, and counted using the established protocol used by the Civic

Laboratory for Environmental Research (CLEAR) (Liboiron, 2017) with some modifications. The processes include: (a) Dissecting station should be on a clean and even surface; (b) Placing the fish on the dissecting pan; (c) Cutting of the stomach from one end to the other allowing the contents to fall into the dissecting pan; (d) Taking out the guts; (e) Cutting of the intestines from the stomach using scissors while keeping anything that spills out into fall into the coffee filter; (f) Pouring carefully and slowly the water over the contents to separate and remove all debris from the stomach.

The subsequent organic digestion protocol was carried out using a protocol modified from Enders *et al.*, 2016; Strand and Tairova (2016); (g) For the digestion, solution 50 ml sodium hypochlorite (6-14 % reactive chlorine) poured into the cups and soaked for 12-24 hours to dissolve the natural food of the fish; (h) Placing the coffee filter in the strainer then putting the remaining debris and pouring water to remove the sodium hypochlorite; (i) examining the collected microplastics using a compound light microscope; (j) identification of the microplastic and non-microplastic.

Data and statistical analyses

The Percentage Frequency of Occurrence (FOC) of microplastics in the gastrointestinal tract of the fishes was computed based on the formula of Hyslop (1980):

$$\% \, FOC = (Ni / N) \times 100$$

Where, *FOC* = Percentage occurrence of the particular microplastics; *Ni* = Total number of stomachs with particular microplastics; *N* = Total number of stomachs with microplastics.

The condition factor of fish was calculated using the formula, according to Bannister (1976).

$$K = \frac{100W}{L^3}$$

Where, K = Fulton's Condition Factor; W = whole body weight (g); L = total length (cm).

Before statistical analysis, all data obtained were tested for homogeneity of variances using Levene's and Kolmogorov–Smirnov's tests to confirm normal distribution. Mann-Whitney U Test was then performed to compare the differences in the condition of all samples with or without microplastic ingestion in the stomach. All statistical tests were performed using the statistical package SPSS (Version 25.0) at 0.05 probability.

Results and discussion

Fig. 1 illustrates the percentage of fish samples with and without the ingestion of microplastics. Out of 180 fish individuals examined, 21 samples (11.67% of the total samples) have microplastics in their gastrointestinal tracts.

The result of the present study is almost similar compared to Phillips and Bonner (2015) report, with 12 of 116 individuals (10.40%), Vendel *et al.* (2017) with 200 of 2233 (9%), Karthik *et al.* (2018) (10.1%) and Anastasopoulou *et al.* (2013) (3.19%) but lower compared to the report of Neves *et al.* (2015) (19.8%), and Lusher *et al.* (2013), 36.50% of the samples were ingested with microplastics (Table 1).

Table 1. Percentage number of samples with microplastics reported by the different authors.

Reference	No. of sample	No. of sample w/ MPs	%	No. of species with MPs	Sampling site
Phillips and Bonner (2015)	116	12	10.40	8	Gulf of Mexico
Vendel *et. al.* (2017)	2233	200	9.00	24	Brazilian Estuaries
Karthik *et. al.* (2018)	79	8	10.10	5	Southeast Coast of India
Anastasopoulou *et. al.* (2013)	1504	48	3.19	5	Ionian Sea
Neves *et al.* (2015)	263	52	19.8	17	Portuguese coast
Lusher *et. al.* (2013)	504	184	36.50	10	English Channel
This study	180	21	11.67	5	Bgry. Britania, Surigao del Sur

The result indicates that microplastics ingestion is as small as 3.19 percent of the fish population. The presence of microplastics in the stomach of the samples depends on the place where the fish are captured. The study area is vulnerable to plastic pollution because it is close to the community, the presence of inlet, and one of the tourist's destinations. According to Free *et al.* (2014) and Wagner *et al.* (2014), proximity to urban centers has been one of the most contributors to microplastics pollution, based on the assumption that microplastics derive from the careless human discharge of plastic debris into aquatic environments.

Microplastics may reach remote oceanic regions, benthic sediments, and shorelines as a result of surface currents and bottom water transport (Bellas, 2016). Jabeen *et al.* (2017) hypothesized that a greater variety of plastic items might be present in the marine environment compared with the freshwater ecosystems, thus, increasing the probability of ingestion of microplastics by sea fish.

Table 2. Mean ± SD of Fultons condition factor (k) for the six (6) most abundant demersal fish species.

Species	K Value				P-Value
	n	Without microplastics	n	With Microplastics	(0.05)
L. nebulosus	18	1.58±0.19	12	1.18±0.07	0.000*
N. forcusus	27	1.22±0.13	3	0.94±0.06	
E. melanostigma	29	1.42±0.18	1	0.84±0.00	
L. gymnocranius	27	1.72±0.50	3	0.77±0.08	
S. vermiculatus	28	1.66±0.30	2	1.02±0.16	
L. vitta	30	1.50±0.12	0	0.00±0.00	
Total/Mean	159	1.51±0.32	21	1.09±0.13	0.000*

*Significant ($P < 0.05$).

The possible sources of fibers in the marine environment could be related to sewage sludge (washing machine effluents) and the fishing industry, which could also be a factor contributing to the reported microplastic values in the area (Browne *et al.*, 2011). Among different beaches studied by Karthik *et al.* (2018), the highest concentrations of microplastics were found from those near to the river mouths. Riverine inputs are the most significant source of marine plastic debris (Rech *et al.*, 2014; Zhao *et al.*, 2015). Domestic discharge, surface runoff, municipal dumping, and factory spillage are identified as the other significant contributors to the microplastic pollution observed in beach sediments (Zbyszewski *et al.*, 2014).

Frequency of occurrence

There are three types of microplastics recorded in this study, namely fiber, microbeads, and rubber (Fig. 2). Fibers occur the most (count = 22; %FO = 78.57%), followed by microbeads (count = 5; %FO = 17.86%) and the least was rubber (count = 1; %FO = 3.57%) (Fig. 3).

Fig. 4 shows that the Spangled Emperor, *Lethrinus nebulosus* had the highest number of microplastics in their gut (13 pcs fiber and 1 pc microbead) followed by the Fork-tailed Threadfin Bream, *Nemipterus japonicus* (3 fibers and 3 microbeads), Emperor, *Lethrinus gymnocranius* (5 fibers), Vermiculated S pinefoot, *Siganus vermiculatus* (1 fiber and 1rubber) and One-blotch Grouper, *Epinephelus melanostigma* (1 microbead). On the other hand, the Brown-stripe Snapper, *Lutjanus vitta* has no microplastic observed in the stomach. This finding corroborates with the report of Neves *et al.* (2015). They reported that out of 73 microplastics ingested by the commercial fish species such as *Scomber japonicas, Trigla lyra, Scomber scombrus, Raja asterias* and others off the Portuguese coast, 48 (65.8%) being fibers and 25

(34.2%) fragments. Bellas (2016) also reported that the detected microplastics in the 37 demersal fish (11 dogfish, 24 red mullets, and two hakes) from the Spanish Atlantic and Mediterranean coasts were mostly constituted by fibers (71%), followed by spheres (only in red mullets, 24%), films (3.2%) and fragmented (1.6%).

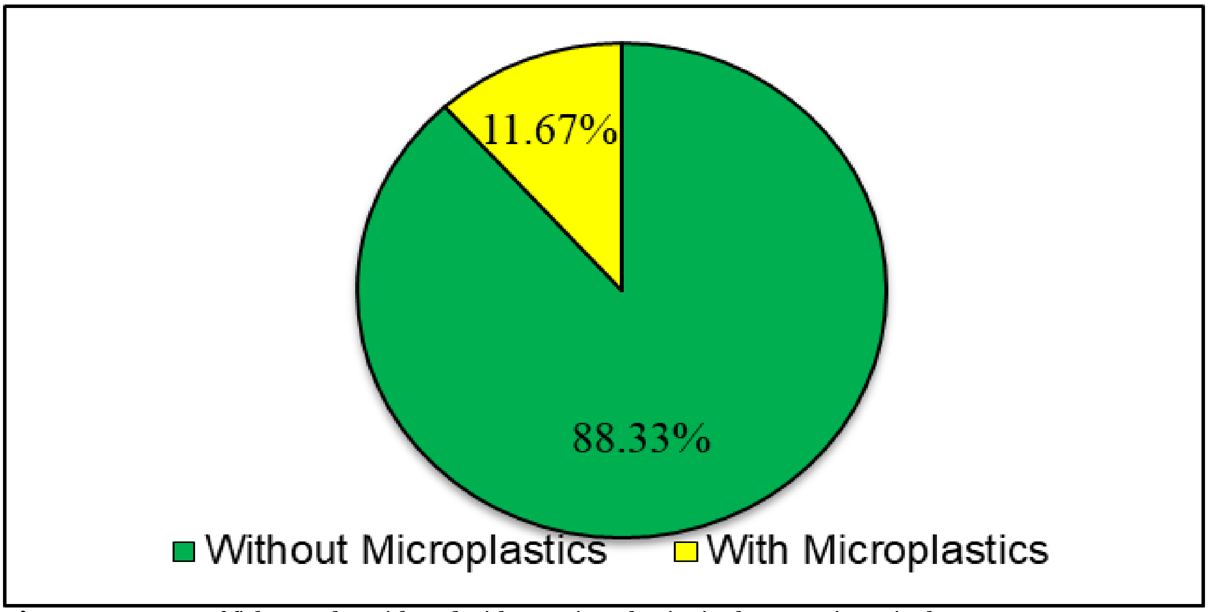

Fig. 1. Percentage of fish samples with and without microplastics in the gastrointestinal tract.

In the study of Lusher *et al.* (2013), the ingested plastic of pelagic (*M. merlangus, M. poutassou, T. trachurus, T. minutus, Z. faber*) and demersal fish (*A. cuculus, C. lyra, C. macrophthalmus, B. luteum, M. variegatus*) from the English Channel consisted primarily of fibres (68.3%) followed by fragments (16.1%) and beads (11.5%). Fibers are the most common microplastics present in marine fish intakes (Avio *et al.*, 2015; Botterell *et al.*, 2019).

Bellas *et al.* (2016) also remarkably noticed that the fiber (96%) was the primary type of microplastics ingested by demersal fish from the Spanish Atlantic and Mediterranean coasts.

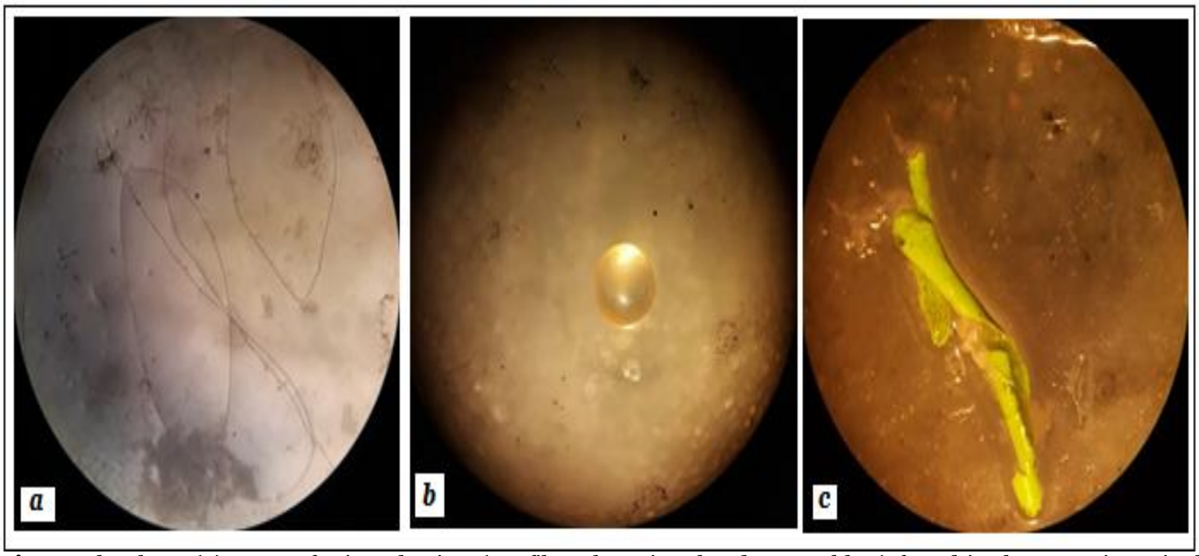

Fig. 2. The three (3) types of microplastics (*a* - fiber, *b* - micro beads, *c* - rubber) found in the gastrointestinal tract of the 21 fish samples.

This finding shows the high chance that fish consume fibers. Similar percentages were also reported by Boerger *et al.* (2010) at 94% and Lusher *et al.* (2013) at 68.3%.

The origin of the fibers in aquatic ecosystems is diverse. According to Pruter (1987) polymers were polyamide and polyester, which are commonly used in the fishing industry.

Fish condition factor

The condition factor of the six (6) most abundant demersal species sampled from the municipal waters of San Agustin, Surigao del Sur in November 2019 to January 2020 is presented in Table 2. The condition factors of 159 fish individuals without microplastics in the gastrointestinal tract ranged from 0.99 to 2.96, with an overall mean of 1.51±0.32, which implies a good and well-proportioned fish.

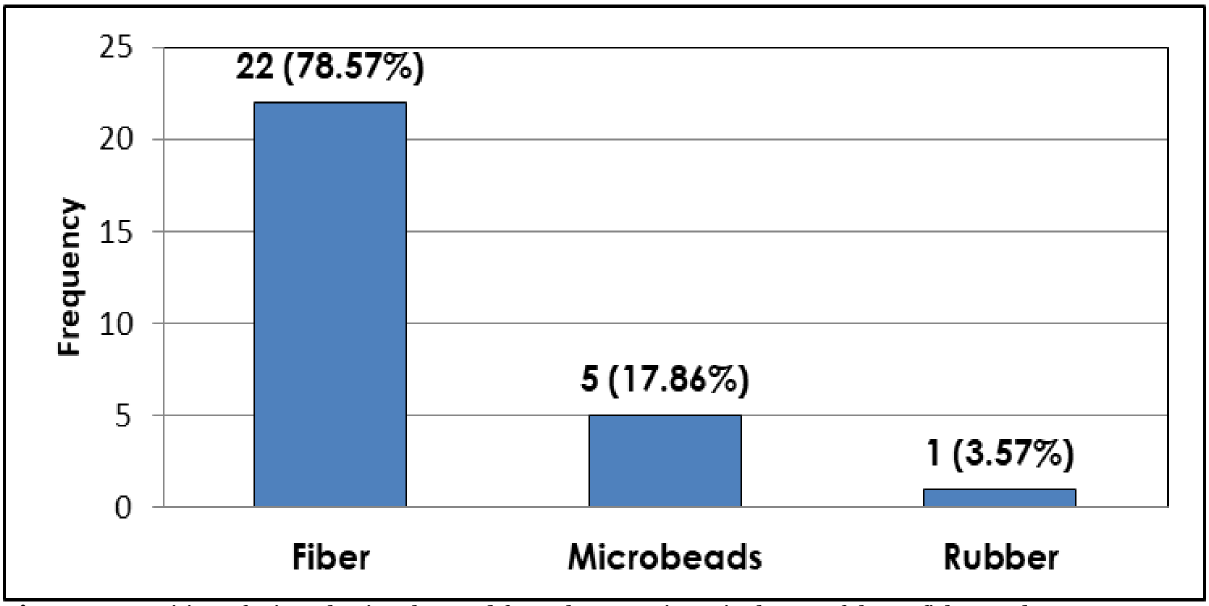

Fig. 3. Composition of microplastics observed from the gastrointestinal tract of the 21 fish samples.

The fish samples with microplastics have lower condition factors ranging from 0.84 to 1.27 with a mean of 1.09±0.13, indicating a poor fish, long and thin. Mann-Whitney U test proved that the condition of fish with microplastics in the gastrointestinal tract significantly differ ($p<0.05$) (Table 2) from the fish samples without microplastics. Similarly, the samples of *L. nebulosus* with microplastics ingestion also statistically differ ($p<0.05$) in the condition of the samples without microplastics in the stomach. The result shows that fish individuals without microplastics in the gastrointestinal tract have a good and better condition than fish samples with the ingestion of microplastics.

The difference in the condition factor in the present study is attributed to microplastics ingestion, environmental conditions, and the abundance of food. According to Deekae *et al.* (2010) several factors affect the condition factor of fishes.

They range from feeding, spawning, food nutrient composition, and fat accumulation. According to King (1997), the variations of condition factor (K) in fish may be due to food abundance, adaptation to the environment, and gonadal development. $K=1$ is the baseline between the slender and robust condition of the organism, and $K>1$ means the fish or crustacea is in a better condition of the robustness of the organism (Hopkins, 1992; Araneda, 2008; Gautam, 2014).Micro and nano plastics may-be both accidentally and deliberately ingested by fish. It can cause various harmful effects in fish: physical damage, change in lipid metabolism, change in behavior, as well as cytotoxicity (Jovanović, 2017). Browne *et al.* (2008) claimed that microplastic could have both physical and chemical effects on the organisms that ingest them.

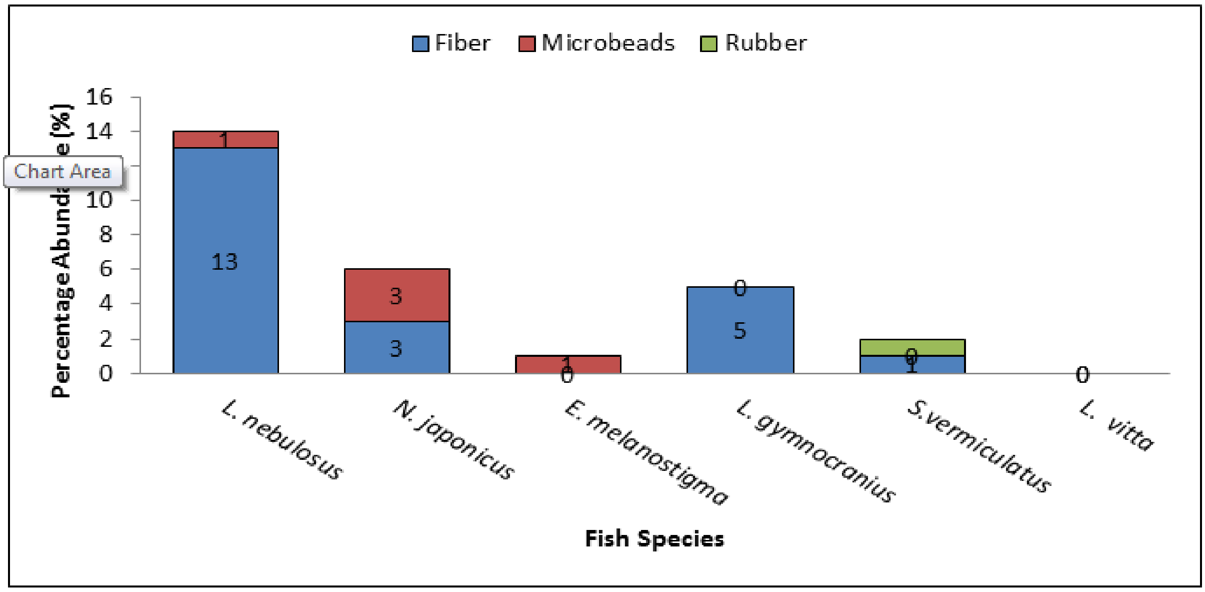

Fig. 4. Percentage frequency of occurrence of microplastics found from the gastrointestinal tract of the six fish species.

Microplastics may move through the gut if swallowed or may be retained in the digestive tract. Fibers may knot or clump and could be hazardous if they block feeding appendages or hinder the passage of food. Hoss and Settle (1990) suggested that if plastic particles were accumulating in high numbers in the intestines of smaller animals, they may have a similar effect to larger items of debris and clog digestive systems (Derraik, 2002; Gregory, 2009; Ryan *et al.*, 2009). The accumulation of waste in the gastrointestinal tract can also cause a false sense of satiation that contributes to decreased consumption of food (Ibrahim, 2017).

Conclusion

This study proved that microplastics are being ingested by the demersal fish species living in the coastal waters of Bgy. Britania, San Agustin, Surigao del Sur. Furthermore, the ingestion of microplastics can decrease the condition factor of the fishes. Thus, plastic pollution in the form of microplastics can be considered as an environmental problem for demersal fishes in the area.

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