

Occurrence of microplastics in the gastrointestinal tract of benthic by-catches from an eastern Mediterranean deep-sea environment

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A B S T R A C T

Concern about microplastic pollution little is known about levels in deep-sea species; to fill this knowledge gap, levels of microplastics in the gastrointestinal (GI) tracts of 34 fish from eight different deep-sea by-catches: blackmouth catshark, lesser spotted dogfish, and velvet belly, armless snake eel, hollowsnout grenadier, phaeton dragonet, royal flagfin, and slender snipe eel were measured. All were collected at the same site (east Sardinia, Mediterranean Sea; 40°10'12.49"N, 9°44'12.31"E) using a bottom gillnet at depths between –820/250 and –1148 ft./350 m. Microplastics (MPs) were retrieved in 16 out of 34 fish. At least one microplastic item was found in 48% (33%, *E. spinax* - 75%, *G. melastomus*) of the samples. The most frequent was polyethylene (PE), with nine items (filaments, films, fragments) found in five specimens. This preliminary study of by-catches adds new data on MPs ingestion by species inhabiting a deep-sea environment of the Mediterranean.

1. Introduction

Global plastics production, excluding polyethylene terephthalate (PET), polyamide (PA), and polyacryl fibers was nearly 370 million tons in 2019 (PlasticsEurope, 2020). Plastic is ubiquitous synthetic organic polymer as marine debris worldwide (Derraik, 2002) and its properties (e.g., lightweight, durable, cheap) make it ideal for a wide variety of applications yet hazardous to the environment (Laist, 1987). Plastic pollution has become a major environmental concern for understanding its extent and its effects on marine environments worldwide (Gola et al., 2021).

Plastic can enter (directly and/or indirectly) marine ecosystems from various sources (e.g., freshwater input, residential and domestic activities, tourism) (Thushari and Senevirathna, 2020); it undergoes degradation (biological, mechanical, photooxidation by ultraviolet light) that changes particle size and density (Galloway et al., 2017). The resulting small particles are microplastics (MPs; 1 µm–<5 mm) similar in size to many biological organisms (Lim, 2021). Microplastics make up 65% of marine debris; the most abundant are pieces < 1 mm (Browne et al.,

2010), which are more difficult and expensive to analyze the smaller they become (Lim, 2021).

Microplastics are classified into primary and secondary MPs (Singh and Sharma, 2008). Primary MPs are manufactured plastic particles, pellets/plastic bead as well, that go into a variety of products (i.e., cosmetics), while secondary MPs are formed during the use and disposal of plastic products or in the degradation of macroplastics into MPs (Singh and Sharma, 2008).

Microplastics are ubiquitous, from high-altitude inland lakes (Zhang et al., 2016; Çomaklı et al., 2020; Pastorino et al., 2021) to remote areas such as deep-sea environments (Zhu et al., 2019; Barrett et al., 2020; Zhang et al., 2020), islands (Imhof et al., 2017; Nel et al., 2020; Tan et al., 2020), the Arctic and the subarctic regions (Obbard et al., 2014; Cózar et al., 2017; Fang et al., 2018; Hurley et al., 2021). Studying the MPs dynamic is essential to better understand the distribution and bioavailability in the water column (Galloway et al., 2017; Li et al., 2018). The vertical transport of MPs differs by plastic and biofilm properties (biofouling) and oceanographic conditions (Kooi et al., 2017). A recent study has shown that near-bed thermohaline currents

(bottom currents) play a key role in the distribution of MPs, especially at depths between $-1968/600$ and -2952 ft./ 900 m (Kane et al., 2020).

The quantification of MPs ingestion by marine organisms has been well documented (Botterell et al., 2020; Garrido Gamarro et al., 2020; Goswami et al., 2020; Hara et al., 2020; Miller et al., 2020; Pereira et al., 2020; Wang et al., 2020; Cho et al., 2021; Hamzah et al., 2021; Pequeno et al., 2021; Thiele et al., 2021; Ugwu et al., 2021), however, few studies have been conducted on deep-sea species and attention has focused largely on species of commercial interest (Bellas et al., 2016; Giani et al., 2019; Mancuso et al., 2019).

Microplastics can be ingested intentionally (confused for prey) and/or accidentally by trophic transfer from contaminated prey (Farrell and Nelson, 2013; Setälä et al., 2014; Nelms et al., 2018), to then be accumulated in the GI tract or excreted (Fossi et al., 2018). Therefore, the feeding behavior it might be useful to explain the ingestion of MPs in demersal fish species (Capillo et al., 2020). For instance, Bour et al. (2018) pointed out that occurrence of MPs in benthic and epibenthic species is likely affected by feeding behavior rather than habitat or trophic level.

The three cartilaginous fish here analyzed, namely blackmouth catshark (*Galeus melastomus*), lesser spotted dogfish (*Scyliorhinus canicula*), and velvet belly (*Etmopterus spinax*), widely distributed in Mediterranean as well as in the Atlantic Ocean, are demersal species living and/or feeding usually below 200 m (-650 ft) (Froese and Pauly, 2021). Overall, these opportunistic benthic scavenger seem to have a similar diet, but which may vary depending on habitats, depth, seasonal and geographical availability and distribution of prey (Valente et al., 2019 and references therein; D'Iglio et al., 2021).

Unlike the elasmobranchs species, other bony fish studied [i.e., armless snake eel (*Dalophis imberbis*), hollowsnout grenadier

(*Coelorinchus caelorhincus*), phaeton dragonet (*Synchiropus phaeton*), royal flagfin (*Aulopus filamentosus*)], are commonly found in about $-656-1640$ ft./ $200-500$ m and feed on a variety of benthic organisms (e.g., polychaetes, gastropods, cephalopods, crustacean), except for slender snipe eel (*Nemichthys scolopaceus*) that usually feed on crustaceans while swimming with its mouth open (Froese and Pauly, 2021).

The aims of this study were: i) measure MPs particles in the gastrointestinal (GI) tract of benthic deep-sea by-catches; ii) report the first evidence of MPs ingestion on three deep-sea species: hollowsnout grenadier (*Coelorinchus caelorhincus*), armless snake eel (*Dalophis imberbis*), and royal flagfin (*Aulopus filamentosus*); iii) elucidate the level of MPs' pollution in the marine biota of the eastern Mediterranean, and in submarine canyon systems.

2. Materials and methods

2.1. Study site

2.1.1. Gulf of Orosei coast

The Gulf of Orosei ($40^{\circ}14'45.40''N$, $9^{\circ}40'07.64''E$; Fig. 1) is surrounded by the foothills of the Gennargentu massif. It stretches for about 75 km along the central east coast of Sardinia, Italy. At its northern end is Cape Comino, which is also the easternmost point of Sardinia. The south coast extends to Cape Monte Santu, which marks the southern end of the Gulf. The area is listed as a Site of Community Importance (SCI) (no. ITB020014) according to European Commission Habitats Directive 92/43/EEC (Directive, 1992). It encompasses about 29,000 ha in total area, 16% of which (roughly 4600 ha) are marine.

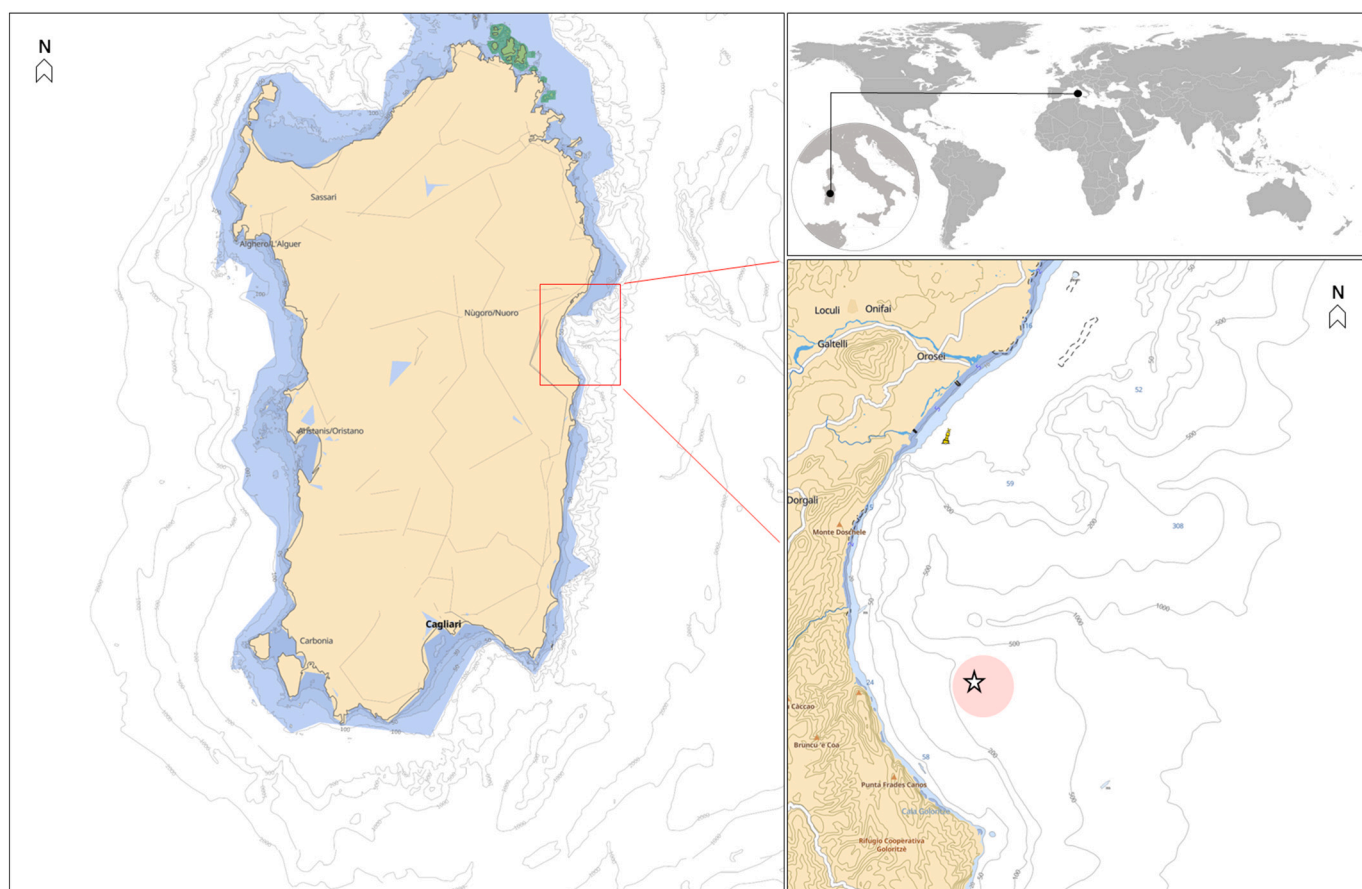


Fig. 1. Map of the study site with sampling area (red circle) and GPS coordinates (star). Color maps modified from Aqua Map©; <https://www.globalterramaps.com>. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.1.2. Seabed morphology

In general, the study area has both rocky and sandy bottoms. The seabed facing the coast rapidly slopes to depths between –65/20 and –131 ft./40 m. The marine sector is characterized by the Cala Gonone–Orosei submarine canyon system, the head of which is about 0.62 miles from the coast. The canyons join in a single element at a depth of about –6561 ft./2000 m for roughly 50 km (Fig. 1). These shelf-incising canyons have great diversity and biomass, with no clear bathymetric connection to a major river system (Harris and Whiteway, 2011).

2.1.3. Sampling

The study area is a 12–km stretch of coastline at the center of the Gulf of Cala Gonone, Dorgali. Deep-sea fish were collected from a single site on 29 May 2020 (40°10'12.49"N, 9°44'12.31"E; Fig. 1) using a bottom gillnet at depths between –820/250 and –1148 ft./350 m. Samples were placed in a cold box and brought to our laboratory for identification according to Iglésias (2013a, 2013b). A total of 34 GI tracts were extracted from eight deep-sea by-catches (Fig. 2; Table 1): blackmouth catshark (*Galeus melastomus*) ($n = 4$), lesser spotted dogfish (*Scyliorhinus canicula*) ($n = 3$), and velvet belly (*Etmopterus spinax*) ($n = 3$) (cartilaginous fish) and armless snake eel (*Dalophis imberbis*) ($n = 2$), hollowsnout grenadier (*Coelorhynchus caelorhynchus*) ($n = 14$), phaeton dragonet (*Synchiropus phaeton*) ($n = 2$), royal flagfin (*Aulopus filamentosus*) ($n = 4$), and slender snipe eel (*Nemichthys scolopaceus*) ($n = 2$) (bony fish). The specimens were photographed (Fig. 2) and measured for total body length (cm), total weight, and total GI weight (g) (Table S1 – Supplementary Materials).

2.1.4. Ethical statement

By-catches were collected dead from the bottom gillnet during normal fishing activity in order to study microplastics occurrence in the Mediterranean fauna of deep-sea environments. They are regulated organisms (live vertebrates or higher invertebrates) exempt from ethical approval.

2.2. Microplastics analysis and assurance/quality control

Creon enzyme (Creon 40.000 at 20 mg per g of tissue; TRIS-buffered pH at 8.00) was used to pretreat the fish samples for digestion of tissues and avert damage to the plastic polymers for chemical testing (von Friesen et al., 2019). Following sonication for 1 h at 30 °C, such digested tissues were continuously mixed at 110 rpm (37 °C) for 24 h. The digestion method based on Creon allows one to reduce microplastic deterioration that could occur with more aggressive methods such as H₂O₂.

After complete dissolution of tissues, digested samples were filtered through 6-µm pore paper disks under a HEPA-filtered laminar-flow fume hood to minimize air exposure and potential airborne pollution and collected filters were stored in glass Petri dishes and dried overnight at 40 °C (Ziajahromi et al., 2017).

Microplastics within the dimensional range 5000–10 µm were determined. To ensure quality control of the analytical process, each sample was analyzed in triplicates. Furthermore, three positive and three negative controls were performed for each batch of analyses.

Negative controls shall be microplastic free within the target dimensional range to be considered acceptable; on the contrary positive controls were considered acceptable if the mean particles recovery of spiking microplastics particles (orange PP and PE fragments mixture spiked in a digested aliquot of tissues, $n = 3$) were recovered over 80% of the initial spiked number.

Stereomicroscopy at 10–80× (SMZ–800 N; software NIS–elements D, Nikon, Tokyo, Japan) was used to sort the filtered samples. Microscopy coupled with Fourier transform infrared spectroscopy (µFT–IR; Nicolet iN10 MX, ThermoFisher Scientific, Waltham, MA, USA) and an MCT–A detector (7.800–650 cm⁻¹ spectral range) cooled with liquid nitrogen and operating in reflection mode was used to chemically analyze the potential targets.

Identification was carried out by 1) determining the spectral match (%) of the targeted items compared to the spectral libraries of aged and



Fig. 2. Deep-sea species caught off the coast of Cala Gonone (east Sardinia, Italy): (a) hollowsnout grenadier (*Coelorhynchus caelorhynchus*); (b) blackmouth catshark (*Galeus melastomus*); (c) royal flagfin (*Aulopus filamentosus*); (d) lesser spotted dogfish (*Scyliorhinus canicula*); (e) velvet belly (*Etmopterus spinax*); (f) slender snipe eel (*Nemichthys scolopaceus*); (g) phaeton dragonet (*Synchiropus phaeton*) and (h) armless snake eel (*Dalophis imberbis*). Photo courtesy of Giuseppe Esposito.

Table 1

Microplastics in deep-sea by-catches from the coast of Cala Gonone (Gulf of Orosei, Sardinia, Italy).

Species	Specimens				MPs		MPs type ^a				
	Total	Positive	Negative	Positive/total	Total	MPs number/individual	PA	PE	PET	PP	PS
<i>Aulopus filamentosus</i>	4	2	2	50%	2	1.00	0	0	1	1	0
<i>Coelorhynchus caelorhynchus</i>	14	6	8	43%	8	1.33	1	2	2	2	1
<i>Dalophis imberbis</i>	2	1	1	50%	2	2.00	0	1	0	1	0
<i>Etmopterus spinax</i>	3	1	2	33%	2	2.00	0	2	0	0	0
<i>Galeus melastomus</i>	4	3	1	75%	4	1.33	0	3	0	0	1
<i>Nemichthys scolopaceus</i>	2	1	1	50%	2	2.00	1	0	1	0	0
<i>Scyliorhinus canicula</i>	3	1	2	33%	1	1.00	1	0	0	0	0
<i>Synchiropus phaeton</i>	2	1	1	50%	1	1.00	0	1	0	0	0
Total	34	16	18	–	22	–	3	9	4	4	2

^a PA denotes polyamide; PE polyethylene; PET polyethylene terephthalate; PP polypropylene; PS polystyrene.

normal MPs (OMNIC™ Picta™ software libraries, ThermoFisher Scientific; and spectral libraries collected and determined on standard plastic materials of certain composition aged and developed by BsRC laboratory); 2) imposing a threshold for spectra back-recognition exceeding 80% of match. The limit of detection (LOD) was a particle size of 10 µm. Recovered items were classified by chemical type, shape, size, and color criteria according to [Galgani et al. \(2014\)](#).

2.3. Statistical analyses

All basic statistics and analyses were carried out using R software (v. 4.0.5; [RStudio Team, 2021](#)). Pearson's correlations were estimated between MPs size and fish dimensions (body length and weight and total GI weight). Cramer's V ([Cramér, 1946](#)) measure was computed to estimate the pairwise associations between three nominal variables: species, MPs color, MPs type. This statistic, based on Pearson's chi-squared statistic, ranges from 0 to 1 and can be read as a correlation value.

3. Results

The average body length and weight of the 34 specimens was 30.26 ± 24.72 cm and 184.00 ± 448.97 g, respectively. The average total GI weight was 5.22 ± 11.33 g. The lightest fish was a blackmouth catshark (*Galeus melastomus*) (5.82 g) and the heaviest was a velvet belly (*Etmopterus spinax*) (2530.78 g). The average dimension of MPs items in the GI tracts was 1277.88 ± 670.44 µm; the smallest (89.78 µm) was a piece of blue polyethylene terephthalate (PET) found in the GI contents

of a slender snipe eel (*Nemichthys scolopaceus*), and the largest was a black polyethylene fiber (PE; 2520.36 µm) in *G. melastomus*. There were weak and not significant correlations (data not shown) between MPs dimension and fish size (total body length and weight and total GI weight).

Microplastics were retrieved from 16 out of 34 specimens ([Table 1](#)). At least one microplastic item was found, on average, in 48% of the fish: from 33% for *E. spinax* to 75% for *G. melastomus*. A total of 1.46 ± 0.47 MPs were found in each positive specimen (mean MPs number per individual; [Table 1](#)), and 22 particles were identified: 15 filaments (68.2%; 312.24–2520.36 µm; 46% blue in color), 6 fragments (27.30%; 89.78–1290.10 µm; 67% blue in color), and 1 film (4.50%; 1584.72 µm; 100% white in color). The average size of microplastic recovered was within 1360.87 µm; range 90–2520 µm.

The most abundant MPs was PE, for a total of nine items of different colors in five different species: hollowsnout grenadier (*Coelorhynchus caelorhynchus*), armless snake eel (*Dalophis imberbis*), velvet belly (*E. spinax*), blackmouth catshark (*G. melastomus*), and phaeton dragonet (*Synchiropus phaeton*) ([Table 3](#); [Fig. 3](#)). All five types of MPs (polyamide (PA), polyethylene (PE), polyethylene terephthalate (PET), polypropylene (PP), and polystyrene (PS)) were found in the GI tract of *C. caelorhynchus* ([Fig. 4](#)).

[Table 2](#) presents the results of Cramer's V statistics ([Mangiafico, 2021](#)). There was a moderate to strong association between species and MPs color (0.54) and type (0.64) and a weak association (0.35) between MPs color and type).

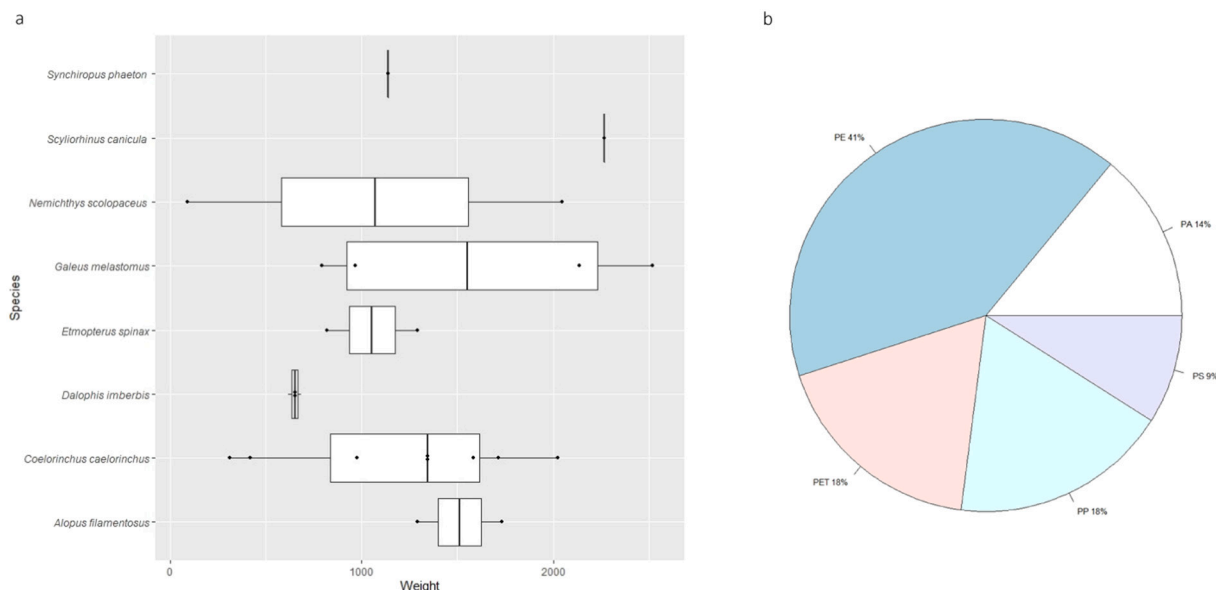


Fig. 3. (a) Box plot of MPs distribution by species; the x axis represent the MPs' weight (µm). (b) Polymeric composition (%) of MPs extracted from the GI tract.

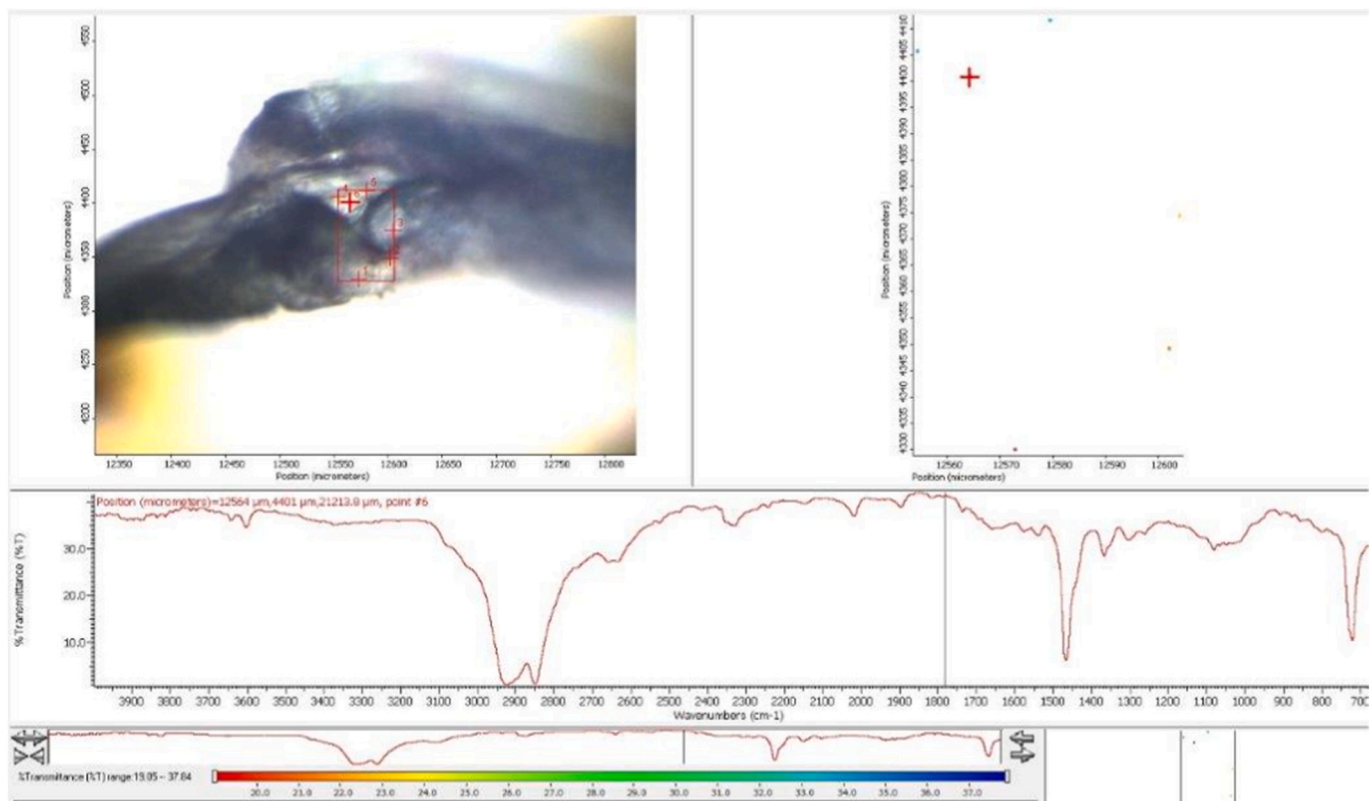


Fig. 4. Microscopic image of a blue filament of polyethylene collected in a fish specimens associated to its FT-IR spectra. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Association between nominal variables.

		Cramer's V
Species	Color	0.54
Species	MPs type	0.64
Color	MPs type	0.35

4. Discussion

Microplastics pollution has gained increasing attention, with a greater focus on its occurrence in marine fish species and its fate in food webs (Setälä et al., 2018). Far fewer studies have been conducted on fish from deep-sea environments of the epipelagic and the hadopelagic zone (see the text references), however, probably because of the high cost involved and the difficulty of sampling (Barnes et al., 2009; Ramirez-Llodra et al., 2011; Pham et al., 2014). Information is scarce on MPs accumulation and characterization in Sardinian deep-sea fish, though recent studies have reported on benthic crustaceans of commercial interest, i.e., Norway lobster (*Nephrops norvegicus*) and blue and red shrimp (*Aristeus antennatus*) (Cau et al., 2019a, 2019b, 2020). Our study, on the contrary, reports the results on the occurrence of MPs in the GI tract of benthic by-catches from an eastern Mediterranean deep-sea environment. However, the scarcity of fishing vessels in the port of Cala Gonone is a strong limit to get some fish samples, especially if they are deep-sea species. In fact, only few fishing vessels operate at high depths; therefore, the small number of fish examined was also attributable to the use of fishing techniques such as bottom gillnets and not by trawling.

4.1. Number of microplastics items in deep-sea fish: an overview

Knowledge of MPs occurrence in by-catch fish of the Sardinian pelagic zone is scarce. Here we report on MPs levels found in by-catch

species from a deep-sea environment of the eastern Mediterranean. However, it is important to point out, that variability in methodologies applied for quantification of microplastics in marine organisms limits the comparability among studies. The average number (\pm SD) of items per positive fish was 1.46 ± 0.47 ; this observation is shared by previous studies in GI tract of deep-sea fish, for instance: in the northern continental slope of the South China Sea (stomach: 1.96 ± 1.12 and intestine: 1.77 ± 0.73 , Zhu et al., 2019); the English Channel (1.90 ± 0.10 , Lusher et al., 2013); the northeast and northwest Atlantic (1.20 ± 0.54 , Lusher et al., 2016; 1.80 ± 0.49 , Wieczorek et al., 2018); the Azores archipelago in the northeast Atlantic [1.4 ± 0.04 (\pm SE); Pereira et al., 2020]; the Spanish Atlantic and Mediterranean coasts (1.56 ± 0.5 , Bellas et al., 2016); the eastern Ionian Sea (1.3 ± 0.2 ; Anastasopoulou et al., 2013); and the North Pacific Central Gyre ($1.15 \pm$ n.a., Davison and Asch, 2011). Differently, higher values (2.10 ± 5.78) were found in mesopelagic and epipelagic species in the last area (Boerger et al., 2010) but lower than in the stomach of longnose lancetfish (*Alepisaurus ferox*) from the North Pacific Ocean (Jantz et al., 2013). Significantly lower values were found in fish from the Balearic Islands, western Mediterranean (0.34 ± 0.07 ; Alomar and Deudero, 2017).

4.2. Types of microplastics in the GI tracts of deep-sea fish

The species analyzed in the present study share similar feeding strategies and interactions with the sea bottom. They are usually found at depths to $-13,123$ ft./4000 m (Froese and Pauly, 2021) and feed on a variety of benthic organisms, including crustaceans and bony fishes (D'Iglio et al., 2021; Froese and Pauly, 2021). We noted a difference in MPs type, shape, size, and color between the two groups (elasmobranchs and bony fish) which may stem from different feeding strategies (Anastasopoulou et al., 2013; Romeo et al., 2015).

In general, the frequency of MPs particles in the GI tract of the deep-sea sharks was in decreasing order: fibers/filaments > fragments

Table 3

Characteristics of MPs in deep-sea by-catches. The size data (mean \pm SD) are expressed in μm .

Species	MPs type ^a	n	Description	Color	Size data
<i>Aulopus filamentosus</i>	PET	1	Filament	Blue	1733.94 \pm 0
<i>Aulopus filamentosus</i>	PP	1	Fragment	Blue	1290.11 \pm 0
<i>Coelorinchus caelorhincus</i>	PA	1	Fragment	Blue	1380.91 \pm 0
<i>Coelorinchus caelorhincus</i>	PE	2	Film – filament	White – black	1281.37 \pm 429
<i>Coelorinchus caelorhincus</i>	PET	2	Filament	Blue, white	1869.29 \pm 221.79
<i>Coelorinchus caelorhincus</i>	PP	2	Filament	Blue, black	862.46 \pm 626.43
<i>Coelorinchus caelorhincus</i>	PS	1	Filament	Black	312.24 \pm 0
<i>Dalophis imberbis</i>	PE	1	Filament	Blue	684.85 \pm 0
<i>Dalophis imberbis</i>	PP	1	Filament	White	617.46 \pm 0
<i>Etmopterus spinax</i>	PE	2	Fragment	Blue, white	1053.93 \pm 334
<i>Galeus melastomus</i>	PE	3	Filament – fragment	Black, pink – white	1815.57 \pm 908.84
<i>Galeus melastomus</i>	PS	1	Filament	Blue	968.32 \pm 0
<i>Nemichthys scolopaceus</i>	PA	1	Filament	Blue	2048.25 \pm 0
<i>Nemichthys scolopaceus</i>	PET	1	Fragment	Blue	89.78 \pm 0
<i>Scyliorhinus canicula</i>	PA	1	Filament	Blue	2267.11 \pm 0
<i>Synchiropus phaeton</i>	PE	1	Filament	Orange	1139.55 \pm 0
Total	–	22	–	–	1213.45 \pm 157.50

^a PA denotes polyamide; PE polyethylene; PET polyethylene terephthalate; PP polypropylene; PS polystyrene.

> films, and the most frequent MPs types were colored PET, PP, PS, PE, and PA as also reported by Alomar and Deudero (2017) and Valente et al. (2019), probably because many of these polymers are also commonly used in the fisheries and aquaculture industry (Lusher et al., 2017). Our data also show that MPs occurrence has shifted towards the elasmobranch *Galeus melastomus*, as reported elsewhere (Anastasopoulou et al., 2013). In addition, high concentrations of cellophane and transparent MPs (~33 and 42%, respectively) have been found in the same species from the continental shelf in the western Mediterranean (Alomar and Deudero, 2017).

As regards bony fishes [hollowsnout grenadier (*Coelorinchus caelorhincus*), armless snake eel (*Dalophis imberbis*), royal flagfin (*Aulopus filamentosus*)], to our best knowledge the present study is the first report on the occurrence of MPs in these three deep-sea species. Slightly different values were found for the slender snipe eel (*Nemichthys scolopaceus*) and the phaeton dragonet (*Synchiropus phaeton*) compared to those reported by Lusher et al. (2016) and Compa et al. (2021), respectively.

The most frequent MPs items in all species were colored filaments and fragments (blue, white or black) from 89.78 to 2520.36 μm in size. Areas of Sardinia at bathymetries to –2624 ft./800 m are less impacted by benthic marine litter than elsewhere in the Mediterranean (Alvito et al., 2018), though concentrations of 5.1 \pm 2.3 fibers per liter have been reported by Musso et al. (2019) with a maximum of ~12 fibers/l around Sardinian surface waters. In this regard, only a small part were MPs, though abundance may vary with season, sampling methods or areas, the last of which also conditioned by proximity to urban and industrial settlements (Lusher et al., 2014; Rebelein et al., 2021, and references therein). For instance, synthetic organic polymers are released into the sea via wastewater discharges from clothes washing (Browne et al., 2011; Napper and Thompson, 2016).

Our preliminary results suggest that the species worst affected are *G. melastomus* and *C. caelorhincus*, though sharks seem to ingest marine debris such as plastics more frequently than teleost fishes (Anastasopoulou et al., 2013). Incidental ingestion was most often observed in these opportunistic scavengers (López-López et al., 2018). This observation was recently noted by Li et al. (2021) who reported that fish passively ingest MPs via breathing, though a small part inevitably accumulates in the gills and the GI tract. The pathway of MPs ingestion can also take place through active capturing, for instance, via transfer from the prey or inadvertently during foraging on fish prey (Chagnon et al., 2018). In addition, fish may also confuse MPs – especially blue items – with their natural prey, i.e., blue copepods (Ory et al., 2017). This may explain why many of the MPs found in the GI tract of the fish were blue in color: there was a moderate-to-strong association between deep-sea species and MPs color and type (0.54 and 0.64, respectively). It is also conceivable, however, that MPs accumulation occurs chiefly indirectly, since visual acuity decreases with increasing sea depth, where the only visible light sources, often bioluminescent flashes, are produced by other organisms inhabiting these environments (Warrant, 2000). It is most likely that they should feed on mainly bioluminescent prey.

4.3. MPs fate at the deepest layers

Plastics and particles produced by its degradation are one of the major pollutants of the world's oceans and seas. While recent studies have provided insights into the way MPs move within the water column down to the deepest layers, much is still largely unclear. Microplastics in deep-sea depositional environments occur worldwide in descending order: contourite drift > trench > submarine canyon > abyssal plain > open continental slope > seamount (Kane and Clare, 2019; Kane et al., 2020). Therefore, as the results from the Tyrrhenian Sea showing, the submarine canyons namely a furrow of the continental slopes (V-shaped transversal profile) are characterized by deep-sea bottom currents and other episodic strong flows. Despite being affected by a rapid and continuous sedimentation, the submarine canyons are not filled with it due to the instability of the deposits that easily feed landslides and turbidity currents. These determine a distribution and burial of large quantities of microplastics in seafloor sediments as well as supply oxygen and nutrients (Pohl et al., 2020). In this regard, given its characteristics (see Section 2.1.2) our sampling site also most likely may be microplastic hotspots: the most abundant were low-density MPs found close to the water surface (ρ_w : ~1,02 g/cm³): PE (0.90–0.99 g/cm³) and PP (0.85–0.95 g/cm³), though high-density MPs such as PET was also found (1.38–1.45 g/cm³). A plausible explanation for MPs at these depths is that the fate of the polymers (floating or sedimentation) is linked to a variety of factors: physical agents (e.g., density, size, shape, etc.) and ocean dynamics (e.g., wind, waves, tides, etc.) (Chubarenko et al., 2016; Kowalski et al., 2016; Zhang, 2017). Furthermore, biofouling can also influence MPs vertical movement (mainly low-density particles), resulting in maximum occurrence at intermediate layers (Kooi et al., 2017), as well as physicochemical parameters including temperature and salinity (Kowalski et al., 2016). Recent studies have shown, however, that near-bed thermohaline currents heavily affect the ultimate fate and spatial distribution of MPs rather than vertical settling from surface accumulations (Kane et al., 2020), as well as turbidity currents through submarine canyons (Pohl et al., 2020).

5. Conclusion

Over 350 million tons of plastic are produced every year (16% in Europe) and most of it ends up as marine debris from surface waters to deep-sea sediments worldwide. The smallest particles, known as MPs, are much more prevalent in the water column and deepest sea bottoms, which become the ultimate sink. Aquatic organisms are gravely threatened as they ingest and/or are entangled by plastic debris, and thus go up the food chain of predators, up to humans.

Overall, our study on MPs' ingestion reported for deep-sea by-catches helps to elucidate the level of pollution in the marine biota of the eastern Mediterranean off the Cala Gonone coast (Sardinia, Italy). Scarce references, especially locally, make it difficult to compare with our data, moreover, to our best knowledge we are reporting the first evidence of microplastic ingestion on three deep-sea species such as hollowsnout grenadier (*Coelorhynchus caelorhincus*), armless snake eel (*Dalophis imberbis*), and royal flagfin (*Aulopus filamentosus*).

Commonly the attention is shifted to fishes of greatest value, even for to understand the degree of pollution so the possible impact on human health. This, however, would lead to underestimate the degree of contamination of these ecosystems, and at the same time the all aquatic organisms living in it; also of those species which have no commercial interest, but which could probably act as excellent bioindicators. The sea and oceanic depths, in general are ecosystems very poor in food and animals; this pushes predator to ingest everything they find like plastic materials (sometimes mistaking them for prey). However, being species living in a remote habitat, but contaminated by MPs, should be an additional and unambiguous signal that the measure is full. The effects that plastic could have on these marine ecosystems are still unknown and could have very serious implications similar to those occurring in the rest of the oceans. These polymers are widespread, and aquatic organisms cannot avoid them in any way; thus, further studies are needed to better understand the distribution and the potential impact of MP pollution in these remote environments such as submarine canyon systems.

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Declaration of competing interest

The authors declare no conflict of interest.

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