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Microplastics in seawater and marine organisms: Site-specific variations over two-year study in Giglio Island (North Tyrrhenian Sea)

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ABSTRACT

Geographical and temporal differences of microplastic occurrence were documented in water and fish collected in 2017 and 2019 from the Giglio Island (North Tyrrhenian Sea) close to the area where the Costa Concordia sank in January 2012. Results on water samples showed a site-dependent difference, suggesting the role of surface current dynamics in the microplastic local distribution, while tested Neuston nets (200 μm and 330 μm mesh size) did not influence microplastic retention efficiency. Fish exhibited in 2019 a higher frequency of specimens positive to microplastic ingestion with respect to 2017, with an occurrence higher than those typically observed in other Mediterranean areas. Both in water and fish, fragments were the dominating shape, polypropylene and polyethylene were the prevalent polymers, without particular difference between sites and years. This study highlights the importance of applying microplastic investigation in biotic and abiotic matrices for an effective monitoring of this pollution in the marine environment.

1. Introduction

Microplastics (MPs) represent a great environmental problem in the oceans worldwide, due to their persistence, ubiquity in all compartments and bioavailability to organisms (Fossi et al., 2018; Morgana et al., 2018; Miller et al., 2020; Suaria et al., 2020).

In the last years, several investigations have focused on the Mediterranean Sea, identified as one of the most affected areas by plastic pollution (Suaria et al., 2016), and covering different subregions, environmental compartments and biota (Fossi et al., 2018; Güven et al., 2017; Cincinelli et al., 2019; Giani et al., 2019; Baini et al., 2018; Avio et al., 2020; Llorca et al., 2020, Adamopoulou et al., 2021). Such studies have also provided responses and guidance useful in the context of the Marine Strategy Framework Directive (MSFD, 2008/56/EC recently implemented with the Commission Decision 2017/848/EU), where the assessment of MPs in water, sediment and biota is among the criteria for the determination of good environmental status (Descriptor 10-Marine Litter).

In this respect, monitoring programs combining analysis of microplastics in both abiotic and biotic compartments represent the best approach for a more comprehensive and effective evaluation of microplastic pollution, their spatial and temporal distribution, potential accumulation zones, transport dynamics and interactions with biota; to date, this kind of combined studies remain quite scarce (Qu et al., 2018; Alfaro-Núñez et al., 2021).

Microplastics abundance in the marine environment depends on several natural factors (e.g weather conditions, geomorphology of investigated areas or prevailing currents) and anthropogenic pressures that may change seasonally, especially at regional and local spatial scales (Galgani et al., 2013). Information obtained from bioindicator species would integrate spatial and temporal presence of microplastics in the environment providing also key data for the assessment of the potential impact of MPs on marine ecosystems (Fossi et al., 2018).

Fish are valuable biological indicators for the occurrence of MPs in

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the marine environment, since they exploit almost every kind of habitat, occupying many ecological niches and representing an important food source for human consumption worldwide. Specific criteria were recently suggested for selection of more appropriate fish species, including biological and ecological characteristics of species, their commercial value and documented ingestion of microplastics (Fossi et al., 2018) and a Bioindicator Index was formulated to improve biomonitoring of MPs ingestion trends in the Mediterranean area (Bray et al., 2019). Single species were already tested and proposed as sentinel organisms (Nadal et al., 2016; Bellas et al., 2016; Giani et al., 2019), but a monitoring strategy based on a multiple-species approach with different characteristics is to be preferred (Bonanno and Orlando-Bonaca, 2018; Fossi et al., 2018; Avio et al., 2020).

In this study, the presence and characteristics of microplastics (shapes, sizes and polymers) were evaluated in surface waters and fish from the Giglio Island (North Tyrrhenian Sea, Italy) in the same areas previously investigated for evaluating the possible impact of microplastic pollution caused by operations for the refloating and removal of the Costa Concordia wreck, sank in 2012 (Avio et al., 2017). The present investigation was aimed to provide evidence of a possible trend on microplastic pollution originated by a specific event, possibly pointing out variations related to time and specific characteristics of the site. This study also highlighted the importance to apply a monitoring strategy for microplastic pollution based on the integration of abiotic and biotic compartments.

2. Materials and methods

2.1. Study area

Giglio Island is located 14 km off the Italian coast, and it is the second largest of the Tuscany Archipelago National Park (North Tyrrhenian Sea, Fig. 1). This Island was interested by the Costa Concordia ship sank in front of Giglio Porto in January 2012: a complex framework of chemical, biological and oceanographic investigations were coordinated by the National Civil Protection and the Italian Institute for Environmental Protection and Research (ISPRA) to assess the environmental impact of the disaster and activities that occurred during and after the Parbuckling project, that required high impact engineering works (Regoli et al., 2014; Bacci et al., 2016; Ciampalini et al., 2016; Squadrone et al., 2018).

For the present study, samplings were carried out in June 2017 and 2019, during two campaigns promoted by Greenpeace in the Mediterranean Sea: “Less Plastic, More Mediterranean” and “MAYDAY SOS Plastic”, respectively.

Two areas were identified for the sampling of water and fish (Fig. 1): one is located in the North-East of the island, in front of Giglio Porto village (GP), the other is in the Western part of the island at Giglio Campese (GC), that was identified as a site not affected by rescue operations of the Costa Concordia wreck (Regoli et al., 2014; Avio et al., 2017).

Water samples were collected along the transects located at 42°22′42.00″N, 10°56′6.00″E (GP) and at 42°20′36.00″N, 10°52′18.00″E (GC) in 2017, and at 42°22′52.51″ N, 10°55′25.88″E (GP) and at 42°21′15.09″N 10°51′24.27″E (GC), in 2019.

Fish were sampled both in 2017 and 2019 at 42°22′04.80″ N, 10°55′16.80″ E (GP) and 42°37′06.36″ N, 10°86′81.37″ E (GC) (Fig. 1).

2.2. Water sampling and analysis of microplastics

In 2017, two different Neuston nets (200 μm mesh size with circular opening Ø 50 cm, and 330 μm rectangular opening 84 cm × 43.5 cm) were trawled in parallel on the water surface at 1–2 knots for 20 min, kept at a distance of about 50 m from the boat to avoid the turbulence induced by the wake of the ship. Neuston nets with different mesh sizes were used to evaluate possible differences in MPs retention efficiency; due to the similar results obtained in 2017, only the 330 μm mesh size Neuston net was used during the sampling in 2019. At the end of the samplings, the nets were rinsed thoroughly from the outside to ensure that both plankton and debris were washed into the end of the net and to prevent any contamination by rinsing water. To quantify the water filtered the nets were equipped with a flowmeter. Considering the low content of organic matter, samples were not digested, but directly examined under a stereomicroscope with an attached digital camera (Olympus SZX7, 8×-56×, Nikon, DSL3). All potential MPs were manually sorted out and photographed. First, the items were classified by shape (fragment, pellet, foam and film) following the criteria reported by Morgana et al., 2018; then, each isolated item was measured and characterized by different size (0.01–0.2 mm; 0.2–0.33 mm; 0.33–1 mm; 1–3 mm; 3–5 mm; >5 mm) in order to taken into account, the contribution of the different mesh size used (200 and 330 μm) and to include

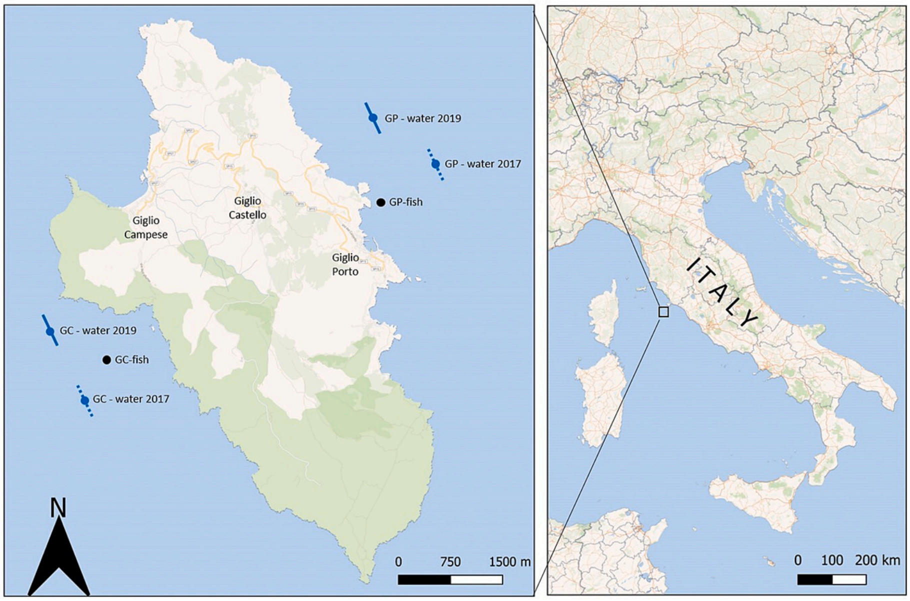

Fig. 1. Localization of the sampling stations in the Giglio Porto (GP) and Giglio Campese (GC) areas, for both fish and water samples collected in June 2017 and 2019.

the size range where fall the particles found in the biota samples (see Section 2.3). Finally, to confirm the polymeric nature of the particles, a PerkinElmer Spectrum Two Fourier Transform Infrared (FT-IR) spectrometer was used equipped with Universal ATR (UATR) accessory with a 9-bounce diamond top-plate (Wave number range: 4000 and 450 cm^{-1}; 4 cm^{-1} resolution; 32 scans). After measurement, the spectrum was compared to reference spectra through libraries supplied by Perkin Elmer, with a $>$ 70 % similarity threshold.

2.3. Fish sampling and analysis of microplastics

Fish were caught by a local fisherman through gillnets with a mesh of 50 mm, deployed overnight at a depth between 30 and 45 m: a total of 32 specimens, representative of 9 species, were obtained in 2017, while 29 organisms, representative of 8 species, in 2019. Due to the fishing method most of the collected species, regardless of site and year of sampling, were typical of the bottom environment (*i.e.*, demersal or benthic) or with a benthopelagic behavior, whereas only one species (*Sphyraena sphyraena*) was exclusive of the pelagic habitat. A detailed list of species with information on sample size, morphometric data and living habitat is given for each sampling campaign in Table 1. Organisms were dissected *in situ* for the collection of the gastrointestinal tracts and frozen at −20 °C or stored in glass jars with 5 % KOH solution at room temperature, depending on the available equipment on board. Once in the laboratory, tissues were processed for the extraction of MPs according to a slight variation of the original method of Avio et al. (2015) or with the pre-digestion with 5 % KOH solution in some cases (Bour et al., 2018). Both the methods included the separation of MPs under density gradient using a NaCl hypersaline solution (1.2 g cm^{-3}), followed by a vacuum filtration onto a cellulose nitrate membrane (Sartorius Stedim Biotech, 8 μm pore size) and the digestion of the residual organic matter in 15 % H_2O_2 solution.

These procedures, both with and without KOH, have been already tested with several species of invertebrates and fish (Avio et al., 2015, 2017, 2020; Bour et al., 2018; Bessa et al., 2019; Cau et al., 2020) and inter-calibrated in a joint exercise between several partners of the JPI Oceans Projects EPHEMARE and BASEMAN.

Filters obtained from the extraction process have been examined through a stereomicroscope (Optika SZM-D), with a maximum 45× magnification and equipped with a digital camera (OPTIKAMB5): items resembling microplastics were manually transferred onto a clean membrane located on a microscope slide (subsequently used as support for the μFT-IR analysis) and measured according to the largest size using an image analysis software (Optika Vision Lite 2.1). Particles as films, fragments, lines and pellets were identified following the criteria for shape characterization given by Avio et al. (2020). In addition, particles were categorized in five size classes (0.01–0.2 mm; 0.2–0.33 mm; 0.33–1 mm; 1–3 mm; 3–5 mm): the upper size limit was fixed to 5 mm, as recommended for the definition of MPs (Galgani et al., 2014) the lower one has been set to 10 μm, due to limitations below such value for the μFT-IR instrument in use and within this range, classes have been selected to allow a dimensional comparison with particles found in surface water samples.

The chemical identification of extracted items has been performed using a Spotlight 200i FT-IR microscope system (Perkin Elmer) equipped with Spectrum Two and driven by Spectrum 10 software. Measurements were carried out in the Mid-IR region (wavenumber range: 4000–600 cm^{-1}) in attenuated total reflectance mode (μATR-FTIR), with the resolution set at 4 cm^{-1} and the optical aperture's dimension of 100 × 100 μm. Spectra were acquired after 32 scans *per* sample and several backgrounds were performed throughout the working session. For the interpretation of spectra, commercial libraries of standard spectra (*i.e.*, PerkinElmer®, selecting Fluka, ATRPolymer, polyATR, FIBERS3, plast1, RP, POLIMERI, PIGMENTI, resin and PERKIN1 libraries) were implemented with custom-made libraries, resulting from the characterization of microplastics during previous studies or compiled within the framework of the JPI-OCEANS project BASEMAN (Primpke et al., 2018). Polymers matching with reference spectra for a Hit Quality Index (HQI) $\geq$ 0.7 were validated, while a lower level (0.60–0.70) was accepted after a careful examination of peaks characteristics (Lusher et al., 2017) and, if necessary, after some treatments applied to the spectra (*i.e.*, ATR correction, baseline correction, smoothing). Synthetic polymers (petroleum-based, biobased and hybrid polymers), copolymers and composites were considered as plastic.

2.4. Quality assurance and quality control

Special care and specific precautions were taken to prevent and check external contamination of water and fish samples as follow. The extraction and characterization procedures for MPs analysis were performed in a dedicated laboratory, where the presence of staff was limited to a maximum of two people at the same time and under a

Table 1

Species, number, habitat and length (mean ± standard deviation) of fish sampled in June 2017 and 2019 from two sites in Giglio Island (Italy) (GP and GC) and analyzed for the occurrence of microplastics in the gastrointestinal tracts.

Year	Sampling site	Species	Common name	Analyzed organisms (n°)	Living habitat	Total length (cm)
2017	Giglio Porto (GP)	*Scorpaena* spp.	Scorpionfish	6	Demersal	20.67 ± 3.78
		Uranoscopus scaber	Stargazer	2	Demersal	23.5 ± 0.71
		Serranus scriba	Pointed comber	2	Demersal	23.5 ± 0.71
		Spondyliosoma cantharus	Black seabream	1	Bentho-pelagic	22
		Phycis phycis	Forkbeard	1	Bentho-pelagic	30
		Mullus barbatus	Red mullet	1	Demersal	22
		Sphyraena sphyraena	Barracuda	1	Pelagic	64
		Muraena helena	Mediterranean moray	3	Benthic	77 ± 3.61
	Giglio Campese (GC)	*Scorpaena* spp.	Scorpionfish	10	Demersal	24.5 ± 4.28
		Uranoscopus scaber	Stargazer	2	Demersal	22.5 ± 0.71
		Spondyliosoma cantharus	Black seabream	2	Bentho-pelagic	19.5 ± 2.12
		Symphodus tinca	Peacock wrasse	1	Benthic	24
2019	Giglio Porto (GP)	*Scorpaena* spp.	Scorpionfish	5	Demersal	26.60 ± 3.59
		Uranoscopus scaber	Stargazer	2	Demersal	25 ± 2.12
		Sphyraena sphyraena	Barracuda	1	Pelagic	71
		Chelidonichthys lucerna	Tub gurnard	4	Demersal	29.30 ± 3.73
	Giglio Campese (GC)	*Scorpaena* spp.	Scorpionfish	7	Demersal	25.07 ± 7.34
		Uranoscopus scaber	Stargazer	3	Demersal	22.17 ± 0.29
		Phycis phycis	Forkbeard	2	Bentho-pelagic	32 ± 2.83
		Sphyraena sphyraena	Barracuda	1	Pelagic	60
		Merluccius merluccius	European hake	1	Bentho-pelagic	36
		Sparus aurata	Gilthead seabream	2	Demersal	24.75 ± 1.06
		Diplodus sargus	White seabream	1	Demersal	14

laminar flow hood when possible. Operators worn nitrile gloves, cotton clothes and lab coats. Work benches were cleaned with ethanol pure grade before starting the activities and between each processing steps. Glass containers (*e.g.* Petri dishes) and metal equipment were used whenever possible and, before use, they were rinsed with ultrapure water, additionally cleaned with compressed air and covered with aluminium foils, which were also kept during stirring, decantation and filtration operations performed for analysis in fish; accessories of the filtration apparatus were subjected to the same treatment. H_2O_2 and KOH solutions used to digest tissues were diluted in ultrapure water, while NaCl solution was prepared in distilled one. Solutions were further filtered on sterile acetate cellulose membranes of 0.45 mm pore size, with the exception for the 5 % KOH, which dissolves membranes.

In addition, blank samples were performed to avoid false results and an overestimation of MPs. For water samples they were carried out both during the recovery of samples from nets on boat and during their processing in laboratory, using petri dishes containing clean acetate cellulose filters wet with ultrapure water and left open during all operations. For analyses on fish, blank samples consisted of glass jars left open on the work bench throughout the collection of the gastrointestinal tracts *in situ* containing NaCl hypersaline solution or 5 % KOH; once in laboratory, they then underwent to the same steps used for biological samples.

Blank samples filters were visually examined under a stereomicroscope: analyses revealed the presence of only microfibers (MFs), excluding any further contamination by other MP shape. MFs were analyzed for color, size and chemical composition and compared with those extracted from environmental samples. Specifically, in blanks related to water samples 19.25 ± 3.59 MFs (2017 campaign) and 3.25 ± 1.26 MFs (2019 campaign) were found, whereas, in blank samples related to biota 14 ± 5 MFs (2017 campaign) and 24 ± 10 MFs (2019 campaign) were extracted. These levels were comparable or higher with the number of fibers found in field samples (10.64 ± 6.65 MFs/m^3 in 2017 and 2.10 ± 0.12 MFs/m^3 in 2019; 9.6 ± 8.02 MFs/fish in 2017 and 7.7 ± 5 MFs/fish in 2019), in addition the chemical characterization highlighted a high percentage (around 80 %) of natural polymers (cellulose-based) in both blank and field samples, according to previous studies from Avio et al., 2020 and Suaria et al., 2020. For these reasons we decided to exclude MFs from the quantification of microplastics in this study: it is plausible that an external contamination by airborne microfibers happened despite precautions, probably during operations performed in the field and in particular for fish samples since dissection was performed on docks as it was no possible to operate on board of the ship.

2.5. Statistical analysis

Statistical analyses were carried out using the Statistical R 3.2.2. software (R Core Team, 2021). Two-way analysis of variance (ANOVA test) with 0.05 level of significance was performed to test differences in MPs abundance in water samples between sites and periods of collection after the evaluation of normality and homogeneity of variance by means of Shapiro-Wilk and Levene test, respectively.

Results on fish were analyzed to highlight differences on frequency of ingestion and abundance of microplastics in relation to sampling sites and periods considering the overall data. As the microplastics data were non-parametric, a Pearson's Chi-squared test was applied to compare data on frequency of ingestion, whereas a Mann-Whitney-*U* test or a Kruskal-Wallis test were used for analyses of the number of MPs ingested per individual; differences were considered significant for $p < 0.05$.

3. Results

3.1. Analysis of MPs in water

Table 2 shows results of MPs occurrence in surface water in the two years and sites using different mesh size nets. In 2017, MPs abundance from 200 μm mesh size Neuston net was 0.048 items/m^3 in the GP site and 0.175 items/m^3 in GC; similar MPs values were obtained with the 330 μm Neuston net: 0.053 items/m^3 in GP site and 0.194 items/m^3 in GC. The same trend was observed in 2019, with 0.048 items/m^3 at GP site and 0.195 items/m^3 observed at GC. Overall, data collected at site GC showed MPs abundance significantly higher than in GP ($p < 0.05$) with both mesh sizes used.

Table 2
Data on abundance of microplastics in water samples collected in June 2017 and 2019 from two sites in Giglio Island (Italy) (GP and GC).

Year	Sampling station	Manta net mesh size (μm)	Abundance (items/m^3)
2017	Giglio Porto (GP)	200	0.048
	Giglio Campese (GC)	200	0.175
	Giglio Porto (GP)	330	0.053
	Giglio Campese (GC)	330	0.194
2019	Giglio Porto (GP)	330	0.048
	Giglio Campese (GC)	330	0.195

A total of 213 MPs were characterized in water samples and Fig. 2 shows the relative % contribution of each shape, size class and polymer typology at GP and GC areas. In 2017 most of the MPs were fragments (Fig. 2A), both in GP and GC, independently from the mesh size used for sampling (GP-200: 83 %, GP-330: 79 % and GC-200: 93 %, GC-330: 96 %). Films were the second most abundant particles in GP, both in samples collected with 200 and 330 μm mesh size net (17 % and 13 % respectively, *vs* 0 and 4 % in GC site). Also in 2019, fragments were the most abundant items in water samples (92 % both in GC and GP), the remaining being films in GP (8 %), and pellets in GC (8 %). Concerning the size classes (Fig. 2B), in 2017 most of the extracted MPs were in the range of 1–3 mm, regardless of the mesh size, with a percentage from 42 % in GP-200 to 62 % in GP-330. Comparing the different mesh sizes used in 2017, MPs < 1 mm were more abundant in the samples collected by using the 200 μm net than in those collected with 330 μm net; a greater prevalence of items in the size 0.33–1 mm was observed for both sampling sites, accounting for 32 % in GP and 29 % in GC with the 200 μm Neuston net, and 15 % in GP and 12 % in GC with the 330 μm. In GP-330 the second highest number of items was found in the 3–5 mm size class, with 28 % prevalence. No differences according to mesh size net were observed for particles smaller than 0.33 mm, occurring with a percentage of 2 % only in GP with both 200 and 330 μm Neuston net. Also in 2019, the majority of particles fell in the range of 1–3 mm followed by 0.33–1 mm in both sites, which contributed for 53 % and 32 % in GP 330 and for 44 % and 37 % in GC 330, respectively. The 0.2–0.33 mm size class accounted for only 4 % of the total items isolated in GP 330 and the 3–5 mm size class was represented by 10 % and 6 % in GP 330 and GC 330, respectively. Regarding the abundance of the smaller particles ranging between 0.01 and 0.2 mm, no items were found in 2017 in both 200 and 330 μm Neuston net and in 2019, for GP and GC sites.

FT-IR analysis revealed the presence of 7 polymers in water samples collected in 2017 and 5 in 2019 (Fig. 2C). In 2017 samples from both sampling devices and sites showed the predominance of polyethylene (PE) with a frequency of 86 and 69 % in GP at 200 and 330 μm, and 73 % and 66 % in GC. Polypropylene (PP) was the second polymer in terms of frequency (14 % in GP and 15 % in GC for 200 μm and 27 % in GC 330 μm) except in GP 330 where both PE and the ethylene-vinyl-acetate copolymer (EVA) items were found at 9 %. EVA was found also in GC, always for 330 μm (4.5 %) while polystyrene (PS) was found in GC 200 and GP 330 μm with a percentage of 8 % and 3 % respectively. In GP 330 polyolefin (PO) and polyamide (PA) accounted for 6 % and 3 % respectively. Ethylene-propylene diene monomer (EPDM) was represented by only 1 % in GC 200.

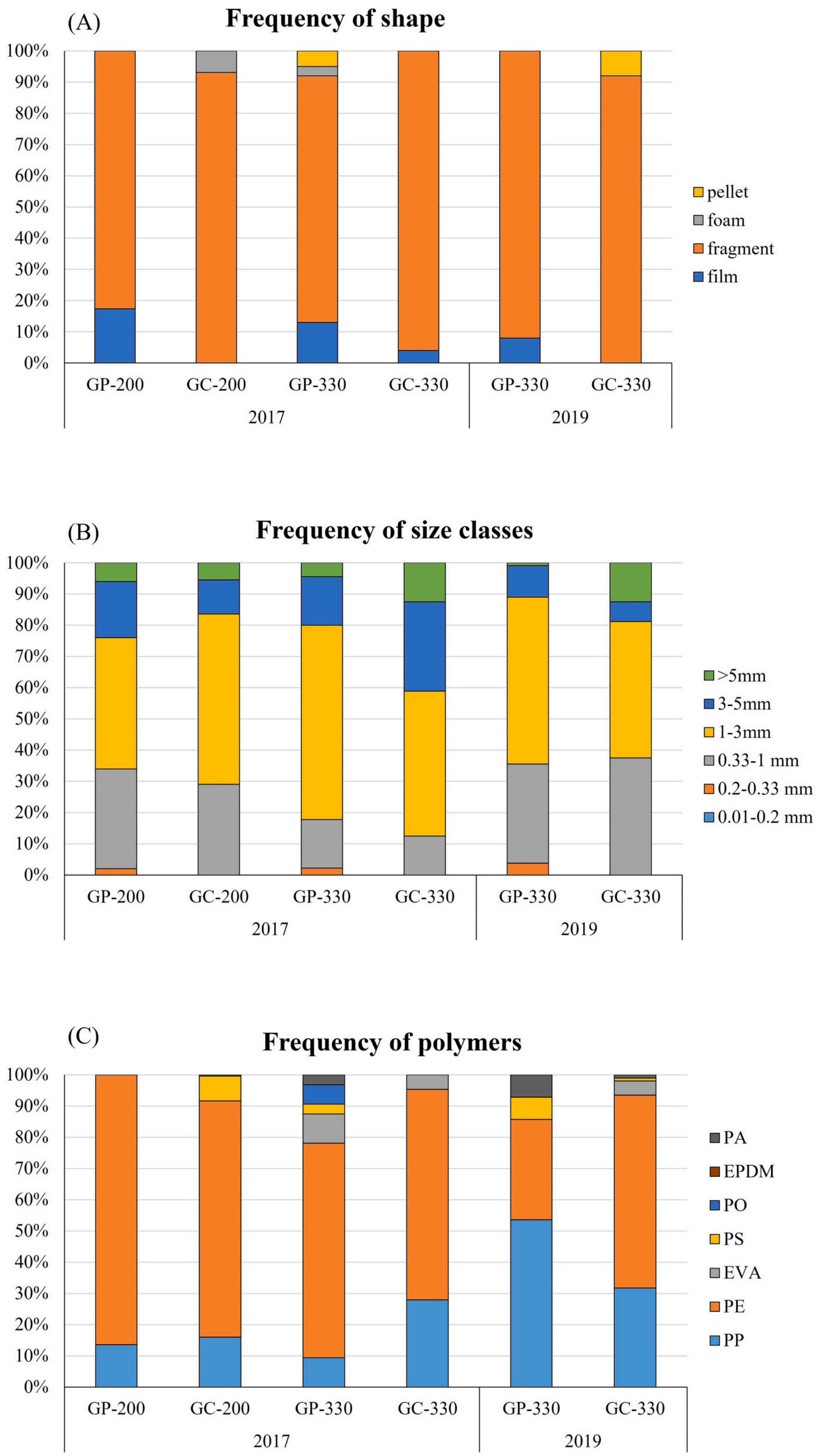

Fig. 2. Relative contribution (%) of each shape (A), size classes (B), and polymers (C) on total MPs extracted from waters collected in Giglio Island from GP and GC sites in June 2017 and 2019.

In 2019, PE remained the most abundant polymer in GC 330 (62 %) followed by PP (32 %); in the GP 330 site the distribution was the opposite with higher frequency of PP (54 %) and PE representing 32 % of the total. In this sampling campaign a 7 % and 1 % of both PS and PA was found in the GP and GC site respectively.

3.2. Analysis of MPs in fish

Table 3 summarizes results on MP ingestion in fish. In 2017, 15 out of 32 analyzed organisms (47 %) had MPs in the gastrointestinal tract and a total of 22 particles were extracted. Among the specimens positive to ingestion, most fish (73 %) contained 1 particle, 2 particles were extracted from the 13 % of analyzed specimens, while the maximum number of 3 and 4 items were ingested by 2 different individuals of *Scorpaena* spp. in GC site. Comparing sampling areas, no statistically significant differences were obtained neither for frequency of ingestion (Pearson's Chi-squared test, $p = 0.2971$) nor for number of particles extracted per individual (Mann-Whitney test, $p = 0.4488$), probably due to the inherent variability and heterogeneity of these samples. Despite this, GC site exhibited a generally higher percentage of organisms positive to MPs ingestion (9 out of 15 analyzed, 60 %) compared to GP site (6 out of 17 analyzed, 35 %). Besides a higher frequency of ingestion, also a major number of MPs was extracted from fish at GC site (a total of 15 particles from 15 analyzed organisms) in respect to those from GP (a total of 7 particles from 17 analyzed organisms), with on average 1.67 ± 1.1 MPs and 1.17 ± 0.4 MPs in specimens positive to ingestion, respectively.

In 2019, a higher frequency of MPs ingestion was observed in comparison to 2017 (65.5 %, 19 out of 29 analyzed organisms) and, as observed in the previous campaign, the GC site highlighted a higher frequency of ingestion (75.5 %) and a greater number of extracted MPs ($n = 29$ with a mean value of 2.23 ± 2.17 MPs in positive individuals) compared to GP site (ingestion in 50 % of organisms, 8 extracted MPs and a mean value of 1.33 ± 0.52 MPs per positive organism). Similarly to 2017, the highest number of MPs extracted in 2019 from a single organism was found in a specimen of *Scorpaena* spp. collected in GC with 9 items in the gastrointestinal tract; in general, 53 % of organisms ingested 1 particle, 32 % 2 items and the 11 % 3 MPs.

A total of 59 MPs were characterized by shape, size and polymeric nature (Fig. 3). In 2017 shape was largely dominated by fragments both at GP (71 %) and GC sites (67 %); films and lines were equally represented in GP (14 %), while lines being 27 % and films 7 % in GC site. No pellets were found in fish in 2017. In 2019, fish from GP area contained films (37.5 %), fragments and lines (25 % each) and pellets (12.5 %); in the GC area, only films and fragments were found in the gastrointestinal tracts, with similar percentage (52 % and 48 %, respectively) (Fig. 3A). The identified size classes revealed that MPs extracted from GP site fish in 2017 and 2019 and from GC in 2019 were mostly smaller than 0.33 mm, with the higher contribution of the lower size class (0.01–0.2 mm, 57 %, 88 % and 55 %, respectively). On the contrary, 73 % of the items found in organisms collected in GC site in 2017 were between 0.33 and 1 mm (53 %) (Fig. 3B). Interestingly, both in 2017 and 2019 the smaller particle and the largest one was found in a same specimen of *Scorpaena* spp. sampled in GC site (a fragment of 0.04 mm and a line of 3.34 mm in 2017, a fragment of 0.036 mm and a film of 4.5 mm in 2019).

In 2017, overall, 6 different polymers were identified through μFT-IR analysis of MPs from fish. Polyester (PEST), EVA and PE were found in organisms of both sampling areas. PE was particularly present (54 %) in organisms at GP, from which also PA was extracted, contributing to the total as PEST and EVA (14 %). Polyvinyl alcohol (PVAL) and PP were identified in fish of the GC site, representing 27 % and 13 % of the total particles.

In 2019, besides PE, PA, PEST, EVA and PP, other 8 polymers were identified: acrylic rubber, thermoplastic copolyester elastomer (TPC), polyvinyl chloride (PVC), polyurethane (PUR), epoxy and alkyd resins, ethylene-propylene-diene-rubber (EPDM), copolymers of styrene-isoprene-styrene (SIS) and of styrene-acrylonitrile (SAN). Acrylic rubber was found in organisms of the GP site (12.5 % of extracted MPs), where the most ingested polymer was PP (37.5 %). The other polymers were extracted from specimens collected at the GC site, showing the highest heterogeneity in polymeric composition, with the main contribution by PE (28 %) followed by PA (17 %), resins and EPDM (14 %

Table 3
Data of microplastic ingestions in fish collected in June 2017 and 2019 from two sites in Giglio Island (Italy) (GP and GC): number of total MPs extracted from the total analyzed organisms, frequency of ingestion (*i.e.* the percentage of specimens with at least 1 particle in the gastrointestinal tract) and average number of MPs calculated on organisms positive to ingestion (mean ± standard deviation).

Year	Sampling site	Species	Total individuals	Total MPs	Frequency of ingestion	MPs/individual (mean ± s.d.)
2017	Giglio Porto (GP)	*Scorpaena* spp.	6	1	17 %	1 ± 0
		Uranoscopus scaber	2	1	50 %	1 ± 0
		Serranus scriba	2	1	50 %	1 ± 0
		Spondyliosoma cantharus	1	1	100 %	1 ± 0
		Phycis phycis	1	0	0	0
		Mullus barbatus	1	0	0	0
		Sphyraena sphyraena	1	0	0	0
		Muraena helena	3	3	67 %	1.5 ± 0.7
	Overall data (GP)		**17**	**7**	**35 %**	**1.17 ± 0.4**
	Giglio Campese (GC)	*Scorpaena* spp.	10	12	60 %	2 ± 1.3
		Uranoscopus scaber	2	2	100 %	1 ± 0
		Spondyliosoma cantharus	2	0	0	0
		Symphodus tinca	1	1	100 %	1 ± 0
	Overall data (GC)		**15**	**15**	**60 %**	**1.67 ± 1.1**
2019	Giglio Porto (GP)	*Scorpaena* spp.	5	2	40 %	1 ± 0
		Uranoscopus scaber	2	1	50 %	1 ± 0
		Sphyraena sphyraena	1	0	0	0
		Chelidonichthys lucerna	4	5	75 %	1.67 ± 0.96
	Overall data (GP)		**12**	**8**	**50 %**	**1.33 ± 0.52**
	Giglio Campese (GC)	*Scorpaena* spp.	7	17	86 %	3.2 ± 3.35
		Uranoscopus scaber	3	1	33 %	1 ± 0
		Phycis phycis	2	3	100 %	1.5 ± 0.71
		Sphyraena sphyraena	1	2	100 %	2 ± 0
		Merluccius merluccius	1	3	100 %	3 ± 0
		Sparus aurata	2	3	100 %	1.5 ± 0.71
		Diplodus sargus	1	0	0	0
	Overall data (GC)		**17**	**29**	**75.5 %**	**2.23 ± 2.17**

Fig. 3. Relative contribution (%) of each shape (A), size classes (B), and polymers (C) on total MPs extracted from fish sampled in Giglio Island from GP and GC sites in June 2017 and 2019.

each) (Fig. 3C).

4. Discussions

In the last years, many investigations were carried out in the Tyrrhenian Sea to assess microplastics pollution, highlighting their ubiquitous presence in waters (Suaria et al., 2016; Baini et al., 2018; Caldwell et al., 2019; Savoca et al., 2019) sediments (Fastelli et al., 2016; Guerranti et al., 2017; Cannas et al., 2017; Mistri et al., 2020; Piazzolla et al., 2020; Missawi et al., 2020) and biota (Avio et al., 2017; Savoca et al., 2019, 2020; Valente et al., 2019; Bottari et al., 2019; Giani et al., 2019; Mancuso et al., 2019; Cau et al., 2019; Capillo et al., 2020; Sbrana et al., 2020; Vecchi et al., 2021; Missawi et al., 2020; Ben Ismail et al., 2022). Among the studies covering the North Tyrrhenian Sea and the Tuscany Archipelago, most focused on its northern part, while the southern one, including Giglio Island, has been less investigated. Giglio Island is of particular interest for MPs pollution since it was firstly investigated in 2014 after the end of the operations for the refloating and removal of the Costa Concordia wreck (Avio et al., 2017). That study was the first to demonstrate a significant impact of a specific anthropic event on MPs ingestion in resident fish, but also highlighted an elevated frequency of this phenomenon in other areas of the island not directly affected by wreck removal operations. In this regard, the present study provided a characterization on MPs presence and composition in the two sites already investigated along the coast of the Giglio Island, thus performing a time- and site- dependent evaluation of microplastics pollution in this Mediterranean area, focusing not only on fish but also analyzing MPs presence in water.

In fact, despite seawater was not analyzed during 2014 investigation, data on number and density of microplastics in water samples obtained in 2017 and 2019 were comparable to those observed from previous investigations in the Tyrrhenian Sea (Collignon et al., 2012, 2014; De Lucia et al., 2014; Fossi et al., 2016), confirming the occurrence of this contaminant in this area of the Mediterranean Sea (Cincinelli et al., 2019; Suaria et al., 2016).

Microplastics abundance in surface waters resulted to be higher, in the North-West side of the island (GC), than in the area where Costa Concordia sank, and removal operations had occurred (GP), with a similar trend both in 2017 and 2019.

The use of different mesh size nets for sampling MPs in surface waters does not seem to affect the abundance of microplastic in both sites. This finding is in agreement with what reported by She et al. (2022) where a rigorous analysis of the differences in data measured with multiple mesh sizes was carried out leading to the conclusion that the differences between datasets with different meshes are not statistically significant. A similar conclusion was achieved also by Tsiaras et al. (2022) that indicate that the different configurations on the mesh size used might not significantly affect the reported final MPs abundance. In contrast, Lindeque et al. (2020) recently reported the ability of 100 μm mesh size to collect a higher number of microparticles compared to 500 or 330 μm; this result might be related to the high percentage of fibers identified by the authors (approximately 80 %), that have been excluded from the counts in this paper. Suaria et al. (2020) pointed out the possible overestimation of microplastic fibers in natural environments, due to the inclusion of man-made cellulosic fibers, known as “semi-synthetic” (*e.g.*, viscose/rayon, lyocell and acetate) in the category of plastics and the lack of a comprehensive chemical characterization of these items; according to Suaria et al. (2020), synthetic polymers account for only the 8.2 % of fibers floating in surface water of open ocean. Results obtained in this study suggested that different mesh size nets can influence the typology of sampled microplastics, in particular for their size (Fig. 2B). Pooling data from the two sites (GP and GC), MPs collected with the 200 μm mesh size contained particles <1 mm for 31.5 % of the total, compared to 15.1 % obtained with the 330 μm manta trawl. This finding indicates that the use of thinner mesh size nets may give more ecologically relevant results, considering that size and shape of microparticles strongly influence the likelihood of ingestion, and that MPs of lower sizes are those mainly ingested by marine organism (Wright et al., 2013; Lehtiniemi et al., 2018; Avio et al., 2020) as pointed out also by our investigation.

Results on the number of microplastics found in fish confirmed that marine organisms generally ingest 1 or 2 items, as also observed by previous studies from the Tyrrhenian Sea, other areas of the Mediterranean or of the world regardless of whether they are vertebrates or invertebrates (Valente et al., 2019; Avio et al., 2020; Bour et al., 2018). In this respect the frequency of ingestion (%), more than the number of MPs per individual, has been suggested as a better indicator of microplastics pollution and bioavailability among habitats or geographical areas since it reflects the likelihood of an organism to encounter microplastics (Avio et al., 2020). This observation is supported by results of the present study, particularly those on *Scorpaena* spp. sampled in both 2017 and 2019 and from both the investigated areas. The high variability observed in the number of MPs ingested per specimen of scorpionfish (represented by the high standard deviations, Table 3), would not allow to highlight differences between sampling sites and periods; on the contrary, using the frequency of ingestion it was possible to identify GC as the area where organisms have been more exposed to MPs occurrence compared to GP site, especially in 2019. The percentage of fish positive to MPs ingestion in GC site in 2019 was 75.5 %, which was consistent with the 77 % obtained on similar species sampled in the same area in 2014 (Avio et al., 2017).

These percentages are rather high compared to those obtained in other studies carried out in the Tyrrhenian Sea: an ingestion frequency of 36 % was measured in red mullet and European hake from harbor of Leghorn (North Tyrrhenian Sea) (Giani et al., 2019), 9.1 % in *Pagellus* spp. sampled in the southernmost part of the Tyrrhenian Sea (Savoca et al., 2019), 56 % in specimens of *Boops boops* along Liguria, Latium and Sardinia coasts (Sbrana et al., 2020) and 68.8 % for deep-water elasmobranch species collected close to Anzio coast where, however, 80 % of microplastics counted in these organisms were fibers (Valente et al., 2019). The frequency of fish ingesting microplastics at GC site was also higher than that observed in the Adriatic Sea (28 %, Avio et al., 2015, 2020), the English Channel (36.5 %), the North Pacific Central Gyre, the Brazilian estuarine environments and various coastal areas of Indonesia and California (30 %) (Avio et al., 2017).

The different degree of MPs contamination between GC and GP sites, highlighted by results from both surface waters and biota, could be explained considering input and intensity of sources: Giglio Island is a sparsely populated area consisting of around 1440 resident inhabitants, where the Giglio Campese town is exposed, in the summer months, to an intense touristic activity with about 150,000 visitors (Chiocchini et al., 2017), while Giglio Porto is subjected to the typical anthropogenic pressure of a small touristic harbor. This may also justify the ingestion of more heterogeneous polymers and the presence of less common plastics in fish of GC site (like PVAL, TPC, EPDM, resins), reflecting the impact of a greater touristic pressure with large usage of synthetic clothes, swimming gears, shopper bags and packaging (Avio et al., 2017). The site-related diversity in polymer composition was less evident in surface water samples where 90 ± 7.6 % of the polymers were represented by PE and PP; these polymers are the most abundantly manufactured in single-use products, and due to their low density may remain longer in the surface layer of seawater (Erni-Cassola et al., 2019). PE and PP were also frequently ingested by fish, confirming their transport through water column and availability for benthic/demersal fish (Coyle et al., 2020). This, along with evidence that almost the 60 % of benthic/demersal specimens of Giglio Island (considering the overall data) contained at least 1 microplastic in the digestive tract, support the consensus that plastic particles, regardless of their initial density, are mostly exported from surface waters toward seabed (Suaria et al., 2020) under the influence of different factors (Coyle et al., 2020). However, the contribution of these factors to the distribution and fate of microplastics in the marine environment remains uncertain, in particular, the extent to which oligotrophic marine systems support biofouling and sinking, the role of biological plastic pump, the effect of de-fouling and particle disaggregation, which eventually make plastic debris to resurface returning to the water column (Erni-Cassola et al., 2019; Wang et al., 2021) and available for pelagic and benthopelagic species.

The hydrographic characteristics of Giglio Island may further influence the introduction and accumulation of plastic in GC site. The general circulation of the Tyrrhenian Sea is characterized by surface and intermediate layers representing a well-defined flux of Atlantic Water (AW; the Tyrrhenian vein): when the Tyrrhenian vein of the AW reaches the channel between the island and the Italian coast, currents are forced in an NW-SE direction toward GC (Cutroneo et al., 2017). This area can be also affected by input of local rivers, flowing out in the Northern and Southern part of Giglio Campese beach. On the other hand, flows of local rivers are closely linked to the rainy period and frequent inversions of current direction are known to occur in summer and spring at Giglio Island (Cutroneo et al., 2017) causing local variations in the potential inputs and drivers of MPs, that could explain the 60 % positive fish to MPs ingestion in GC site in 2017, a percentage still high compared to the above mentioned studies on other areas, but lower compared to that found in GC in 2014 and in 2019. Results on fish confirmed the link between microplastics occurrence in organisms and the contribution of specific anthropogenic activities (Avio et al., 2017). In fact, a significant reduction both in the frequency of ingestion and numbers of MPs in biota of GP area was observed in 2017 (35 % and 1.17 ± 0.4 MPs/fish) and in 2019 (50 % and 1.33 ± 0.52) compared to the 94.7 % organisms positive to ingestion with an average of 4 ± 1.83 particles recorded in 2014 (Avio et al., 2017). The high levels of MPs observed in fish in 2014 have reasonably been associated to the physical impact on the bottom of the operation area with the installation of 6 platforms, 11 anchor blocks, 25,000 tons of cement grout bags, the presence of approximately 4000 tons of debris, along with the frequent movement of sediments during activities, that favored the resuspension of MPs and the consequent exposure for demersal species (Avio et al., 2017). The significant reduction of MPs ingestion observed in 2017 and 2019 is possibly connected with the cleaning operations of the seabed, started immediately after the wreck removal.

5. Conclusion

This study highlighted the importance of integrating information on microplastic presence in different matrices for an effective investigation of microplastic pollution in marine environments. Analysis of surface waters provides a snapshot of microplastic pollution revealing the potential contribution of both hydrographic conditions and local inputs. On the other hand, the presence of MPs in biota reflects their bioavailability, with more ecologically relevant implication: beside confirming the elevated ingestion of MPs in fish from the Giglio Island due to local characteristics, this study also confirmed the possibility to use these organisms as bioindicators to monitor the impact and the recovery of specific microplastic pollution events.

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CRediT authorship contribution statement

LP, **FG**, **SG**, **GU** and **FR**, conceptualization and design of the study; **LP**, **EC**, **RM**, **AN**, **LV**, **SM**, analyses and methodologies; data collection; **LP**, **FG**, **EC**, **SG** data curation, statistical analyses and data elaboration; **LP**, **FG**, **EC**, **SG**, **FR** writing original draft, review and editing; **MC** analysis supervision; **FG**, **SG** and **FR**, funding acquisition and project administration. All authors contributed to the article and approved the submitted version.

Declaration of competing interest

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

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Data availability

Data will be made available on request.

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