

Article

Microplastics' Occurrence in Edible Fish Species (*Mullus barbatus* and *M. surmuletus*) from an Italian Marine Protected Area

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Abstract: This study reports on the presence of microplastics in the gastrointestinal tracts and livers of demersal fish (the mullet, *Mullus* spp.) from a Marine Protected Area (Porto Cesareo) along the Ionian Sea coast (Apulia, Southern Italy). The results showed microplastic ingestion in more than 60% of specimens analyzed with an average of three items per fish and average levels in red mullets being almost twice as high as the average in the congeneric striped red mullets. The dominant polymers identified by Attenuated Total Reflectance Fourier Transform Infrared spectroscopy (ATR-FTIR) and Prolysis Gas Chromatography/Mass Spectrometry (Py-GCMS) analysis were polyethylene and polystyrene. Results can be used to set baseline levels for the assessment of microplastic pollution useful for the implementation of the Marine Strategy Framework Directive (MSFD) descriptor 10 in the Italian coast of Ionian Sea.

Keywords: microplastic; fish; Ionian Sea; Marine Protected Areas; MSFD; Py-GC/MS analyses



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1. Introduction

The Mediterranean basin, with about one-third of endemic fauna, is recognized as one of the world's most biologically rich, yet endangered, areas. Habitat loss and degradation, overfishing, biological invasions, and chemical pollution represent some of the threats that are affecting Mediterranean biodiversity. A great concern has been recently expressed for new types of persistent pollutants that are seriously affecting the marine environment, namely, marine plastic litter. Special interest has been paid to the so-called microplastics (MPs), the smaller fraction (<5 mm) of plastic litter, which represents over 92% of all plastic items found at sea [1].

Marine Protected Areas (MPAs) have been shown to be useful in facing many anthropogenic impacts. However, there is nothing they can do to limit the impacts posed by threats acting at a scale wider than that of the reserve boundaries, namely, climate change and ocean acidification, invasion of alien species, or spreading of pathogens and chemical pollution [2]. If the local-scale actions put in place through the implementation of an MPA can be determined to prevent the new entry of debris in a marine environment, nothing can be done to avoid the spread of MPs from other areas (e.g., [3–5]). Because the fluid nature of seas and estuaries allows for the transport of contaminants across long distances, MPAs at varying distances from pollution sources may be under threat [6]. Plastic abundances on beaches, sea surfaces, and sea beds are even drastically increased in the last 5 years in the

remote Atlantic Marine Protected Areas, reaching levels found at industrialized areas and affecting their associated food webs, from primary consumers to top predators [7].

Waste and, in particular, plastics can affect marine biota in several manners, causing a general debilitation of the organisms, even to death.

Interaction with plastic litter can result in entanglement and smothering. Plastic pieces can be ingested and, according to the litter size and species, they may be egested or accumulate in the gastrointestinal tract, resulting in physical and mechanical damage and harm, such as lacerations and ulcerating wounds in the digestive tract, blockage of the gastrointestinal tract, and reduction in food intake related to pseudo-satiation [8–13].

Furthermore, toxicity, carcinogenesis, endocrine disruption, oxidative stress, dysfunctions in immune defenses and neuro-transmission, and genotoxicity can be triggered by the release of plastic additives (i.e., phthalates and bisphenol A) or other environmental pollutants and microbes absorbed on their hydrophobic surface [9,12,14–22].

Marine litter has been listed by the European Union Marine Strategy Framework Directive (MSFD) as a specific descriptor (D10) to take into account to achieve the Good Environmental Status (GES).

More specifically, criteria D10C3 and D10C4 (Commission Decision 2017/848) require establishing threshold values both for the amount of litter and micro-litter ingested by marine animals and for the adverse effects on organisms, respectively.

As for the other types of chemical pollutants, the assessment of floating and submerged marine litter depends greatly on oceanographic characteristics, such as currents, winds, and basin conformation, and alone give little information about the potential effects on biodiversity and ecosystem functioning [23]. The use of bio-indicators allows integrating a long-term interaction of several environmental conditions and threats, including marine litter. Bioindicators could, in fact, provide information about the presence of micro-litter in the environment by quantifying the presence and abundance within organisms and the potential to cause harm to biodiversity by measuring any related biological effects [17].

Several studies have tested the suitability of the red mullet (*Mullus barbatus*) and the striped red mullet (*Mullus surmuletus*) as a small-scale bioindicator of the presence of microplastic in the Mediterranean benthic habitats. Indeed, these species represent an important target for local fisheries, have a narrow home range, feeding mainly on benthic species, and they are subjected to the ingestion of plastic pieces [20,24–30], fulfilling then the requirements suggested by Fossi et al. (2018) to properly select sentinel species (background, habitat, trophic and behavior information, spatial distribution, commercial importance, sensitivity to litter ingestion).

Among the spectroscopic techniques generally used to detect MPs in tissues, there are μ -Raman [16,31], μ -FTIR [20,24,26], and more recently dark-field hyperspectral microscopy [32,33]. Py-GCMS is an emerging analytical technique for smaller MPs and offer the opportunity to quantify their occurrence [34–36]. The samples were taken from a Marine Protected Area and were not fed with selected MPs; so, it was not possible to know beforehand the polymer type MPs were made of. As a result, pyrolysis GCMS, which is a very sensitive mass-based technique, and FTIR, which offers the best compromise in terms of time, cost, and simplicity for MPs' identification in the microns' range, with both working for all kinds of polymers, were used.

The aim of this study was to assess the occurrence and composition of microplastics ingested by marine animals by using sentinel species, namely, the two congeneric fish species of *Mullus*, in an MPA of the FAO macro-region (i.e., GSA-19) where these aspects have never been investigated.

2. Materials and Methods

2.1. Study Area and Sampling

Samples were collected within the framework of the SAMPEI project from the MPA of Porto Cesareo, one of the largest Italian marine reserves (16,654 ha) located in the SE Ionian Sea macro-region.

A total of 50 individuals of Mullidae (*M. surmuletus* and *M. barbatus*) were collected by gillnets (trammels) at about a 20–30-m depth in the winter of 2016.

Fish were transported to the lab where total length and total mass were recorded for each specimen. Stomach, intestines, and liver were dissected out, starting from the upper part of the esophagus, and weighed. Gastrointestinal (GI) contents and the liver of each individual were separately placed inside coated Petri dishes, frozen and kept at $-25\text{ }^{\circ}\text{C}$ until the next processing steps.

All the experimental procedures were in accordance with the requirements of the Ethical Committee of the University of Salento for scientific activities with marine organisms.

2.2. Microplastic Analyses

The protocol of Avio and co-workers (2015) was adopted to extract microplastic from tissues of sample fish. Specifically, the GI contents of each individual were thawed and dried in oven at $50\text{ }^{\circ}\text{C}$ overnight.

The dried samples were mashed with a pottery mortar and pestle in order to obtain a fine powder of MPs, organic matter, and other contents (e.g., sands). The powder was put in a beaker with 100 mL of hypersaline solution of NaCl (1.2 g/cm^3), stirred, and decanted for 1 h. Then, 50 mL of supernatant was collected with a micropipette and directly filtered under vacuum on quartz fiber filters (Whatman[®] QM-A quartz filters with pore size of $2.2\text{ }\mu\text{m}$, Merck KGaA, Darmstadt, Germany).

Filtered materials were digested with a few mL of hydrogen peroxide (15%) and dried in an oven at $50\text{ }^{\circ}\text{C}$ overnight before the microscopic observation.

Filters were observed under a stereoscopic microscope, Nikon SMZ 1270 (NIKON CORPORATION, Konan, Minato-ku, Tokyo, Japan) with an image analysis system (DS-Fi2 camera, NIKON CORPORATION, Konan, Minato-ku, Tokyo and NIS Elements Software), in order to characterize plastic items according to both size class ($5000\text{--}1000\text{ }\mu\text{m}$; $1000\text{--}500\text{ }\mu\text{m}$; $500\text{--}100\text{ }\mu\text{m}$; $100\text{--}50\text{ }\mu\text{m}$; $50\text{--}10\text{ }\mu\text{m}$; $<10\text{ }\mu\text{m}$ measured at their largest cross section) and shape (line, fragment, fiber, pellet).

A subset of four livers of *M. surmuletus* was randomly chosen for the detection of microplastics in order to assess if ingested microplastics passing through the gut can reach the liver.

Microplastics' extraction from the liver followed the procedure above described for the gastrointestinal contents.

In order to confirm the occurrence of microplastics in collected individuals of Mullidae, 26 samples (i.e., 52%) were randomly selected from the full sample size for ATR-FTIR spectroscopy and pyrolysis GC-MS analysis.

2.3. Quality Assurance and Control

In order to reduce air contamination by plastic threads, especially from fibers, and ensure the quality of the data, rigorous laboratory procedures were carried out. Only metal, pottery, and glass instruments, previously rinsed with pre-filtered ($0.45\text{ }\mu\text{m}$), bi-distilled water, were used, and cotton laboratory coats were worn. All technical procedures (dissection, extraction, and filtration) were carried out under a vertical laminar flow hood and filters were stored in covered Petri dish before being transported out of the fume hood for the counting of microplastics under the stereomicroscope.

Procedural blank samples (samples without tissues processed in parallel with the tissues samples) were used whenever a new hypersaline solution was prepared. Control filters were treated in the same way as the rest of the samples and checked for contamination under the microscope and, in case of airborne contamination, the same typology of microplastics, according to shape, color, and size was removed from the results.

2.4. Polymer Identification

ATR-FTIR single measurements were performed with an Agilent 680 spectrometer and accessories, as already described [37]. Spectra were collected from 4000 cm^{-1} to 600 cm^{-1} .

Resolution was set at 4 cm^{-1} . Generally, 64 scans were co-added for every spectrum, and for zero-filling factor 4, Blackmann–Harris three-term apodization was used. The background was measured with the same settings against air or against the investigated substrate. The ATR diamond/ZnSe crystal was cleaned with ethanol or 2-propanol and a background scan was performed between each sample. Each sample was compressed against the diamond until a good contact between the sample and ATR crystal was reached. Absorption bands identified were recorded and compared to absorption bands of each polymer reported in the literature and obtained from our in-house spectral library. A minimum of four matching absorption bands were required for accepted identification.

Py-(THM)-GC/MS analyses were performed by a Curie-point pyrolyzer (CPP) (Pilodist, Bonn, Germany) coupled with a 6890N (Agilent, Santa Clara, CA, USA) chromatograph in combination with a 5973 inert (Agilent) mass spectrometer. The inlet conditions were set at $280\text{ }^{\circ}\text{C}$ with the split vent closed for maximum sensitivity. The transfer line connecting the GC to the mass analyzer was heated at $280\text{ }^{\circ}\text{C}$ to minimize peak broadening. In the mass analyzer, the source (electron ionization) operated at a standardized 70 eV for reproducible spectra and source was kept hot at $250\text{ }^{\circ}\text{C}$ to limit source contamination. GC conditions were HP-INNOWAX capillary column (internal diameter (i.d.) = 0.25 mm , $0.25\text{ }\mu\text{m}$ film thickness, length 30 m) connected to a deactivated fused silica guard column ($l = 2\text{ m}$, i.d. = 0.25 mm), and He carrier gas flow was 1.2 mL min^{-1} . A few micrograms of the sample, without any further treatment, were inserted into a ferromagnetic tube and placed directly in the pyrolysis chamber. Pyrolysis conditions were pyrolysis temperature $590\text{ }^{\circ}\text{C}$, pyrolysis time 9.9 s , and pyrolysis chamber temperature $200\text{ }^{\circ}\text{C}$. The chromatographic conditions for the separation of pyrolyzed compounds were $40\text{ }^{\circ}\text{C}$ (2 min), $2\text{ }^{\circ}\text{C min}^{-1}$ until $50\text{ }^{\circ}\text{C}$, and $5\text{ }^{\circ}\text{C min}^{-1}$ until $260\text{ }^{\circ}\text{C}$ (15 min). MS conditions were linear quadrupole, EI ionization 70 eV, cycle time 2.94 scans per second, and mass range $30\text{--}550\text{ m/z}$. Collected data were processed and analyzed by MS Chemstation (Agilent).

3. Results

A total of 30 individuals of *M. surmuletus* and 20 specimens of *M. barbatus* were analyzed; all the specimens were adult with size $> 12\text{ cm}$ (Table 1).

Table 1. Biological parameters estimated in all individuals of *M. surmuletus* and *M. barbatus*. Data are displayed as mean \pm standard error (SE) for total length (TL) and fresh weight (FW).

Species	TL (cm)			FW (g)		
	Min	Max	Mean (SE)	Min	Max	Mean (SE)
<i>M. surmuletus</i>	14.5	20.5	18 ± 0.5	31	135	79 ± 0.6
<i>M. barbatus</i>	15	18	16.7 ± 0.3	56	82	70 ± 3

The percentage of individuals with microplastic in the gastrointestinal contents was very high for both species, reaching 57% and 75% for *M. surmuletus* and *M. barbatus*, respectively.

Average levels of MP items observed in red mullets (4.10 ± 1.04) were almost twice as high as in the congeneric striped red mullets (2.27 ± 0.62), with a total of 82 and 68 MPs counted, respectively.

The dimensional distribution analysis showed the dominance of particles between 10 and $100\text{ }\mu\text{m}$ across in the stomach contents of both species (Figure 1a). A higher variability in the microplastic dimensional pattern was found in *M. barbatus* than that found in *M. surmuletus*.

Mesoplastics (5–25 mm) were found only in one specimen of *M. barbatus*, accounting for only 4% of the total of plastic items (Figure 1a).

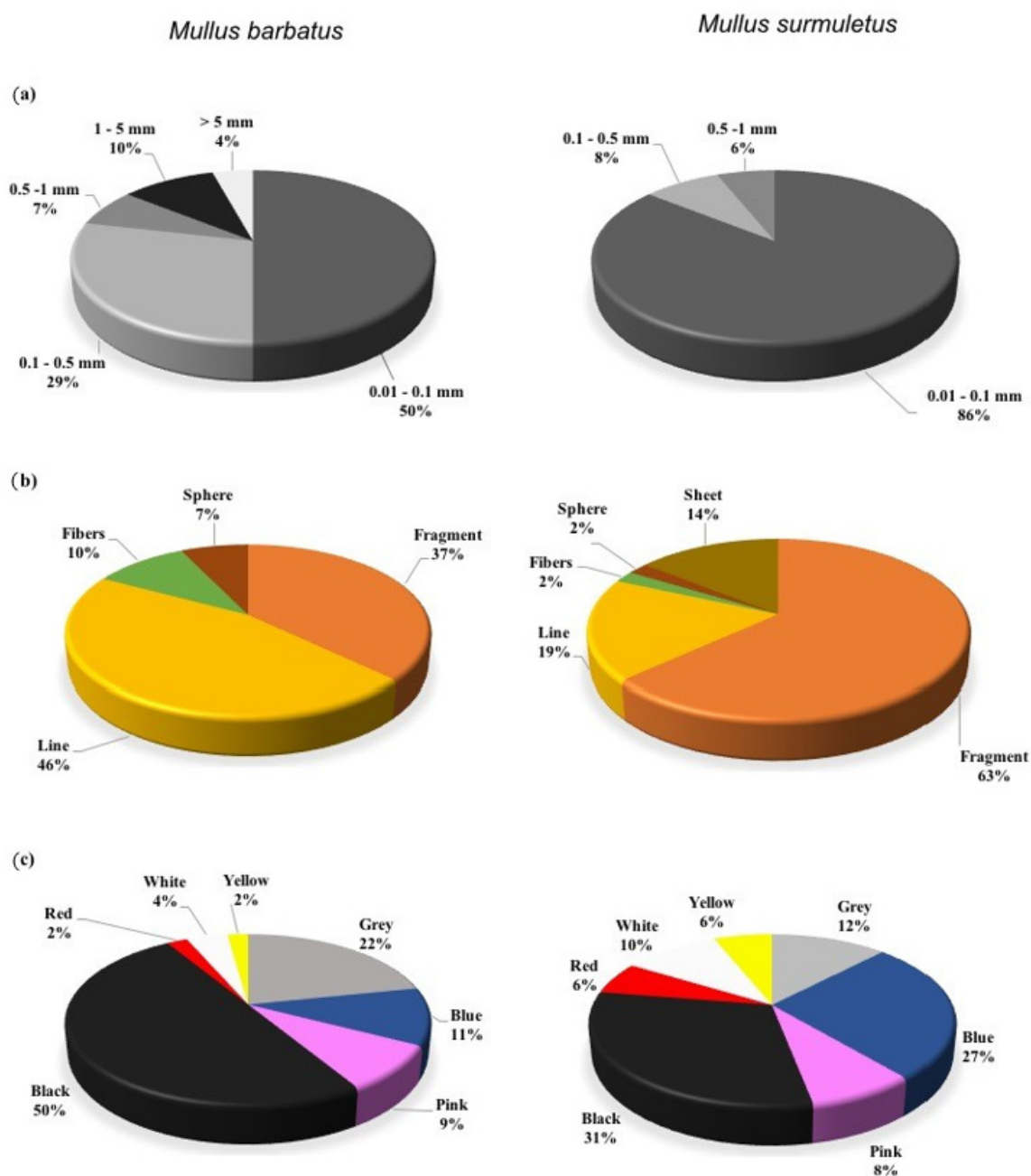


Figure 1. Pie charts showing (a) dimension class, (b) shape, and (c) colors of microplastics found and their relative abundance for the total sample of *M. barbatus* (left) and *M. surmuletus* (right).

Regarding the shape of the isolated microplastics, the fragment and line represent the most frequently ingested items, both for the red mullet (37% and 46%, respectively) and striped red mullet (63 and 19%). Fibers were more abundant in red mullets (10%), whereas they represented only 2% of microplastics found in the congeneric striped red mullet. Sheet-like plastic pieces were found only in the striped red mullet (14%) (Figure 1b).

Colors showed a very similar pattern of distribution for both the species (Figure 1c). Black particles were the most frequent color, representing half of the total microplastics detected (50%) for the red mullet and only 31% for the congeneric striped red mullet. Blue and gray particles were the following most abundant items found in both species.

Microscopic images of the extracted microplastics are provided in the Supplemental Materials (Figure S1).

Samples were first characterized with ATR-FTIR, analyzing the filtrate directly on the filter. A minimum of four matching absorption bands were required for accepted identification and, in particular, the doublet at 1462–1467 cm^{-1} and the peaks at 2915 and 2845 cm^{-1} (Jung et al. 2018) were noticed. Polyethylene was the only polymer that we were able to identify in 33% of the GI contents analyzed.

Mass spectrometry (MS) coupled with gas chromatography (GC) is an alternative approach that has attracted recent attention for qualitative and quantitative analysis of microplastics [36,38]. Thermal desorption and pyrolysis were used to thermally decompose polymers into products amenable to the GC-MS analysis [38–41]. It has been demonstrated that the pyrogram, i.e., the chromatogram of the polymer degradation products when a pyrolysis inlet is used [39], is a “chemical fingerprint” of the analyzed microplastic. In order to simplify the data interpretation and to avoid relying on pyrogram interpretation for literature data only [39], samples of the raw quartz filter (process blanks) and filters containing powdered polymers (polyvinylchloride PVC, polyethylene PE, polypropylene PP, nylon, polystyrene PS, polyethylene terephthalate PET, polymethylmetacrylate PMMA) were prepared and analyzed using the Py-GCMS under the same conditions. As a result, the markers’ ions for these polymers that represent the great majority of the currently identified microplastics in marine litter were identified and are reported in Table 2. These degradation products are those that were considered necessary to conclude the presence of a specific chemical type of MPs in the samples.

Table 2. Polymer decomposition products of selected polymers by Curie-point pyrolysis GC-MS.

Polymer	Main Decomposition Products	M (<i>m/z</i>)	Indicator Ions (<i>m/z</i>)	RT *
PE	alkanes (ex. $\text{CH}_3(\text{CH}_2)_8\text{CH}_3$)	142	85, 71	6.8
	α -alkenes (ex. $\text{CH}_2=\text{CH}(\text{CH}_2)_7\text{CH}_3$)	140	97, 83	7.3
PP	α,ω -alkadienes (ex. $\text{CH}_2=\text{CH}(\text{CH}_2)_6\text{CH}=\text{CH}_2$)	138	95, 81	8
	2,4-dimethylhept-1-ene	126	126, 70	3.5
PP	2,4,6-trimethyl-1-nonene (meso form)	168	111, 112, 83, 69	10.1
	2,4,6-trimethyl-1-nonene (racemic form)	168	112, 111, 83, 69	10.3
	2,4,6,8-tetramethyl-1-undecene (isotactic)	210	111, 83, 69	17.3
	2,4,6,8-tetramethyl-1-undecene (heterotactic)	210	111, 83, 69	17.6
PS	2,4,6,8-tetramethyl-1-undecene (syndiotactic)	210	111, 83, 69	17.9
	styrene	104	104, 78	11.7
	α -methylstyrene	118	117, 118, 103	14.1
PVC	C=C(Ph)-C-C-Ph (dimer)	208	208, 91	36.7
	C=C(Ph)-C-C(Ph)-C-C-Ph (trimer)	312	312, 194, 91	51.2
PVC	Chlorobenzene	112	112, 114, 77	10.3
	Styrene	104	104, 78	11.7
	Indene	116	116, 115	18.1
	Naphthalene	128	128	24.2
	1-methylnaphthalene	142	142, 141, 115	26.7
PET	2-methylnaphthalene	142	142, 141, 115	27.4
	Acetophenone	105	120, 105, 77, 51	22.1
	Vinyl benzoate	148	148, 105, 77, 52	22.2
	Benzoic acid	122	122, 105, 77	32.6
PMMA	Divinyl terephthalate	218	175, 104	34.7
Nylon	Methyl methacrylate	100	100, 69, 41, 85	4.2
	ϵ -caprolactam	113	113, 85, 83	29.9

* Measured on 30 m HP Innowax column.

The Py-GCMS analysis of GI contents' filtrate not only permitted us to identify the presence of different polymers but revealed the presence of plastic in 80% of individuals. In particular, Py-GCMS revealed that polyethylene (80%) and polystyrene (33%) were the most common plastic polymers (Table 3). As an example, Figure 2 shows the total ion pyrogram of sample MS16, evidencing the presence of PMMA, PS, and PE. It is possible to see, along with the peak attributable to the different identified polymers, different peaks due to the pyrolysis of organic matter still not digested; however, their presence does not hinder the identification of polymer type.

Table 3. Percentage of samples containing microplastics according to the polymer type (%) identified with Py-GC-MS analysis in *M. surmuletus* and *M. barbatus*.

Polymer Type	Composition (%)
Polyethylene (PE)	80
Polystyrene (PS)	33.4
Polymethylmethacrylate (PMMA)	6.7
Polyvinylchloride (PVC)	6.7

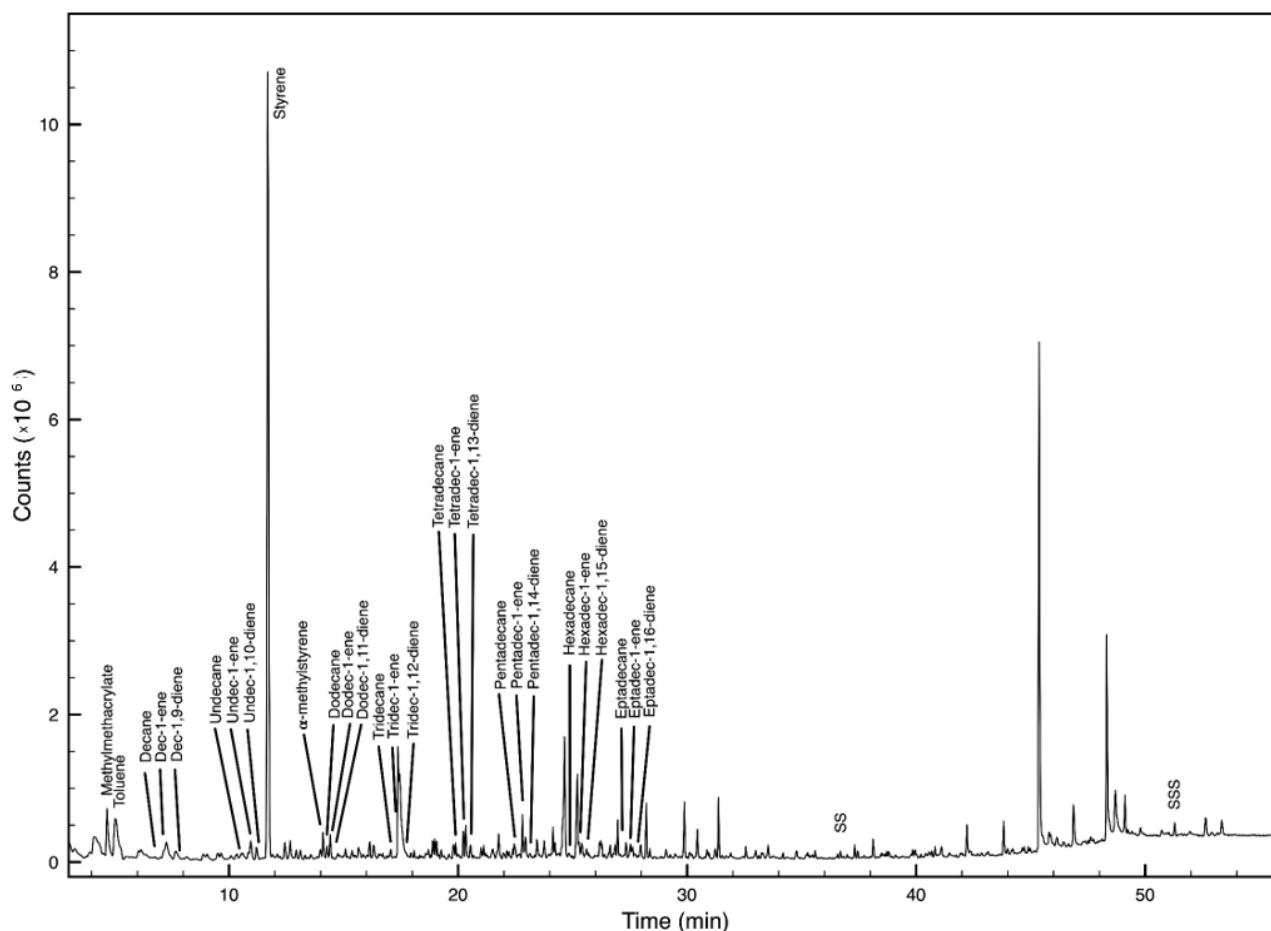


Figure 2. Total ion counts' pyrogram of MS16 relevant to an *M. surmuletus* sample. Main peaks of PMMA, PS, and PE are labeled. Pyrogram cut at 56 min.

A subset of four livers of *M. surmuletus* was randomly chosen for the detection of microplastics. As expected, the dimensions of MPs able to enter liver cells were in the nano scale; therefore, they could not be identified visually or spectroscopically by ATR-FT-IR. In addition, the high load of organic residual, not completely removed by the digestion with 15% H₂O₂, was detrimental for these analyses. To overcome these difficulties,

we decided to introduce a small piece of quartz filter, onto which possible MPs were trapped, into the analysis cup. Considering that it was reasonable that the filtrate was homogeneously dispersed on the filter, this blind method can be considered as a random sampling of a homogeneous filter. Actually, this was confirmed, repeating three times the pyrolysis of the same filter (obviously sampling different parts of the same filter, as pyrolysis is a microdestructive analytical method), which gave pyrograms containing the same information.

Interestingly, chemical analysis by Py-GC-MS revealed the presence of plastic polymers in three of the four livers analyzed. Figure 3 shows the TIC pyrogram of sample MS4 where both PE and PS were identified, confirming the usefulness of this analytical tool for the detection of MPs in fish tissues. This was an interesting result, considering that liver tissues are even more difficult to be digested and that the amount of microplastics was definitely lower than those observed in GI contents, with the PE and PS peaks being less intense with respect to the peaks attributable to the matrix.

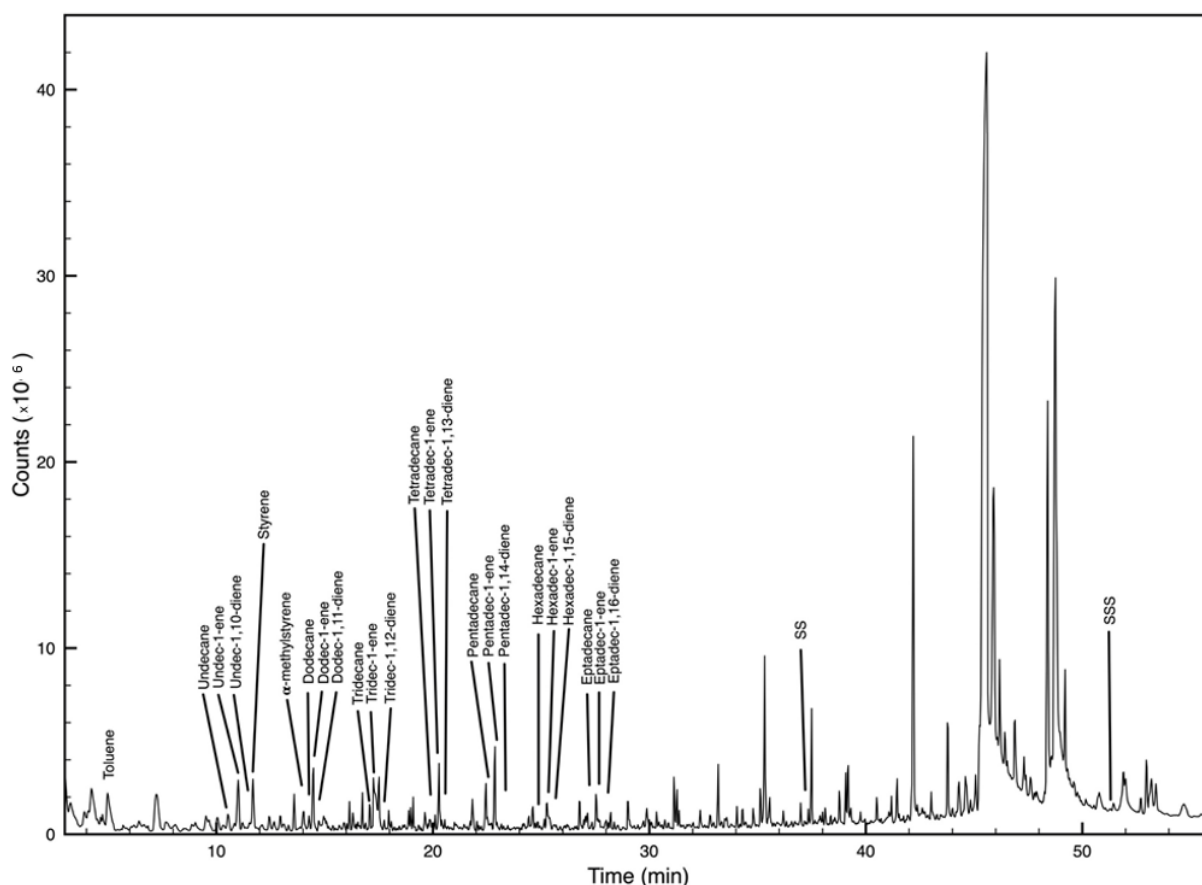


Figure 3. Total ion counts' pyrogram of MS4F relevant to the liver of an *M. surmuletus* sample. Main peaks of PS and PE are labeled. The presence of intense peaks due to the not digested organic matter is evident. Pyrogram cut at 56 min.

4. Discussion

This study reports, for the first time, the presence of microplastics in the gastrointestinal contents of demersal fish (*Mullus* spp.) from a Marine Protected Area.

Ingestion of microplastics was evident, as more than 60% of specimens analyzed had ingested microplastics, with an average of three items per fish.

The ingestion of plastic debris by *M. barbatus* was higher than that reported in previous surveys. Concerning the Ionian Sea, Giani et al. (2019) found an occurrence of 15.5% in a total of 58 individuals of *M. barbatus*, with a mean of 1.25 (± 0.44) particles per fish. Anastasopoulou et al. (2018) [42] analyzed MPs' ingestion, separating information on micro

(<1 mm) and macro (>1 mm) litter, reporting an occurrence of 32% (in 25 individuals) with a mean value of 1.5 (± 0.8) “micro”-particles/individual, whereas only 8% (in 50 individuals) of individuals had ingested “macro”-litter, with a mean value of one particle per fish.

Focusing the attention on surveys carried out worldwide, the available results show a high heterogeneity both for the occurrence of microplastics and for the number of samples. In decreasing order, previous studies on *M. barbatus* reported ingestion of 66% with a mean value of 1.4 particles per fish in the Aegean–Levantine Sea (207 individuals) [27]; 64% (11 specimens, Adriatic Sea, mean = 1 MPs/ind. [20]); 29% in 38 fish (Adriatic Sea, mean = 1 MPs/ind. [30]); 19% in 128 individuals (north/western Mediterranean Sea, mean = 1.9 items/ind. [25]); 17% (Tyrrhenian Sea, mean = 1 MPs/ind. [30]); 14% (South Adriatic Sea, mean = 1.2 MPs/ind. [29]); and 0% (North Adriatic Sea [28]).

Results from this study provide further evidence of the ingestion of MPs by *M. surmuletus*. Our finding is similar to previously reported ingestion values in *M. surmuletus* from both the Northern Adriatic Sea (mean value = 2.7 MPs/individuals, 70% of occurrence [40]) and the Portuguese coast (mean value = 1.66 ± 0.57 MPs/individuals, 100% of occurrence [24]) but higher than those found near the Balearic Islands (27.30% of occurrence with a mean value of 0.42 ± 0.04 MPs/individual [26]).

As stated above, a high heterogeneity for the occurrence of microplastics exists in studies carried out on these demersal species. This high variability may depend not so much on the density of marine litter in the environment but on the different methods used to assess litter ingestion, making comparisons of data difficult. The aforementioned studies used filters with different mesh sizes or, in some cases, the direct visual sorting under stereomicroscope of the stomach contents without any filtering step, thus resulting in different cutoff size for microplastic. For example, only particles with a diameter above 8 μm , the lowest cutoff value [20], or above 300 μm in the case of the highest cutoff [25] were counted. In this study, the filtration mesh size was 2 μm , representing, together with the survey of Giani et al. (2019) [30], the smallest cutoff value for microplastic ingestion by fish to date.

The fine mesh filters used in this study may explain the high percentage of ingestion of microplastic by fish. As highlighted by Guven et al. (2017), the percentage of microplastic ingestion depends more on the mesh of filters/sieves used rather than the areas sampled, since a lower % of ingestion was often found in fish from more plastic-polluted water than fish sampled in cleaner waters. At the same time, when the mesh size of the filter/sieve was smaller, the ingestion rate increased [27]. Therefore, the ingestion estimates provided in this study did not mean that the MPA of Porto Cesareo is more polluted than other Mediterranean areas but simply that the methodology used is among the most precise and complete to date.

Contrary to previous studies where fibers represented the dominant shape of plastic debris in fish stomach contents of the *Mullus* species [24,25,27,30], in this study, we found particles mainly consisting of lines and fragments. Some authors have even excluded fibers from the results because of the high risk of airborne contamination, underrating the real plastic contamination [41,43]. In this study, the precautions taken during all stages of the protocol, from extraction to analysis of MPs, provided realistic information about the proportion of microplastics' fibers ingested by fish.

Colors of microplastics were similar to other studies with blue and black being the most abundant [25–27,30].

As to the analytical methods used, it is possible to confirm that GC-MS coupled to thermal methods of sample introduction, such as pyrolysis, offered great sensitivity also in the nanoscale range. The presence of interfering substances not removed in the preprocessing steps made more difficult the identification of microplastics in samples. However, this drawback proved more challenging in ATR-FTIR analysis rather than Py-GCMS measurements, possibly due to the greater selectivity assured by mass spectrometry. Our results can be considered a de facto method for the analysis of those micro- and nanoplastics that are usually beyond the detection limits and spatial resolution of spectroscopic techniques.

This is confirmed by different recent published papers in which the quantitative aspects also were addressed [38].

5. Conclusions

The results presented in this study represent a baseline for microplastic research useful for the implementation of the Marine Strategy Framework Directive descriptor 10 in the Italian coast of the Ionian Sea. The high number of MPs found in this study suggests that more efforts should be addressed in achieving consistency in analyzing techniques, taking into account the importance of using fine mesh filters to provide a more realistic view of the problem and a stronger basis for comparisons among existing and future studies of plastic ingestion in fish.

Further studies on biota and sediments could be useful to better understand the effects and the dynamics of MPs inside the MPA with the aim of providing potential management actions.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/microplastics1020021/s1>, Figure S1: Examples of microplastics extracted from the gastrointestinal contents of *M. surmuletus* and *M. barbatus*.

Author Contributions: Conceptualization, S.F. and A.T.; formal analysis, S.F. and G.E.D.B.; investigation, S.F. and M.P.; resources, G.E.D.B., C.M. and A.T.; writing—original draft, S.F.; writing—review and editing, S.F., M.P., G.E.D.B. and A.T. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: Written informed consent has been obtained from the patient(s) to publish this paper.

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