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One is not enough: Monitoring microplastic ingestion by fish needs a multispecies approach.

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Abstract

The development of monitoring programmes based on bioindicators is crucial for assessing the impact of microplastic ingestion on marine organisms. This study presents results from an Italian pilot action aimed at investigating the suitability of a monitoring strategy based on a multispecies approach. The benthic-feeder *Mullus barbatus*, the demersal species *Merluccius merluccius*, and the pelagic-feeder species of the genus *Scomber* were used to assess the environmental contamination by microplastics in three different marine areas, namely Ancona (Adriatic Sea), Anzio (Tyrrhenian Sea), and Oristano (Western Sardinia). Microplastic ingestion frequencies were higher in samples from Anzio (26.7%) and Ancona (25.0%) than Oristano (14.4%), suggesting a relationship between microplastic bioavailability and the proximity to urban settlements and river flows. Furthermore, microplastic ingestion was affected by the feeding habits of the examined species. The detected differences reinforce the hypothesis that a multispecies approach is needed to evaluate microplastic ingestion by marine animals.

Keywords: marine litter; micro-litter; bioindicator; feeding habits; Mediterranean Sea; MSFD

1. Introduction

Since the 1950s, the establishment of a consumption-based human society determined the release of significant quantities of waste into the environment (Zalasiewicz et al., 2016). Most are made of plastic (Woodall et al., 2014), a widespread set of synthetic polymers with low biodegradability (Palmisano and Pettigrew, 1992). Despite the low toxicity associated with most plastic materials (Worm et al., 2017), plastic pollution is one of the major environmental threats of current times (Horton et al., 2017). Indeed, plastics are often loaded with hazardous chemicals, such as additives, dyes, and flame retardants (Deanin, 1975; Lithner et al., 2011; Fries et al., 2013). Furthermore, the environmental persistence of plastics results in a progressive fragmentation process, impacting items exposed to ultraviolet radiation, chemical oxidation, mechanical abrasion, and biological agents (Liu et al., 2020; Turner et al., 2020). As a result, all the environmental compartments are today contaminated by the presence of microplastics (MPs), generally defined as tiny pieces of plastic smaller than 5 mm in size (Arthur et al., 2009).

Oceans and seas represent the main sink for MPs (Hale et al., 2020). In the aquatic media, MPs can sorb many kinds of organic and inorganic pollutants, including toxic and carcinogenic substances (Pelamatti et al., 2021; Rai et al., 2022). At the same time, MPs overlap the size range of prey of a wide variety of marine animals that can ingest these small particles either accidentally, or intentionally – by confusing plastic particles with natural or potential preys, as well as due to secondary ingestion (*i.e.*, items already ingested by prey) (Anbumani and Kakkar, 2018; Fossi et al., 2018; Prinz and Korez, 2020). Therefore, MPs are hazardous contaminants that can act as vectors of chemicals through the marine food webs, posing threats to ecosystem functioning (Carbery et al., 2018). Several laboratory experiments linked plastic ingestion to negative physiological effects, such as energy depletion, starvation, increased immune response, and decreased fecundity (von Moos et al., 2012; Wright et al., 2013; Rios-Fuster et al., 2021). Field studies also reported physical impacts, including blockage of the digestive tract, abrasions, and difficulties in breathing (Sharma and Chatterjee, 2017).

Several legal and policy frameworks address the "marine litter" topic, such as the Global Partnership on Marine Litter (GPML, https://www.gpmarinelitter.org/), the Honolulu Strategy, and the G7 Countries Agenda (Löhr et al., 2017). Even within the UN Sustainable Development Agenda, 4 of 17 Sustainable Development Goals (SDGs) pose targets concerning the reduction and mitigation of plastic pollution in marine environments by 2030 (https://sdgs.un.org/). In this context, the UN Environmental Assembly (UNEA-5.2 https://www.unep.org/environmentassembly/) aims to forge an international agreement for a global transition to a circular economy. At the Mediterranean level, the Regional Plan on Marine Litter Management sets legally binding targets by 2025 to deal with marine litter from both land- and sea-based sources (UN Environment/Mediterranean Action Plan, https://www.unep.org/unepmap/). Similarly, the 2008/56/EC Marine Strategy Framework Directive (MSFD) fixes the aim of achieving the Good Environmental Status (GES, "*the environmental status of marine waters where these provide ecologically diverse and dynamic oceans and seas which are clean, healthy and productive*") by defining the criteria D10C3 as *"The amount of litter and micro-litter ingested by marine animals is at a level that does not adversely affect the health of the species concerned*" (Commision Decision 2017/848/EU).

To drive and confirm the strength of the programs of measures implemented by legislative frameworks, the UNEP/MAP recognized the importance of developing appropriate monitoring strategies based on bioindicators for assessing the occurrence and the impacts of MP ingestion on marine organisms (Galgani, 2017). The MSFD

Technical Group on Marine Litter (TG-ML) established essential criteria for the selection of bioindicators of marine litter ingestion, such as sample availability, regular litter consumption, and sufficient knowledge of the biology of the involved species, including habitat, trophic level, feeding behavior, spatial distribution, commercial importance, and conservation status (Fossi et al., 2018). The seabird *Fulmarus glacialis* in the Northern European seas and the loggerhead sea turtle *Caretta caretta* in the Mediterranean basin are the target species for monitoring macro-litter (> 5 mm) ingestion (van Franeker et al., 2011; Matiddi et al., 2017; 2019). In contrast, a micro-litter monitoring strategy is not yet defined, though fish species are good candidates for assessing this impact (Bray et al., 2019).

Several recent studies show that MP ingestion occurs in many fish species, including bony fishes and elasmobranchs of commercial importance that showed to be regular litter consumers (Wang et al., 2020). Previous studies successfully proposed monitoring programs based on the assessment of MP ingestion by examining a single target species (*e.g.*, Tsangaris et al., 2020). However, it is known that the feeding habits of different fish species may determine differences in MP ingestion rates (Miller et al., 2020; Justino et al., 2021). Moreover, the distribution of MPs in the marine environment varies according to their different shape, size, and chemical composition (Palazzo et al., 2021). Several environmental factors (such as waves, tides, and currents) at different geographical scales contribute to determining different accumulation pathways for different MP types (Li et al., 2020). In this view, it is likely that more than one fish species should be selected for describing MP contamination of the marine environment. In particular, the target species should have different feeding behaviors and habitat uses to investigate different marine compartments within the examined area (Matiddi et al., 2021).

This study presents results from an Italian pilot action, which aims to investigate the adequacy of a monitoring strategy based on the assessment of MP ingestion by fish species with different feeding habits. This activity was planned by a national consortium involving the Italian National Institute for Environmental Protection and Research (ISPRA), the National Research Council – Institute of Anthropic Impacts and Sustainability in Marine Environment (CNR-IAS), and the Marche Polytechnic University – Department of Life and Environmental Sciences (UNIVPM-DiSVA). Three marine areas characterized by different MP contamination sources and circulation patterns were investigated to highlight site-specific variations in MPs ingested by different fish species. The selected species are the benthic-feeder *Mullus barbatus*, the demersal species *Merluccius merluccius*, and the pelagic-feeder species of the genus *Scomber*. These species were designated because of their different feeding habits and considering commercial importance, their availability in the selected areas, and the well-documented occurrence of MP ingestion (Giani et al., 2019; Avio et al., 2020; Bianchi et al., 2020; Capillo et al., 2020). The red mullet *M. barbatus* is a benthivorous species that feeds on the sea bottom by swallowing sediment with the prey and expelling the sediment through the gills (Labropoulou and Eleftheriou, 1997). Therefore, it could be regarded as an indicator of MP ingestion within the strictly benthic compartment. Differently, the European hake *M. merluccius* is a demersal top predator that frequently moves from the sea floor to mid- and surface-waters, where it typically feeds upon fast-moving preys, such as cephalopods and fish (Carpentieri et al., 2005). Finally, the Atlantic mackerel *S. scombrus* and the Atlantic chub mackerel *S. colias* are pelagic species preferring zooplankton and small pelagic fish (Lopes et al., 2020), and therefore might represent the pelagic compartment.

The main aim of this study is to provide further information for improving the future development of marine micro-litter monitoring systems by: i) investigating possible differences in MP ingestion by different fish species within the same area; ii) highlighting the diversity of MPs ingested by the same species in three different marine areas; iii) verifying the ecological relevance of a multi-species monitoring approach.

2. Materials and methods

2.1 Study areas and sampling activities

Fish were sampled in August-October 2019 at 3 sampling sites within 3 different Italian coastal areas: Ancona in the Adriatic Sea, Anzio in the Tyrrhenian Sea, and Oristano on the Western side of Sardinia (Figure 1). The marine area off the city of Ancona is characterized by low depths and it is affected by the inputs of the Po River and other relevant runoffs. The Po River is the main Italian river, which flows into the Adriatic Sea ≈ 150 km north of the sampling area. The effect of the West Adriatic current (flowing toward the South) together with the intense urbanization of the coast and the presence of commercial seaports make this area a prospective site of plastic accumulation within the Mediterranean Sea (UNEP/MAP, 2012; Liubartseva et al., 2016; Giani et al., 2019). Similarly, the Tyrrhenian Sea area off Anzio coast is affected by the discharge of the Tiber River (distance from the mouth ≈ 50 km), the second largest river catchment in Italy, and shows a high coastal urbanization. On the other hand, the basin is characterized by a wider bathymetric range, a complex bottom topography, and a highly variable circulation that isolates this area from other Mediterranean sub-regions during the warm season (Inghilesi et al., 2012), determining the potential accumulation of waste from local inputs. Finally, the Gulf of Oristano is not closely affected by large rivers and important urban settlements. Therefore, due to the distance of the island of Sardinia from the mainland, the plastic contamination in this area is mainly due to the transport conveyed by the prevailing winds, which blow from West/North-West, and by the inflow of Modified Atlantic Water (MAW) (Camedda et al., 2021; Palazzo et al., 2021).

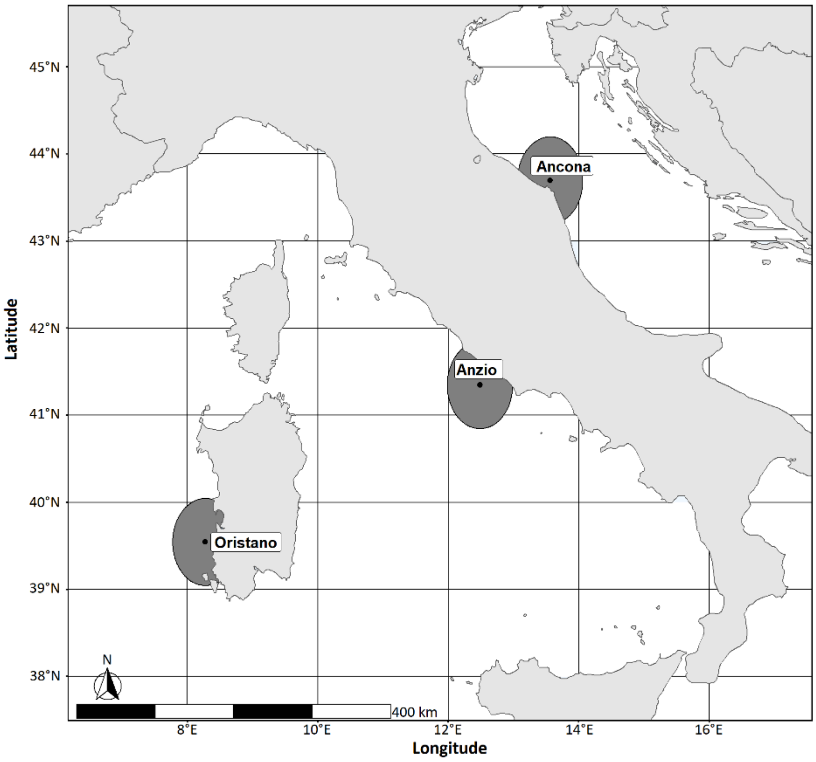

Figure 1. Map of sampling areas: Ancona (Adriatic Sea), Anzio (Tyrrhenian Sea), and Oristano (Western Sardinia).

In each of the three areas, fish were caught by local fisheries using trawling nets for commercial fishery, with a hemp-coloured cod-end and a 40 mm square mesh. During each fishing trip (geographical centroids: Ancona – 43°41′45″N, 013°33′32″E; Anzio – 41°18′31″N,012°28′43″E; Oristano – 39°30′19″N,008°20′29″E), the nets were towed for 3 hours at an average speed of 3 knots (estimated swept area ≈ 0.8 km^2). A total of 264 specimens were collected: 84 from Ancona (24 *M. merluccius*, 30 *M. barbatus*, and 30 *S. scombrus*), 90 from Anzio (30 *M. merluccius*, 30 *M. barbatus*, and 30 *S. colias*), and 90 from Oristano (30 *M. merluccius*, 30 *M. barbatus*, and 30 *S. colias*). Table 1 reports information on the mean values (± se) of total length, total weight, gastrointestinal weight, and relative condition factor (Kn) recorded for each species within each sampling area.

	M. merluccius	***M. barbatus***	***Scomber* spp.**
a) Total length [cm]			
Ancona	30.0 ± 1.5	16.5 ± 1.2	22.2 ± 0.9
Anzio	28.9 ± 1.3	15.5 ± 0.7	27.2 ± 1.3
Oristano	23.0 ± 1.6	13.2 ± 0.7	26.8 ± 2.0
All Locations	27.4 ± 3.1	15.1 ± 1.6	25.4 ± 2.7
b) Total weight [g]			
Ancona	198.9 ± 26.1	57.1 ± 13.4	93.3 ± 12.3
Anzio	172.5 ± 23.3	36.6 ± 4.8	157.6 ± 24.7
Oristano	114.2 ± 22.0	32.9 ± 5.6	209.2 ± 49.7
All Locations	159.3 ± 42.4	42.2 ± 13.8	153.4 ± 57.7
c) GI weight [g]			
Ancona	7.1 ± 3.5	1.6 ± 1.0	6.5 ± 2.6
Anzio	6.3 ± 5.3	1.4 ± 0.4	7.2 ± 1.9
Oristano	5.9 ± 3.3	2.0 ± 0.7	12.8 ± 6.8
All Locations	6.4 ± 4.1	1.7 ± 0.8	8.8 ± 5.2
d) Relative condition factor (Kn)			
Ancona	1.05 ± 0.10	1.11 ± 0.09	1.14 ± 0.19
Anzio	1.01 ± 0.06	0.88 ± 0.06	0.85 ± 0.04
Oristano	1.23 ± 0.12	1.29 ± 0.13	1.17 ± 0.07
All Locations	1.10 ± 0.13	1.09 ± 0.19	1.05 ± 0.19

Table 1. Morpho-anatomical data (mean ± sd) of fish samples (*Merluccius merluccius*, *Mullus barbatus*, and *Scomber* spp.) collected in August-October 2019 within three Italian marine areas (Ancona, Anzio, and Oristano): **a)** total length [cm]; **b)** total weight [g]; **c)** gastrointestinal (GI) weight [g]; **d)** relative condition factor (Kn). Kn was computed according to the expression $Kn = TW \cdot (a \cdot TL^{b})^{-1}$, where a and b are the log-transformed length-weight relationship parameters, TW is the total wet weight expressed in grams, and TL is the total length expressed in cm.

After the collection onboard or at landing, all the individuals were immediately stored at -20 °C until further analyses. Specimens showing diseases, evidence of net feeding, or regurgitation were discarded.

2.2 Lab analyses

Lab analyses were performed in the laboratories of ISPRA (Anzio), CNR-IAS (Oristano), and DiSVA (Ancona) following the guidelines provided in Matiddi et al. (2021). Following this protocol, the MPs in this study were defined as "*All sorts of small particles of plastic, less than 5 mm in size in two of the three dimension or diameter that pass through a 5 mm mesh screen but are retained by a lower one, according to the chosen size class*", fixing the lower size limit to 100 μm due to operational limits.

Total length and total wet weight of each individual were recorded after thawing at room temperature. Then, fish were dissected to extract the entire gastrointestinal tract (GI), which was weighed to the nearest 0.1 g. Each GI was placed into individual glass jars and filled with 15% H_2O_2 to digest the biogenic component. Extraction of micro-items larger than 100 μm was performed using a vacuum pump for filtering the digestates on glass microfiber (Whatman GF/D^{TM}; pore size: 2.7 μm) or cellulose nitrate (Sartorius 11301; pore size: 8 μm) membranes. MP identification was made using a dissecting microscope. Shape type (filament, film, fragment, granule, or pellet), colour, and size class (class 1: 100 μm – 330 μm; class 2: 330 μm – 1 mm; class 3: 1 mm – 5 mm) was recorded for each collected item. Polymeric characterization was determined using Fourier Transformed Infra-Red Micro-Spectrometry (μFT-IR). Fibres from textiles were not considered. The discrimination between fibres and filaments was based on an accurate shape analysis of each thread-like item. Filaments were defined as rod-like particles with regular diameter, while microfibers were characterized as items with a ribbon-like shape, not regular diameter, and frayed ends. Polymeric characterization was performed for all the questionable items.

2.2.1 Quality assurance and quality control

All the precautions suggested by Matiddi et al. (2021) were adopted during every analytical step to reduce secondary contamination. Dissecting procedures were performed in a clean room where airflow and staff presence were limited. Workbenches, instruments, and tools were cleaned with ethanol or rinsed with ultrapure water before every use. Only glass and metal labware was used whenever applicable and the wearing of synthetic clothes was avoided. All dissecting tools, containers, and sieves were additionally cleaned with compressed air. Aluminium foils were used to cover labware and samples exposed to airborne contamination. Covered Petri dishes were used to store and preserve membranes.

A contamination control was treated in parallel to each batch of 10 samples. Blank controls only revealed the presence of microfibers made of natural polymers (cellulosic-made or wool; mean ± se = 2.61 ± 0.79 items · control^{-1}). Since natural microfibers were not considered in this study, no results adjustment techniques were adopted.

2.3 Data and statistical analyses

Relative fish condition factor (Kn) was computed for each individual. Kn calculation was performed according to the expression $Kn = TW \cdot (a \cdot TL^{b})^{-1}$, where *a* and *b* are the log-transformed length-weight relationship parameters, TW is the total wet weight expressed in grams, and TL is the total length expressed in cm. Frequency of Occurrence (FO) was computed as no. of individuals with ingested MPs · no. of individuals examined^{-1}. The abundance of ingested MPs was expressed as the average (± standard error, se) no. of ingested MPs.

Differences in FO were tested using Generalized Linear Models (GLMs) assuming a binomial error structure and a logit link function. Differences in MP abundance were analyzed using a negative-binomial GLM. Relationships between biological parameters (namely total length, total weight, GI weight, and Kn) and the occurrence of ingested MPs were investigated through binomial Generalized Linear Mixed Models (GLMMs), setting sampling area as random effect. Correspondence analysis was performed to highlight differences in the types of ingested MPs by species and sampling area. Association between variables was tested through chi-square statistics. The significance level was set at $p < 0.05$.

Statistical analyses were performed with R4.1.0 (R Core Team, 2021) using packages lme4 (Bates et al., 2015), FactoMineR (Le et al., 2008), factoextra (Kassambara and Mundt, 2020), ggplot2 (Wickham, 2016), and gplots (Warnes et al., 2020).

3. Results

In all three marine areas, MP ingestion occurred in all the examined species, with an overall frequency of occurrence of 22.0%. FO was higher in samples from Anzio (26.7%) and Ancona (25.0%) than Oristano (14.4%).

Comparing species, the highest FO was recorded in *Scomber* spp. (33.3%), followed by *M. merluccius* (20.2%), and *M. barbatus* (12.2%). The 58 individuals with ingested MPs contained a total of 73 items (mean ± se = 1.26 ± 0.09 items · individual^{-1}). The max numbers of MPs extracted from an individual were: 2 for *M. barbatus*, 2 for *M. merluccius*, and 5 for *Scomber* spp. FOs and MP abundances (average ± se) for species by sampling area are available in Table 2.

	M. merluccius	***M. barbatus***	***Scomber* spp.**	**All species**
a) FO (%)				
Ancona	33.3	16.7	26.7	25.0
Anzio	20.0	10.0	50.0	26.7
Oristano	10.0	10.0	23.3	14.4
All Locations	20.2	12.2	33.3	22.0
b) MP abundance (considering all the examined individuals)				
Ancona	0.38 ± 0.12	0.17 ± 0.07	0.30 ± 0.10	0.27 ± 0.05
Anzio	0.23 ± 0.09	0.13 ± 0.08	0.73 ± 0.20	0.37 ± 0.08
Oristano	0.13 ± 0.08	0.13 ± 0.08	0.30 ± 0.11	0.19 ± 0.05
All Locations	0.24 ± 0.06	0.14 ± 0.04	0.44 ± 0.08	0.28 ± 0.04
c) MP abundance (considering only individuals with ingested MP)				
Ancona	1.12 ± 0.12	1.00 ± 0.00	1.12 ± 0.12	1.10 ± 0.07
Anzio	1.17 ± 0.17	1.33 ± 0.33	1.47 ± 0.29	1.38 ± 0.19
Oristano	1.33 ± 0.33	1.33 ± 0.33	1.29 ± 0.19	1.31 ± 0.13
All Locations	1.18 ± 0.12	1.18 ± 0.12	1.33 ± 0.15	1.16 ± 0.09

Table 2. Microplastic ingestion events recorded in fish samples (*Merluccius merluccius*, *Mullus barbatus*, and *Scomber* spp.) collected within three Italian marine areas (Ancona, Anzio, and Oristano) in August-October 2019: **a)** Frequency of occurrence (FO = no. of individuals with ingested MPs · no. of total individuals examined^{-1}); **b)** average ± se no. of ingested MPs considering all the individuals examined; **c)** average ± se no. of ingested MPs considering only the individuals with ingested MPs.

Comparing marine areas, the highest abundance of filaments was extracted in samples from Anzio (42.4% of the total number of MPs found). Fragments were always among the most represented shape categories (39.4% at Anzio, 47.8% at Ancona, 29.4% at Oristano). Pellets and granules were isolated only in samples from Oristano. Considering colours, most of the MPs were black (23.29%) or blue (19.19%). Further colours frequently found were green (16.44%) and white (16.44%), followed by transparent (10.96%) and red (9.69%). Other colours were rarer (4.11%). Following the proposed size classification of MPs, most items ranged between 100 and 330 µm (56.16%). Items in the other two size classes (330 µm - 1 mm, and 1 mm - 5 mm) had equal frequencies (21.92% both). A detailed summary of MP types found ingested by species and sampling area is reported in Figure 2.

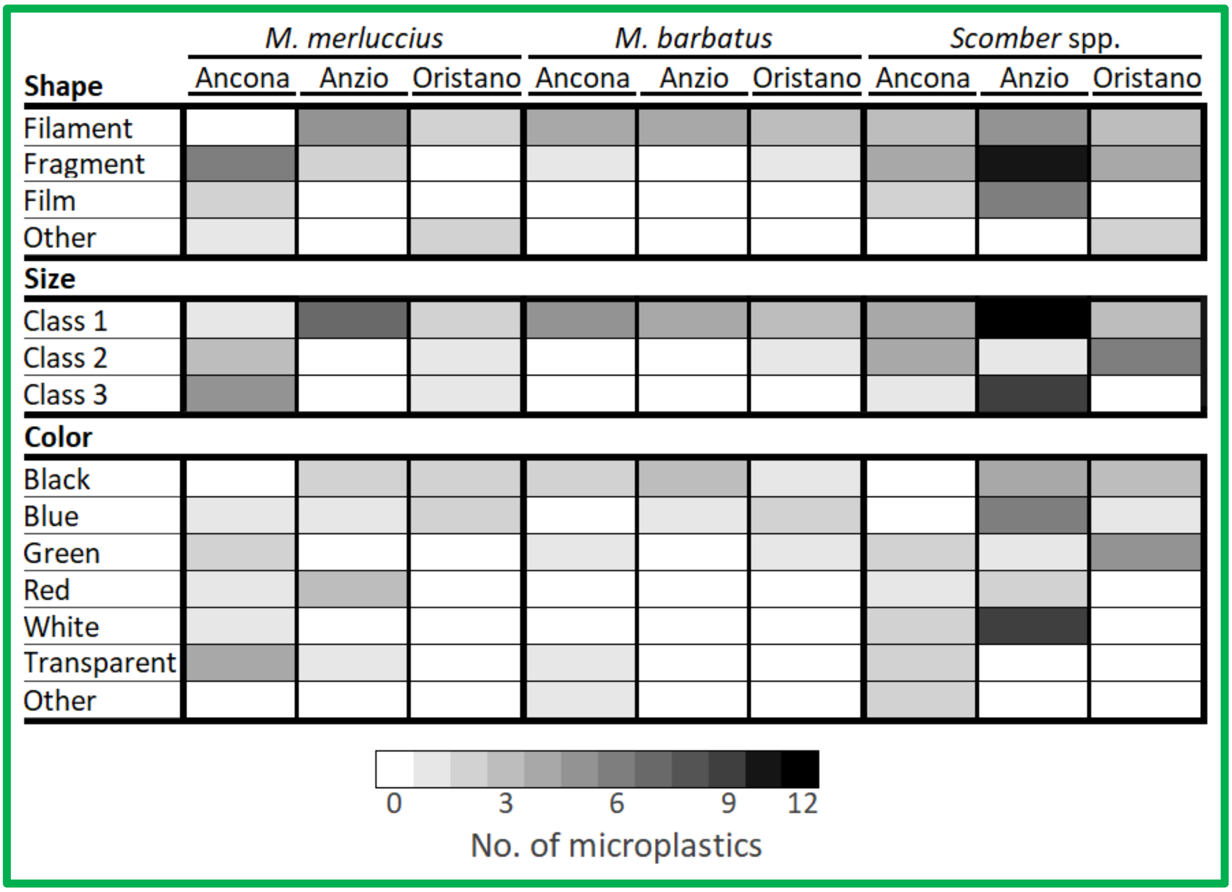

Figure 2. Heatmap representing the diversity of microplastic types in the gastrointestinal tract of fish samples (*Merluccius merluccius*, *Mullus barbatus*, and *Scomber* spp.) collected in three Italian marine areas (Ancona, Anzio, and Oristano) in August-October 2019.

The polymeric composition of 48 out of the 73 recovered MPs was determined through spectroscopic analyses (65.7%). The most represented polymer was polyethylene (PE=33.33%), followed by polypropylene (PP=14.58%), polyester (PEST=12.50%), polyacrylate (PAK=8.33%), polyethylene terephthalate (PET=6.25%), and nylon (PA=6.25%). Other particles found were made of copolymers (PE/PP and Ethylene/Metacrilate=16.67%), or other plastic types such as EDPM rubber and MATER-BI bioplastic. Polymer frequencies by species and sampling areas are available in Table 3.

	Polymer							
	PE	**PP**	**PEST**	**PAK**	**PET**	**PA**	**COP**	**OTH**
a) Sampling area								
Ancona	25.0	-	20.8	0.8	-	12.5	16.7	16.7
Anzio	40.0	25.0	-	5.0	15.0	5.0	10.0	-
Oristano	40.0	40.0	20.0	-	-	-	-	-
b) Species								
M. merluccius	44.4	-	-	11.1	-	22.2	22.2	-
M. barbatus	-	-	57.1	-	28.6	-	-	14.3
Scomber spp.	37.5	21.9	6.25	6.25	3.13	3.13	12.5	9.4

Table 3. Percentages of polymers found ingested in fish samples collected in August-October 2019 by: **a)** sampling areas; **b)** fish species. Abbreviations: polyethylene, PE; polypropylene, PP; polyester, PEST; polyacrylate, PAK; polyethylene terephthalate, PET; nylon, PA; copolymers, COP; other, OTH.

Statistical analysis of absence/presence data showed a two-group partitioning of the samples, the former including samples with FOs equal to 10.0% (*i.e., M. barbatus* from Anzio and Oristano, and *M. merluccius* from Oristano), and the latter including all the other samples with FOs equal to or higher than 16.7% (Figure 3; Table 1S). No significant differences among samples were detected considering the number of ingested MPs (all p-values > 0.05).

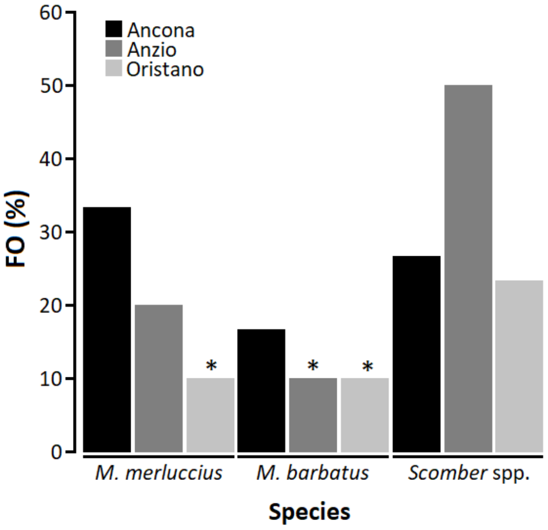

Figure 3. Barplot of the frequency of microplastic ingestion (FO) detected in fish samples (*Merluccius merluccius*, *Mullus barbatus*, and *Scomber* spp.) collected within three Italian marine areas (Ancona, black; Anzio, dark grey; and Oristano, light grey) in August-October 2019. Bars with * indicate significant lower values according to a Generalized Linear Model (GLM) computed by assuming a binomial error structure and a logit link function.

Relationships between biological parameters (total length, total weight, GI weight, and Kn) and the occurrence of ingested MPs were investigated through GLMMs. Only a slightly significant correlation for the effect of GI weight on frequency of ingestion by *M. merluccius* was found (p-value = 0.031; Table 2S).

Correspondence analysis showed a significant association between the combined variable "species-sampling area" and the shape ($X^2 = 46.771$; df = 24; p-value <0.01) and size ($X^2 = 42.604$; df = 16; p-value <0.001) of ingested MPs. Figure 4 showed how the types of MPs ingested by the examined species were different according to both their feeding habits and the sampling location. Figure 4a highlighted different proportions of MP shape-types ingested by *M. merluccius* in the three sampling areas, while the between-sites differences for the other two species seemed less relevant. Figure 4b showed a high prevalence of small MPs (size class 1: 100 µm - 330 µm) in *M. barbatus.*

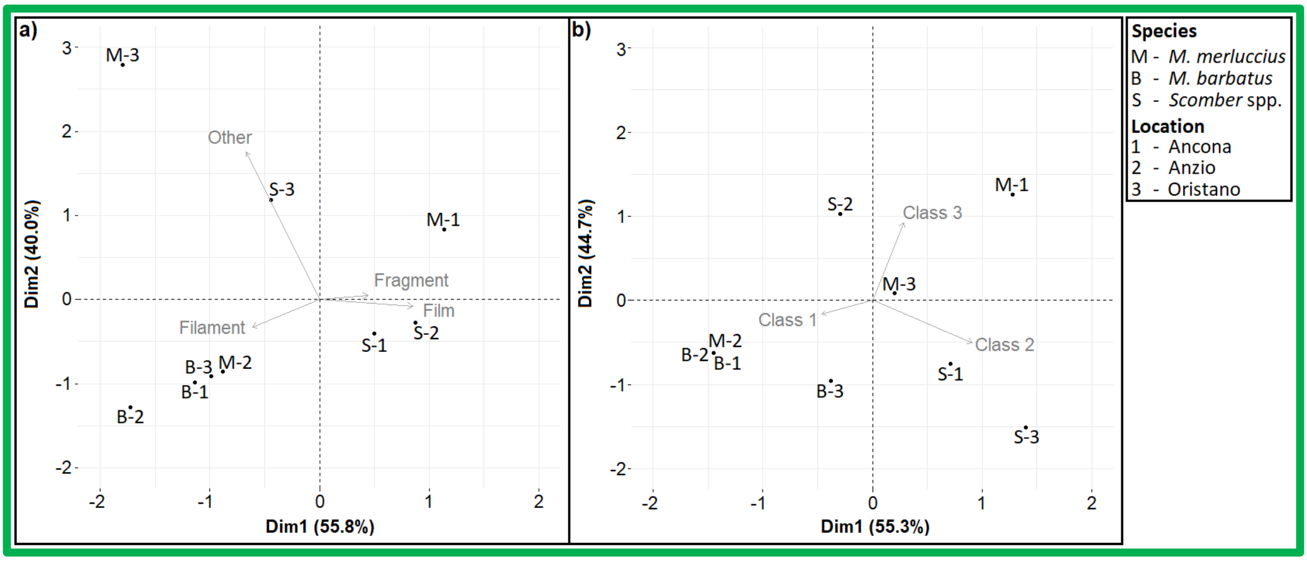

Figure 4. Biplots from correspondence analysis describing the diversity of microplastics ingested by different fish species (*Merluccius merluccius*, *Mullus barbatus* and *Scomber* spp.) sampled within three Italian marine areas (Ancona, Anzio, and Oristano) in August-October 2019: **a)** shape categories (*i.e.,* filament, film, fragment, and other); **b)** size classes (size class 1: 100 µm – 330 µm; class 2: 330 µm – 1 mm; class 3: 1 mm – 5 mm). Abbreviations: B, *M. barbatus*; M, *M. merluccius*; S, *Scomber* spp.; 1, Ancona; 2, Anzio; 3, Oristano. Percentages of explained variance are labelled on the relevant axis.

4. Discussion

The impact of plastic pollution on marine ecosystems is one of the main environmental issues of emerging concern (Thushari and Senevirathna, 2020). Although monitoring programs of environmental matrices are currently underway (*e.g.*, in the case of seawater or beach litter within the European MSFD), the fulfilment of new tools for assessing MP bioavailability is crucial for understanding the threat posed by marine litter to marine life. As a result of snapshot in time, the present study shows that MP ingestion occurs in all the examined species within the three sampling areas, confirming that MP ingestion by marine fish is widespread (Savoca et al., 2021). The overall FO (22.0%) is relatively low compared to other studies from European seas (median FO = 42.0%; Wootton et al., 2021), but we must notice that our results are not fully comparable with other studies due to the exclusion of fibres, which must be considered in future specific investigations aimed at describing their diversity within different marine compartments (Athey and Erdle, 2022). Nevertheless, the detected differences among species and sampling areas provide interesting insights for developing a monitoring strategy based on fish species as bioindicators of MP impact on biota.

4.1 Frequency of occurrence

Coastal anthropogenic pressures are recognized as key predictors of spatial variations in MP ingestion rates (Sbrana et al., 2020; Tsangaris et al., 2020). In the present study, FO is slightly lower in samples from Oristano (14.4%) than Ancona and Anzio (25.0% and 26.7%, respectively. This difference could be explained considering that Anzio and Ancona marine areas have more densely populated coasts (with urban settlements hosting more than 100,000 inhabitants compared to the 30,000 inhabitants living at Oristano; ISTAT, 2018), are crossed by important shipping lanes, and are affected by the flows of the two main Italian rivers (the Tiber River and the Po River, respectively) (Inghilesi et al., 2012; Liubartseva et al., 2016). The relationship between the proximity to the contamination sources and MP bioavailability seems to suggest that the marine environment is continuously subject to diffuse contamination through new MP inputs. Then, the definition of the assessment areas and the sampling strategy should be carefully planned to obtain reliable estimates of temporal variations of MP loads from land-based activities (Bai et al., 2022).

However, it is important to notice that the differences among the three locations emerge only considering all the species together. Indeed, *M. barbatus* from Anzio and Oristano have the same FO. Likewise, no significant FO differences distinguished the *Scomber* spp. samples collected in the three marine areas. A similar picture of the overall conditions (*i.e.*, Anzio ≈ Ancona > Oristano) is obtained from the analysis of *M. merluccius*, but only the integration with the data from the other two species returns a comprehensive description of the contamination status (see Subsection 4.4). Our results show that different species can show different MP ingestion frequencies within the same location. Nevertheless, each single species has different FOs within different locations. This evidence suggests that the environmental contamination by MPs cannot be described using a one-time assessment based on the examination of only one species.

As requested by the MSFD for EU Member States*,* a reliable strategy for monitoring the impact of litter ingestion on marine organisms should provide an ecologically relevant assessment. Therefore, study areas must be selected according to the several factors that may affect MP distribution, such as the presence of contamination sources (e.g.*,* industrial, fluvial, urban) and oceanographic features (*i.e.*, wind stress, water depth, waves, tides, and currents). Moreover, the assessment of MP ingestion should be based on the analysis of a reasonable number of species that can be regarded as indicators of MP ingestion in different marine compartments (Avio et al., 2020). On the other hand, the number of individuals analyzed per single species must be sufficient to allow reliable statistical analysis (Matiddi et al., 2021). A monitoring program should then optimize the work effort (in terms of sampling activities, lab analysis, and staff employment) to obtain a proper characterization of MP ingestion events occurring in a determined area. This study suggests that the analysis of about 30 individuals of at least three different fish species (for a minimum of ≈ 90 samples from each sampling area) might provide a fair quantity of information. However, more extensive targeted studies will need to determine the efforts required to obtain reliable data on spatial and temporal variability, as well as in terms of statistical power. For instance, the number of individuals needed may depend on the degree of contamination of the assessment area (Matiddi et al., 2021). Furthermore, the number of target species that should be examined to obtain an ecologically relevant assessment could be larger and variable according to the complexity of the resident trophic web (Avio et al., 2020).

4.2 Abundance of ingested MPs

The number of MPs ingested by individuals is low, with a maximum value of 5 recorded in a *S. colias* specimen from Anzio. This result is in line with previous studies showing that in regions with a high incidence of plastic contamination MPs are ingested in relatively low quantities by many specimens of different species (*e.g.*, Moore et al., 2001; Lusher et al., 2013; Foekema et al., 2013; Valente et al., 2019). The low number of MPs per individual associated with high FOs suggests that the excretion rates of MPs are greater than ingestion rates (Sbrana et al., 2020). As a result, the residence time of MPs in the gastrointestinal tract of fish is relatively short, and it does not lead to their bioaccumulation over time or biomagnification along food chains. However, this could not be true either in severely contaminated areas – where MP encounter rates exceed the rates of depuration, or for MPs with smaller sizes (the operational lower size limit in this study is 100 µm) (Avio et al., 2017; Covernton et al., 2021; Miller et al., 2021).

In the perspective of a monitoring program, the absence of bioaccumulation and biomagnification processes ensures that fish can be considered as appropriate bioindicators of MP ingestion at local scale, since it is unlikely that they retain MPs ingested far from sampling locations (Sbrana et al., 2020). On the other hand, it is difficult to highlight species-specific, spatial, and temporal differences in MP ingestion through MPs abundance or MP concentration values (Covernton et al., 2021). Therefore, FO appears to be the most reliable index for evaluating the incidence of MP ingestion for monitoring purposes and for studies comparison (Avio et al., 2020). Nevertheless, it should be considered that the retention time of MPs varies according to the range of specific biological traits and the diet composition of the examined species (Valente et al., 2019). This could affect the estimation of MP ingestion probabilities through FO values and future studies will need to understand how biological traits of different fish species may alter the retention time of ingested MPs. Moreover, these considerations are valid for the size range of MPs that are commonly documented in biota (*i.e.*, >100 µm; Covernton et al., 2021) and further considerations would be made for MPs of smaller sizes.

4.3 Relationships between biological parameters and MP ingestion

Previous studies describe some relationships between the biological parameters of fish and MP ingestion. Alomar and Deudero (2017) and Valente et al. (2019) highlight a positive correlation between GI fullness of elasmobranch species and the number ingested MPs, suggesting that the retention time of MPs and food items could be the same. In this study, we observed a significant correlation between the GI weight and the frequency of MP ingestion by *M. merluccius*. This could be due to the strict predatory habit of this species (Carpentieri et al., 2005), which is potentially exposed to secondary ingestion through contaminated prey. Indeed, the ingestion of many preys (in turn exposed to MP ingestion) could lead to an increase in the probability of MP secondary ingestion and affect the diversity of ingested MP types. However, we have no tools to unequivocally confirm this speculation, which should be considered in future studies that should also provide new methodological approaches allowing MP detection and diet analysis simultaneously.

Further research will also be needed to consider the potential influence of fish conditions on MP ingestion. In this study, we did not find significant direct correlation between the occurrence of ingested MPs and the relative condition factor of individuals. Although MP ingestion seems not to affect individual body conditions in these species (Compa et al., 2018; Sbrana et al., 2020), considering *M. merluccius* and *Scomber* spp., we noted a correspondence between Kn and FO at the site-specific level. In particular, *M. merluccius* from Anzio (FO = 20.0%; Kn = 1.01 ± 0.06) and Ancona (FO = 33.3%; Kn = 1.05 ± 0.10) have lower values of Kn and significant higher values of FO compared to *M. merluccius* from Oristano (FO = 10.0%; Kn = 1.23 ± 0.12). Similarly, *Scomber* sp. from Anzio (FO = 50.0%; Kn = 0.86 ± 0.05) have very low Kn values and a very high FO, while the relative condition factor and FOs computed for samples from Ancona (FO = 26.7%; Kn = 1.14 ± 0.19) and Oristano (FO = 13.3%; Kn = 1.18 ± 0.07) are similar. Considering the degree of coastal anthropogenic pressure impacting Ancona and Anzio marine areas (see Subsections 2.1 and 4.1), MP ingestion seems to reflect the overall environmental conditions of the marine compartments exploited by the examined species.

Since a capillary data collection is crucial to increase the knowledge about the possible predictors and effects of MP ingestion by fish, the recording of data on biological parameters (*e.g.*, sex, maturity, and weight of liver and gonads) and the evaluation of fish conditions should be strongly recommended during monitoring activities.

4.4 MP typologies

The results of this study show that the types of MPs ingested by the examined species are different both according to the feeding habits and the sampling areas. Comparing species, *M. barbatus* tends to ingest smaller MPs, mostly filaments made of high-density polymers such as PEST, PET, and EDPM that, due to their physical characteristics, are more susceptible to wind mixing, vertical transport, and therefore sinking to the sea bottom (Woodall et al., 2014; Bergmann et al., 2017; Courtene-Jones et al., 2017; 2018). Therefore, it is not surprising to find these types of MPs in the GI of benthic feeder species like *M. barbatus*, which search for food in the sediment (Labropoulou and Eleftheriou, 1997). In contrast, the analysis of MPs ingested by *M. merluccius* and *Scomber* spp. reveals a predominance of polymers with lower densities (such as PE, PP, and Ethylene/Metacrilate copolymers) and a wider variety of shape-types and sizes. Overall, *Scomber* spp. showed the highest variability in ingested MP types in each sampling area. On the other hand, the between-sites differences were more relevant in *M. merluccius.* This could be likely related to the feeding habits of these species. Lopes et al. (2020) found that MP ingestion by *Scomber* spp. is significantly related to its diet composition, suggesting that a size-selection mechanism might drive MP ingestion in these species. Differently, the predatory behavior of *M. merluccius* might result in a higher incidence of secondary ingestion (see Subsection 4.3). Indeed, *M. merluccius* can be exposed to different MP types depending on the selectivity of its prey, which can vary among areas and life stages (Mahe et al., 2007).

All these results reinforce the hypothesis that a multispecies approach is needed to assess MP ingestion. There are many site-specific factors governing the distribution of MPs, including the presence of different contamination sources (*e.g.*, industrial, fluvial, urban) and oceanographic features (*i.e.*, wind stress, water depth, waves, tides, and currents). Furthermore, the bioavailability of different MP types could be affected by species-specific biological and ecological traits such as feeding habits, which vary considerably between different distribution areas. Further studies will need to clarify the relative importance of the several mechanisms affecting the complex interaction patterns between MPs and marine organisms. Particular attention must be paid to the selection of target species for monitoring programs. In fact, only an exhaustive description of the MP ingestion events occurring in an area will allow the collection of relevant and comparable data through time and space.

5. Conclusions

The development of monitoring programs is essential to reveal the effectiveness of measures that could be applied by policy to reduce plastic pollution, such as the ban of single-use plastic and the implementation of a wide educational plan to increase the awareness of new generations. Through a snapshot in time, this study provides a set of suggestions that can improve the future development of monitoring programs of MP ingestion, such as the ones required by the Marine Strategy Framework Directive for the European Seas. Our results confirm that fish species are suitable bioindicators of MP ingestion at a local scale., The relationship between MP ingestion frequency and the proximity to MP contamination sources (such as urban settlements, river flows, and shipping lanes) suggests that the marine environment is continuously subject to new MP inputs. Therefore, monitoring programs should investigate marine areas at different distances from land-based and sea-based human activities, including both highly anthropized sites and theoretically pristine areas. Furthermore, this study shows that different fish species can show different MP ingestion frequencies within the same marine area and even that biological and ecological traits of different species may affect the bioavailability of different MP types. Therefore, an ecologically relevant assessment of MP ingestion cannot be based the examination of only one species. A multispecies approach should be developed considering that the choice of target species can vary according to the complexity of the resident trophic web. Further research is needed to select appropriate target species within different marine areas and to develop a common strategy to obtain suitable and comparable data through time and space.

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CRediT authorship contribution statement

Tommaso Valente: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing – Original Draft; **Tania Pelamatti:** Investigation, Writing – Review and Editing; **Carlo Giacomo Avio:** Methodology, Investigation, Data curation; Writing – Review and Editing; **Andrea Camedda:** Data curation, Writing – Review and Editing; **Maria Letizia Costantini:** Supervision, Writing – Review and Editing; **Giuseppe Andrea de Lucia:** Supervision, Writing – Review and Editing; **Carlo Jacomini:** Writing – Review and Editing; **Raffaella Piermarini:** Resources, Project administration; **Francesco Regoli:** Supervision, Writing – Review and Editing; **Alice Sbrana:** Investigation, Writing – Review and Editing; **Daniele Ventura:** Supervision, Writing – Review and Editing; **Cecilia Silvestri:** Supervision, Project administration, Resources, Writing – Review and Editing; **Marco Matiddi:** Conceptualization, Supervision, Project administration, Resources, Writing – Review and Editing.

Declaration of competing interest

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

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Data statement

Data will be made available on request.

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