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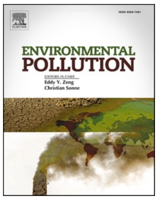

Trophic niche influences ingestion of micro- and mesoplastics in pelagic and demersal fish from the Western Mediterranean Sea☆

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ABSTRACT

Plastic pollution has been extensively documented in the marine food web, but targeted studies focusing on the relationship between microplastic ingestion and fish trophic niches are still limited. In this study we investigated the frequency of occurrence and the abundance of micro- and mesoplastics (MMPs) in eight fish species with different feeding habits from the western Mediterranean Sea. Stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) was used to describe the trophic niche and its metrics for each species. A total of 139 plastic items were found in 98 out of the 396 fish analysed (25%). The bogue revealed the highest occurrence with 37% of individuals with MMPs in their gastrointestinal tract, followed by the European sardine (35%). We highlighted how some of the assessed trophic niche metrics seem to influence MMPs occurrence. Fish species with a wider isotopic niche and higher trophic diversity were more probable to ingest plastic particles in pelagic, benthopelagic and demersal habitats. Additionally, fish trophic habits, habitat and body condition influenced the abundance of ingested MMPs. A higher number of MMPs per individual was found in zooplanktivorous than in benthivore and piscivorous species. Similarly, our results show a higher plastic particles ingestion per individual in benthopelagic and pelagic species than in demersal species, which also resulted in lower body condition. Altogether, these results suggest that feeding habits and trophic niche descriptors can play a significant role in the ingestion of plastic particles in fish species.

1. Introduction

It is now evident how human beings have profoundly changed the environment in recent decades. This new era renamed "Anthropocene" is characterized by climate change, loss of biodiversity and several sources and kinds of pollution. Plastic pollution in particular has become ubiquitous (MacLeod et al., 2021), leading to a growing scientific interest, especially for its impact on the marine environment (Bergmann et al., 2017; GESAMP, 2019). It has been estimated that more than 92% of all plastic items floating in the oceans are smaller than 5 mm, namely microplastics (van Sebille et al., 2015).

In recent years, concerns about the impacts of microplastics in the oceans include ingestion by marine organisms from different habitats, ranging from invertebrates to marine mammals (Jacobsen et al., 2010; Cole et al., 2013; Fossi et al., 2014; Bour et al., 2018; Giani et al., 2019; Thiele et al., 2021). Despite the global distribution of this phenomenon, the Mediterranean basin results as a critical accumulation zone for plastic items (Cózar et al., 2015; Vlachogianni et al., 2020). This is likely related to its nature of semi-closed basin with densely populated coasts high maritime traffic and its long water residence time (Lacombe et al., 1981).

Ingestion rates of plastic particles reported for Mediterranean fish species vary widely depending on the ecological features of the species (e.g., feeding habits, habitat, and trophic level) and the different

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foraging areas (Bellas et al., 2016; Compa et al., 2018; Digka et al., 2018; Pellini et al., 2018; Giani et al., 2019). Considering the growing evidence demonstrating the prevalence of plastic ingestion and its harmful impacts on fish species (Limonta et al., 2019; Wang et al., 2020), including the hypothesys of potential risks to human health (Wright and Kelly, 2017), identifying the main causes of different levels of ingestion among species is a crucial step of ecological relevance (Santos et al., 2021).

Most studies focus on documenting the presence of plastic particles in fish or on linking the theoretical feeding habits of marine organisms with the ingestion of microplastics. However, very few studies analysed the relationship between trophic niche features (such as trophic level, trophic niche width, and trophic niche diversity) and microplastic ingestion (Garcia et al., 2021; da Silva et al., 2022).

Selective predator species are known for feeding on a limited choice of prey and are likely to assume only the most advantageous ones, whereas generalist species, like filter-feeders and omnivores with higher trophic adaptability (*sensu* Gerking, 1994), are known for ingesting a wide range of preys with limited selection when foraging. Since the trophic transfer of microplastics has been documented in several studies, we compared plastic ingestion found at different trophic levels to consider both the direct ingestion of plastic items and the possibility of their trophic transfer from prey to predator.

Trophic niche features can be inferred through stable isotope analysis of carbon and nitrogen (δ^{13}C and δ^{15}N), which for decades has been successfully used in trophic ecology studies to determine the origin of the sources used, the trophic position and to quantify the width and diversity of isotopic niche (Fry, 2006; Jackson et al., 2011; Layman et al., 2012). Hence this represents a promising approach to study the trophic drivers of microplastics contamination.

Fish are good bioindicators of microplastic ingestion due to their availability, easy collection, high commercial and ecological value (Fossi et al., 2018; Bray et al., 2019). Therefore, in order to guide the selection of different sentinel species, it is necessary to understand whether the ingestion of microplastics varies according to the different habitat and the trophic niche features of the species. Moreover, as suggested by other authors, it is necessary to include more than one species as a bioindicator of plastic ingestion (Valente et al., 2022), Accordingly, in the present study, we selected four species (*Boops boops*, *Scomber scombrus*, *Merluccius merluccius* and *Mullus barbatus*) already confirmed as bioindicators for microplastics ingestion in different habitat (Garcia-Garin et al., 2019; Giani et al., 2019; Sbrana et a., 2020; Valente et al., 2022) and other four species based on several criteria such as habitat, trophic habits, commercial importance and sensitivity to litter ingestion based on the existent literature (Fossi et al., 2018). All the eigth fish species were collected from the North Tyrrhenian Sea (Western Mediterranean Sea): four pelagic (*Engraulis encrasicolus*, *Sardina pilchardus*, *Scomber scombrus* and *Trachurus trachurus*), three benthopelagic (*Boops boops, Merluccius merluccius* and *Micromesistius poutassou*) and one demersal species (*Mullus barbatus*). The European anchovy, *E. encrasicolus* (Linnaeus, 1758) and the European sardine, *S. pilchardus* (Walbaum 1792), represent two of the major resources of Mediterranean fisheries (Whitehead, 1990; Stamatopoulos, 1993). Several studies on these species have shown their ability to switch between filtering or particulate feeding mode, making these two species highly opportunistic (O'Connell, 1972; Bulgakova, 1996; van der Lingen et al., 2006, 2009; Borme et al., 2009; Costalago et al., 2015). The occurrence of microplastics ingestion in E. encrasicolus and *S. pilchardus* has been reported in many studies from different Mediterranean areas (Collard et al., 2015; Compa et al., 2018; Digka et al., 2018; Renzi et al., 2019). The Atlantic mackerel, *S. scombrus* (Linnaeus, 1758), occupies a crucial position in the Mediterranean trophic chain, as they are an important component of the diet of large pelagic fish (sharks, swordfish and tuna) (Zardoya et al., 2004). The feeding behavior and diet of *S. scombrus* are very complex; it can change annually, seasonally or daily, and with prey size and abundance (Óskarsson et al., 2016; Trenkel et al., 2014). Microplastic ingestion in this species has been previously reported (Lopes et al., 2020). The blue whiting, *M. poutassou* (Risso, 1827), is a key species in the marine food web as a food source for many species of fish (hake, mackerel, small sharks and horse mackerel) and various marine mammals (common dolphin, striped dolphin and bottlenose dolphin) (Würtz and Marrale, 1993; Rellini et al., 1994) but the microplstic ingestion in the Mediterranean area has been poorly assessed. The bogue, *B. boops* (Linnaeus, 1758), has been indicated as one of the potential small-scale bioindicators for microplastics in coastal waters for the Mediterranean Sea (Fossi et al., 2018; Bray et al., 2019; Tsangaris et al., 2020; Bottari et al., 2022). The European hake, *M. merluccius* (Linnaeus, 1758), represents one of the most important predators in the Mediterranean Sea (Carpentieri et al., 2005) and it has been reported to ingest microplastics (Giani et al., 2019). The red mullet, *M. barbatus* (Linnaeus, 1758), is a common demersal fish inhabiting sandy and muddy bottoms for trophic reasons, feeding mainly on benthic organisms (Demestre et al., 1998). The Atlantic horse mackerel, *T. trachurus* (Linnaeus, 1758) is a pelagic fish classified as omnivorous with a preference for faunal species (Stergiou and Karpouzi, 2002) and has been extensively studied for plastics ingestion (Chenet et al., 2021; Maaghloud et al., 2021).

Given the high concentration of plastic particles in the different ecosystems of the Mediterranean Sea and the key role of the selected fish species in the marine food web, the specific aims of this study were: i) to evaluate the frequency of occurrence (%) and abundance (items/individual) of plastic particles (either microplastics, <5 mm and mesoplastics, 5–25 mm) ingested by each fish species, also categorized by size, shape, colour and polymer; ii) to investigate the relationship between fish trophic characteristics (trophic levels, trophic habit, isotopic niche width and diversity), fish habitat (demersal, pelagic and benthopelagic), body condition and plastic particles occurrence and abundance; iii) to propose trophic niche parametrers based on stable isotopes analysis as an additional parameter to properly select bioindicator species for plastic ingestion. We hypothesized that fish species showing wider isotopic niches and higher trophic diversity are more likely to ingest plastic particles.

2. Materials and methods

2.1. Study area and fish sampling

This study was carried out in waters surrounding Elba Island (Tuscan Archipelago, Italy), located in the North Tyrrhenian Sea (NW of the Mediterranean Sea) which is part of the Geographical Sub-Area 9 (GSA9) identified by the FAO's General Fisheries Commission for the Mediterranean (GFCM) (Fig. 1). This region was identified as an area of great interest for commercial fishing (Sartor et al., 2003). The coastline of GSA9 is among the most urbanized and industrialized along the Italian coastline. Concerning plastic pollution, the Tuscan Archipelago area was found to be one of the spots for of floating macro- and microplastics in the Mediterranean Sea (Suaria et al., 2016; Baini et al., 2018).

Fish specimens were collected through bottom trawl and trammel fishing vessels operating in the North Tyrrhenian Sea in summer 2018. Sampling was carried out in a short-term time window to avoid possible environmental variables in the different trophic niches of the selected species. Sardines, anchovies, bogues, mackerel and horse mackerel were collected by professional fishermen using a pelagic trawl at a shallow coastal area. Red mullet, European hake and blue whiting were captured with a bottom trawl between 50 and 250 m of depth. Once fish were identified, habitat and trophic habit categories were assigned to each species, according to the relevant literature (see Table S1). All the sampled specimens were adult. For each specimen, the following information was recorded: fish biological parameters (total length of the specimen (cm), fork length (cm) and total weight (g)) as well as detectable deformations and external body conditions. The gastrointestinal (GI) tract of each specimen from the esophagus to the end of the intestine was removed and the muscle was stored for stable isotope

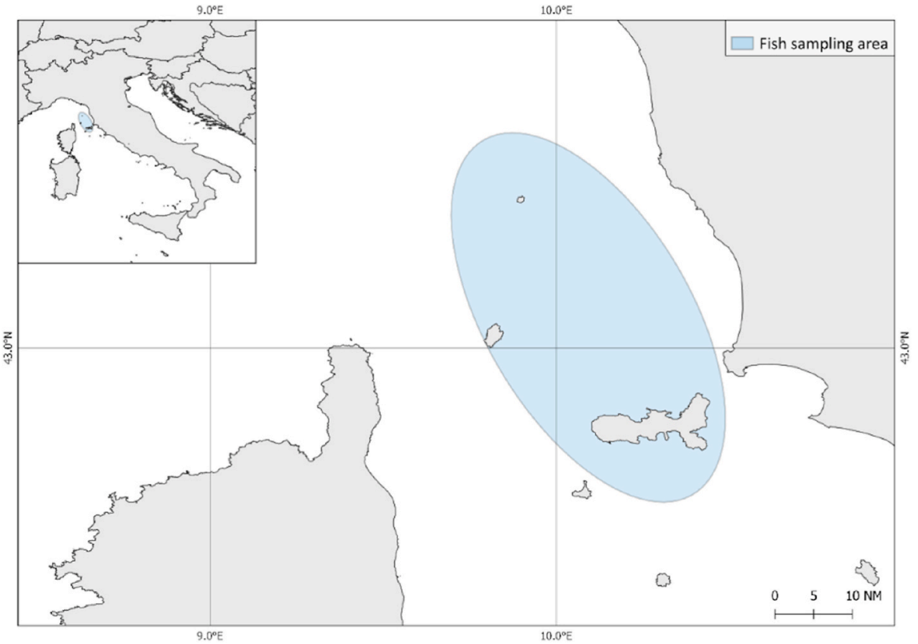

Fig. 1. Fish sampling area in the Tuscan Archipelago, North Tyrrhenian Sea (GSA-9).

analysis. Tissues were stored at −20 °C in rinsed aluminium foil for the subsequent laboratory analysis. Fulton's condition factor (K) was calculated for each individual following the equation $K = W*100\ l^{-3}$, where, W is the fish weight (g), l is the standard length (cm) and 100 is a factor used to bring the value of K near unity (Fulton, 1904).

2.2. *Plastic particles extraction and characterization*

All the recent studies underline the importance of the use of harmonized and standardized protocols for the detection of micro- and mesoplastics (MMPs) in biota to compare different studies and methodologies. For this reason, in our study we adopted the method suggested by Tsangaris et al. (2021) for the extraction of plastic items larger than 100 μm in fish species using KOH 10%. This method was a result of the interlaboratory exercise performed within the Interreg Med Plastic Busters MPAs Consortium. For a detailed description of the method of MMPs extraction, digestion of organic matter and particle characterization, see Supplementary material.

Polymer analysis was performed by Fourier Transform Infrared spectroscopy on an Agilent Cary 630 FTIR spectrometer, polymer recognition was accepted when a match to reference spectra had a level of confidence >80%. The smaller items and fiber shape particles were analysed with μFT-IR (Nicolet iN10 Thermo Fisher Scientific, Madison, WI, USA) to confirm the polymeric composition. The analysis was performed with an infrared microscope with a single mercury cadmium telluride (MCT) detector cooled with liquid nitrogen and equipped with a motorised stage. The analyses were carried out by acquiring the signal in reflection mode with the following setup: 16 scans at 4.0 cm^{-1} resolution and 4000-400 cm^{-1} spectral range, with the OMNIC™ Picta software (Thermo Fisher Scientific Inc.).

For each species, the results were expressed as: i) frequency of occurrence (%) of ingested plastics for each organism (percentage of analysed individuals with ingested plastic items); ii) abundance (number of ingested particles per individual for each species). Frequency of occurrence of the parameters expressing the characteristics of MMPs (shape, colours and dimension) were tested for discriminant factor analysis in R v4.0.3 (R Core Team, 2018).

2.3. *Quality assurance and quality control (QA/QC)*

Each analytical phase was conducted in a laminar flow cabinet and micro-filtered water (0.22 μm) was used in each step to minimize contamination by MMPs (especially fibers) present in the environment. All materials used and the fish GI tracts were rinsed three times with filtered water before use. To monitor any contamination during sample preparation and filtration, one procedural blank has been prepared for each two samples (flask containing only filtered water solution and KOH) which was subsequently filtered by the same process as for samples. In case of aerial contamination of procedural blanks, the same type of MMPs, depending on the shape, colour and size, was removed from the results. A Petri dish with a filter inside was placed under the laminar flow cabinet to monitor airborne contamination at each stage of the analysis. Only sterilized glass items were used for all the processes. It is important to point out that most procedural blanks (>90%) were clean. Those with potential airborne contamination at most contained a single fiber.

2.4. *Isotopic analysis*

To assess trophic niche features, a subsample composed by an equal number of individuals with and without ingested MMPs across the size range of each fish species was processed for isotopic analysis. Dorsal muscle was removed from each individual using a scalpel and tweezers, oven-dried at 60 °C for 48 h to constant weight, and ground with a micro mill to obtain a fine powder. Each sample was then encapsulated in tin cups and analysed for $\delta^{13}C$ and $\delta^{15}N$ by means of an isotope ratio mass spectrometer (Thermo Delta IRMS Plus XP) combined to an elemental analyser (Thermo Flash EA1112) (see Supplementary material for more details).

2.5. *Data analysis*

Trophic positions (TP) of fish species were estimated at individual level following Post (2002) equation: $TP_f = [(\delta^{15}N_f - \delta^{15}N_b)/\Delta_n] + \lambda$. $\delta^{15}N_f$ and $\delta^{15}N_b$ are the nitrogen isotopic signatures, respectively for the fish and the reference baseline. The mean signature of mesozooplankton samples previously collected around the Tyrrhenian Sea was considered as baseline for pelagic and benthopelagic species, and the value was 3.4

± 0.8‰ (unpublished data). The mean $\delta^{15}N$ value of the decapod *Goneplax rhomboides*, 4.3 ± 0.8‰, was considered as baseline of benthic detritus-based trophic web (Fanelli et al., 2009) and used for demersal species. Δ_n is the trophic enrichment assumed for each trophic level (3.4‰ in accordance with Post, 2002) and λ is the baseline's trophic position, which is two for both mesozooplankton and the decapod. Differences in trophic position between individuals with and without ingested MMPs were tested for each species through Student t-test. The relationship between fish trophic position and MMPs abundance (items/individual) found was evaluated through linear regression, using the fish trophic position as independent variable and abundance in each species as dependent variable.

To assess trophic niche features of groups of individuals with and without ingested MMPs, we calculated seven descriptive metrics of the isotopic niche. Particularly, the corrected standard ellipse area and the Bayesian standard ellipse area (respectively SEAc and SEAb) were estimated to describe the isotopic niche width (Jackson et al., 2011). Layman isotopic metrics (Layman et al., 2007) were calculated to characterize the isotopic niche and were: $\delta^{13}C$ Range (CR), $\delta^{15}N$ Range (NR), mean Distance to Centroid (CD), mean Nearest Neighbour Distance (NND) and its Standard Deviation (SDNND) (see Supplementary material for more detailed description of the metrics). All Layman metrics were bootstrapped (n = 10000, indicated with a subscript 'b') and their mode was reported along with the 95% credible interval (Jackson et al., 2011, 2012).

Differences between groups with and without ingested MMPs were tested for SEAb and the bootstrapped Layman metrics using a probability test (see Jackson et al., 2011). All the metrics were calculated in R v4.0.3 (R Core Team, 2018), using the SIBER package v2.1.5 (Stable Isotope Bayesian Ellipses in R) (Jackson et al., 2011).

To test for differences in MMPs abundance among fish species, fish living in different habitats and displaying different trophic habits, Generalized Linear Models (GLM) with Poisson distribution were applied to our data, setting MMPs abundance as response variable and the above-mentioned variables as explanatory variables. Furthermore, the influence of fish condition on MMPs abundance was also investigated, by applying the Generalized Linear Mixed Model (GLMM) with Poisson distribution, where the species were included as a random factor.

To assess the influence of trophic niche descriptors on MMPs occurrence (%) in fish with different trophic habits, a Hurdle Poisson GLMM was used. It consists of a two-part model which assumes a mixing distribution concerning both zeros and positives: the first part is a binomial probability model that verifies if a zero or non-zero result occurs, the second part is a truncated count data distribution that describes the positive outcomes. The zero-inflated model included the mean trophic position in the fixed part, to take into account also the information about the trophic position of the fish.

Finally, the relationship between trophic niche descriptors and the occurrence of MMPs characterized by size, colour and shape was modeled using a multinomial model, which is an extension of the polytomous case of a standard logistic regression. The total MMPs counts of each size, colour and shape by species were included in the model as a response variable and the mean trophic position of each species as the explanatory variable. All GLM, GLMM and multinomial models were run in R v4.0.3 (R Core Team, 2018).

3. Results

3.1. Plastic particles occurrence (%) and abundance

A total of 139 plastic particles were isolated in 98 out of the 396 fish analysed (25%) (Table 1). The particle dimensions ranged from 0.11 to 27 mm and 95% of the items were microplastics.

Among the eight target species, *Boops boops* revealed the highest

Table 1

List of analysed fish species with details on habitat and feeding habits (based on the relevant literature, see Table S1), sample size (n), range of the main biometric variables (total and fork length (cm), total and gastrointestinal weight (g)), mean Fulton's condition factor (K), MMP occurrence (% of analysed individuals with ingested plastic items) and total abundance of micro- and mesoplastic particles ingested (n).

Species	Family	Acronym	Habitat	Feeding habit	Sample size (n)	Total length (cm)	Fork length (cm)	Total weight (g)	GI weight (g)	Fulton's condition factor (K)	MMP occurrence (%)	Total micro-plastics (n)	Total meso-plastics (n)
Boops boops	Sparidae	BBO	benthopelagic	zooplanktivorous	68	15.5–25	13.2–19.2	39.4–99.2	1.7–10.3	1.46 ± 0.22	37	38	4
Engraulis encrasicolus	Engraulidae	EEN	pelagic	zooplanktivorous	77	11.3–13.9	10.1–12.8	9.05–17.96	0.3–1.9	0.80 ± 0.11	21	20	3
Merluccius merluccius	Merlucciidae	MME	benthopelagic	piscivorous	36	27.7–40.7	22.2–36.5	120.5–478.6	2.2–11.5	0.97 ± 0.08	8	3	0
Micromesistius poutassou	Gadidae	MPT	benthopelagic	zooplanktivorous	25	17.7–26.4	16.2–24.0	30.2–136	0.8–3.4	0.95 ± 0.14	24	5	1
Mullus barbatus	Mullidae	MBA	demersal	benthivorous	36	15.8–19	12.7–15.5	43–75.03	1–3.5	1.97 ± 0.16	17	6	0
Sardina pilchardus	Clupeidae	SPC	pelagic	zooplanktivorous	69	12.1–16.2	11.0–15.1	14.2–35.2	0.9–3.8	1.02 ± 0.23	35	35	2
Scomber Scombrus	Scombridae	SSC	pelagic	zooplanktivorous	42	21.5–28.9	20.5–27.0	92–210.7	5–19.6	1.08 ± 0.07	29	14	1
Trachurus trachurus	Carangidae	TTR	pelagic	piscivorous	43	13–24	12.0–21.8	20.9–103.3	1.1–3.5	1.06 ± 0.12	14	6	1

occurrence; 37% of individuals were found with MMPs in their GI tract, followed by *Sardina pilchardus* (35%). *Merluccius merluccius* showed the lowest occurrence of 8% (Table 1). *B. boops* and *S. pilchardus* showed higher MMPs abundance (number of particles/individuals) than most of the analysed species (i.e., *Engraulis encrasicolus*, *Mullus barbatus*, *M. merluccius* and *Trachurus trachurus*; GLM p-value <0.05, Table S2a), counting an overall average 0.6 ± 1.1 and 0.5 ± 0.8 items ind^{-1}, respectively (Fig. 2a).

Fish body condition was related with MMPs ingestion; specimens with a better physiological status (higher values of Fulton's condition factor) revealed a lower abundance of MMPs ingestion (GLMM p-value $= 0.02$, Table S3).

The most dominant particle size class, ranging from 1 to 2.5 mm, was found in *E. encrasicolus*, *M. merluccius* and *S. pilchardus* (respectively 57%, 49% and 67% of the isolated particles belonged to this class), followed by microplstics ranging from 0.5 to 1 mm in *Scomber scombrus* and *M. barbatus* (33 and 50%, respectively), and microplstics <0.5 mm in *B. boops* and *T. trachurus* (38 and 43%, respectively) (Fig. 2b). *M. merluccius*, the most selective predator, showed the highest percentage of plastic particles below 0.3 mm in length and exhibited less variability of MMPs size classes.

Concerning the shape, fibers represented 54% of the total isolated particles. Film shape was found only in the GI contents of *S. pilchardus*, *B. boops*, *S. scombrus*, which represented, as already mentioned, the species with the highest occurrence of MMPs (%) (Fig. 2c).

MMPs were also characterized according to colour, with transparent particles being the most abundant, accounting for 35% of the total. Transparent particles were the most widespread in pelagic and benthopelagic species (40% for *S. pilchardus*, 30% for *E. encrasicolus* and around 50% for *B. boops*), while black particles were dominant in the demersal and benthopelagic species (67% for *M. barbatus* and for *M. merluccius* respectively) (Fig. 2d).

Results of the discriminant factor analysis based on shape, colour and dimension of the MMPs, highlighted that the species *T. trachurus* exhibited a different pattern of plastic ingestion compared to the other species, as it ingested particles such as pellets, fragments, and brown plastic items. The analysis was significant for the Monte Carlo test (calculated from 999 replicates was 0.097 with p-value $= 0.007$) demonstrating that the species examined could ingest MMPs with different characteristics.

All the isolated items were identified by FT-IR and μFT-IR. Fig. 3 shows, in percentage, the revealed polymers. Most of the plastics identified were polyethylene (PE) (31%), followed by PET (30%), polypropylene (PP) (19%) and rayon (11%). Examples of the identified polymer spectra and images can be seen in Fig. 3.

3.2. Relationship between fish features, trophic niche metrics and MMPs ingestion

3.2.1. Occurrence (%)

Some of the assessed trophic niche metrics were found to influence MMPs occurrence in fish displaying different trophic habits or living in different habitats. Considering the investigated trophic habits (zooplanktivorous, benthivorous and piscivorous) as random factor, fish feeding on a wider diversity of sources (wider CR of the isotopic niche) showed higher MMPs occurrence, accounting for a 1.44% increase when CR increased by 1‰ (p-value = 0.03; see Hurdle Poisson GLMM output in Table S4a). Similarly, taking into account the habitats (demersal, pelagic and benthopelagic) as random factor, fish with a wider isotopic niche (SEAc, NR and CR) and higher trophic diversity (CD) showed higher MMPs occurrence, which accounted for 4.51% when SEAC increased by 1‰^{2}, and accounted for 1.60%, 1.89% and 6.33% when respectively NR, CR and CD increased by 1‰ (p-value <0.05 in all cases, see Hurdle Poisson GLMM output in Tables S4b–e).

A significant relationship was also found between fish trophic position (TP) and the MMPs size classes. Results of the multinomial model revealed that at increasing TP, the probability to ingest MMPs of size classes 0.5–1 mm increased compared to the probability to ingest MMPs

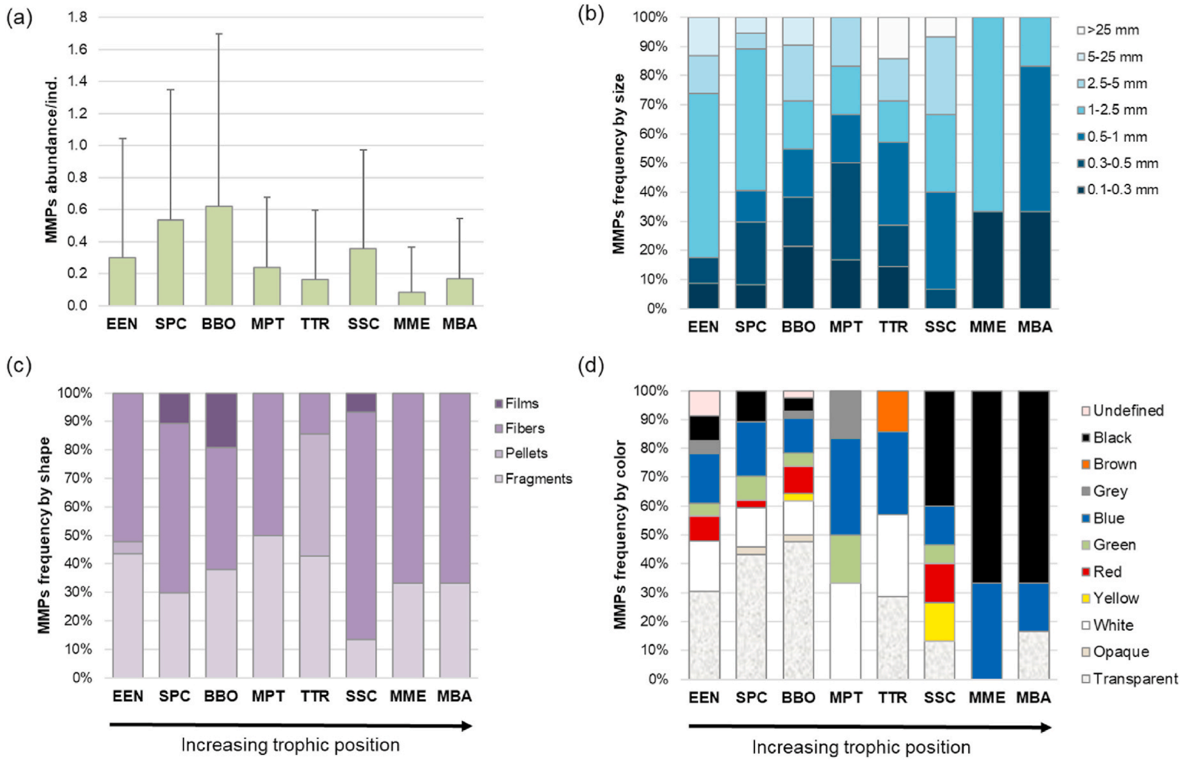

Fig. 2. Mean (±s.d.) number of MMPs ingested by the eight Mediterranean commercial fish species investigated (a) and corresponding MMPs occurrence (%) by size (b), shape (c) and colour (d). For species acronyms see Table 1. Species are ordered by increasing trophic position. Statistics of data reported in plot (a) is presented in Table S2.

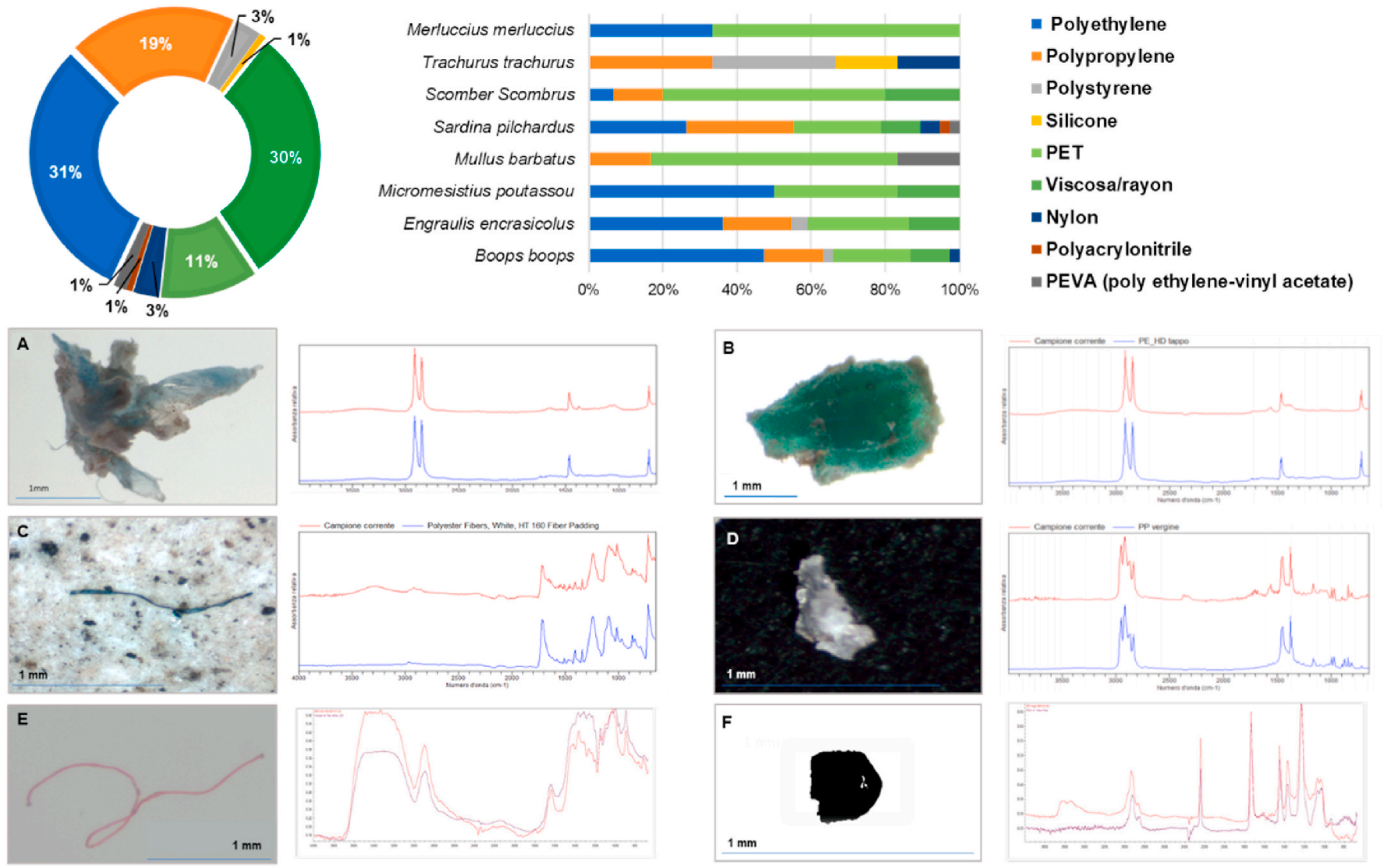

Fig. 3. Polymer composition of the analysed MMPs revealed by FT-IR for each species and examples of polymer spectra identified: Polyethylene (A, B), Polyester (C), Polypropylene (D), Rayon/viscosa (E) and PEVA (F).

of size classes <0.5 and 1–2.5 (p-value <0.05, see multinomial model output in Table S5a). The same model highlighted that overall, at increasing TP, the probability to ingest black MMPs was significantly higher than the probability to ingest transparent MMPs (p-value = 0.002, see multinomial model output in Table S5b).

3.2.2. *Abundance (items/ind.)*

MMPs abundance varied between fish species displaying different trophic habits or living in different habitats. Particularly, MMPs were more abundant in zooplanktivores than in benthivores (2.6-fold) and piscivores (3.5-fold) (GLM *p*-value <0.05, Table S2b). Accordingly, a significant negative relationship between fish trophic position and MMPs abundance (items/individual) was highlighted. The zooplanktivorous species at the lower trophic levels (*E. encrasicolus*, *S. pilchardus* and *B. boops*) ingested more plastic items per individual than bentivorous and/or selective predator species at the higher trophic levels (*M. barbatus* and *M. merluccius*) (F-value = 6.89, r^2 = 0.53, p-value = 0.039) (Fig. 4). In addition, MMPs were more likely to be ingested by benthopelagic species than by demersal ones (0.4-fold) (GLM *p*-value = 0.04, Table S2c).

The isotopic niche, expressed as a standard ellipse area (SEAc), varyied in width among species (Fig. 5). SEAc position within the isotopic space (the δ^{13}C -δ^{15}N biplot, Fig. 5) changed in accordance with fish trophic position, which ranged between 3.1 ± 0.4 for *E. encrasicolus* and 4.2 ± 0.4 for *M. barbatus* (Table 2).

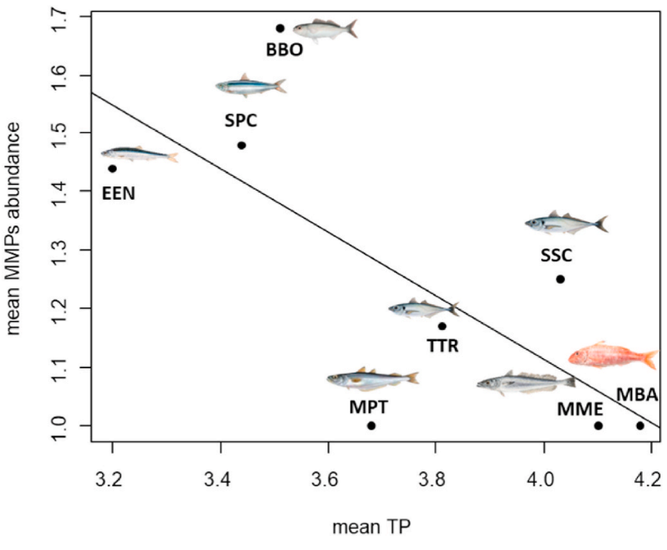

Fig. 4. Scatterplot between the mean trophic level (TL) calculated by isotope analysis and the average number of MMPs per individual of each species (EEN = *E. encrasicolus*, BBO = *B. boops*, SPC = *S. pilchardus*, TTR = *T. trachurus*, SSC = *S. scombrus*, MPT = *M. poutassou*, MME = *M. merluccius* and MBA = *M. barbatus*). Significant Correlation for Spearman Ranks (p = 0.039).

Overall, trophic features in terms of trophic position (TP), isotopic niche width (SEAc and SEAb), nitrogen and carbon range (NR and CR), trophic diversity (CD), redundancy (NND) and evenness (SDNND), did not change significantly between groups with and without ingested MMPs for the majority of the fish species. The only exceptions were given by *E. encrasicolus* and *M. merluccius*. *E. encrasicolus* specimens with MMPs showed a wider niche (expressed as SEAb), wider δ^{15}N range (NR) and higher trophic diversity (CD) than specimens without MMPs in the GI tract (Table 2). The opposite result was found for *M. merluccius*, although this should be taken with caution due to the small sample size.

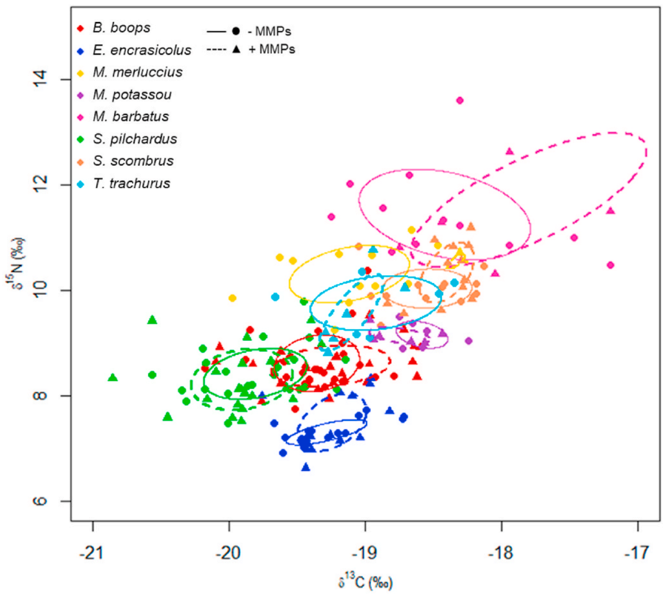

Fig. 5. Standard ellipse areas (SEAc) of fish species. Specimens of each species are differentiated according to the presence (+MMPs) or absence (-MMPs) of ingested plastics.

4. Discussion

4.1. Relationship between fish features, trophic niche metrics and MMPs ingestion

4.1.1. Occurrence (%)

The ingestion of plastic items was detected in all the eight investigated species. Twenty-five percent of the analysed specimens showed plastic items in their GI tract. Despite the relatively low number of species analysed per each habitat and feeding habit category, the findings of our case-study suggested that fish trophic niche metrics influenced the occurrence of MMPs ingestion, while fish trophic habits, habitat and condition affected the abundance of ingested MMPs. Our results reveal that different species from the same study area can show different microplastic occurrence and even that biological and ecological characteristics of the species may affect the bioavailability of different plastic items as confirmed by other authors (Valente et al., 2022).

As hypothesized, fish species with wider isotopic niche resulted to be more likely to ingest MMPs. As a result, *Boops boops* and *Sardina pilchardus* showed the highest occurrence of MMPs in their GI tracts (37% and 35%), in line with previous studies conducted in the same area of the Western Mediterranean Sea (Compa et al., 2018; Garcia-Garin et al., 2019; Sbrana et al., 2020; Tsangaris et al., 2020). The isotopic niche of these two zooplanktivorous species, in fact, displayed the widest carbon range (CR). Accordingly, even when looking at the species within each habitat, the benthopelagic *B. boops* and the pelagic *S. pilchardus*, showed the highest niche width (highest SEAc, CR and NR) and trophic diversity (CD).

This result suggests that the more the diet of a species is diversified, and the wider the diversity of basal sources used (CR) is, the higher is the

Table 2
Mean TP (trophic position, ±s.d.), SEAc (‰^{2}), SEAb (‰^{2}) and bootstrapped ($_b$) Layman metrics (NR, CR, CD, NND, SDNND) along with 95% credible intervals. Data are reported for groups of individuals of each fish species with and without ingested MMPs (+ and -). * Indicates significant differences between groups with and without ingested MMPs.

Species	MMPs and n° individual	TP	SEAc	SEAb	NR$_b$	CR$_b$	CD$_b$	NND$_b$	SDNND$_b$
Boops boops	- (25)	3.5 ± 0.2	0.5	0.5 (0.3–0.74)	2.6 (1.1–2.6)	1.4 (0.8–1.4)	0.5 (0.3–0.6)	0.2 (0.1–0.2)	0.2 (0.1–0.3)
	+ (25)	3.5 ± 0.1	0.5	0.5 (0.3–0.73)	1.6 (1.0–1.6)	1.5 (1.1–1.5)	0.5 (0.4–0.6)	0.2 (0.1–0.2)	0.1 (0.1–0.2)
Engraulis encrasicolus	- (17)	3.1 ± 0.4	0.2	0.2 (0.1–0.26)	0.8 (0.5–0.8)	1.0 (0.6–1.0)	0.3 (0.2–0.4)	0.1 (0.0–0.1)	0.1 (0.0–0.1)
	+ (14)	3.2 ± 0.2	0.4	**0.4*** (0.2–0.64)	**1.7*** (1.0–1.7)	0.9 (0.5–0.9)	**0.5*** (0.4–0.6)	0.2 (0.0–0.2)	0.2 (0.1–0.3)
Merluccius merluccius	- (13)	4.1 ± 0.4	0.8	0.7 (0.4–1.21)	1.9 (0.8–1.9)	1.5 (0.6–1.5)	0.6 (0.4–0.7)	0.3 (0.1–0.4)	0.2 (0.1–0.4)
	+ (3)	4.1 ± 0.0	0.0	**0.02*** (0.0–0.07)	**0.3*** (0.0–0.3)	**0.1*** (0.0–0.1)	**0.1*** (0.0–0.1)	0.2 (0.0–0.3)	**0.0*** (0.0–0.0)
Micromesistius poutassou	- (6)	3.7 ± 0.1	0.2	0.1 (0.1–0.33)	0.6 (0.1–0.6)	0.5 (0.1–0.5)	0.3 (0.1–0.3)	0.2 (0.0–0.3)	0.1 (0.0–0.3)
	+ (6)	3.7 ± 0.0	0.1	0.1 (0.0–0.23)	0.4 (0.1–0.4)	0.5 (0.1–0.5)	0.2 (0.1–0.3)	0.1 (0.0–0.3)	0.1 (0.0–0.3)
Mullus barbatus	- (14)	4.2 ± 0.4	1.7	1.5 (0.8–2.82)	3.1 (0.9–3.1)	2.0 (0.8–2.0)	0.9 (0.5–1.7)	0.5 (0.1–0.6)	0.3 (0.2–0.7)
	+ (6)	4.2 ± 0.4	2.8	2.4 (1.0–6.04)	3.4 (0.7–2.3)	2.3 (0.5–2.3)	1.2 (0.5–1.6)	1.1 (0.0–1.4)	0.5 (0.0–1.5)
Sardina pilchardus	- (25)	3.5 ± 0.1	0.6	0.5 (0.3–0.78)	2.3 (1.0–2.3)	1.4 (0.9–1.4)	0.5 (0.4–0.6)	0.2 (0.1–0.2)	0.2 (0.1–0.3)
	+ (23)	3.4 ± 0.2	0.7	0.6 (0.4–0.98)	1.9 (1.4–1.9)	1.5 (0.8–1.5)	0.6 (0.4–0.7)	0.3 (0.1–0.2)	0.2 (0.1–0.3)
Scomber scombrus	- (12)	3.9 ± 0.1	0.4	0.4 (0.2–0.65)	1.5 (0.3–1.5)	0.9 (0.5–0.9)	0.4 (0.2–0.5)	0.3 (0.1–0.3)	0.2 (0.1–0.3)
	+ (12)	4.0 ± 0.2	0.3	0.3 (0.2–0.55)	1.6 (1.0–1.6)	0.6 (0.2–0.6)	0.5 (0.3–0.6)	0.2 (0.1–0.2)	0.1 (0.1–0.3)
Trachurus trachurus	- (6)	3.9 ± 0.2	0.9	0.7 (0.3–1.68)	1.2 (0.3–1.3)	1.3 (0.1–1.3)	0.6 (0.3–0.6)	0.4 (0.0–0.8)	0.3 (0.0–0.6)
	+ (6)	3.8 ± 0.2	0.4	0.3 (0.2–0.91)	1.9 (0.4–1.9)	0.6 (0.1–0.6)	0.6 (0.1–0.8)	0.4 (0.0–0.6)	0.3 (0.0–0.8)

probability to incur in MMP ingestion. This is in accordance with Zhang et al. (2022), who indicated that the wide range of consumed food sources may be one of the factors inducing the microplstic ingestion in marine fish species in the western Yellow Sea. Consequently, this implies that higher trophic plasticity induces higher risk of MMP ingestion. The absence, or lower amount of MMPs in predator species such as *M. merluccius*, suggests that such small particles (<2.5 mm) if transferred from the prey, do not accumulate in predator species as also reported by Chagnon (Chagnon et al., 2018).

A similar outcome to this study, relating plastic ingestion with trophic niche width, was found for the diving seabird Long-tailed Duck *Clangula hyemalis*. This species showed higher ingestion of microplastic debris compared to other seabird species showing narrower isotopic niche width (Morkūnas et al., 2021).

Two very similar pelagic species such as sardine and anchovy showed different MMPs occurrence, respectively 35% and 21%. Several factors could determine these differences; like the morphology of the filtration system (Collard et al., 2017) and the different feeding strategy. Anchovy can switch more frequently from filter feeding to selective predation (Bulgakova, 1996; Tanaka and Takada, 2016; Collard et al., 2017) and showed a smaller isotopic niche width compared to sardine. Other studies have shown similar results, showing *S. pilchardus* as the most impacted species compared to the *E. encrasicolus* (Collard et al., 2017; Compa et al., 2018; Renzi et al., 2019; Rios-Fuster et al., 2019).

According to our findings, MMPs occurrence by size and colour seemed to be overall related to the trophic position of the species. In the analysed species the increased probability to ingest MMPs of size class 0.5–1 mm at higher trophic position might be more related to its feeding strategy and its preferential prey dimension other than MMP size classes availability in the marine environment. However, the direct comparisons between the size of isolated microplastics and the main prey ingested by fish species revealed some remarkable differences. If we look at the species at the lowest trophic position, *E. encrasicolus*, 60% of the isolated microplastics were between 1 and 2.5 mm in length, while the preys more frequently ingested by this species are small copepods between 0.3 and 0.6 mm (Borme et al., 2009). Anchovies usually do not target organisms <200 μm, ingesting large prey more frequently than sardines (Nikolioudakis et al., 2014) and switching from filter feeding to particulate feeding at a threshold prey size of 700 μm (James and Findlay, 1989). Consequently, it seems likely that the difference between the typical size of the prey and the ingested microplastics could result from a conscious choice of some specimens to actively select certain particles mistaken for exceptional food available with less energy effort.

The scenario changes radically looking at the other analysed species: the dimensions of ingested microplastics in sardine were smaller (about 30% of items were <0.5 mm length) than in anchovy and showed some overlap with the main preys of this species. This is maybe due to the frequent use of filter-feeding mode and the highest filtration area with closest gill rakers of sardines compared to anchovy increases the capacity to grab microplastics (Garrido et al., 2007; Garrido and van Der Lingen, 2014; Collard et al., 2017).

As the size of individuals increases, the size of the ingested particles should increase as well (Provencher et al., 2019). Jâms et al. (2020), in fact, found a strong positive relationship between marine organisms body length ranging from invertebrates to marine mammals, and plastic size.

However, no overlap has been observed between the main prey of piscivorous species such as *M. merluccius*, and *T. trachurus*, and the size of plastics isolated in their GI tracts. Small particles (<500 μm) were present in different percentages in all specimens of both species. It is therefore likely, that the smallest plastic particles could have been accidently ingested during feeding (through contaminated prey) or breathing activity, as evidenced by other authors (Li et al., 2021). Whereas in *B. boops* the accurate size range of its main preys is not well known. It has been observed how this zooplantivorous species in Mediterranean waters frequently feeds on portions of bigger organisms, such as the jellyfish *Pelagia noctiluca* (Milisenda et al., 2014) that has been found to accumulate plastic particles in both umbrella and oral arms (Albano et al., 2021). This active predation could also be confirmed by the similar colour of *Pelagia noctiluca* (transparent) and 50% of microplastics ingested by the bogue. A great variety of colours were found in the isolated particles. The occurrence of transparent particles dominates in species at lower trophic positions (*E. encrasicolus*, *S. pilchardus* and *B. boops*), whereas the occurrence of black particles dominates in species at higher trophic positions (*S. scombrus*, *M. merluccius* and *M. barbatus*). However, this result cannot be generalized since spatial variation in the ingested particles colours could reflect differences in the sources of plastics (de Haan et al., 2019). The prevalence of transparent particles may suggest that fish ingest MMPs without taking into account their colour, as transparent or white particles are not visibly different to fish (Peters and Bratton, 2016). For other dominant colours such as blue, it has been suggested that the incidence of ingested items could be due to these particles resembling some copepod species (Ory et al., 2017). Similarly, we hypothesize that the ingestion of brown plastics and pellets by *T. trachurus* may be due to a misidentification of these peculiar items with some of its common preys such as crustaceans or molluscs (Bayhan et al., 2013). However, to confirm this assumption, or to understand whether ingestion is unintentional, further analysis is necessary.

Comparing our results obtained in the Mediterranean Sea with data acquired in other oceans, a similar ingestion rate has been detected in the South Pacific Gyre (Markic et al., 2018) and in the North Pacific Gyre (Boerger et al., 2010). While North Sea and Arctic Sea species revealed very low microplastics ingestion occurrence, most of them ranged between 1 and 5% (Liboiron et al., 2016; Hermsen et al., 2017; Saturno et al., 2020) and 34% respectively (Morgana et al., 2018; Collard and Ask, 2021).

Regarding the shape of detected MMPs, no significant relationships emerged between their occurrence and trophic niche features, but fiber shape was dominant, representing 54% of the total isolated MMPs. For demersal species (*M. barbatus*) the occurrence rise up to about 67%. These results are in accordance with previous studies that reported fibers as the dominant shape of plastic items in the fish GI tract (Bellas et al., 2016; McGoran et al., 2017) and in the water column (Rios-Fuster et al., 2022). It has been estimated that plastic microfibers account for 35% of the total environmental microplastics (Cristaldi et al., 2020). However, fibers are not always considered microplastics and are thus excluded from the results as they could originate from airborne contamination (Dris et al., 2016; Giani et al., 2019). Moreover, some authors support the theory that fish species do not actively capture microfibers; instead, they passively uptake microplastics while sucking in microfibers while breathing (Li et al., 2021). Our results confirm the widespread distribution of fiber-shaped plastic particles in marine fish species as an emerging pollutant. Further research concerning this pollution should aim to the development of advanced techniques to solve this problem such as the efficient monitoring of the contamination sources and the improvement of cost-effective and efficient remediation technologies.

4.1.2. Abundance

Abundance of MMPs particles found in fish (items/individual) was significantly related to species trophic habit, habitat, trophic position and body condition, despite the inherently low number of plastic items per individual found in our wild samples.

Zooplanktivorous species revealed a higher abundance of ingested MMPs than benthivorous and piscivorous species. This is in agreement with the significant negative relationship found between microplastics abundance and trophic position. Zooplanktivorous species at the lower trophic levels (anchovy, sardine and bogue) showed more ingested MMPs per individual than benthivorous and/or selective predator species at the higher trophic levels (red mullet and European hake). However, we should take our results carefully since in the literature there are studies based on different approaches (e.g. stomach content analysis) which basically reported the opposite results, with planktivorous species (European sardine and bogue) showing lower microplastic concentration in the GI tract than fish depending on larger prey such as *Scomber scombrus* (Lopes et al., 2020). Our result, together with the high MMP abundance found in benthopelagic and pelagic species respect to demersal species might be related to the interplay of a series of factors. For example, it might be related to the floating capacity of MMPs which persist for a long time in the water column before sinking, hence to the availability of such plastic source. Additionally, the heterogeneous distribution of plastic items throughout the water column, with the highest concentration in the upper levels and an exponential decrease with depth could contribute to the different MMP uptake from the water column (Goldstein et al., 2013; Reisser et al., 2015). Also, species which feed by suction, have been demonstrated to ingest MMPs unintentionally (Li et al., 2021). Another factor influencing the abundance of ingested MMPs seems to be fish body condition. Our results show that lower biological condition index (Fulton's condition factor), was significantly related to the high abundance of ingested MMP particles. Our outcome is in line with several recent studies that underline how significantly higher Fulton's factor values were observed for fish that had not ingested plastics (Compa et al., 2018; Cardozo et al., 2018; Filgueiras et al., 2020; Li et al., 2021). Santos and co-workers hypothesize that since starvation induces poor foraging performances, the risk of plastic ingestion increases with increasing starvation. As a consequence, an increase in opportunistic feeding behaviours might be expected to the point of considering plastic items as the preferred food option (Santos et al., 2021). We argue that this could probably be related to the higher capability of healthy fish to select optimal feeding sources compared to starving fish that tend to feed on a broader variety of resources to increase the probability to ingest more food, hence increasing the probability to incur in MMP ingestion. Hileman et al. (1994) have pointed out that avoidance of unpalatable prey decreased with increased starvation, with hungrier predators feeding on unpalatable prey more often. Nevertheless, some authors oppose the correlation between plastic ingestion and body condition factor, arguing that since only single particles per individual were often found, there is no possibility of accumulation and therefore minimal effect on fish health (de Vries et al., 2020a, b).

Therefore, our hypothesis is that there may be two possible routes of uptake for microplastics ingestion: the unintentional or accidental ingestion of smaller items and the active selection of larger particles, the latter probably related to the ease of capture and the state of health of every single specimen.

After all, based on our results, we found that isotopic niche features were linked to plastic ingestion in fish species, suggesting the application of our framework to the feeding ecology context and to species-specific foraging in marine food webs.

5. Conclusion

In this study we evaluated microplastic ingestion and fish feeding habits using trophic niche metrics in fish species from different habitats. We highlighted how trophic factors and different habitats increase the risk of microplastic ingestion in marine fish species. Based on their feeding strategy and habitat preferences, *Engraulis encrasicolus* (pelagic), *Sardina Pilchardus* (pelagic) and *Boops boops* (benthopelagic) are suggested as the most suitable bioindicator fish species for monitoring MMPs ingestion in Mediterranean Sea.

In conclusion, our results highlighted the difficulty in predicting the ingestion of MMPs in fish species, since it is driven by a complex mix of factors. We underline how a fish specimen in good health status (based on Fulton's condition factor), with a higher trophic level and a very selective feeding strategy is less likely to ingest microplastics.

We have provided evidence of the interaction between feeding habits of fish species and plastic ingestion; however, the complexities of these relationships, that possibly experience a certain variability at a larger temporal and geographical scale, need further examination. Linking these two different fields, future research should focus on monitoring strategies which consider both seasonal and spatial variation of the complex realtionship between fish trophic ecology and plastic patterns in different environmental compartments. Indeed, further studies would benefit from increasing the variety of species examined per habitat and trophic habits to allow stronger generalization of outcomes linked to the considered biological or ecological characteristics. To achieve this, the application of stable isotope analyses is essential to improve our knowledge of plastic ingestion by marine species.

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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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2023.121632.

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