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Ask the shark: blackmouth catshark (*Galeus melastomus*) as a sentinel of plastic waste on the seabed

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Abstract

The presence of plastic waste in the marine environment has driven the scientific community to make significant efforts to study and mitigate its possible effects. One of the critical aspects is to determine if and how an increase in ingestion events may occur as a result of the accumulation of plastic waste on the seabed. In this study, *G. melastomus* is examined for its ability to indirectly provide information on the amount of macroplastics accumulating on the seafloor. Plastic ingestion is explored by describing the feeding habits of the species, which have the potential to provide very useful information regarding biological or ecological issues. The diet of *G. melastomus* mainly consisted of cephalopods, bathypelagic fishes, and decapod crustaceans, increasing in diversity during growth. The generalist-opportunistic feeding behaviour of this species leads to the incidental ingestion of plastic particles ($N = 47$, with a mean ($\pm$ SD) of 1.47 ± 0.28 per specimen) which can be indirectly associated to the presence of macroplastics on the seafloor. Indeed, our results indicate a significant relationship between the amount of macroplastics present on the seabed and the frequency of ingestion of plastic particles by blackmouth catshark. We propose *G. melastomus* as an excellent candidate for developing a valid monitoring strategy for the presence of plastics on the seabed, as requested by the EU Marine Strategy Framework Directive.

Keywords Plastic pollution · Mediterranean Sea · Biomonitoring · *Galeus melastomus* · Diet

Introduction

Over the last century, plastic production has grown exponentially as a result of its versatility, durability, malleability, and low price, becoming part of everyday life and delivering social benefits (Geyer et al. 2017; Boucher et al.

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2020). The development and diffusion of plastic materials have led to inevitable consequences with repercussions on waste management, becoming a topic of discussion in recent years (Thompson et al. 2009; GESAMP 2019; Markic et al. 2020). The release of plastic wastes affects both terrestrial and aquatic ecosystems, contributing to environmental pollution (Kumar et al. 2021). Irrational production, inappropriate disposal at landfills, and insufficient recycling lead to plastic becoming waste. And the waste, sooner or later, comes to sea (Eriksen et al. 2014). In the case of improper discharge, the plastic enters waterways and into the oceans, causing marine plastic leaks (Boucher et al. 2020). A total of 35% of plastic leakage is attributed to the cities and towns located directly on the coast (Boucher and Billard 2020). The remaining 65% is generated from the inland and comes into sea water by surface run-off (Boucher and Billard 2020). An important contribution of plastics also comes from rivers, with the highest leakage rates from catchment areas (basins) hosting large rivers (Hurley et al. 2018).

The abundance, persistence, and ubiquity of plastic in seawaters have made this pollutant one of the most controversial topics in recent years, representing a serious threat to marine organisms. A wide range of animals can ingest plastic particles once they reach the sea (Cau et al. 2019; Markic et al. 2020; Capone et al. 2020; Santos et al. 2021), either intentionally—by mistaking plastic particles for natural prey, or accidentally—by ingesting items already consumed by their prey (Bergmann et al. 2015). Most of the plastic seems to be accumulated on the seafloor in form of microplastics (MiP < 5 mm), or macroplastics (MaP 25–1000 mm), or mesoplastics (MeP 5–25 mm) (GESAMP 2019) with very heterogeneous and complex patterns (Reisser et al. 2015; Franceschini et al. 2019; Tsiaras et al. 2021). These accumulation areas increase the risk of coming into contact with the pollutant, especially for species that live or feed near the seafloor (Alomar et al. 2020; Franceschini et al. 2021).

Plastic ingestion is associated with a variety of negative health effects. Macroplastics may provoke blockages or internal bleeding in the intestines of the species (Van Franeker et al. 2011; Camedda et al. 2014; Provencher et al. 2017; Domènech et al. 2019). The smaller pieces of plastic (MiP or MeP), on the other hand, can have indirect effects such as decrease of mobility, reduction of feeding and growth, and worsened body condition (Valente et al. 2019; Markic et al. 2020; Sbrana et al. 2020). In addition, plastic additives may induce physiological alteration, such as endocrine functions (Flint et al. 2012; Rochman et al. 2014). Several studies have sought to understand the relationship between ingestion of plastic and environmental contamination, with very promising results. A study conducted in coastal areas of the Western Mediterranean Sea found a correlation between the amount of plastic in the digestive tract of the demersal to semi-pelagic species, *Boops boops,*

and the level of pollution in the environment, using an index based on several factors, including population density, river inputs, distance from the coast, and shipping routes (Sbrana et al. 2020). More recent studies revealed that proximity to hotspots of macroplastics accumulation significantly correlates with microplastic ingestion by *Nephrops norvegicus* (Linnaeus, 1758) (Cau et al. 2019, 2020; Franceschini et al. 2021). It is worth noting that there is a growing trend of evidence that marine plastics pollution contaminates key stocks for fisheries, with potential negative consequences to human health, resources status, and socio-economic sectors (Rochman et al. 2015; Lusher et al. 2017). Numerous studies have suggested also that plastics in the marine environment can adversely affect sharks (Anastasopoulou et al. 2013; Cartes et al. 2016; Alomar and Deudero 2017; Bernardini et al. 2018; Valente et al. 2019). In marine food webs, elasmobranchs play an important role as apex predators and seem more frequently affected by plastic ingestion than bony fishes within deep-water habitats (López-López et al. 2018). Therefore, sharks and rays could be considered as sentinel species for marine pollution biomonitoring studies (Alves et al. 2016; Fossi et al. 2018).

The Mediterranean Sea, considered as one of the most threatened environments in the world, is also subject to this ubiquitous pollutant (Eriksen et al. 2014; Cózar et al. 2015; Suaria et al. 2016; Zambianchi et al. 2017; Liubartseva et al. 2018; Tsiaras et al. 2021). The total plastic accumulated in the Mediterranean Sea is estimated at 1,178,000 tonnes, with an annual marine leakage of 229,000 tonnes. Based on these data, Italy (and Rome locality) appears to be one of the three top contributors of plastic leakage in the Mediterranean Sea (Boucher and Billard 2020).

The spread and potential impacts of plastic waste in the marine environment are a global emergency that is pushing the scientific community to make a huge effort to understand and help stem these phenomena (Thompson et al. 2009; GESAMP 2019; Santos et al. 2021). As plastic is undergoing, even in the marine environment, a phenomenon of miniaturization and degradation that makes it increasingly less traceable (Chamas et al. 2020), it is crucial to enhance the monitoring of macroplastics and their role in the process of environmental contamination. At the same time, plastic can impact biota through accidental ingestion (Savoca et al. 2019). Therefore, it is paramount to understand whether and how the accumulation of plastic waste on the seafloor can lead to increased ingestion events. However, given that marine pollution has also reached very high levels in the Mediterranean and larger areas, it is very complicated and costly to make spatial and temporal assessments of the different levels of contamination (Galgani 2015).

The blackmouth catshark *Galeus melastomus* (Rafinesque, 1810) is a demersal bottom dwelling species, inhabiting continental shelf breaks, and slope habitats (common

depth range: 150–1200 m). As a generalist meso predator, it plays an important role in the deep-water food web in providing a link between the upper and lower trophic levels (D'iglio et al. 2021). *G. melastomus* is the most abundant species of Selacii in the Mediterranean Sea (Bradai et al. 2012). The species has a considerable part of its population living in the Mediterranean beyond the deepest boundary trawled by vessels (Peristeraki et al. 2020). Indeed, the species and especially its reproducers, can live up to 1600 m, being abundant still at 1400 m (Carrassón et al. 1992; Cartes et al. 2016). In this way, the deepest part of its spatial distribution is outside the range of activity of bottom trawlers and protected by direct fishing exploitation, contributing to the stability of the whole stock. This species is therefore an ideal candidate for monitoring plastics distribution on different fishing grounds (Fossi et al. 2018).

In this context, this study aims to: I) examine the stomach contents of *G. melastomus* to reconstruct its feeding ecology; II) evaluate and characterize the plastics ingested by the species, to detect sources of plastic pollution; III) relate plastics on the seabed and the ingestion of plastics to determine whether it can provide useful information about the amount of macroplastics accumulating on the seafloor.

Materials and methods

Study area and collection of samples

Specimens of *G. melastomus* were collected in the central Tyrrhenian Sea (FAO Geographical Sub Area 9—Western Mediterranean Sea), in summer 2020–2021. This area has a very narrow continental shelf, characterized by fine sands and muddy bottoms (Ardizzone et al. 2018). The coastal area is heavily populated with large urban centres, industrial settlements, and important ports, being close to Rome city. The Tiber River run-off significantly affects the chemical-physical traits of the area, also bringing contaminants such as plastic debris (Crosti et al. 2018). Samples were collected both on board of a commercial bottom trawl fishing vessel and during the MEDITS (MEDiterranean International bottom Trawl Survey) experimental trawl surveys. Based on a stratified random sampling design, 29 fishing hauls were collected over 29 sampling locations (Fig. 1), covering the major fishing grounds in the study area. In this way, sampling sites mirror the fishing footprint of the Italian fleet operating in the GSA9 (Russo
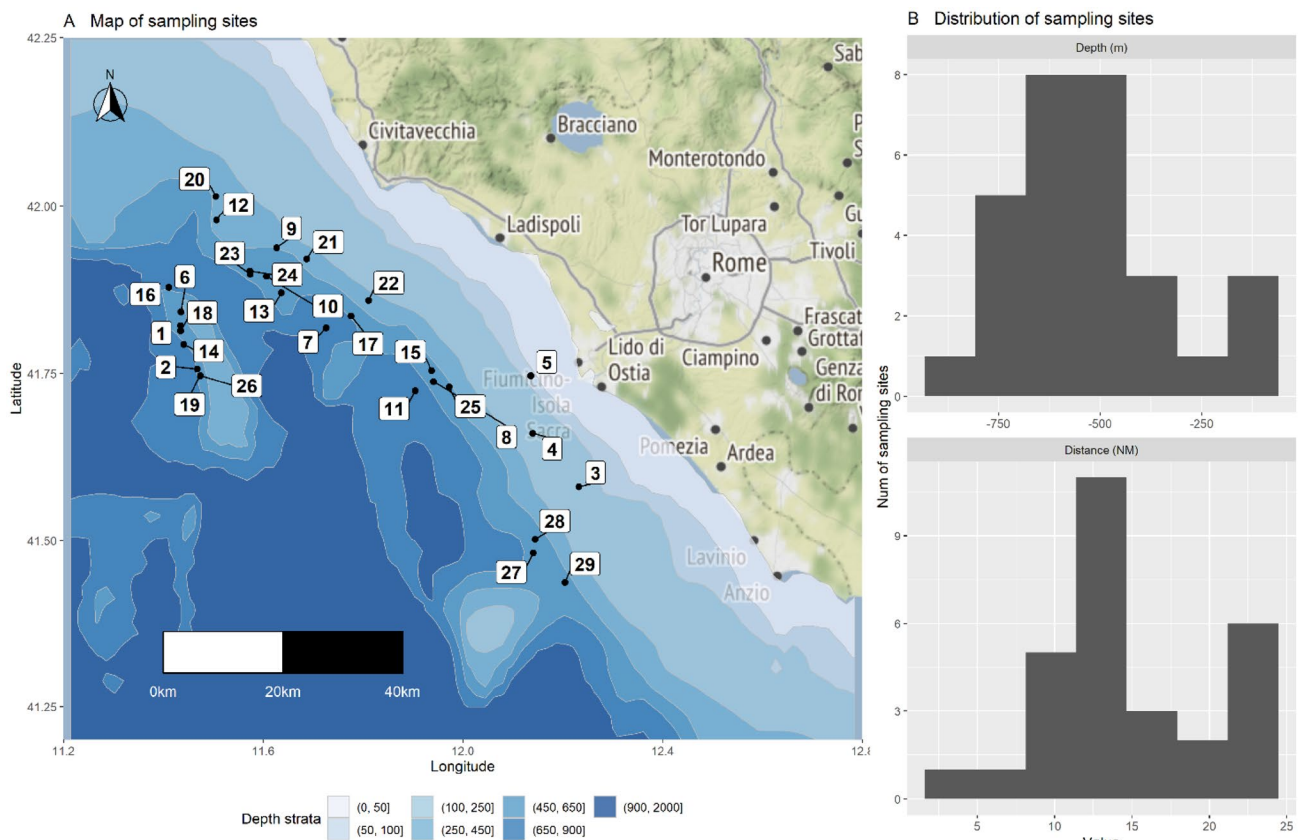

Fig. 1 Map of sampling sites of *Galeus melastomus* (**A**), histogram of haul distribution by depth (**B**) and haul distance from the coast (**C**) in the central Tyrrhenian Sea

et al. 2019). The specimens of *G. melastomus* collected at each sampling site were immediately separated during the sorting of catch, frozen, and stored at -20 °C on board of the vessel and, successively, transferred to the laboratory for analyses.

Laboratory analyses

Once thawed to room temperature, basic measurements were recorded for each fish, including total length (TL, mm), body wet weight (TW, g), gastrointestinal wet weight (GIW, g), sex (F: Female and M: Male) and maturity stage (1: immature virgin, 2: maturing, 3a: mature, 3b: mature/extruding) assigned according to (Follesa and Carbonara 2019). Gastrointestinal tracts (stomach and intestine with their contents and wall) were removed by dissection from each fish and stored individually in glass beakers. The total volume of stomach contents was recorded for each individual. Preys within the stomach were identified at the lowest possible taxonomic level (decapod crustaceans, fish, and cephalopods were usually identified to species) using specific taxonomic literature (e.g. Clarke 1986; Riedl and Abel 1991; Falciai and Minervini 1992; Tuset et al. 2008). For each *G. melastomus*, the following diet data were recorded: the percentage of stomach volume, the number, and the weight of prey items. Finally, plastics were identified and classified in shape: fragment, film, sphere, and including the distinction between fibre and filament, as reported by Matiddi et al. (2021); colours, and subdivided into three size classes (330 μm–1 mm; 1–5 mm; 5–25 mm). Plastics identifications were performed considering: (i) the resistance of the particles to the contact with tweezers; (ii) the absence of biological structures; (iii) either typical skewed shapes and crooked edges or uniform thickness; (iv) distinctive colours. Furthermore, we considered plastic items those that were showing a dark sticky mark when touched with a hot needle (Hermsen et al. 2018).

During sample processing within the laboratory, best practice contamination control measures were applied to reduce the risk of contamination. In all laboratory analyses, a 100% cotton lab coat was worn at all times, and all equipment and surfaces were thoroughly washed with 70% ethanol and/or rinsed with water (GESAMP 2019). Plastics found in the samples were compared with equipment used during all procedural steps, based on their characteristics (polymer, colour, and type). Additionally, fibres detected in samples were excluded from the analysis due to the inability to prevent efficiently their background contamination (Torre et al. 2016).

After that, polymer detection was obtained using Nicolet iS10 Fourier Transform Infrared Spectroscopy with Attenuated Total Reflection (FT-IR ATR, Thermo Fisher Scientific, Madison, WI, USA). FT-IR was used to identify objects greater than 1 mm in size in 10% or more of the particles collected in this study, according to guidelines developed by the MSFD technical group on marine litter (Galgani et al. 2013). The identification was based on the comparison among the analysed IR spectra and known polymer spectra libraries ("HR Spectra Polymers and Plasticizers by ATR", "HR Hummel Polymer and Additives", "HR Polymer Additives and Plasticizers"), provided with software OMNIC 9.8.286 (Thermo Fisher Scientific Inc.). The level of certainty of the comparison between the sample spectrum and the polymer library database for all analyses was set up to a minimum of 70%.

Seafloor macroplastics were collected through MEDITS trawl campaign (143 sampling sites from 2013 to 2019). The survey follows a stratified random sampling design, proportional to bathymetric strata (i.e. 10–50, 51–100, 101–200, 201–500, and 501–800 m). Information on the plastic litter was recorded in terms of total weight, the total number following the procedural protocol by Fiorentino et al. (2013). The amount of marine litter occurring at each sampling site was defined according to MEDITS protocol as the distance-weighted mean of the amount of litter in the sampling sites of the MEDITS campaign. We adopted this approach considering that: (1) the MEDITS protocol for litter assessment represents an acknowledged and powerful source of information for the distribution of MaP litter in the Mediterranean Sea; (2) the distribution of the MEDITS sampling sites always allowed to use from 3 to 14 MEDITS sites to estimate the amount of litter in the sampling sites of this study.

Data and statistical analyses

The feeding incidence (%FI = individuals with identifiable prey remains/total number of fishes × 100) was used to evaluate the rate of feeding activity, whereas the degree of stomach fullness was estimated by the fullness index = GIW/TW, being GIW the gastrointestinal wet weight and TW the body wet weight. To assess the importance of prey items in the diet of *G. melastomus*, the following dietary indices were calculated: relative abundance (%N = number of individuals of prey i/total number of prey × 100), weight percentage (%W = weight of prey i/total weight of all prey × 100), volume percentage (%Vol = volume of prey i/ total volume of all prey × 100), and frequency of occurrence (%FO = number of stomachs containing prey i/ total number of stomachs containing prey × 100). Based on the Costello graphical method (Costello 1990) modified by Amundsen et al. (1996), the feeding behaviour of *G. melastomus* at different length classes (size) was determined by plotting prey-specific abundance (%Vol) against the frequency of occurrence (%FO) on a two-dimensional graph. The position of prey types in the two-dimensional plot can be used to infer precise information regarding prey importance, feeding strategy, and niche size contribution, according to Amundsen et al. (1996).

Correspondence analysis (CA) was used to graphically inspect the variation in diet (preys volume abundance) and relate the preference towards different food items according to depth, size, and sex. Also, non-parametric multivariate PERmutational ANalysis of VAriance (PERMANOVA) was applied to test for differences in *G. melastomus* diet according to different dwelling depths, size, and sex. To do so preys volume abundance was used as the response variable, using depth, size, and sex as explanatory factors.

For each prey species a trophic level (TrL) was assigned using data from the FishBase database for fishes (http://www.fishbase.org) or SeaLifeBase (http://www.sealifebase.org) for invertebrates. To estimate individual TrL of *G. melastomus*, the mean TrL of prey items was proportionally weighted based on their contribution to the diet (weighted mean of TrL by the volume occupied by each prey), aiming at estimating the instantaneous Trophic level of each "meal" (Cortés 1999), a snapshot of the short-term diet of *G. melastomus* individuals. Trophic levels of each prey were listed in Table 2.

Microplastic abundance was expressed as the number of microplastic items found in every single gut. The frequency of occurrence (FO%) of ingested microplastics was computed as the percentage of individuals examined containing at least one microplastic upon the total sample.

Generalized Additive Models (GAMs), a non-parametric extension of GLMs that includes smooth functions (a piecewise polynomial curve) of explanatory variables (Leathwick et al. 2006), were used to evaluate whether different factors including fish biometric parameters, the weighted mean of the trophic level of each prey, characteristics of the sampling sites, and the amount of MaP on the seafloor explain variations in the frequency of plastics ingested by *G. melastomus*. The R packages mgcv (Wood 2017) was used to fit a series of models in which the number of plastic items ingested by each specimen of *G. melastomus* represented the dependent variable and the following predictors:

- Fish length
- Fish sex
- Depth of the sampling site
- Distance from the coast of the sampling site
- Trophic Level
- The stomach fullness
- Amount of MaP as the number of items per Km^2;
- Amount of MaP as the weight of items per $Km.^2$

All these potential predictors were evaluated using a model selection on all the possible combinations of them. The Quasi-Poisson distribution was used to model the distribution of the dependent variable (number of plastic items ingested). The Quasi-Poisson distribution is a generalization of the Poisson distribution and is commonly used to model overdispersal count (discrete) variables when the variance is greater than the mean (Zeileis et al. 2015). Model selections were based on the information-theoretic approach (Burnham and Anderson 2007) by comparing models AICs (Akaike's Information Criterion; Akaike 1974).

Results

A total of 164 *G. melastomus* were collected in the central Tyrrhenian Sea: 31 stomachs were empty or regurgitated (19%) and 133 contained food (%FI = 81%). Overall, stomach fullness ranged between 0.01 and 0.36 with a median value of 0.06. The analysis of the length-frequency distribution for this species indicated that the samples contained individuals of three distinct length classes, defined according to the natural breaks in the observed distribution (15–25 cm, 26–35 cm, 36–55 cm). Details on biometrics and stomach contents are presented in Table 1, where: the number of individuals, sex, fullness, and frequency of empty–full stomachs are summarized for each length class.

Table 2 presents the prey list and dietary index values (%Vol, %N, %FO, and TrL) for each food item. A total of 28 prey taxa were found in the stomach contents. Most of the diet consisted of Cephalopoda (Histioteuthidae and Sepiolidae), fishes (both bathypelagic species, as Myctophidae and Stomiiformes benthic species, such as Pleuronectiformes and demersal species, such as Gadiformes), and Crustacea (Decapoda and Euphausiacea). In general, the number of preys was lower in the smaller individuals (length class: 15–25 cm) than in the other length classes (pie charts of

Table 1 Information on biometric characteristics [n. of individuals; sex (Female: F and Male: M)], and stomach fullness, the percentage of empty stomachs (% Empty), and full stomachs (% Full) recorded for each length class (15–25 cm; 26–35 cm; 36–55 cm) of *Galeus melastomus* collected in the central Tyrrhenian Sea

Length class (cm)	N. individuals	Sex		Fullness	Prey items	
		F	M		% Empty	% Full
15 – 25	23	13	10	0.11	30.43	69.57
26 – 35	41	18	23	0.07	17.07	82.93
36 – 55	100	78	22	0.07	16.00	84.00

Table 2 Diet composition of *Galeus melastomus* and percentage values of dietary indexes calculated for each prey item: volume (%Vol); abundance (%N); frequency of occurrence (%FO); and trophic level according to FishBase or SeaLifeBase (TrL)

Prey	%Vol	%N	%FO	TrL	Prey	%Vol	%N	%FO	TrL
Cephalopoda					**Osteichthyes**				
Oegopsida					Anguilliformes				
Histioteuthidae					Nettastomatidae				
Histioteuthis bonnellii	1.15	22.32	1.22	4.2	*Nettastoma melanurum*	0.19	0.26	1.22	3.5
Sepiida					Aulopiformes				
Sepiolidae					Chlorophthalmidae				
Heteroteuthis dispar	2.56	0.78	2.44	3.6	*Chlorophthalmus agassizi*	1.18	0.26	1.22	3.17
Rossia macrosoma	2.56	0.78	2.44	4	Gadiformes				
Sepietta oweniana	2.56	0.78	2.44	3.5	Macrouridae				
Cephalopoda und	20.71	22.32	40.85	–	*Hymenocephalus italicus*	0.20	0.26	0.61	3.4
Malacostraca					Merlucciidae				
Amphipoda					*Merluccius merluccius*	0.40	0.52	1.22	4.4
Hyperiidae sp.	0.33	1.04	3.05	2	Myctophiformes				
Decapoda					Myctophidae				
Aristeus antennatus	0.31	1.15	0.61	3.3	*Ceratoscopelus maderensis*	1.50	1.11	3.35	3.3
Parapeneus longirostris	7.02	1.04	3.66	3.7	*Lampanyctus crocodilus*	1.50	1.16	3.35	3.2
Pasiphaea sivado	5.16	2.09	7.93	3.2	*Myctophum punctatum*	1.50	1.11	3.35	3.4
Polycheles typhlops	0.51	1.15	0.61	3.6	*Symbolophorus veranyi*	1.50	1.11	3.35	3.25
Processa canaliculata	0.06	1.15	0.61	3.1	Pleuronectiformes				
Crangonidae und	0.51	1.15	1.22	3.2	Bothidae				
Natantia und	8.66	1.15	23.17	–	*Arnoglossus thori*	0.02	8.96	0.6	3.7
Penaeidae und	7.02	1.04	3.66	3.5	Soleidae				
Sergestidae und	5.22	5.22	9.15	–	*Microchirus sp.*	0.07	4.48	0.91	3.3
Anomura					*Microchirus variegatus*	0.07	4.48	0.91	3.3
Anapagurus laevis	0.31	0.26	0.61	–	Stomiiformes				
Euphausiacea					Stomiidae				
Euphausiidae					*Chauliodus sloani*	0.46	0.65	2.44	4.06
Meganyctiphanes norvegica	3.41	2.61	11.59	3	*Stomias boa*	0.46	0.65	2.44	3.95
					Osteichthyes und	22.90	8.96	34.76	–

Fig. 2). The largest individuals (length class: 36–55 cm) consume a broad range of different prey items.

In Fig. 3, the frequency of occurrence (%FO) is plotted against prey-specific abundance (%Vol) and subdivided into length classes (15–25 cm, 26–35 cm, 36–55 cm), based on the Costello graphical method (Costello, 1990) modified by Amundsen et al. (1996). The majority of the prey points are depicted at the lower left corner of the diagrams or near the y-axis, indicating that many preys were consumed at low frequency and abundance. Five taxa of prey have been identified in the smallest size class; two out of these 5 groups (Myctophidae and Chlorophthalmidae) occupy the upper portion of the y-axis. As shown in Fig. 3, the first group (Myctophidae) consists of prey with a high specific abundance and occurrence, while the second group (Chlorophthalmidae) includes prey with a low occurrence and high abundance (upper left). A total of seven groups of prey are found in the size class 26–35 cm,

which occupied the lower middle part of the x and y axes. Prey groups raise to nineteen in the largest size class, located at the lower portion of the axes.

In the CA plot, the first two axes accounted for 48.85% of the variance (Fig. 4). The positive portion of axis I and II (I quadrant) condensed only a small portion of prey, including two groups of crustaceans (Processidae and Hyperiidae), and two fish (Merlucciidae and Myctophidae). A large number of individuals and prey were located in IV quadrant. The groups Histioteuthidae, Penaeidae, and Chlorophthalmidae occupied the regions between the II and III quadrants. Convex hulls were plotted and coloured according to depth, size, and sex. Preys in the stomach contents differed significantly according to length class (pseudo-$F = 4.64$; $p < 0.01$) and depth distribution (pseudo-$F = 2.73$; $p < 0.02$). The results of the PERMANOVA did not reveal differences between sexes (Table 3).
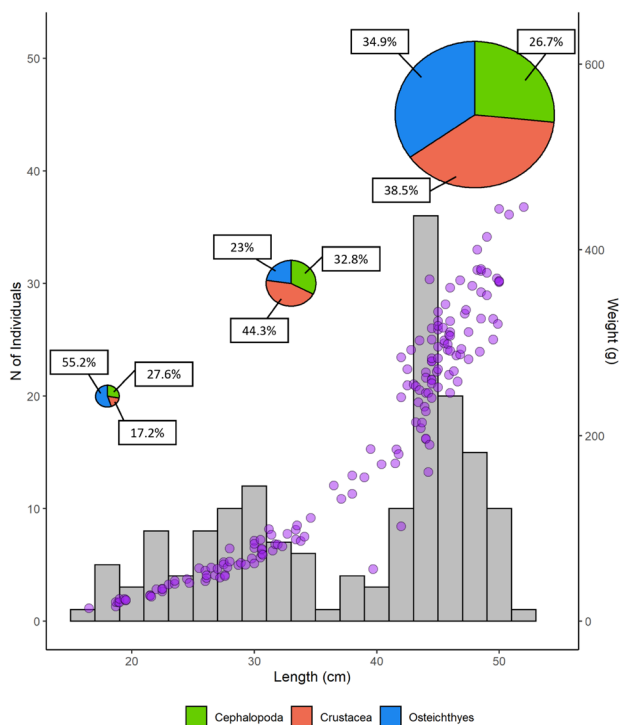
Fig. 2 Frequency distribution (grey) and length–weight relationship (purple) of *Galeus melastomus* collected in the central Tyrrhenian Sea. Pie charts indicate the percentage of prey species (%FO) present in individuals' stomach contents, and they are sized based on the number of prey species present in each length class (15–25 cm, 26–35 cm, 36–55 cm)

Concerning plastics, the FT-IR analysis revealed the polymeric nature of all isolated plastic particles (20 particles), corresponding to Polyethylene (PE: 50%), Polypropylene (PP: 25%), Polyamide (PA: 12.5%), and Polystyrene (PS: 12.5%). Plastics were found in the stomach contents of 33 individuals, with a percentage of occurrence of about 20%. Individuals of *G. melastomus* ingested 47 plastics in total, ranging from 1 to 11 items, with a mean of 1.47 ± 0.28 ($\pm$ SD) (Fig. 5). Plastic size class between 1 and 5 mm was the most abundant (44.7%) and included all shape categories. The most abundant plastic-shaped category was filaments (53.2%), followed by fragments (25.5%), films (17%), and spheres (4.3%) (Fig. 5). Most plastic items were transparent (59.6%), then black (17%), white (14.9%), green (4.3%), blue (2.1%), and red (2.1%).

The analysis of the frequency of occurrence of plastic items by shape, size, and according to sex, size, distance from the coast, and depth reveals that transparent filament is the most common ingested item, ($N = 14$), followed by transparent films ($N = 9$), black filament ($N = 8$), transparent fragments ($N = 5$), and white spheres ($N = 2$). The largest amount of plastic was found in fish that were caught within the buffer of 10–15 nautical miles from the coast, in the

depth stratum between 450 and 650 m, in the length class of 36–55 cm, and in female (Fig. 5).

The GAM model selection returned as best model the following relationship:

Num of Items $\sim$ s (Distance) + s (TrophicLevel) + s (Fullness) + s (L1n) + s (L1w).

where Distance is the distance from the coast of the haul, Trophic level is the weighted mean of the trophic level of each prey (Table 2). Fullness is the degree of stomach fullness, which could be important to consider that empty stomachs could contain neither food nor plastic, and L1n and L1w are the amounts of MaP in number of items (*n*) and weight (*w*).

This model allowed explaining as much as 53.7% of the total variance in the data, and it fits adequately the pattern in the input data (Fig. 6). Distance from the coast and amount of MaP litter as weight was significantly related to the dependent variable, but with opposite effects (Fig. 6B-D-E). In particular, the former was associated with a decreasing and monotonic (almost linear) effect on the number of ingested plastics, while the latter was associated with an increasing and monotonic effect. The other predictors, although retained in the model, were not significant.

Discussion

The diet analysis of *G. melastomus* confirmed that several mesopelagic and bathypelagic species belonging to fish, cephalopods and crustaceans, provide the primary food source for this species, and contributed to filling the knowledge gap highlighted by D'Iglio et al. (2021) for the central Tyrrhenian Sea. Food habits of blackmouth catshark revealed changes during its ontogenetic development, with an increasing diversity along size. Due to this, the diet of small and young individuals mainly consisted of fish, whereas the diet of intermediate-sized individuals (26–35 cm) mostly included decapod crustaceans. The diet of large individuals resulted in a more balanced composition with similar proportions of crustaceans, cephalopods, and fish.

The diet of *G. melastomus* exhibited a high diversity in species composition, since it mainly included bathypelagic (Myctophidae) and demersal (Chlorophthalmidae) fish in the smaller individuals. Prey diversity increased in the individual of intermediate size, and additionally included demersal crustaceans (Penaeidae) and cephalopods (Sepiolidae), together with pelagic Euphausiidae and Sergestidae. The most diversified diet was observed in adult individuals, and comprised demersal crustaceans (e.g. Penaeidae, Aristeidae, Polychelidae), as well as pelagic crustaceans (e.g. Euphausiidae, Pasiphaeidae, Sergestidae and Hyperiidae amphipods), which may have been captured during their
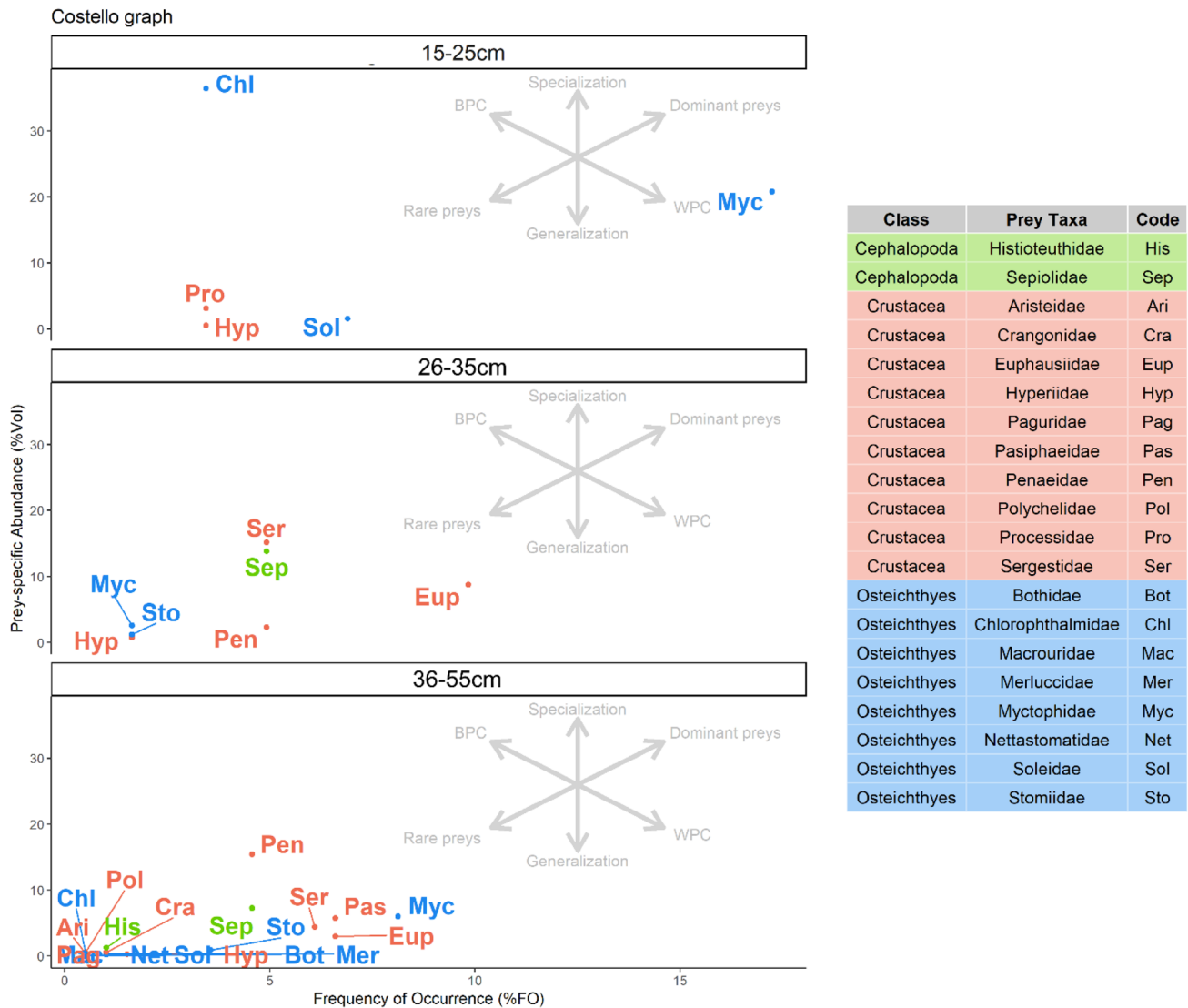
Costello graph

Fig. 3 *Galeus melastomus* collected in the central Tyrrhenian Sea: frequency of occurrence (%FO) was plotted against prey-specific abundance (%Vol) and subdivided into length classes (15-25 cm, 26-35 cm, 36-55 cm), based on the Costello graphical method (Costello 1990) modified by Amundsen et al. (1996) (*BPC* between phenotype component, *WPC* within phenotype component). Preys are subdivided into three groups (Cephalopoda, Crustacea, and Osteichthyes)

daily migrations in mid-water (Onsrud and Kaartvedt 1998; Elder and Seibel 2014). Moreover, adult individuals prey on many bathypelagic (Stomiidae, Myctophidae) and demersal fish (e.g. Merlucciidae, Macrouridae, Soleidae, Bothidae). Finally, cephalopods included both bathypelagic (*Histioteuthis bonnellii*) and demersal species (*Rossia macrosoma* and *Heterotheutis dispar*), as well as they are an important food source for other bathyal selachians (Bello 1997; Rey et al. 2005; Fanelli et al. 2009; Valls et al. 2011; Darna et al. 2019; D'Iglio et al. 2021).

The presence of typically benthic preys, such as fish of the families Bothidae and Soleidae, as well as the crustaceans of the family Paguridae, indicate that *G. melastomus* is

a benthic feeder and scavenger, as reported by other authors (Anastasopoulou et al. 2013; Barría et al. 2018). In addition, the presence of bathypelagic species highlighted that *G. melastomus* behaves as a supra-benthic predator capable of moving from the bottom to catch prey in the bathypelagic environment, confirming the behaviour previously reported for *G. melastomus* in other areas of the Tyrrhenian Sea (D'Iglio et al. 2021; Ricci et al. 2021).

Small individuals were the only ones that displayed preys with a high specific abundance and occurrence, as the Costello model revealed, and such evidence is an indication of their specialization. In fact, preys with a low specific abundance and presence were only occasionally consumed by
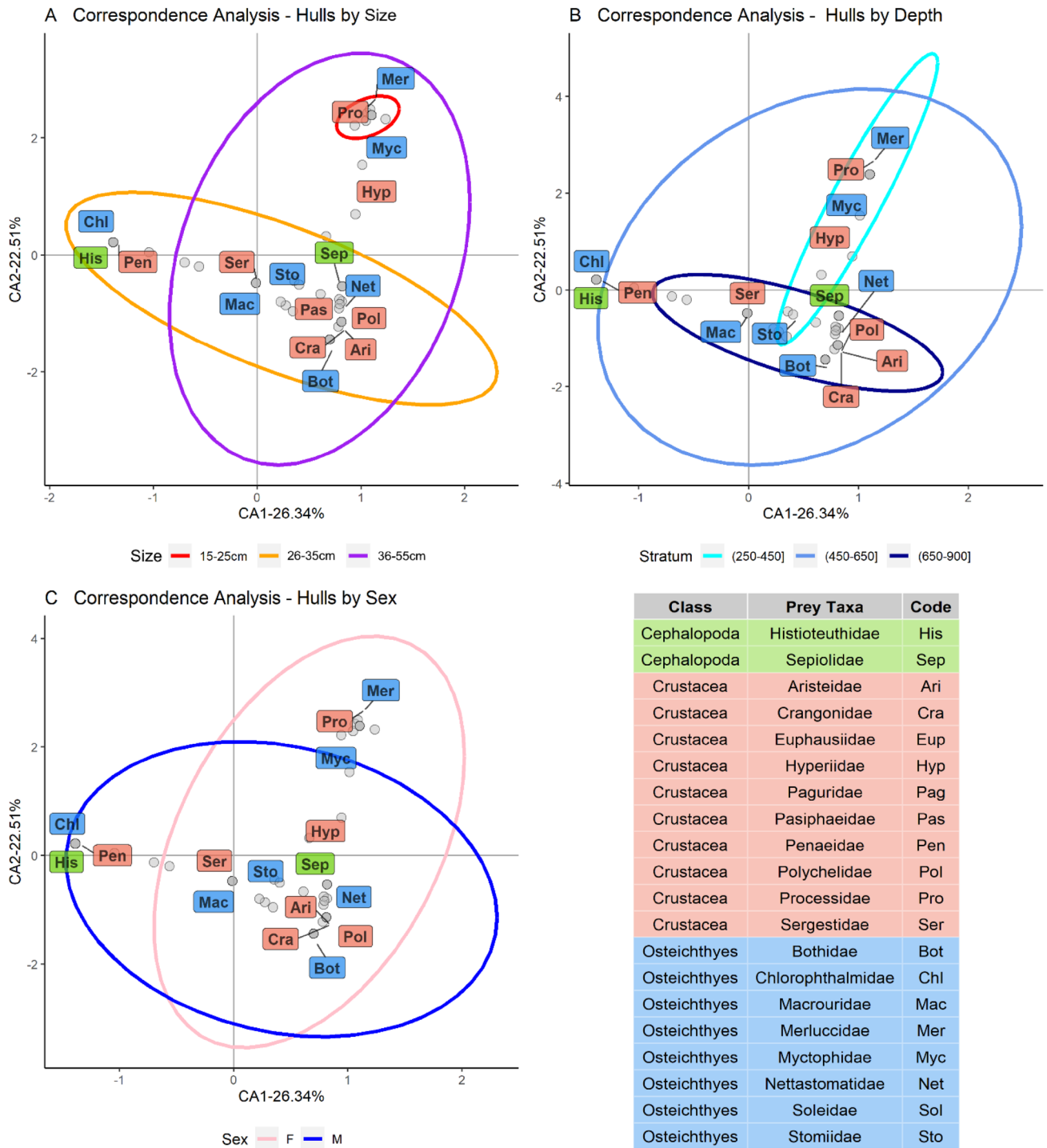
Fig. 4 *Galeus melastomus* collected in the central Tyrrhenian Sea: Correspondence analysis (CA) graphically inspect the variation in diet and relate the preference for different food items based on size (15–25 cm, 26–35 cm, 36–55 cm) (**A**), depth (**B**), and sex (Female: F; Male: M) (**C**). Preys are subdivided into three groups (Cephalopoda, Crustacea, and Osteichthyes)

a few individuals. In contrast, individuals of intermediate and large size did not display a close trophic relationship with specific prey species, since no dominant species were found in their diet. This result suggests that, for most of its

life cycle, blackmouth catshark has a generalist behaviour and, accordingly to the prey ecological habits, the results confirmed that it actively catches preys on the bottom, as well as in the supra-benthic layer, feeding on all available

Table 3 Results from non-parametric multivariate PERmutational ANalysis of VAriance (PERMANOVA) applied to *Galeus melastomus* diet data as prey volume abundance per food items by depth, size, and sex

PERMANOVA	SumOfSqs	R2	pseudo-*F*	Pr(>*F*)
Depth	1.0899	0.0198	2.7303	0.002*
Length	1.8542	0.0338	4.6450	0.001*
Sex	0.3629	0.0066	0.9092	0.567

*Indicates significant *p* values

preys, as also reported by other authors (Anastasopoulou et al. 2013; Barria et al. 2018; D'Iglio et al. 2021).

Further differences in diet related to depth distribution and size are also revealed by the CA analysis and were consistent with the behaviour of both preys and predator. Based on the prey composition of the stomach contents, the differences among the size groups were confirmed and could be explained by the vertical movement of blackmouth catshark. Indeed, the dynamics of the species suggest that younger individuals move through the water column towards surface waters. Specifically, the smaller blackmouth catshark are more active near the continental plateau (250–450 m depth), while the adult individuals are found at depths between 450 and 900 m. Our results are also in agreement with other studies which highlighted variations in the blackmouth catshark habits and composition of their preys during their growth in relation to the availability of the resources (Carrassón et al. 1992; Olaso et al. 2005; Fanelli et al. 2009).

Our study allowed to show that blackmouth catshark ingest a wide variety of plastics in terms of shape, colour, and size, which are in a clearly higher proportion than in the study of Carrassón et al. (1992). Particularly, there was a high proportion of microplastics (1–5 mm), and transparent filaments and films were the most abundant particles found in the samples. Films and filaments are the most abundant plastic particles in seawater and sediments (Gago et al. 2018). In areas densely populated such as near Rome, the sources of filaments might be more abundant, also for the presence of Tiber River mouth, which discharge in the Tyrrhenian Sea pollutant like plastic litter (Crosti et al. 2018). The transport of filaments can also accumulate in deep areas, as a consequence of their greater sinking capacity if compared to spheres and fragments (Chubarenko et al. 2016). Conversely, low-density particles (polystyrene spheres found in the samples) tend to float (Hidalgo-Ruz et al. 2012), but they can even sink after biofouling. Therefore, this can change the weight of the specific particle, making it easier to detect them within deep-sea sediments (Eriksen et al. 2014; Alomar and Deudero 2017; Valente et al. 2019). Finally, several polymers of polyamide have been found in the stomach contents of *G. melastomus*. As this polymer is the main component of the nylon used in the ropes and

fishing nets (Deopura et al. 2008), the wastes from fishing gears appeared a considerable cause of plastic pollution in the Tyrrhenian Sea, in line with other studies in deep-sea areas (Pruter 1987; Murray and Cowie 2011; Lusher et al. 2013; Neves et al. 2015; Güven et al. 2017; Welden and Cowie 2017).

In summary, significant sources of plastic pollution were identified through the GAM model, which was used to disentangle the role of different factors in the ingestion of plastic by *G. melastomus*. Based on this, two predictors were identified as major contributors affecting the number of plastic items ingested. Indeed, out of all the potential sources of variability considered (i.e. fish size, sex, depth of capture, distance from the coast, short-term trophic level, stomach fullness, and amount of MaP in terms of the number of items and total weight), only the distance from the coast and the total weight of MaP on the seafloor significantly affect the number of plastic items ingested by *G. melastomus*. The decreasing amount of plastics associated with the distance from the coast could be interpreted as the move away from the source of pollution. This variable was one of the major factors that negatively affect the amount of waste on the seabed along the coast (Coll et al. 2012; Steer et al. 2017; Sbrana et al. 2020; Franceschini et al. 2021). Accordingly, the coast of the Tyrrhenian Sea (GSA9) hosts a series of fishing grounds along a narrowed shelf that receives plastic waste through some important sources including the rivers Tiber and Arno (Inghilesi et al. 2008; Ludwig et al. 2009; Montuori et al. 2016; Crosti et al. 2018).

Similarly, the ingestion by *G. melastomus* is positively correlated with the amount of MaP present on the bottom This second factor deserves an additional reflection since, in this study, the amount of MaP was associated with the ingestion of smaller particles and not directly associated to the MaP. It is well known that plastic waste at sea is undergoing a process of fragmentation and miniaturization, so it is logical to expect that large quantities of MaP can generate corresponding quantities of MeP and MiP (Crawford and Quinn 2017; Chamas et al. 2020). Similar studies have already identified positive relationships between MiP ingestion and MaP hotspots at the sea bottom (Alomar et al. 2020; Franceschini et al. 2021). Particles, however, increase their ability to be transported as they become smaller (Zhang 2017), and thus oceanographic factors can greatly affect the spread and accumulation of different sizes of plastic particles. In addition, waste is stored in deposition centres (low-hydrodynamic areas). Finally, trawling contributes to the resuspension of litter on the bottom, slowing down plastic deposition. Leaving aside the oceanographic dynamics of plastic pollutants, the biomonitoring of their abundance and distribution as revealed by the analysis of stomach contents in non-commercial species, such as the blackmouth catshark,
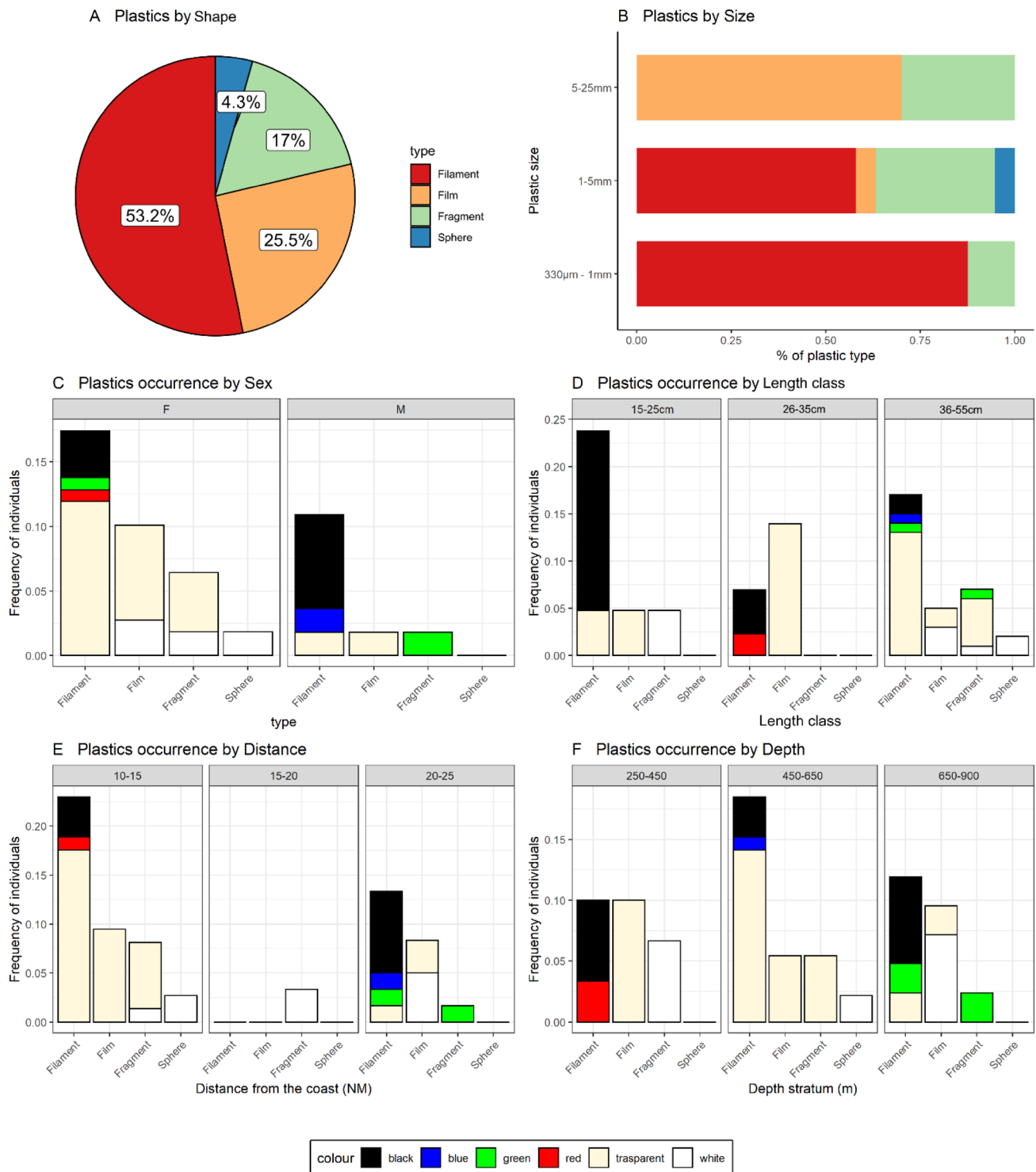
Fig. 5 Characterization of plastics found in *G. melastomus* stomach contents in the central Tyrrhenian Sea. **A** Pie chart by plastic-shape; **B** histograms by plastic size; **C** plastic occurrence by sex (Female: F; Male: M); **D** by length class (15–25 cm, 26–35 cm, 36–55 cm); **E** by distance from the coast; **F** and depth strata, subdivided into colours

could represent an important approach to assessing the risk of contamination of fisheries landings of commercial species. The results of this analysis did not reveal any

adverse effects on shark health, although we did not check the volume occupied by plastics in the stomach contents. However, it is still difficult to determine what are the real

A

B GAM results

Predictor	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	-2.88	0.775	-3.715	0

Predictor	edf	Ref.df	F	p-value
s(Distance)	1.000	1.000	9.115	0.003
s(TrophicLevel)	1.482	1.817	0.733	0.377
s(fullness)	3.101	3.523	2.305	0.060
s(L1n)	2.175	2.523	2.628	0.069
s(L1w)	1.000	1.000	5.636	0.020

Deviance.explained	Adj.Rsq
53.47 %	0.2432989

C Observed VS predicted number of ingested plastic items

D Effects of Distance

E Effects of plastic litter (Weight)

Fig. 6 Summary of results of the best-fit Generalized Additive Model (GAM) used to evaluate whether different factors including fish biometric parameters (fullness), the weighted mean of the trophic level of each prey, characteristics of the sampling sites (depth and distance from the coast) and the amount of macroplastics on the seafloor (number and weight of plastic items) explain variations in the frequency of plastics ingested by *G. melastomus* collected in the central Tyrrhenian Sea. **A** Comparison between theoretical (Quasi-Poisson distribution) and observed distribution of plastic ingestion (Number of items per individual of *G. melastomus*); **B** Tables of GAM results; **C** Observed versus GAM predicted values of plastic ingestion (Number of items per individual of *G. melastomus*); **D**, **E** The effects of the significant predictors, independent of the other predictors represented as response variable shape. The degree of smoothing is indicated in the y-axis label. Confidence intervals (95%) around the response curve are represented in grey

threats to a species that ingest plastic. In this way, it would be useful to further research using also an ecotoxicological perspective. Moreover, a future study could examine whether the pattern returned by the blackmouth catshark could serve as a proxy for those of other species, including key resources for fisheries, given that the samples collected during this study were taken over important fishing grounds (Russo et al. 2018).

In conclusion, this study provides a contribution to knowledge on trophic ecology and on the contamination of the diet by plastic. Several novel insights have been gained into the feeding ecology of *G. melastomus* in the central Tyrrhenian Sea, which is an area where this predator is very abundant and commonly caught as a by catch in bottom trawl fisheries, but adequate information is lacking. The dietary approach, which begins with a description of the organism's feeding habits, allows further exploration of plastic ingestion with great potential and versatility for most biological or ecological studies (Baker et al. 2014; Mahesh et al. 2019). Measurement of instantaneous trophic levels should be further investigated, even though the results are not significant. In our study the estimates for the trophic level were assigned based on FishBase or SeaLifeBase. These estimates were derived from a number of food items using a randomized resampling routine. However, if we consider some relevant dataset on Stable Isotopes Analysis (SIA) for deep-sea species in the Mediterranean Sea, the trophic levels of benthos feeders adopted in this study are likely to be underestimated. We are aware that stable isotope allows us to estimate trophic levels more accurately and would be fundamental to deepen this topic. Unfortunately, the isotopic values (especially $\delta^{15}N$) for some species are not recorded, while for other species the values are not available for the Central Tyrrhenian Sea. The opportunistic feeding behaviour of *G. melastomus* affects the incidental ingestions of numerous plastic particles likely confused for other preys, or indirectly ingested by feeding, in line with the results of previous research (Anastasopoulou et al. 2013; Cartes et al. 2016; Alomar and Deudero 2017; Valente et al. 2019).

It is widely acknowledged that the use of a single bioindicator species for monitoring plastic ingestion cannot be used to all European marine waters, even in areas with very different characteristics and levels of plastic pollution. Instead, it is recommended to use specific bioindicators based on habitat type and spatial distribution (Fossi et al. 2018). For instance, some crustaceans such *as Nephrops norvegicus* (in the Sea of Sardinia) and *Aristeus antennatus* (around the Balearic Islands) are considered effective indicators of the presence and impact of microlitter (mainly microplastics) in the Mediterranean deep sea (Carreras-Colom et al. 2018; Cau et al. 2019). Additionally, individuals of different sizes could be exposed to particles of different sizes (Worm et al. 2017). In this way, bioindicator species should be carefully selected according to the spatial domain being studied. Given this, and considering the generalist behaviour of blackmouth catshark, its abundance and distribution, and interaction with plastic hotspots, we consider, in line with Fossi et al. (2018), this species as a suitable candidate for developing a monitoring programme for the presence of plastics on the seabed, as requested in the Marine Strategy Framework Directive for European waters.

Declarations

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