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Microplastics in mussels and fish from the Northern Ionian Sea

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ABSTRACT

Microplastic ingestion by marine organisms presents an emerging threat to marine ecosystems; microplastics in different marine species are currently reported worldwide. This study aims to assess microplastic ingestion in four, highly commercial, marine species from Greek waters in the Northern Ionian Sea (Mediterranean Sea). Microplastics were found in mussels (*Mytilus galloprovincialis*) and all three fish species (*Sardina pilchardus*, *Pagellus erythrinus*, *Mullus barbatus*) examined. The frequency of occurrence of ingested microplastics was 46.25% in mussels, while among fish species, *S. pilchardus* showed the highest frequency of microplastic ingestion (47.2%). Microplastic abundance ranged from 1.7–2 items/individual in mussels and from 1.5–1.9 items/individual in fish. The majority of ingested microplastics were fragments, while their color and size varied. Fourier Transform Infrared Spectroscopy (FT-IR) indicated polyethylene as the most common polymer type in mussels and fish. Results can be used to set baseline levels for the assessment of microplastic pollution in the Ionian Sea.

1. Introduction

Microplastics are defined as plastic particles < 5 mm in size and constitute part of marine litter, a major global environmental problem (Arthur et al., 2009; Cole et al., 2011; Rezania et al., 2018). Microplastics are widely spread in the marine environment and are found in all marine compartments i.e. sea surface (Suaria et al., 2016; Zhu et al., 2018), shorelines (Browne et al., 2011; Liebezeit and Dubaish, 2012) and seabed (Woodall et al., 2014; Alomar et al., 2016). Recently, it has been estimated that 5 trillion microparticles are floating globally, while up to 890,000 plastic particles km^{-2} were predicted in the Mediterranean surface waters (Eriksen et al., 2014). Microplastics can be ingested by various marine organisms: zooplankton (Cole et al., 2014; Setälä et al., 2014), worms (Wright et al., 2013), shellfish (Li et al., 2018), fish (Lusher et al., 2013; Romeo et al., 2015; Bellas et al., 2016; Compa et al., 2018), seabirds (Codina- García et al., 2013) and cetaceans (Fossi et al., 2016), raising concerns on their potential effects on physiology and welfare of marine biota (Lusher, 2015). Microplastic ingestion can result in physical damage of tissues or organs (GESAMP, 2016). In addition, chemical additives used in plastic manufacturing, as well as persistent organic pollutants and metals adsorbed on microplastic surfaces once in the marine environment, are likely to be taken up by marine organisms during microplastic ingestion with potential toxic effects (Teuten et al., 2009; Rochman et al., 2014). Microplastic ingestion and their potential to increase the concentration of harmful chemicals in species destined for human consumption, raises concerns also on human health (Galloway, 2015; GESAMP, 2016; Wright et al., 2017). When microplastics are ingested, additives and adsorbed chemicals can be released in the gastrointestinal fluids and potentially transfer to edible tissue (Browne et al., 2013; Rochman et al., 2013; Wright et al., 2017). Furthermore small plastic particles may enter the circulatory system, resulting in translocation and redistribution to most commonly consumed tissues (Browne et al., 2008; GESAMP, 2016). In the European Union, marine litter (which includes microplastics), is addressed by one of the 11 Descriptors to achieve a Good Environmental Status (GES) set by the Marine Strategy Framework Directive (MSFD) (2008/56/EC). Microplastic ingestion by marine organisms is among the elements to be used for the assessment of GES for MSFD Descriptor 10 – Marine Litter (Decision 2017/848/EU) according to the MSFD criterion D10C3 "*The amount of litter and micro-litter ingested by marine animals is at a level that does not adversely affect the health of the species concerned*". Currently, indicator species for monitoring microplastics ingestion have not been established with the exception of the northern fulmar *Fulmarus glacialis* adopted by OSPAR in the North Sea (OSPAR, 2015).

Microplastic ingestion has been mostly shown in laboratory

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experiments while less studies report microplastic occurrence in marine organisms from the field (Lusher, 2015; GESAMP, 2016). Field studies can contribute to the definition of baseline levels of microplastics in marine biota with the view to support future monitoring programs for the assessment of microplastic pollution. Many studies record microplastics in mussels (*Mytilus edulis*, *Mytilus galloprovincialis*) and fish (e.g. *Scomber scombrus*, *Gadus morhua*, *Trachurus trachurus*, *Xiphias gladius*, *Mulus barbatus*, *Boops boops*, *Sardina pilchardus*, *Engraulis encrasicolus*, *Dicentrarchus labrax*, *Diplodus vulgaris*, *Platichthys flesus*, *Alepes djedaba*, *Epinephelus coioides*, *Sphyraena jello*, *Platycephalus indicus*, *Galeus melastomus*) (Foekema et al., 2013; Lusher et al., 2013; De Witte et al., 2014; Van Cauwenberghe and Janssen, 2014; Romeo et al., 2015; Nadal et al., 2016; Li et al., 2016; Bellas et al., 2016; Alomar and Deudero, 2017; Akhbarizadeh et al., 2018; Phuong et al., 2018; Bessa et al., 2018; Compa et al., 2018; Qu et al., 2018) as these organisms are used for human consumption and in addition, mussels and several fish have been effectively applied as indicator species for chemical pollution monitoring worldwide (Farrington et al., 2016; Robinson et al., 2017). Most studies on microplastic ingestion in Mediterranean waters have been carried out in the Western Mediterranean Sea (Fossi et al., 2018). Information on microplastic ingestion in the Ionian Sea is lacking, while one previous study in the deep waters of the Eastern Ionian Sea has shown macro litter (5–60 mm) ingestion in deep sea fishes (Anastasopoulou et al., 2013).

The present study investigates microplastic ingestion in mussels (*Mytilus galloprovincialis*), sardines (*Sardina pilchardus*), common pandoras (*Pagellus erythrinus*) and red mullets (*Mullus barbatus*) from Greek waters in the Northern Ionian Sea (Mediterranean Sea). The Northern Ionian Sea is in the transition zone between the Adriatic and Ionian Seas. The Adriatic-Ionian coastline, being long and complex, creates a high diversity of hydrodynamic and sedimentary environments. River outflows into the North Ionian Sea include Kalamas (Greece) and Butrinto (Albania) rivers. Shoreline tourism and recreational activities including poor waste management practices, fisheries, aquaculture, and shipping are the main anthropogenic activities related to marine litter inputs in the Northern Ionian Sea (Vlachogianni et al., 2017). The selection of species included organisms of different habitats and feeding strategies i.e. a sessile mollusc (mussel), a pelagic (sardine) and two demersal fish (common pandora and red mullet), reflecting environmental conditions in different marine compartments. All species are of high commercial value. Furthermore mussels, sardines and red mullets are among proposed indicators for microplastics in the Mediterranean Sea, due to their wide spatial distribution, commercial importance, habitat and feeding strategies, as well as documented microplastic ingestion (Fossi et al., 2018). Mussels are recommended as local scale indicators of microplastics in the water column along the coastline, while sardines and red mullets are proposed as small scale indicators of microplastics in open waters and on the seafloor respectively (Fossi et al., 2018). The aim of this study is to quantify microplastic ingestion in the Northern Ionian Sea and to explore possible variations in number and type of ingested microplastics among species of different ecological traits (habitat, feeding strategy, sessile/mobile state).

2. Materials and methods

2.1. Study area and sampling

Mussels (*Mytilus galloprovincialis* Lamarck, 1819) and fish (*Sardina pilchardus* (Walbaum, 1792); *Pagellus erythrinus* (Linnaeus, 1758); *Mullus barbatus*, Linnaeus, 1758) were sampled around Corfu Island in the Northern Ionian Sea (Fig. 1) in the framework of the "DeFishGear" project. The mussels were collected by hand from the pier in the port of Corfu and from a long line type mussel culture farm in Thesprotia, in summer 2015 (Fig. 1). Sampling depths were up to 1 and 3 m in Corfu Port and Thesprotia farm, respectively, and the sampling distance between the sites was approximately 28 km. Fish were caught by trawling off Corfu Island, during spring 2015, and trawling depths were 47.2 m and 60.4 m for sardines, 67.8 m and 140.1 m for common pandora and 62.4 m for red mullet. Whole mussels and fish were stored at −20 °C prior microplastic detection. Shell length (SL) of mussels, total length (TL) and total wet weight (TWW) of fish were recorded.

2.2. Microplastic extraction

Mussels and fish were thawed at room temperature before dissection. As it has been seen in previous preliminary experiments that among mussel tissues, digestive glands followed by gills, showed higher number of microplastics (Tsangaris et al., 2015), these tissues have been selected for microplastic analysis in this study. Digestive glands and gills of mussels were dissected out and wet weight was recorded. Fish stomach and intestine were dissected out and cut opened, their contents were weighed (wet weight) and initially examined using a stereomicroscope for items resembling microplastics (e.g. items with no cellular or organic structures, non-breakable items). In order to test differences in microplastic abundance between tissues, gills and digestive glands of mussels as well as stomach and intestine of fish were separately treated. To degrade organic matter and enable detection of microplastic particles, both mussel tissues and fish gastrointestinal contents were subjected to hydrogen peroxide digestion according to Mathalon and Hill (2014) with minor modifications (i.e. density separation by NaCl after digestion was omitted as there was only a small amount of organic matter remains). Gills, digestive glands, stomach and intestine contents of each individual were placed into glass beakers in 1:20 (w/v) H_2O_2 (30% H_2O_2, Chem-Lab, Germany) and heated on a hot plate at 55–65 °C until H_2O_2 was evaporated. If organic matter was not fully removed by the time H_2O_2 was evaporated to nearly 1 ml, 1–2 ml of H_2O_2 was added until nearly all of the organic matter was digested. Samples were then diluted with 100 ml of purified water (Milli-Q), stirred and filtered under vacuum on fiber glass filters (pore size 1.2 μm, Whatmann, GE Healthcare, UK) which were placed in Petri dishes and dried at room temperature.

Recovery of microplastics by the applied extraction protocol was tested on samples of fish gut tissue enriched with specific number (10 particles/sample) of different types of virgin plastic particles (polyethylene PE, polypropylene, PP, polyvinyl chloride, PVC, polystyrene, PS and polyethylene terephthalate, PET) in the size range of 0.3–1 mm. The number of particles detected after applying the extraction procedure was used to calculate % recovery of microplastics. Microplastic recovery was 100% for PP and PET, 90% for PVC, 80% for PE and 60% for PS.

2.3. Microplastic observation and quantification

Filters were examined under a stereomicroscope (Olympus, SZE and SZX7) for items resembling microplastics. Using a digital camera (Luminera) and the INFINITY ANALYZE software, items were photographed, counted and categorized according to maximum length, color, and type (fragment, fiber), following guidelines produced by the MSFD technical group on marine litter (Galgani et al., 2013). Number of microplastics was recorded separately for gills, digestive gland, stomach and intestine and then summed for each individual. Microplastic abundance was expressed for each species as a) average number of microplastic items per individual in all individuals examined b) average number of microplastic items per individual in individuals containing microplastics and c) average number of microplastic items per gram wet weight of mussel tissue or fish gastrointestinal content, in individuals containing microplastics. This approach intended to facilitate comparisons to literature. Frequency of occurrence of ingested microplastics for each species was calculated as the percentage of the individuals examined containing microplastics.

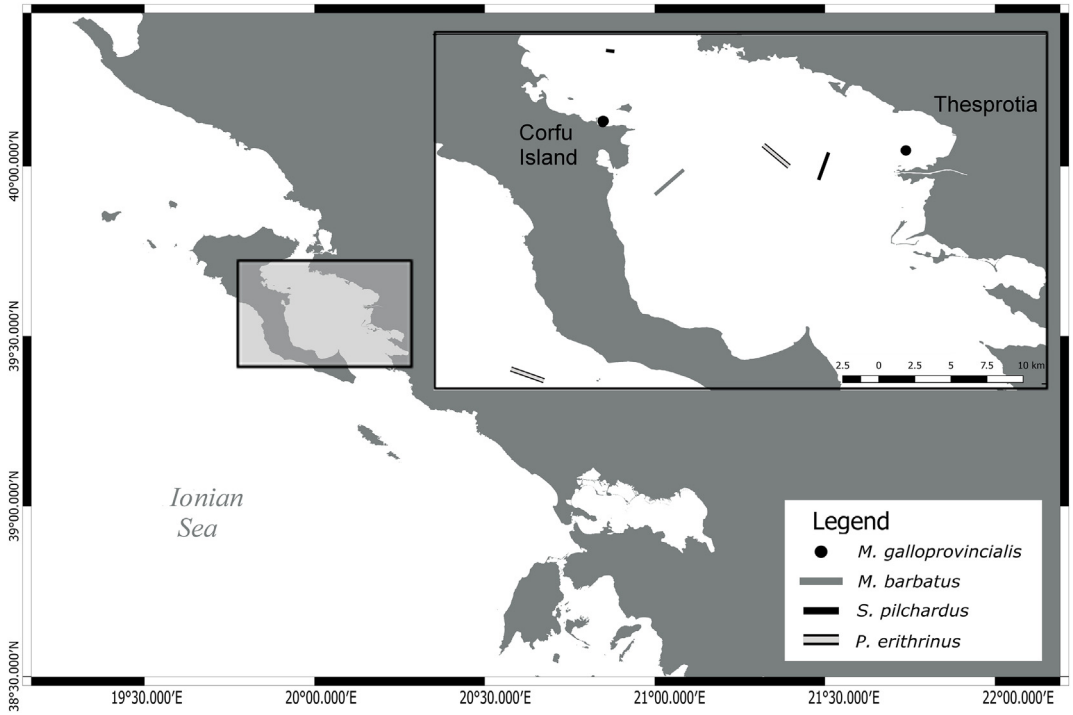

Fig. 1. Sampling area in the Northern Ionian Sea and location of sampling sites for each species.

2.4. FT-IR analysis

Fourier transform infrared spectroscopy (FT-IR) was used to confirm the synthetic polymer origin of the resembling microplastic items. FT-IR analysis was carried out on an Agilent Cary 630 FTIR spectrometer using a self-generated polymer library (i.e. spectra of reference polymer types provided by industry). The level of certainty when comparing sample spectrum to that of the self-generated library database was set up to 80%. At least a 10% proportion of fragments and filaments recorded, were analysed by FT-IR as suggested by the guidelines produced by the MSFD technical group on marine litter (Galgani et al., 2013).

2.5. Contamination precautions and quality control

All glassware was rinsed thoroughly with purified water. For initial examination of fish gastrointestinal content, the stereomicroscope observation area was isolated using a plastic cover (Torre et al., 2016). Mussel and fish samples were covered by foil paper during digestion and when not in use. A glove bag was used as working area for sample rinsing and filtration. Filters were covered with glass lids during observation under a stereomicroscope. Procedural blank samples were used in all steps and items similar to those found in blank samples were excluded, as they were considered airborne contamination. Procedural contamination was < 10% of the mean microplastic number in the samples (Galgani et al., 2013).

2.6. Statistical analysis

All results are presented as mean ± standard error of the mean (SEM). The Kruskal Wallis test or the Mann-Whitney *U* test were applied to determine differences in microplastic numbers among tissues, species, habitats (demersal versus pelagic fish), mobile/sessile state (fish versus mussels), trawling depths and mussels sampling sites, as data did not comply with the assumptions of normality and homogeneity of variance checked by the Shapiro-Wilk test and Levene's test respectively. The likelihood ratio Chi-square test was used to compare types of ingested microplastics (shapes, class sizes and colors) among species, habitats (demersal versus pelagic fish), mobile/sessile state (fish versus mussels) and mussel sampling sites. Relations between body length (shell length of mussels, total length of fish) and microplastic number or size were tested using the Spearman's rank correlation. Significance level was set at $p < 0.05$. Statistical analyses were performed with SPSS Statistics 17.0.

3. Results

3.1. Microplastic ingestion

A total of 80 *M. galloprovincialis* (SL: 4.67 ± 0.72 cm), 36 *S. pilchardus* (TL: 11.04 ± 0.6 cm, TWW: 9.63 ± 1.46 g), 19 *P. erythrinus* (TL: 15.42 ± 3.31 cm, TWW: 55.83 ± 30.89 g) and 25 *M. barbatus* (TL: 12.93 ± 2.77 cm, TWW: 28.54 ± 23.18 g) were analysed. Microplastics (125 items in total) were found in 37 mussels, 17 sardines, 8 common pandoras and 8 red mullets (Table 1). Frequency of occurrence of ingested microplastics was 46.3% in mussels, 47.2% in sardines 42.1% in common pandoras and 32.0% in red mullets (Table 1).

Average number of microplastics per individual (calculated by summing number of microplastics in the examined tissues) in individuals containing microplastics was 1.9 ± 0.2 items/individual in mussels, 1.8 ± 0.2 items/individual in sardines, 1.9 ± 0.2 items/individual in common pandoras, and 1.5 ± 0.3 items/individual in red mullets and showed no significant differences among species (Kruskal-Wallis test, $p > 0.05$) (Table 1). Similarly, there were no differences among species when microplastic abundance was calculated considering all examined individuals (Kruskal-Wallis test, $p > 0.05$) (Table 1). In both fish and mussels, microplastic ingestion ranged from 0 to 4 items per individual. In fish, the maximum number of ingested items per individual consisting of 2 fibers and 2 fragments was detected in sardine. The average number of microplastics per individual was similar among fish species (in individuals containing microplastics and in all individuals examined), but when calculated on gastrointestinal content weight basis (Table 1), the average number of microplastics

Table 1
Frequency of occurrence of ingested microplastics (% of individuals containing microplastics) and abundance of microplastics in mussels and three fish species sampled near Corfu Island in the Northern Ionian Sea. Microplastic abundance (mean ± SE) for each species is expressed as a) average number of microplastic items per individual in all individuals examined b) average number of microplastic items per individual in individuals containing microplastics and c) average number of microplastic items per gram wet weight of mussel tissue or fish gastrointestinal content, in individuals containing microplastics.

Species	*M. galloprovincialis*		*S. pilchardus*	*P. erithrinus*	*M. barbatus*
Location	Port	Farm	Offshore	Offshore	Offshore
Number of individuals examined	40	40	36	19	25
Number of individuals containing microplastics	19	18	17	8	8
MP frequency of occurrence (%)*	47.5	45.0	47.2	42.1	32.0
MP number	32	36	30	15	12
MP longest dimension length range (μm)	40–737	55–620	39–857	32–1272	41–800
MP abundance					
a) Number of items per individual in all individuals examined	0.8 ± 0.2	0.9 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.5 ± 0.2
b) Number of items per individual in individuals containing microplastics	1.7 ± 0.2	2.0 ± 0.2	1.8 ± 0.2	1.9 ± 0.2	1.5 ± 0.3
c) Number of items per gram weight in individuals containing microplastics	5.3 ± 0.5[a]	2.5 ± 0.3[b]	34.9 ± 7.9[a]	27.8 ± 24.6[b]	11.2 ± 2.8[b].

MP: microplastics.
[a,b]Indicate significant difference between mussel sampling locations or between fish species (Mann-Whitney *U* Test).
* Based on full stomach and intestine.

was significantly higher in sardine than in common pandora and red mullet (Mann-Whitney test, $p < 0.05$), as stomach and intestine content weight of sardines was lower than this of the other two species (Mann-Whitney test, $p < 0.05$). Comparison of microplastic ingestion between demersal and pelagic fish showed significantly higher microplastic number per gram wet weight of stomach and intestine content in the latter (Mann-Whitney test, $p < 0.05$). Comparison of microplastic ingestion between mussels and fish showed no significant differences in microplastic numbers per individual (in individuals containing microplastics and in all individuals examined) (Mann-Whitney test, $p > 0.05$).

Average number of microplastics per individual (in individuals containing microplastics and in all individuals examined) was similar between trawling depths (Mann-Whitney test, $p > 0.05$), as well as between mussel sampling sites (Mann-Whitney test, $p > 0.05$). However, as soft tissue weight in mussels collected from Corfu port, was lower than those from the mussel farm (Mann-Whitney test, $p < 0.05$), when microplastic abundance was calculated per gram tissue weight (Table 1), the average number of microplastics in mussels collected from Corfu port was significantly higher than those collected from the farm (Mann-Whitney test, $p < 0.05$). Average microplastic number was similar in gill and digestive gland of mussels (Mann-Whitney test, $p > 0.05$) as well as in stomach and intestine of fish (Mann-Whitney test, $p > 0.05$). There was no relationship between number of ingested microplastics and body length tested for each species (Spearman's r, $p > 0.05$).

3.2. Microplastic characterization (shape, size, color and polymer type)

Fragments were the most abundant microplastic shape category both in mussels and fish (Fig. 2a); in mussels, 77.8% of the total microplastics recorded were fragments and 22.2% were fibers. The percentage of fragments and fibers in fish was 80% and 20% for sardines, 73.3% and 26.7% for common pandoras and 83.3% and 17.7% for red mullets, respectively. The proportion of microplastic shape categories was similar among species (mussel, sardine, common pandoras red mullet) as well as between mussels from the two sampling sites (likelihood ratio Chi-square test, $p > 0.05$).

Microplastics were classified to 4 size categories, according to their largest dimension: < 0.1 mm, 0.1 mm–0.5 mm, 0.5 mm–1.0 mm, 1.0 mm–5.0 mm. In both, mussels and fish, microplastics between 0.1 mm and 0.5 mm were the most abundant size class (52.6% and 67.6% respectively) (Fig. 2b). Particles > 1 mm were found only in common pandoras. The proportion of size class categories differed among species (likelihood ratio Chi-square test, $p < 0.05$), sardines and mussels showing higher number of smaller size (< 0.1 mm, 0.1 mm–0.5 mm) ingested microplastics, as well as between mussels and fish (likelihood ratio Chi-square test, $p < 0.05$), but not between mussel sampling sites (likelihood ratio Chi-square test, $p > 0.05$). No correlation was found between microplastic size and organism length

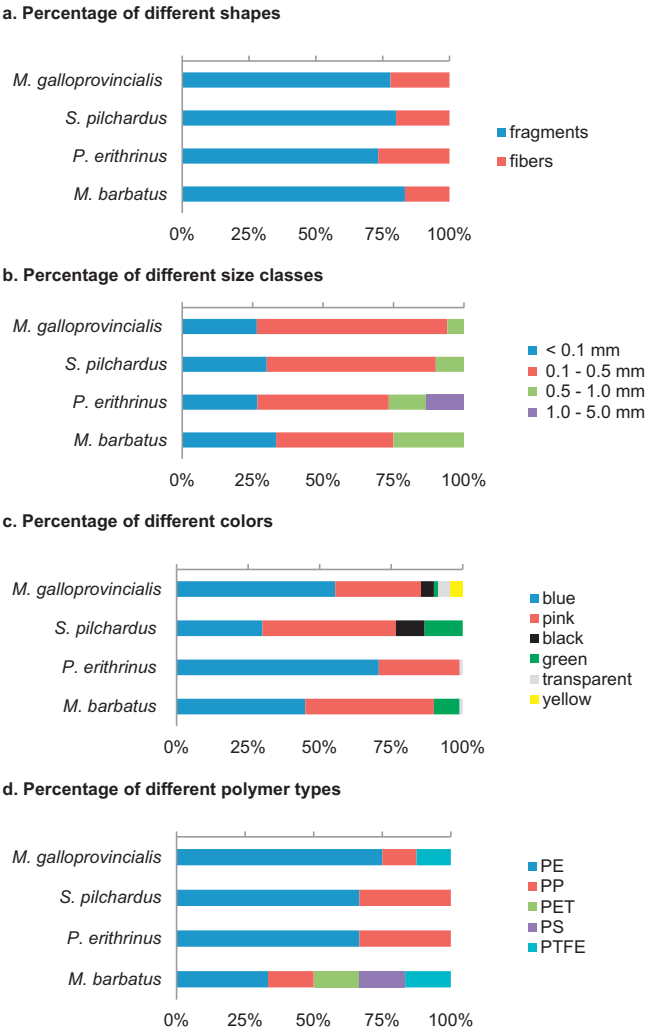

Fig. 2. Shape, size, color and polymer type of microplastics detected in mussels (*Mytilus galloprovincialis*) and three fish species (*Sardina pilchardus*, *Pagellus erythrinus*, *Mullus barbatus*) sampled near Corfu Island in the Northern Ionian Sea.

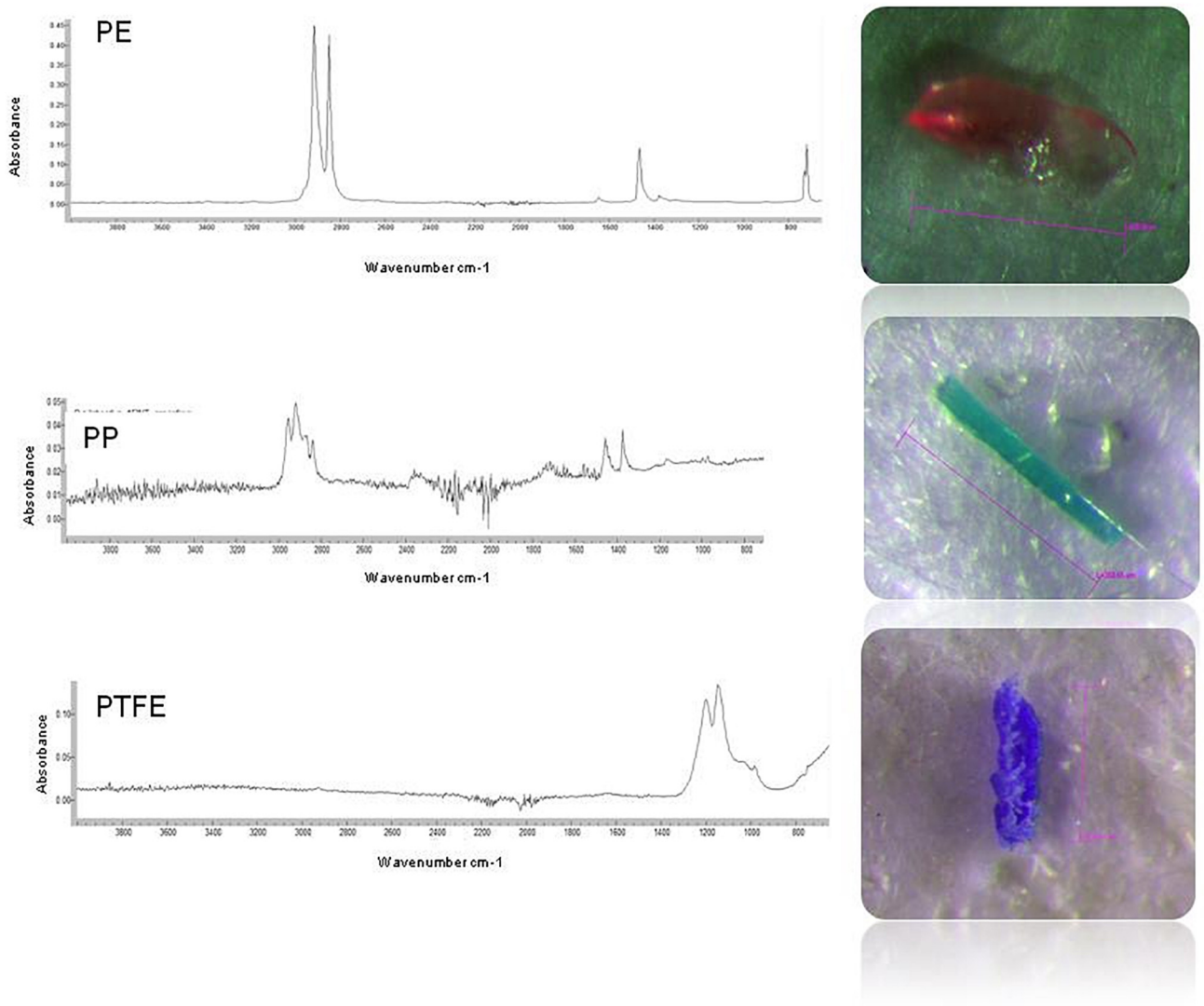

Fig. 3. Examples of microplastics found in mussel gills and digestive glands, and fish stomachs and intestines with FTIR spectrum.

(Spearman's r, $p > 0.05$).

The most common colors of microplastics in mussel samples were blue (54.4%) and pink (29.4%), some were black (4.4%), yellow (4.4%) or transparent (4%) and a few were green (1.4%) (Fig. 2c). Similarly, in fish, the dominant colors were blue (42.1%) and pink (40.3%), while a few particles were green (8.7%), black (5.2%) and transparent (3.5%) (Fig. 2c). No significant differences were detected among species, however mussels collected from the farm revealed a significantly higher proportion of blue colored microplastics in comparison to mussels from Corfu port (likelihood ratio Chi-square test, $p < 0.05$).

Fish habitat (demersal/pelagic fish) or species sessile/mobile state (mussels/fish) was not associated to shape, size class and color categories of ingested microplastics (likelihood ratio Chi-square test, $p > 0.05$).

The following polymer types were identified: polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), polystyrene (PS) and polytetrafluoroethylene (PTFE) (Fig. 3). FT-IR analysis was performed in 20% of the ingested microplastics thus numbers were not adequate for statistical analysis. In both, mussels and fish, PE was the most common polymer type detected (Fig. 2d). More specifically, in mussels, 75% of the microplastics analysed were identified as PE, 12.5% as PP and 12.5% as PTFE. In fish, 55.5% of plastics analysed were identified as PE, 27.7% as PP, 5.5% as PET, 5.5% as PS and 5.5% as PTFE. The categories PET, PS and PTFE were detected only in *M. barbatus* individuals.

4. Discussion

4.1. Microplastic ingestion in mussels, sardines, red mullets and common pandoras

The present study detected microplastic ingestion in mussels (*M. galloprovincialis*) and three fish species (*S. pilchardus*, *P. erythrinus* and *M. barbatus*) from Greek waters of the Northern Ionian Sea. These species are commercial, common and abundant in Mediterranean waters. Our findings provide further evidence that they are all susceptible to microplastic ingestion (frequency of occurrence: 32.0 to 47.2%) as documented in other areas of the Mediterranean Sea and worldwide (Table 2). Microplastic abundance was similar among species (1.5 ($\pm$ 0.26) to 2 ($\pm$ 0.26) items/individual in individuals containing microplastics) while mussels and sardines showed the highest frequencies of microplastic occurrence (46.3% in mussels and 47.2% in sardines).

Average number of ingested microplastics in mussels of this study (0.9 $\pm$ 0.2 items/individual in all individuals examined and 1.9 $\pm$ 0.2 items/individual in individuals containing microplastics) is similar to values reported in Giglio Island, Italy (1–2 items/individual in individuals containing microplastics: Avio et al., 2017) and in the French

Table 2
Microplastic ingestion reported in the literature for *Mytilus* sp., *S. pilchardus*, *P. erythrinus and M. barbatus* (MP: microplastic, w.w.: wet weight, d.w.: dry weight, i.c.m.: individuals containing microplastics, a.i.: all individuals).

Species	No of individuals	% with plastic	MP number	Reporting unit	Method	Area	Reference
M. galloprovincialis (wild & commercial)	61		0.04–0.34	Items/g w.w.	Whole tissues HNO_3 (69%) digestion or HNO_3 (65%): $HClO_4$ (68%) digestion, hot needle test	Portugal, Italy, Spain	Vandermeersch et al., 2015
M. galloprovincialis (transplanted)	120	10–36	1.0–2.0	Items/individual i.c.m.	Whole tissue desiccation, grounding, density separation, filtration, H_2O_2 (15%) digestion, FTIR	Giglio Island, Italy	Avio et al., 2017
M. edulis (wild & commercial)	162		1.1–6.4 0.7–2.9	Items/individual items/g w.w.	Whole tissues H_2O_2 (30%) digestion, NaCl flotation, μFTIR	Coastal waters of the U.K.	Li et al., 2018
M. edulis (wild & commercial)	30		0.4–8.1	Fibers/10 g	24 h gut clearance, whole tissues HNO_3 (65%): $HClO_4$ (68%) digestion	Belgian coast	De Witte et al., 2014
M. edulis (wild & cultured)	12–30/site 22 sites		1.5–7.6 0.9–4.6	Items/individual items/g w.w.	Whole tissues H_2O_2 (30%) digestion, NaCl flotation, μFTIR	Coastal waters of China	Li et al., 2016
M. edulis (wild)			0.2 ± 0.3	Items/g w.w.	24 h gut clearance, whole tissues HNO_3 (69%) digestion, Raman	French, Belgian and Dutch North Sea coast	Van Cauwenberghe et al., 2015
M. edulis (commercial)	8		10.4 ± 3.42, 0.9 ± 0.99, 1.3 ± 2.38 /2.0 ± 0.42, 0.2 ± 0.21, 0.3 ± 0.59	Fibers, particles, films/ individual a.i. /Fibers, particles, films/g w.w.	Whole tissue Corolase 7089 enzyme digestion	Estuary of Forth River Edinburgh, UK	Catarino et al., 2017
M. edulis (wild)	12		37,000	Items/kg d.w.	Whole tissue freeze-dry, grounded, proteinase k- H_2O_2 (30%) digestion incubation	South pier of IJmuiden, the Netherlands	Karlsson et al., 2017
M. edulis (wild & commercial)	45		106–178	Fibers/individual	Whole tissues H_2O_2 (30%) digestion, NaCl flotation	Nova Scotia beaches	Mathalon and Hill, 2014
M. edulis (wild)	20		19–105	Items/g d.w.	Whole tissues freeze-dried, grounded, nitric acid, H_2O_2 (30%) digestion, FTIR	Dutch coast	Leslie et al., 2017
M. edulis (wild and cultured)	120		0.60 ± 0.56 0.23 ± 0.20	Items/individual a.i Items/g d.w. a.i	Whole tissues KOH 10% digestion, KI flotation, μFTIR	French Atlantic coast	Phuong et al., 2018
M. gallopro-vincialis (wild & cultured)	80	45.0–47.5	1.7–2.0 (0.8–0.9) 2.46–5.26	Items/individual i.c.m. (a.i.) Items/g w.w. i.c.m.	Gill and digestive gland H_2O_2 (30%) digestion, FTIR	Northern Ionian Sea coast	This study
S. pilchardus	105	0–0.33	0–0.53 ± 0.35	Items/individual a.i.	Microscopic observation of stomach content, FTIR	Spanish Mediterranean coast	Compa et al., 2018
S. pilchardus	12	0	0	Items/individual	Microscopic observation of stomach content, FTIR	off Portuguese coast	Neves et al., 2015
S. pilchardus	7	57	3.75 (2.14)	Items/individual i.c.m. (a.i.)	Stomach and intestine H_2O_2 (35%) digestion, FTIR	Mediterranean coast of Turkey	Güven et al., 2017
S. pilchardus	99	19	1.78 ± 0.7	Items/individual i.c.m.	Gastrointestinal tracts desiccation, grounding, density separation, filtration, H_2O_2 (15%) digestion, FTIR	Central and North Adriatic Sea	Avio et al., 2015
S. pilchardus	20	45%	11	Total number	Stomach content, 9% NaClO, filtered and rinsed 99% methanol, centrifuged, Raman	English Channel, NW Med. Sea, NE Atlantic	Collard et al., 2017
S. pilchardus	36	47.2	1.8 (0.8)	Items/individual i.c.m. (a.i.)	Gastrointestinal tracts content H_2O_2 (30%) digestion, FTIR	Northern Ionian Sea	This study
P. erythrinus	54	52	1.21 (0.63)	Items/individual i.c.m. (a.i.)	Stomach and intestine H_2O_2 (35%) digestion, FTIR	Mediterranean coast of Turkey	Güven et al., 2017
P. erythrinus	19	42.1	1.9 (0.8)	Items/individual i.c.m. (a.i.)	Gastrointestinal tracts content H_2O_2 (30%) digestion, FTIR	Northern Ionian Sea	This study
M. barbatus	128	18.8	1.9 ± 1.29	Items/individual i.c.m.	Stomach content dried, NaOH 1 M digestion, hot metal tip test	Spanish Mediterranean coast	Bellas et al., 2016
M. barbatus	11	64	1.57 ± 0.78	Items/individual i.c.m.	Gastrointestinal tracts desiccation, grounding, density separation, filtration, H_2O_2 (15%) digestion, FTIR	Central and North Adriatic Sea	Avio et al., 2015

(*continued on next page*)

Table 2 (*continued*)

Species	No of individuals	% with plastic	MP number	Reporting unit	Method	Area	Reference
M. barbatus	207	66	2.12 (1.39)	Items/individual i.c.m. (a.i.)	Stomach and intestine H_2O_2 (35%) digestion, FTIR	Mediterranean coast of Turkey	Güven et al., 2017
M. barbatus	25	32	1.5 (0.5)	Items/individual i.c.m. (a.i.)	Gastrointestinal tracts content H_2O_2 (30%) digestion, FTIR	Northern Ionian Sea	This study

Atlantic coast (0.6 ± 0.6 items/individual in all individuals: Phuong et al., 2018), but lower than those reported in coastal waters of China (1.5 to 7.6 items/individual in all individuals: Li et al., 2016) and coastal waters of UK (1.1–6.4 items/individual in all individuals: Li et al., 2018). The coast of China shows high microplastic contamination (Qiu et al., 2015) which could relate to the differences in microplastic ingestion between the study of Li et al. (2016) and our study. On the other hand, the study in UK mussels (Li et al., 2018) reports total debris items of which only 50% were microplastics. When comparing to the studies by Vandermeersch et al. (2015) in estuaries of Portugal, Italy and Spain, by De Witte et al. (2014) in the Belgian coast, and by Van Cauwenberghe et al. (2015) in French, Belgian and Dutch North Sea coasts (Table 2), that report ingested microplastics in mussels on wet weight basis, microplastic ingestion values of our study are an order of magnitude higher. In the latter three studies, results are reported per weight of whole mussel tissues, which possibly causes the observed differences from our results that are calculated per weight of gills and digestive glands containing higher microplastic numbers among mussel tissues (Tsangaris et al., 2015). Furthermore, the above mentioned studies use pooled samples and thus report mean values in all individuals tested, while we calculated number of microplastics on wet weight basis considering only individuals containing microplastics. Other studies report microplastic ingestion in mussels on dry weight basis (Leslie et al., 2017; Karlsson et al., 2017), thus are not comparable to our results. Frequency of occurrence of ingested microplastics is not reported by most studies in mussels possibly due to the use of pooled samples of animals for microplastic extraction. Frequencies of microplastic occurrence in mussels in our study (45.0–47.5%) are higher than those reported by Avio et al. (2017) in Giglio Island, Italy (10–36%), who also use individual mussels for microplastic extraction. The observed differences could be due to the use of different mussel sampling strategies i.e. wild and cultured mussels were sampled in our study, while mussels transplanted at two depths (surface, bottom) for four weeks were used in the study by Avio et al. (2017).

Microplastic ingestion in fish was overall comparable to values reported for the same species in other Mediterranean areas (Table 2). Microplastic numbers per individual, considering individuals containing microplastics, were similar in sardines (1.8 ± 0.2) and red mullets (1.5 ± 0.3) of the present study and those recorded in sardines (1.8 ± 0.7) and red mullets (1.6 ± 0.8) from the Central and North Adriatic Sea (Avio et al., 2015) and in red mullets (1.9 ± 1.3) from the Spanish Mediterranean coast (Bellas et al., 2016), but lower than in sardines (3.8 ± 2.1) and red mullets (2.1 ± 1.4) from the Mediterranean coast of Turkey (Güven et al., 2017). On the contrary, in common pandoras, our values (1.9 ± 0.2) were slightly higher than those reported in the Mediterranean coast of Turkey (1.2 ± 0.6) (Güven et al., 2017). To our knowledge, there are no other studies in common pandoras. Considering microplastic number in all individuals examined, our values in sardines (0.8 ± 0.2) are slightly higher than those reported along the Spanish Mediterranean coast (0 to 0.53 ± 0.35) (Compa et al., 2018). The frequency of occurrence of ingested microplastics in all fish species examined in this study ranged between 32 and 47%. Similar results for these species have been also reported by other authors (Table 2) with the exception of one study that did not reveal any microplastics in sardines caught off the Portuguese coast (Neves et al., 2015). It should be noted that comparisons among studies are currently hampered by the variability of methods used for microplastic extraction and plastic identification, as well as, inconsistency in reporting of results (e.g. number of microplastics per individual or per weight of tissue in individuals containing microplastics, or in all individuals examined). Differences in microplastic ingestion reported among studies on the same species could be due to actual variations in microplastic contamination among study areas or could relate to the different protocols used by different research teams. For example, the above mentioned studies in mussels and fish used either direct microscopical examination or oxidative, acid and alkaline

digestion for microplastic extraction and FTIR or hot metal tip test for plastic identification (Table 2), thus differences in analytical protocols could bias quantitative comparisons. There is an urgent need to harmonise methodologies to allow for an accurate assessment of the levels of microplastic ingestion and to increase comparability between studies (Rochman et al., 2017; Lusher et al., 2017a). This will enable the use of microplastic ingestion in appropriate indicator species for different marine compartments as a mean to assess microplastic levels in the marine environment at regional and larger scales (Fossi et al., 2018).

In this study, mussels and sardines, which are filter feeders reflecting microplastic quantities in the water column at coastal and pelagic habitats respectively, showed the highest frequencies of microplastic ingestion. Pelagic fish have been found to ingest more microplastics than fish of other habitats (Güven et al., 2017) and also exhibit higher frequencies of microplastic ingestion than demersal fish (Rummel et al., 2016). However, this is not always the case as other studies report no differences in frequency of microplastic ingestion between pelagic and demersal fishes (Lusher et al., 2013; Neves et al., 2015; Phillips and Bonner, 2015), while higher microplastic abundance in benthopelagic than demersal fish has recently been reported (Bessa et al., 2018). In the present study, the pelagic sardines showed higher microplastic ingestion frequencies and higher number of microplastics per gram of gastrointestinal content than the demersal red mullets and common pandoras. Feeding type can also play a critical role on the amounts of microplastics that are ingested (Setälä et al., 2016; Mizraji et al., 2017). Mesocosm experiments show that filter feeding animals or animals utilizing at least partly the water column while feeding, can take up higher amounts of microplastics than deposit feeders (Setälä et al., 2016). Sardines are generally thought to be “filter feeding” pelagic fish (Garrido et al., 2007). Their fine branchial apparatus allows them to filter small particles with great efficiency (Collard et al., 2017) which is consistent with our results. Mussels, that are also filter feeders with high capacity of microplastic ingestion (Setälä et al., 2016), showed similar levels of microplastic ingestion to sardines. These findings may relate to the prevalence of small size microplastic items (< 1 mm) in surface waters of the study area (Digka et al., 2018) since mussels and sardines were found to ingest higher numbers of the smaller size microplastics (< 0.1 mm, 0.1 mm–0.5 mm) than the demersal fish species. Thus, our results suggest that filter feeders either in pelagic or coastal habitats may be more vulnerable to microplastic pollution in the Northern Ionian Sea.

Except for habitat and feeding behaviour, other factors including species sessile/mobile state (mussels versus fish), trawling depth, body length and tissue examined (stomach versus intestine of fish and gill versus digestive gland of mussels) showed no effect on microplastic ingestion. Concerning fish size, there are indications that larger individuals with better physical condition are less likely to ingest microplastics (Compa et al., 2018; Bessa et al., 2018). However, other studies report no relationships between fish size and microplastic ingestion (Güven et al., 2017; Davison and Asch, 2011; Foekema et al., 2013) which is in accordance to our results. Larger sample size and wider size range of the tested organisms may have been more suitable for examining relations of microplastic ingestion and size of the organisms, but this was not the main scope of this study.

Phuong et al. (2018) also reported no influence of mussel size on microplastic ingestion. In our study, this resulted in lower number of microplastics per gram wet weight in farmed than in wild mussels from the Corfu port, since farmed mussels were larger than the wild ones. However, frequency of microplastic ingestion was similar in wild and farmed mussels (47.5% and 45% respectively). These findings suggest that sampling site and/or sampling conditions (wild versus farmed mussels) had no effect on microplastic ingestion in our study. This is in agreement with the findings of Phuong et al. (2018) in wild and cultured mussels from the French Atlantic coast. Other studies report similar microplastic number per weight between farmed and wild mussels in Mediterranean and North Sea coasts (De Witte et al., 2014; Vandermeersch et al., 2015). On the contrary, Li et al. (2016) reported lower levels of microplastics in farmed compared to wild mussels both per individual and per weight, along coastal waters of China. Mussels are considered good bioindicators of microplastics in coastal areas and it has been recently shown in a large spatial scale study in China coastal areas, that microplastic abundance in mussels is related to microplastic levels in seawater (Qu et al., 2018). The absence of spatial differences in values of microplastic ingestion in mussels of our study could relate to similar microplastic levels in seawater among the two sampling sites.

4.2. Microplastic characterization

The composition of ingested microplastics in mussels and fish examined from the Northern Ionian Sea showed higher levels of fragments in comparison to fibers. In consistency with this finding, fragments were the dominant shape category of microplastics in seawater at the same area (Digka et al., 2018). In accordance to our results, Avio et al. (2015), excluding textile fibers from counts of microplastics, found that microplastics were mostly fragments in fish from Adriatic Sea. Similarly, in the study of Phuong et al. (2018) in mussels from the French Atlantic coast, the majority of microplastics were fragments reaching 82%, which is close to our result (77.8% fragments). However, most studies in mussels (De Witte et al., 2014; Li et al., 2016) and fish (Lusher et al., 2013; Bellas et al., 2016; Güven et al., 2017; Compa et al., 2018; Bessa et al., 2018) report higher percentage of fibers than fragments. A possible explanation for the difference in shape categories of ingested microplastics among the Northern Ionian Sea and other areas could be linked to different sources and waste management strategies (Rochman et al., 2015). Litter inputs in the Northern Ionian Sea include poor waste management practices, both in land and on the shoreline, where tourism and recreational activities are intense (Vlachogianni et al., 2017). This may lead to high amounts of plastic inputs in the sea (e.g. plastic bags, plastic bottles, plastic cups) that can break to microplastic fragments. In addition, mismanaged plastic can be more rapidly fragmented on land and enter the sea as microplastics (Corcoran et al., 2009; Kalogerakis et al., 2017). The proportion of plastic fragments in ingested microplastics was similar among species, habitats and sampling sites, suggesting ubiquitous distribution in the study area.

Concerning microplastic size, the predominant size class was 0.1–0.5 mm followed by particles < 0.1 mm. Still, microplastics < 0.1 mm may have been underestimated, as recovery rates decrease with decreasing particle size (Avio et al., 2015). The prevalence of particles < 0.5 mm is in agreement with the size distribution of floating microplastics in Mediterranean waters (Suaria et al., 2016). Sardines and mussels ingested more microplastics of the smaller size class (< 0.1 mm, 0.1 mm–0.5 mm) which can be explained by their filter feeding strategy.

In all species tested, blue was the most common color for ingested items. This predominance of blue plastic items has been previously reported in fish (Romeo et al., 2015; Güven et al., 2017; Peters et al., 2017; Compa et al., 2018). Significantly higher frequencies of blue microplastics found in mussels from the farm compared to those from the port are likely to derive from materials used in the mussel's farm infrastructures. Plastics are used in all stages of long-line type of mussel culture including ropes, lines, suspension buoys and mesh bags (Lusher et al., 2017b). Blue plastic mesh bags and floating barrels are widely used in the Greek mussel industry which explains the high proportion of blue items ingested by the farmed mussels.

The dominant polymer type in the examined mussels and fish was PE (75% and 55.5% respectively), in accordance to findings of Avio et al. (2017) in the Adriatic Sea and other studies in European Seas (Phuong et al., 2018; Collard et al., 2015). Polyethylene is the most common plastic on a global scale and consequently the most dominant plastic debris in the Mediterranean Sea and worldwide, deriving mainly from plastic bags and bottles (Cózar et al., 2014; Suaria et al., 2016;

Cózar et al., 2017). It is well documented that plastics as PE and PP float in the water surface, as their density is lower than this of the water. On the other hand, polymer types like PET and PTFE, which are denser than water, are more likely to sink, and thus to be ingested by benthic organisms. This is in accordance with our findings that indicate that PET and PTFE is ingested by red mullet.

4.3. Microplastic extraction methods

For the detection of microplastics in marine organisms, tissues or gastrointestinal contents are microscopically examined directly (Lusher et al., 2013) or after extraction by a digestion treatment (Miller et al., 2017). Diverse digestion methods are currently being used to degrade organic matter and facilitate detection of plastic particles, including acid (Van Cauwenberghe and Janssen, 2014; Vandermeersch et al., 2015), KOH (Foekema et al., 2013), hypochlorite (Collard et al., 2015) hydrogen peroxide (Mathalon and Hill, 2014) and protease (Cole et al., 2014) digestion. Methods that digest tissues without damaging microplastics are considered optimal. In the present study, hydrogen peroxide digestion was sufficient to allow detection of microplastics in mussel tissues and fish gastrointestinal contents. This digestion method has been recently used by several studies to detect microplastics in mussel and fish (Li et al., 2016; Avio et al., 2017; Güven et al., 2017). Preliminary tests using hydrogen peroxide on virgin microplastics in powder form (PE, PP, PS, PET) showed no alterations in the appearance of plastic particles (Tsangaris et al., 2015) in accordance with findings of Karlsson et al. (2017). Average recovery rates for PE, PP, PVC, PS and PET particles in the size class of 0.3–1 mm was 86%, which falls in the range of recovery rates reported for microplastics after hydrogen peroxide digestion of tissue samples (70–95%) (Miller et al., 2017).

While in fish gastrointestinal tracts are commonly investigated for microplastics, in small invertebrates such as mussels, shrimps and lugworms, whole bodies of the animals are used for microplastic extraction (Devriese et al., 2015; Li et al., 2015; Van Cauwenberghe et al., 2015; Phuong et al., 2018). In this study, the use of mussel digestive gland and gills, tissues involved in food intake and digestion, resulted in higher number of microplastics per gram tissue than those reported in the literature for whole mussel tissues (De Witte et al., 2014; Vandermeersch et al., 2015; Li et al., 2016, 2018; Phuong et al., 2018), suggesting this approach may facilitate microplastic detection, particularly if microplastic numbers are low. Also, this approach is well fitted for hydrogen peroxide digestion, since the time required for digestion depends largely on the amount of tissue to be digested (Li et al., 2015).

5. Conclusion

This study provides information on the quantities and types of microplastics in mussels (*M. galloprovincialis*), sardines (*S. pilchardus*), common pandoras (*P. erythrinus*) and red mullets (*M. barbatus*) from Greek waters of the Northern Ionian Sea. Our results show higher levels of microplastic ingestion in mussels and sardines suggesting filter feeding and pelagic species are more prone to microplastic pollution in the study area. The majority of ingested microplastics in all species were fragments, in consistency with microplastics in seawater at the study area, which can relate to poor waste management practices in the Northern Ionian Sea. Polyethylene was the most frequent polymer type found in all species, reflecting the global production and use of this material. Microplastic ingestion values are comparable to those reported for the same species in other Mediterranean areas. Still, harmonization of methods is necessary to allow precise comparisons of microplastic ingestion among different studies at subregional and regional scale. In addition, further research is needed to understand the impacts of microplastic ingestion in natural populations, the potential adverse effects of microplastics as contaminant vectors and their potential transfer in the food web that currently raise human health concerns.

Given the commercial value, wide distribution and abundance of *M. galloprovincialis*, *S. pilchardus* and *M. barbatus* in the Mediterranean Sea, as well as the existing evidence on microplastic ingestion, they are among the species considered as bioindicators for monitoring microplastics in the Mediterranean Sea. Our findings add information on microplastic ingestion and further support their potential use as bioindicators for microplastics. Furthermore, our results can contribute to the definition of baseline levels of microplastic ingestion in the studied species. Once indicator species are established, baseline values are needed to set threshold values tor the assessment of GES with respect to the MSFD criterion on litter and micro-litter ingestion by marine animals.

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