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—
Dipartimento
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della Vita
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DISVA

“Less Plastic More Mediterranean”
2017 Campaign on board the Rainbow Warrior
Greenpeace Ship

“MICROPLASTICS IN FISH AND INVERTEBRATES
ALONG THE TYRRHENIAN COAST”

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Introduction

Microplastic pollution is recognized as a world-wide phenomenon with nearly 300.000 tons of debris floating at sea surface (Eriksen et al., 2014; Suaria et al., 2016). The main inputs of plastics into the sea derive from beaches and land-based sources like rivers, wastewater discharges or transport of land litter by wind; maritime activities contribute with materials lost by professional and recreational fishing and debris dumped by commercial, cruise or private ships. Plastic accumulation in the marine environment produces several negative repercussions: from the aesthetic impact of litter and economic costs for beach cleaning, to adverse biological and ecological effects (Avio et al., 2017a).

In the recent years a great interest is being directed toward microplastics, particles with a grain size lower than 5 mm, which are manufactured ex novo for their use in cosmetics, industrial or medical applications, or derive from macroscopic debris after chemical, physical and biological fragmentation (Barnes et al., 2009). The presence of microplastics has been documented in marine organisms from different taxa and trophic levels, spanning from planktonic species, invertebrates, fish, top predators and cetaceans (Fossi et al., 2014; Ryan et al., 2016). Because of their small dimensions, the uptake of microplastics in invertebrates can occur through respiration across gills or by feeding activities (Avio et al., 2015a). In addition to direct uptake, marine vertebrates can ingest microplastics also through trophic transfer from prey's consumption; at now a number of field studies were performed in order to expand our knowledge on baseline levels of microplastics in wild organisms, with general comparisons between geographical areas, species, biological or ecological characteristics (Lusher et al., 2013; Rochman et al., 2015; Avio et al., 2015b).

Microplastics are now being defined as emerging pollutants and the European Marine Strategy Framework Directive (MSFD, 2008/ 56/EC) included marine litter and microplastics among the descriptors of the Good Environmental Status. Traditional pollutants are often monitored through the quantitative comparison of tissue levels measured in organisms from different areas or sampling periods. In recent years, International Expert Committees and Organizations (like ICES, GESAMP, UNEP-MEDPOL, JPI Oceans) supported activities to define standardized protocols and enhance scientific knowledge for monitoring microplastics in marine ecosystems.

In summer 2017 the international tour "LESS PLASTIC MORE MEDITERRANEAN" has been carried out by GREENPEACE in order to gather direct data on pollution from plastic in our seas and inform public opinion about this environmental emerging issue. During the Italian tour of the campaign (started on June 24th from Genova and finished in Ancona on 9th of July) different marine organisms were collected in collaboration with local fishermen, with the main aim to provide useful information on the microplastics distribution in the Tyrrhenian biota.

This report summarizes the main results obtained in fish and invertebrates collected along the Tyrrhenian Sea during the campaign. A special focus was given to results obtained on the Giglio Island, which offered the unique opportunity to investigate the variation of microplastic pollution in a well-defined case study, after the huge maritime operations related to the removal of the Costa Concordia wreck, sunk on January 2012. Results obtained in the present study have been compared with those already published in a previous paper, where microplastics were characterized in different benthic fish sampled in the same area in August-September 2014, after 2.5 years of huge engineering operations for the parbuckling projects and immediately after the Costa Concordia removal (Avio et al., 2017b).

Fish and Invertebrates sampling

During the ship tour, marine organisms were collected along the Tyrrhenian Sea in the areas of Genoa (06/23/2017), Talamone (06/26/2017), Giglio Island (06/26/2017), Ventotene Island (28/06/2017) and Naples (30/06/2017). All the specimens have been sampled in collaboration with local fishermen (Figure 1).

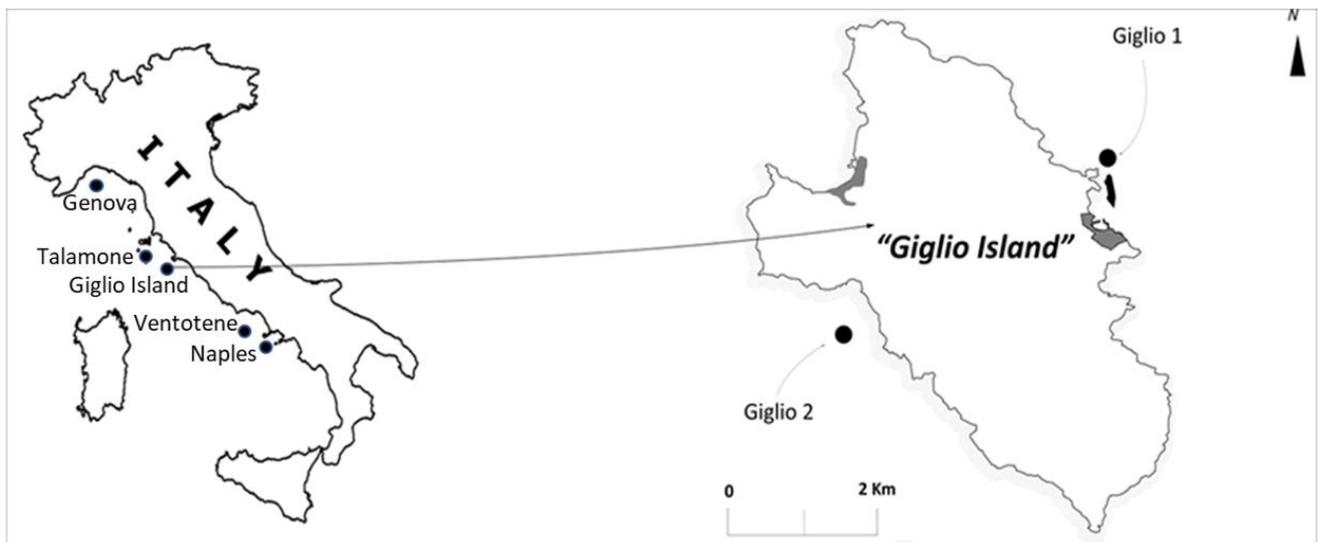


Figure 1. Localization of the organisms sampling areas along the Tyrrhenian Sea.

A total of 201 organisms (157 fish and 44 invertebrates belonging to 18 species of vertebrates and 3 species of invertebrates) were collected (Table 1). Depending on the availability, from 15 to 50 fish were analyzed for each sampling site, while a number between 5 and 11 specimens of invertebrates (mussels, mantis shrimps, striped prawns) were analyzed for the sites of Genoa and Naples only. In Giglio Island, fish were sampled from two specific sites (Fig. 1), corresponding to the same areas already investigated in a previous monitoring study performed in September 2014 and aimed to evaluate MPs occurrence after the removal of Costa Concordia wreck (Avio et al., 2017b): fishing grounds were respectively North-East of the island (Giglio wreck area: 42°22'04.80'' N, 10°55'16.80'' E, close to the wreck area) and South-West on the opposite site of island (Giglio reference area: 42°37'06.36'' N, 10°86'81.37''E).

Table 1. Sampling sites, species and common name (English/Italian) of organisms collected during the campaign “Less plastic more Mediterranean”.

VERTEBRATES			
Site	Species	Common name English-Italian	n. analysed organisms
Genova	<i>Engraulis encrasicolus</i>	anchovy - acciuga	15
Genova	<i>Mullus barbatus</i>	red mullet - triglia	11
Genova	<i>Merluccius merluccius</i>	European hake - merluzzo	10
Genova	Total	-	36
Talamone	<i>Scorpaena sp.</i>	scorpionfish - scorfano	7
Talamone	<i>Sarpa salpa</i>	sarpa fish - salpa	4
Talamone	<i>Mullus barbatus</i>	red mullet - triglia	4
Talamone	<i>Lichia amia</i>	leerfish - leccia	2
Talamone	<i>Pagellus erythrinus</i>	common pandora - pagello	2
Talamone	<i>Dactylopterus volitans</i>	flying gurnard- pesce civetta	2
Talamone	<i>Uranoscopus scaber</i>	stargazer - pesce prete	1
Talamone	<i>Phycis phycis</i>	forkbeard - musdea	1
Talamone	<i>Scomber scombrus</i>	mackerel - sgombro	1
Talamone	Total	-	24
Giglio Wreck area	<i>Scorpaena sp.</i>	scorpionfish - scorfano	6
Giglio Wreck area	<i>Uranoscopus scaber</i>	stargazer - pesce prete	2
Giglio Wreck area	<i>Serranus scriba</i>	pointed comber - sciarrano	2
Giglio Wreck area	<i>Spondyliosoma cantharus</i>	black seabream - tanuta	1
Giglio Wreck area	<i>Phycis phycis</i>	forkbeard - musdea	1
Giglio Wreck area	<i>Mullus barbatus</i>	red mullet - triglia	1
Giglio Wreck area	<i>Sphyrna sp.</i>	barracuda	1
Giglio Wreck area	<i>Muraena sp.</i>	Mediterranean morai - murena	3
Giglio Wreck area	Total	-	17
Giglio Reference area	<i>Scorpaena sp.</i>	scorpionfish - scorfano	10
Giglio Reference area	<i>Uranoscopus scaber</i>	stargazer - pesce prete	2
Giglio Reference area	<i>Symphodus tinca</i>	peacock wrasse - tinca	1
Giglio Reference area	<i>Spondyliosoma cantharus</i>	black seabream - tanuta	2
Giglio Reference area	Total	-	15
Ventotene	<i>Phycis phycis</i>	forkbeard - musdea	3
Ventotene	<i>Micromesistius poutassou</i>	blue whiting - pesce melù	7
Ventotene	<i>Pagellus erythrinus</i>	common pandora - pagello	4
Ventotene	<i>Scyliorhinus canicula</i>	catshark - gattuccio	1
Ventotene	Total	-	15
Napoli	<i>Engraulis encrasicolus</i>	anchovy - acciuga	10
Napoli	<i>Scomber scombrus</i>	mackerel - sgombro	10
Napoli	<i>Merluccius merluccius</i>	European hake - merluzzo	10
Napoli	<i>Mullus barbatus</i>	red mullet - triglia	10
Napoli	<i>Chelidonichthys lucerna</i>	tub gurnard - gallinella	10
Napoli	Total	-	50

INVERTEBRATES			
Site	Species	Common name English-Italian	n. analysed organisms
Genova	<i>Mytilus galloprovincialis</i>	mussel - mitilo	11
Genova	<i>Squilla mantis</i>	mantis shrimp - canocchia	6
Genova	<i>Penaeus kerathurus</i>	striped prawn - mazzancolla	6
Genova	Total	-	23
Napoli	<i>Mytilus galloprovincialis</i>	mussel - mitilo	11
Napoli	<i>Squilla mantis</i>	mantis shrimp - canocchia	5
Napoli	<i>Penaeus kerathurus</i>	striped prawn - mazzancolla	5
Napoli	Total	-	21

Microplastic extraction and characterization

Gastrointestinal tracts of fish and the whole tissues for invertebrates were processed with a validated procedure (Avio et al., 2015b); a schematic presentation of the main steps are summarised in Figure 2.

The method is based on trituration of dried samples followed by separation under density gradient and filtration under vacuum, partial digestion in 15% H₂O₂ visual sorting and FT-IR characterization (Fig 2-5).

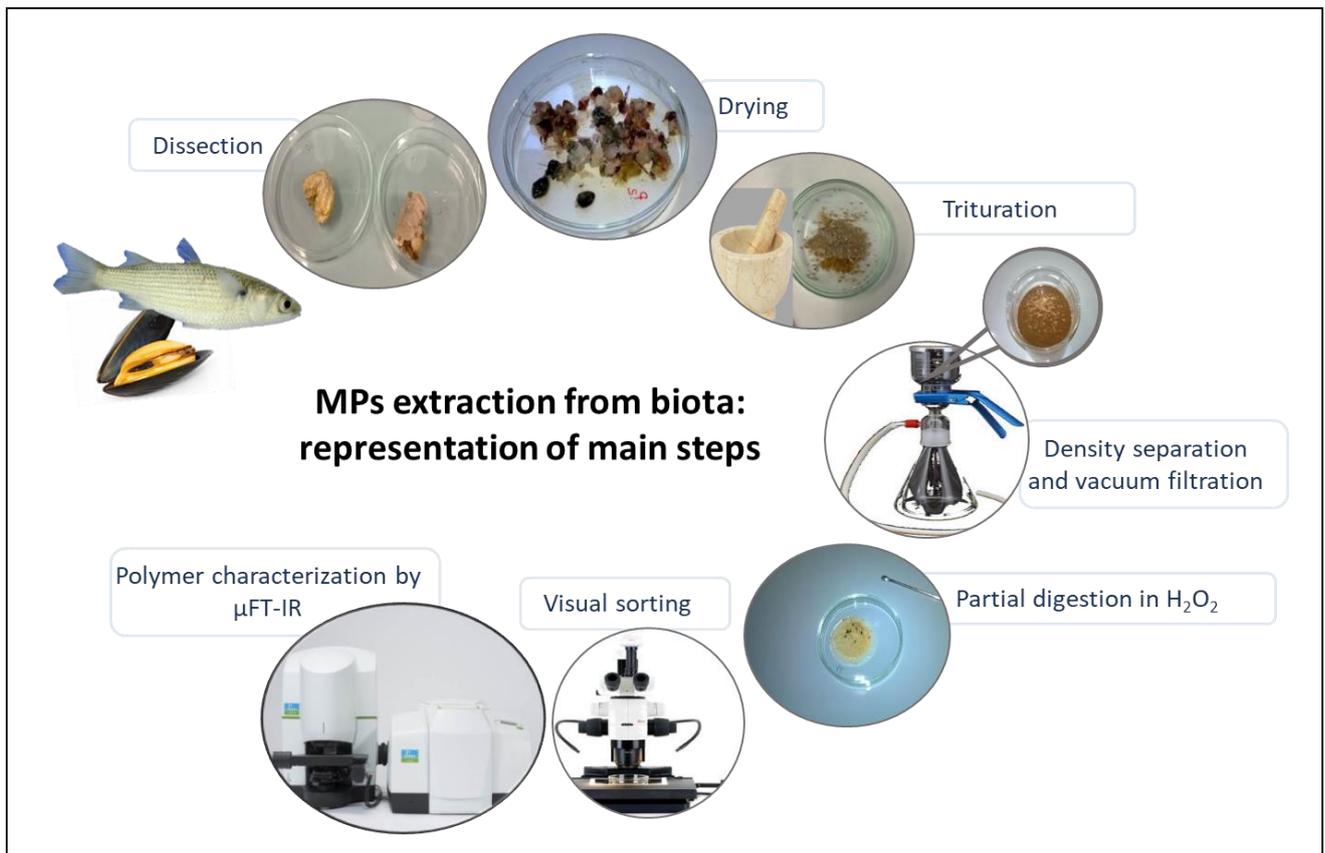


Figure 2. Microplastic extraction from the biota: representation of the main steps.



Figure 3. Representation of the filtration apparatus positioned into the hood, to prevent environmental contamination of MPs. Main steps of the extraction method include filtration of the supernatant, recovery of the membrane and overnight digestion of organic material in 15 % H_2O_2 .

Extracted particles were microscopically observed, photographed, measured at their largest cross section through a stereomicroscope, and categorized according to both size classes (1-5 mm; 0.5-1 mm; 0.1-0.5 mm; 0.01-0.1 mm) and shapes (fragments, film, pellet, line). Pictures in Figure 4 show some examples of fragments, films and lines microscopically analysed for determining the shape and size of extracted microparticles.

Criteria for shape characterization were the following: fragments were considered the irregular shaped particles, like crystals, powder and flakes, rigid, thick, with sharp crooked edges; pellets were particles with spherical shape, like common resin pellets, spherical microbeads and microspheres; films appeared in irregular shapes, thin and flexible; lines were characterized by regular diameter along the particles and not frayed ends. Fibers, appearing like ribbon, with not regular diameter along the particles and frayed ends; results on fibres are not presented in this report.

In order to prevent any confounding factors related to the selection of microparticles that are not made of plastic (such as organic or inorganic material), the subsequent μ FT-IR analyses must be performed for each extracted particles; indeed, it is widely demonstrated that from 5 to 50% of microparticles, selected as microplastic during the visual sorting, turn out to be not synthetic origin, after the FT-IR analyses (Suaria et al., 2016).

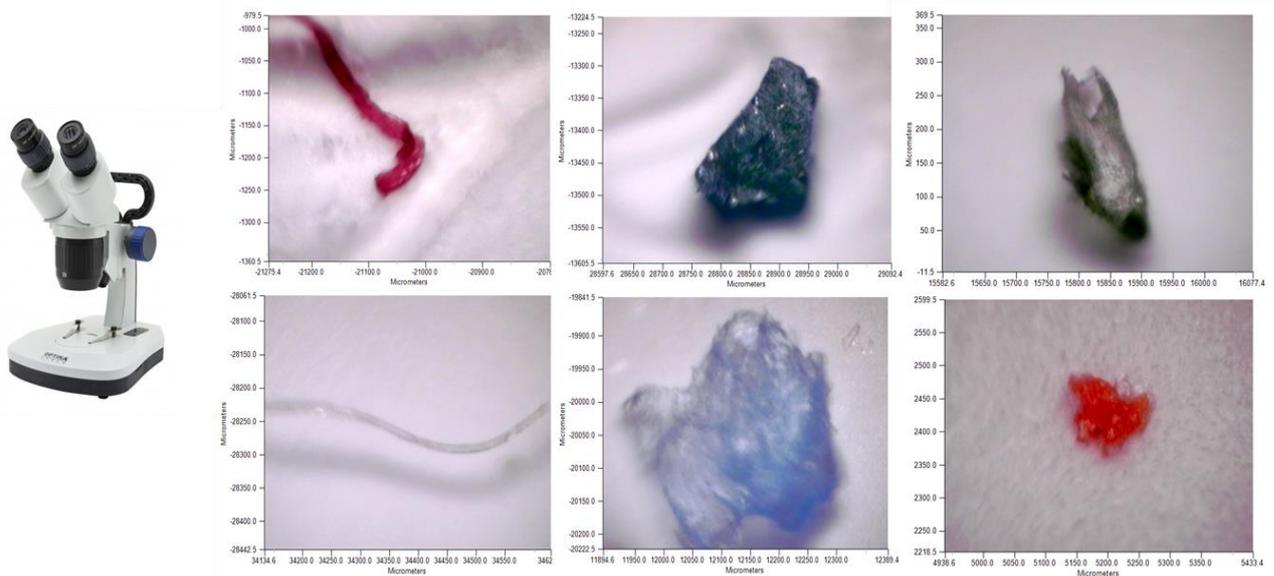


Figure 4 Example of line, film and fragment microparticles extracted from organisms and microscopically characterized in terms of color, shape and size.

Particles were characterized using a Spotlight i200 FT-IR microscope (Perkin Elmer). Pictures in Figure 5 showed spectroscopic infrared analyses of extracted microplastics; following back-ground scans, 16 scans were performed for each particle with a resolution of 4 cm^{-1} . Spectrum 10 software was used for the output spectra and the identification of polymers was performed by comparison with a library of standard spectra. Only polymers matching reference spectra for more than 70% were accepted.

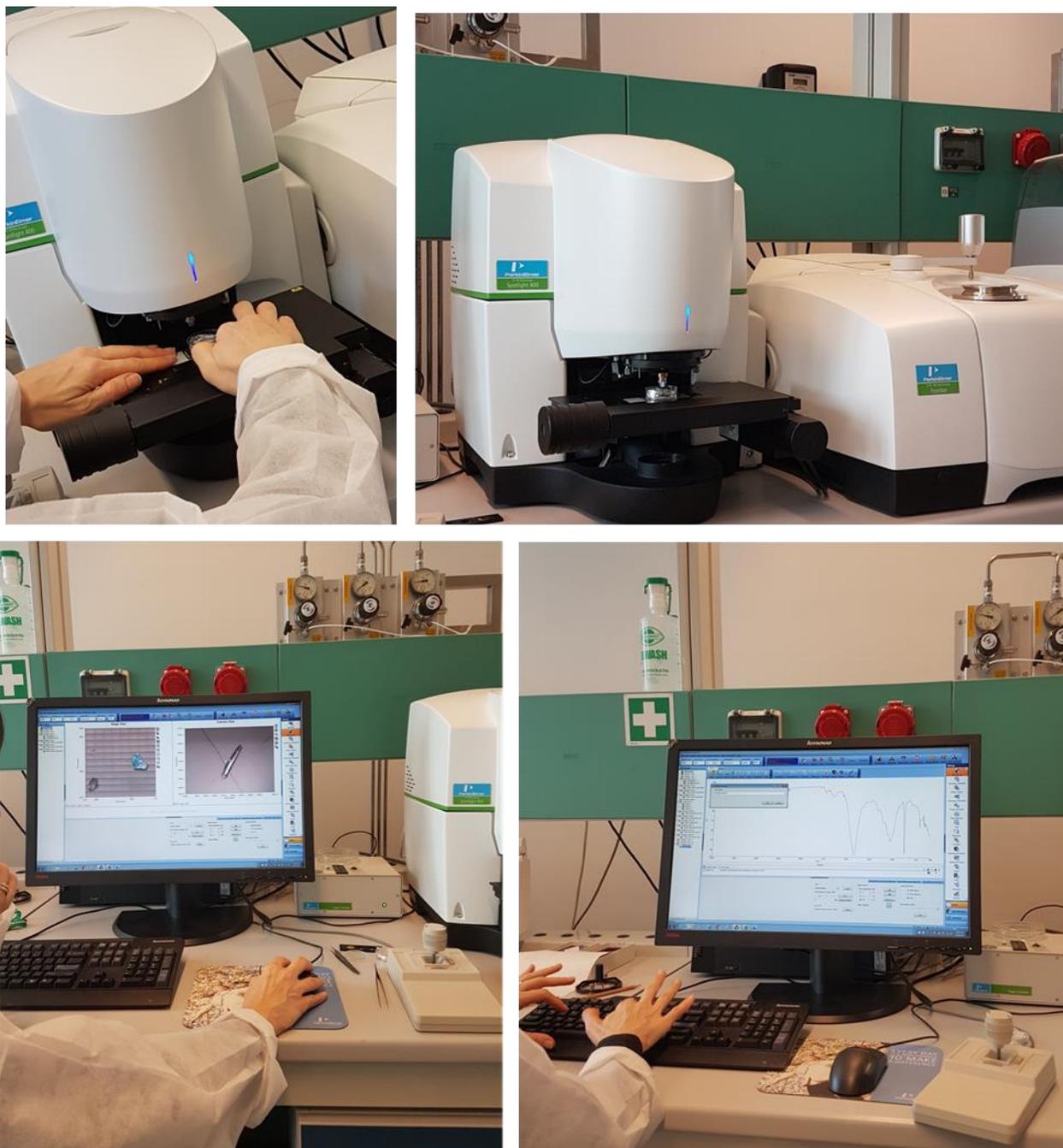


Figure 5. Analyses of microparticles through infrared spectroscopy (μ FTIR): scans and spectra determination for polymeric characterization.

Results

Table 2 summarises all the results obtained on microplastic extraction analyses: number of analysed organisms for each species and site, number and frequency (%) of those specimens positive to plastic ingestion (i.e. containing at least 1 particle), number of total particles extracted from each species and site, average number of MPs (items) extracted in individuals positive to ingestion; in some areas, the groups “Other” include the averaged results for those species for which only one individual was collected, and that were not found in any of the other investigated sites (Table 2).

On a total of 157 fish analysed from all the Tyrrhenian sites, 46 specimens contained at least 1 particle, thus revealing an overall frequency of approximately 30% of organisms positive to microplastics ingestion. Similar percentages (on a lower number of specimens) were obtained for invertebrates, with 13 organisms containing microplastics on a total of 44 analysed (29.5%). Overall these values of frequency are comparable to those described for the central and southern sectors of Adriatic Sea, with 25-30% of marine organisms containing microplastics.

The greater number of organisms has been collected the sites of Genova and Napoli (59 and 71, respectively). Here, the higher frequency of specimens positive to microplastic ingestion was obtained in striped prawns from Genova (50%), and in striped prawns, tub gurnard and mackerel from Napoli (60, 40 and 60% respectively). In Genova, all the species were positive to microplastic ingestion, while in Napoli only anchovies and European hake did not contain particles. The number of particles extracted from all the positive organisms typically ranged between 1 and 2, with an average of 1.13 ± 0.4 items in specimens from Genova, and 1.45 ± 0.8 items in those from Napoli.

In Talamone and Ventotene Island, only fish could be sampled (no invertebrates), with numbers of analysed organisms lower than those of other sites (24 and 15 respectively). At Talamone, 9 specimens contained microplastics and the frequency of ingestion in positive species ranged from 50% in flying gurnard, up to 100% in leerfish and common pandora: despite a similar frequency might appear as particularly high, it is worthy to remind that only 2 organisms were analysed for these species. In this respect, at Ventotene Island, only 1 common pandora (on 4 analysed and on a total of 15 fish) was positive to microplastic ingestion. Also in these sites, the number of particles extracted from the positive organisms ranged between 1 and 2, with an average of 1.33 ± 0.8 items in specimens from Talamone, and 1.0 ± 0.0 items in the only one from Ventotene Island.

Fish sampled at Giglio Island exhibited the highest frequency of specimens positive to microplastic ingestion, ranging from 35% in proximity of the wreck area up to 60% of specimens containing microplastics in the reference area chosen on the South -West part of the island; in this area, fish also exhibited a more elevated content of particles (1.66 ± 1.11 items/individual, $n=15$) compared to those from sampled in the wreck area (1.16 ± 0.4 items/individual, $n=15$, Table 2, Figure 6). The generally high occurrence of microplastics in fish from the Giglio Island may reflect the elevated levels of these particles in Tyrrhenian basin (Suaria et al., 2016) but also the close proximity of catching areas to the shoreline, which is an important sink compartment for microplastics, particularly in highly touristic and anthropized areas.

The campaign Less Plastic more Mediterranean gave us the possibility to investigate the variations of microplastic pollution in a well-defined case study offered by the huge maritime operations related to the removal of the wreck. Results of 2017 were compared with those already obtained in a previous work when fish had been collected in a reference area and around the wreck in 2014 after the end of rescue operations, when the ship was finally towed away to Genova (Avio et al., 2017b). At that moment, benthic fish sampled in the wreck area exhibited a frequency of 95% of organisms with ingested microplastics and an average content of 4 ± 1.8 particles (Figure 6). Overall, these results suggest that, compared to the previous monitoring study performed in 2014, a significant improvement has occurred in the area impacted by the operations for the wreck removal, with a lower number of organisms positive to the MPs ingestion (35% versus 95%) and lower number of ingested particles (1.16 ± 0.4 versus 4 ± 1.8 particles).

More comparable, but still higher results had been obtained in 2014 also in the Giglio reference area, which had previously shown a frequency of 77% positive fish (compared to 60% in 2017) and a content of 2.5 ± 2 particles (compared to 1.66 ± 1.1 in 2017). In this case, the difference could be related to the different sampling period which, in 2014 occurred in mid-September, at the end of summer touristic season.

Table 2. Data of analysed organisms collected along the Tyrrhenian coast; sites, species, number of analysed organisms for each species, number of organisms positive to MPs ingestion, frequency (%) of positive, total number of extracted MPs and average number of items in individuals containing microplastics.

Site	Species	analysed organisms (number)	organisms positive to ingestion (number)	frequency (%)	tot. extracted MPs / tot. specimens	items/ind (mean \pm sd)
Genova	<i>E. encrasicolus</i>	15	5	30	5/15	1,0 \pm 0,0
Genova	<i>M. barbatus</i>	11	2	18	3/11	1,5 \pm 0,5
Genova	<i>M. merluccius</i>	10	2	20	2/10	1,0 \pm 0,0
Genova	<i>M. galloprovincialis</i>	11	2	18	2/11	1,0 \pm 0,0
Genova	<i>S. mantis</i>	6	1	17	2/6	2,0 \pm 0,0
Genova	<i>P. kerathurus</i>	6	3	50	3/6	1,0 \pm 0,0
Genova	Total	59	15	25	17/59	1,13 \pm 0,4
Talamone	<i>Scorpaena sp.</i>	7	0	0	0/7	0
Talamone	<i>S. salpa</i>	4	0	0	0/4	0
Talamone	<i>M. barbatus</i>	4	0	0	0/4	0
Talamone	<i>L. amia</i>	2	2	100	2/2	1,0 \pm 0,0
Talamone	<i>P. erythrinus</i>	2	2	100	4/2	2,0 \pm 1,4
Talamone	<i>D. volitans</i>	2	1	50	1/2	1,0 \pm 0,0
Talamone	Other	3	2	67	2/3	1,0 \pm 0,0
Talamone	Total	24	7	29	9/24	1,33 \pm 0,8
Giglio Wreck area	<i>Scorpaena sp.</i>	6	1	17	1/6	1,0 \pm 0,0
Giglio Wreck area	<i>U. scaber</i>	2	1	50	1/2	1,0 \pm 0,0
Giglio Wreck area	Other	9	4	44	5/9	1,25 \pm 0,0
Giglio Wreck area	Total	17	6	35	7/17	1,16 \pm 0,4
Giglio Reference area	<i>Scorpaena sp.</i>	10	6	60	12/10	2,0 \pm 1,3
Giglio Reference area	<i>U. scaber</i>	2	2	100	2/2	1,0 \pm 0,0
Giglio Reference area	Other	3	1	33	1/3	1,0 \pm 0,0
Giglio Reference area	Total	15	9	60	15/15	1,66 \pm 1,1
Ventotene	<i>P. phycis</i>	3	0	0	0/3	0
Ventotene	<i>M. poutassou</i>	7	0	0	0/7	0
Ventotene	<i>P. erythrinus</i>	4	1	25	1/4	1,0 \pm 0,0
Ventotene	<i>S. canicula</i>	1	0	0	0/1	0,0
Ventotene	Total	15	1	7	1/15	1,0 \pm 0,0
Napoli	<i>E. encrasicolus</i>	10	0	0	0/10	0
Napoli	<i>S. scomber</i>	10	6	60	12/10	2 \pm 1,3
Napoli	<i>M. merluccius</i>	10	0	0	0/10	0
Napoli	<i>M. barbatus</i>	10	3	30	4/10	1,33 \pm 0,6
Napoli	<i>C. lucerna</i>	10	4	40	5/10	1,25 \pm 0,5
Napoli	<i>M. galloprovincialis</i>	11	3	27	3/11	1 \pm 0,0
Napoli	<i>S. mantis</i>	5	1	20	1/5	1 \pm 0,0
Napoli	<i>P. kerathurus</i>	5	3	60	4/5	1,3 \pm 0,6
Napoli	Total	71	20	28	29/71	1,45 \pm 0,8

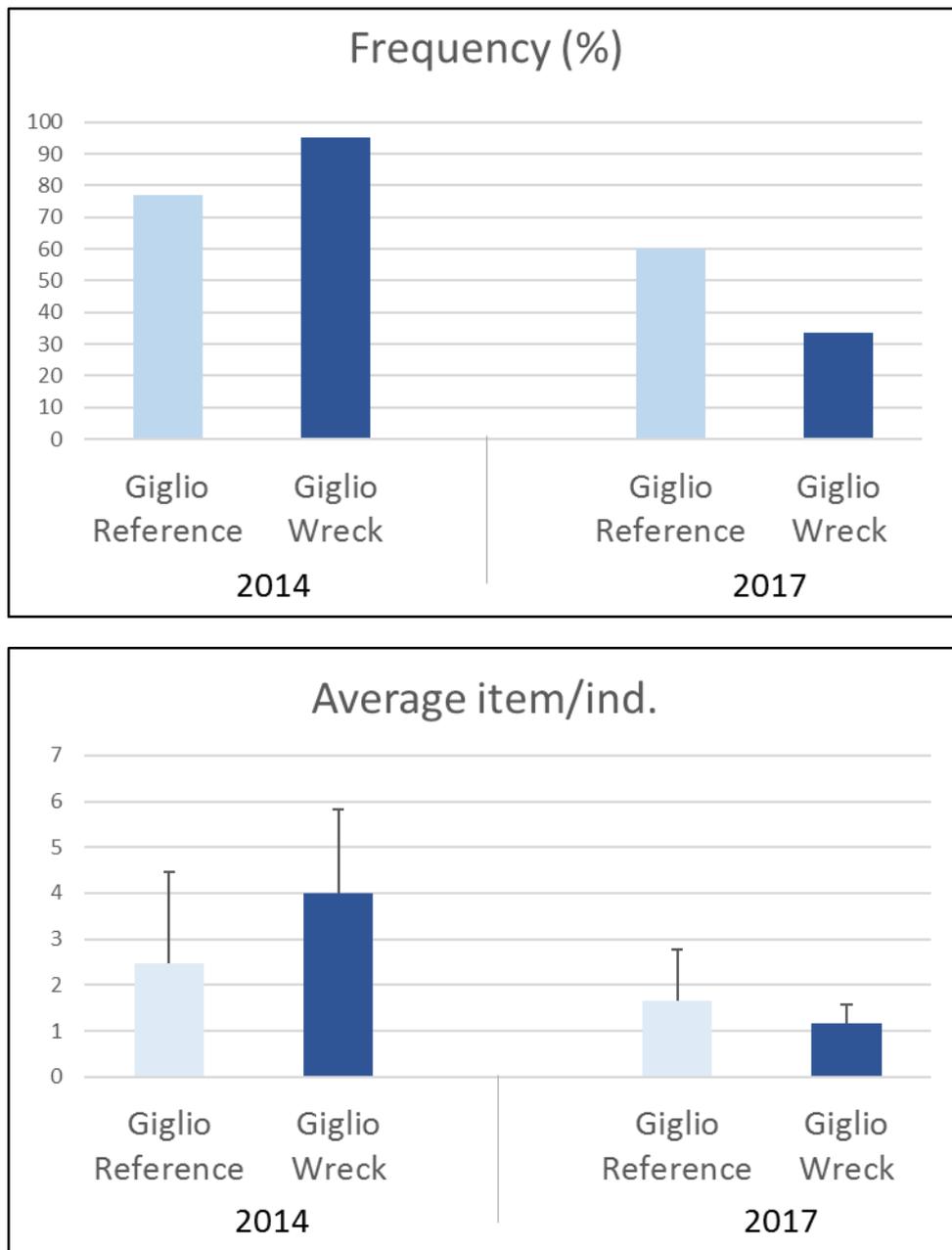


Figure 6. Comparison between results obtained in 2014 and 2017 at two different areas of Giglio Island; frequency (%) of fish positive to the microplastics ingestion and average item per individual (mean \pm standard deviation).

Figure 7 shows the main characteristics in terms of shape, size and polymer typology for the plastic particles isolated from all of the organisms and sampling sites.

The shape was quite homogeneous being largely dominated by fragments (typically >55%), followed by lines and films which (together) prevailed only in organisms from Napoli (Figure 7).

The most common size classes were those smaller than 0.5 mm which generally accounted for at least 60% of all ingested microplastics: once again, the only exception was for fish from Napoli, where 50% of extracted particles were between 1 and 5 mm and related to the elevated abundance of lines and films (Figure 7).

The μ FT-IR analyses provided typical spectra allowing to reveal the polymeric composition of extracted MPs (Figures 7-11). Results indicated a certain heterogeneity of the polymer composition, especially in those sites represented by a large number of analysed organisms (Genova and Napoli); polyethylene (PE) is the most represented chemical typology followed by polyester, ethylene-vinyl acetate (EVA), polyamide (PA) and polypropylene (PP), which might reflect the influence of both specific local activities, or hydrographic conditions.

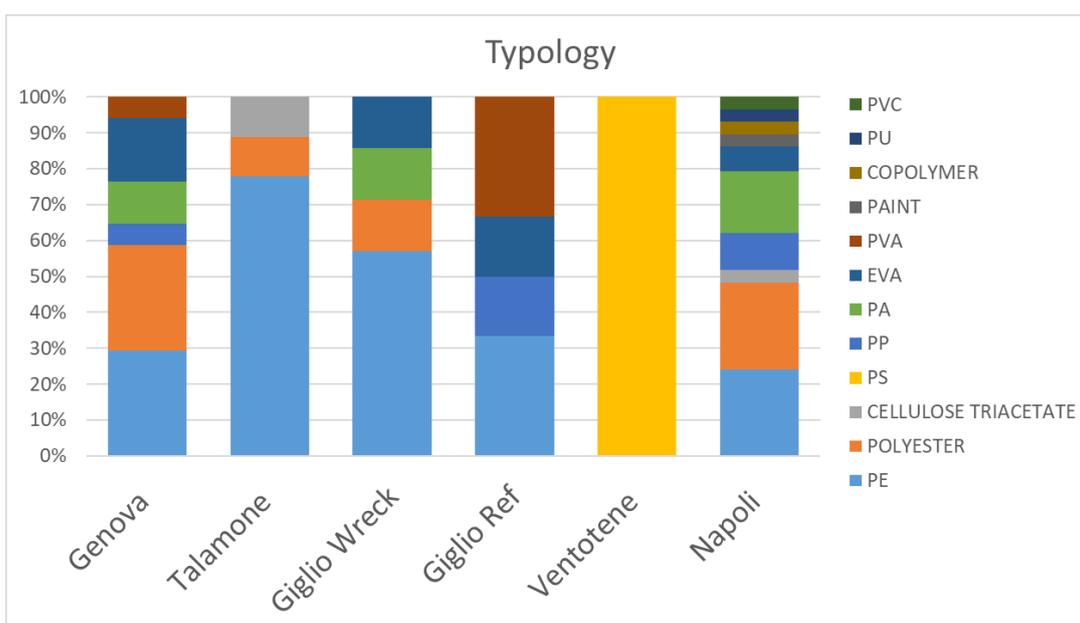
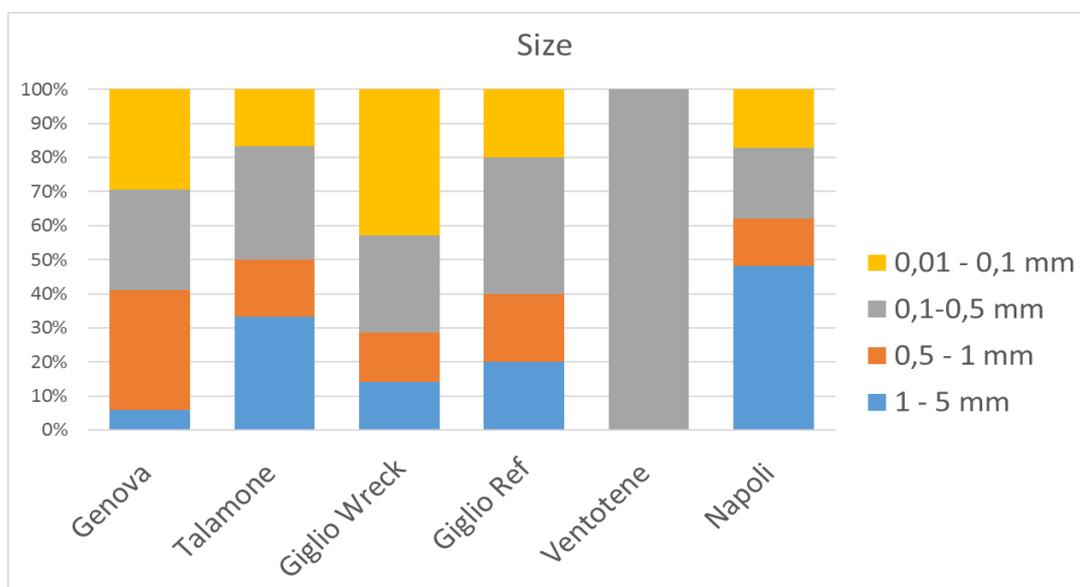
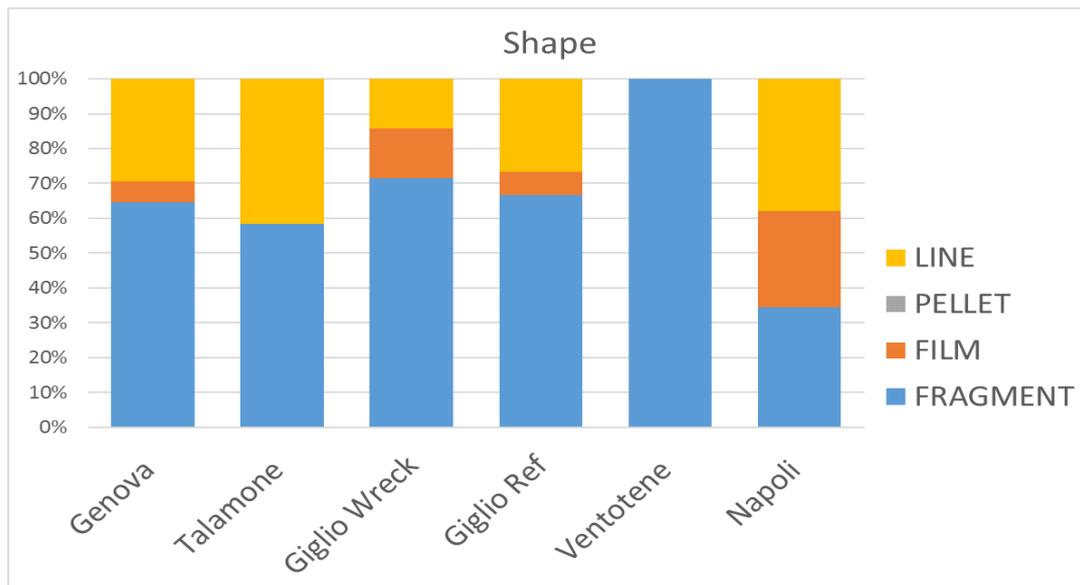


Figure 7. Shape, size and typology of microplastics extracted in organisms from the Tyrrhenian Sea.

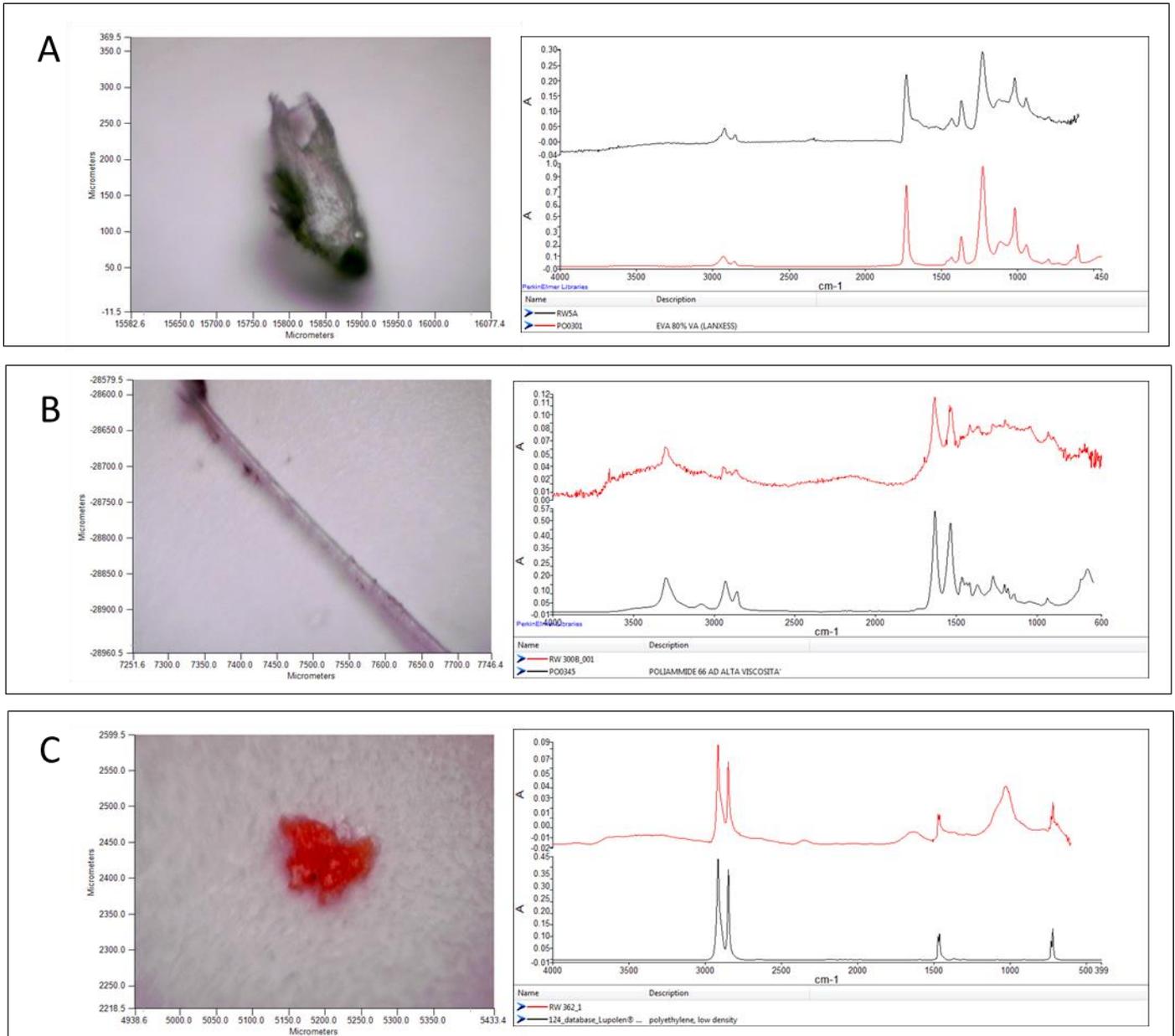


Figure 8. Examples of FT-IR spectra of EVA (A), polyamide (B) and polyethylene (C) found in organism from Genova. The red spectra show the reference spectra.

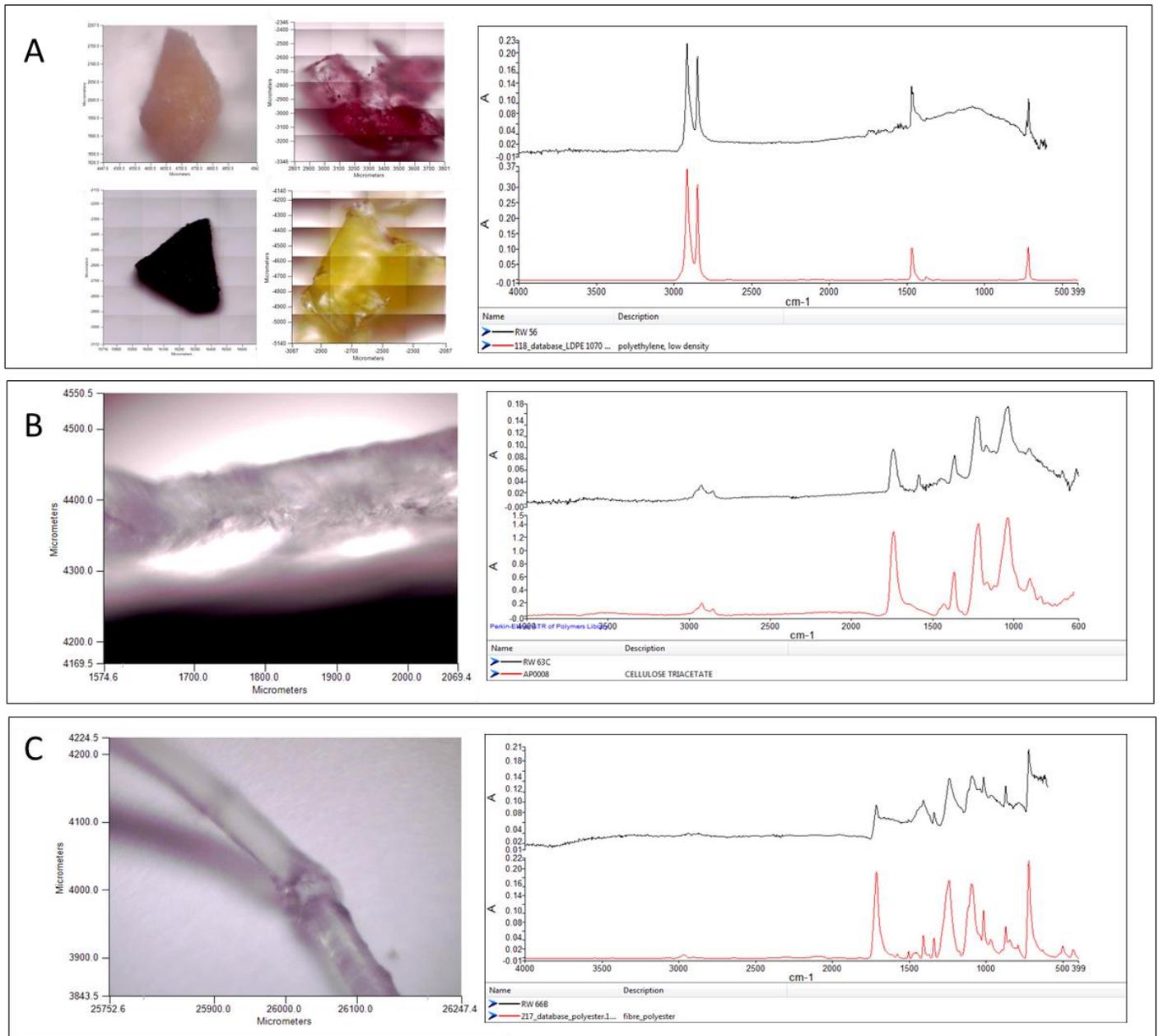


Figure 9. Examples of FT-IR spectra of polyethylene (A), cellulose triacetate (B) and polyester (C) found in organism from Talamone. The red spectra show the reference spectra.

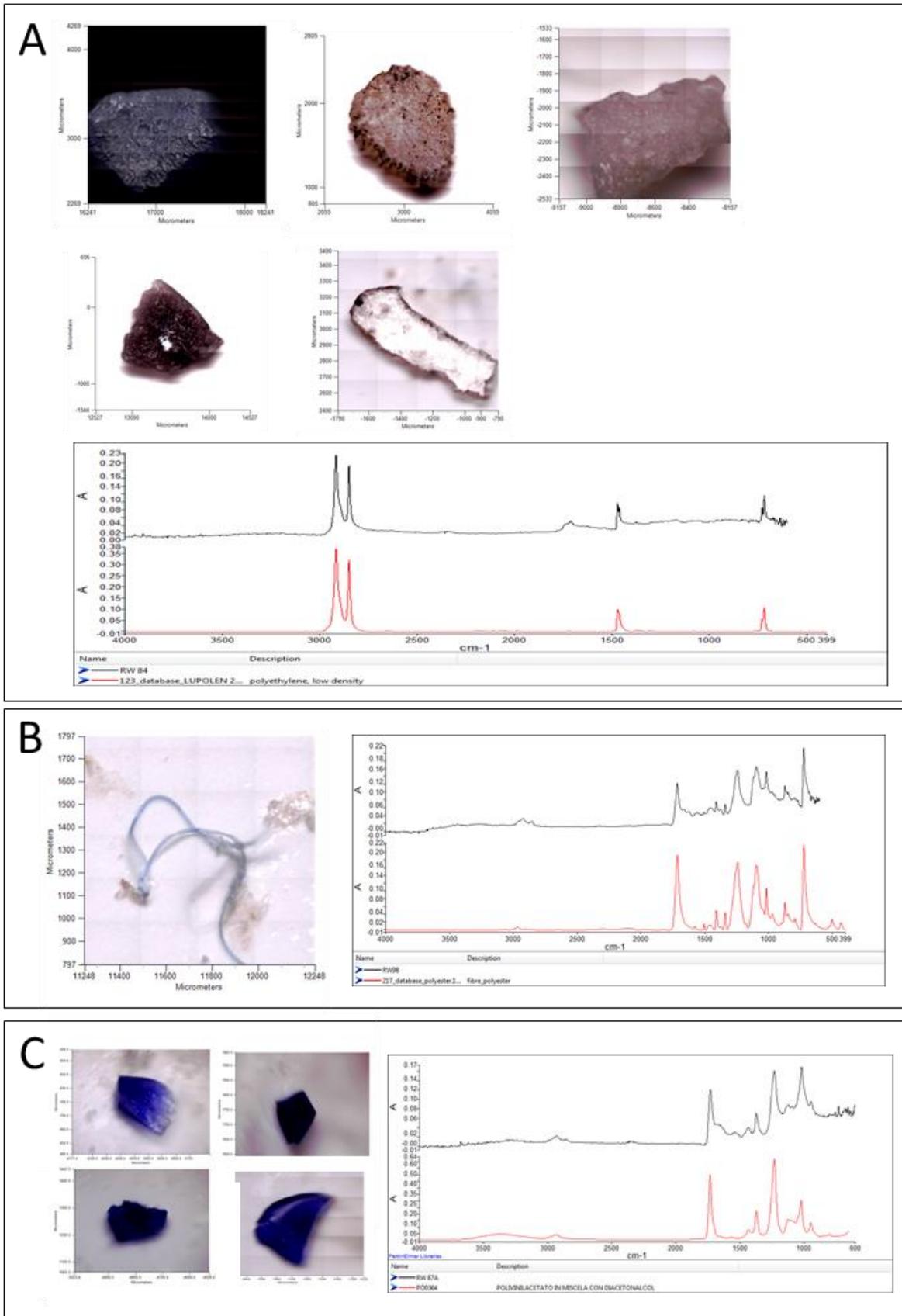


Figure 10. Examples of FT-IR Spectra of PE (A), polyester (B) and polyvinyl acetate (C) found in fish from Giglio Island. The red spectra show the reference spectra.

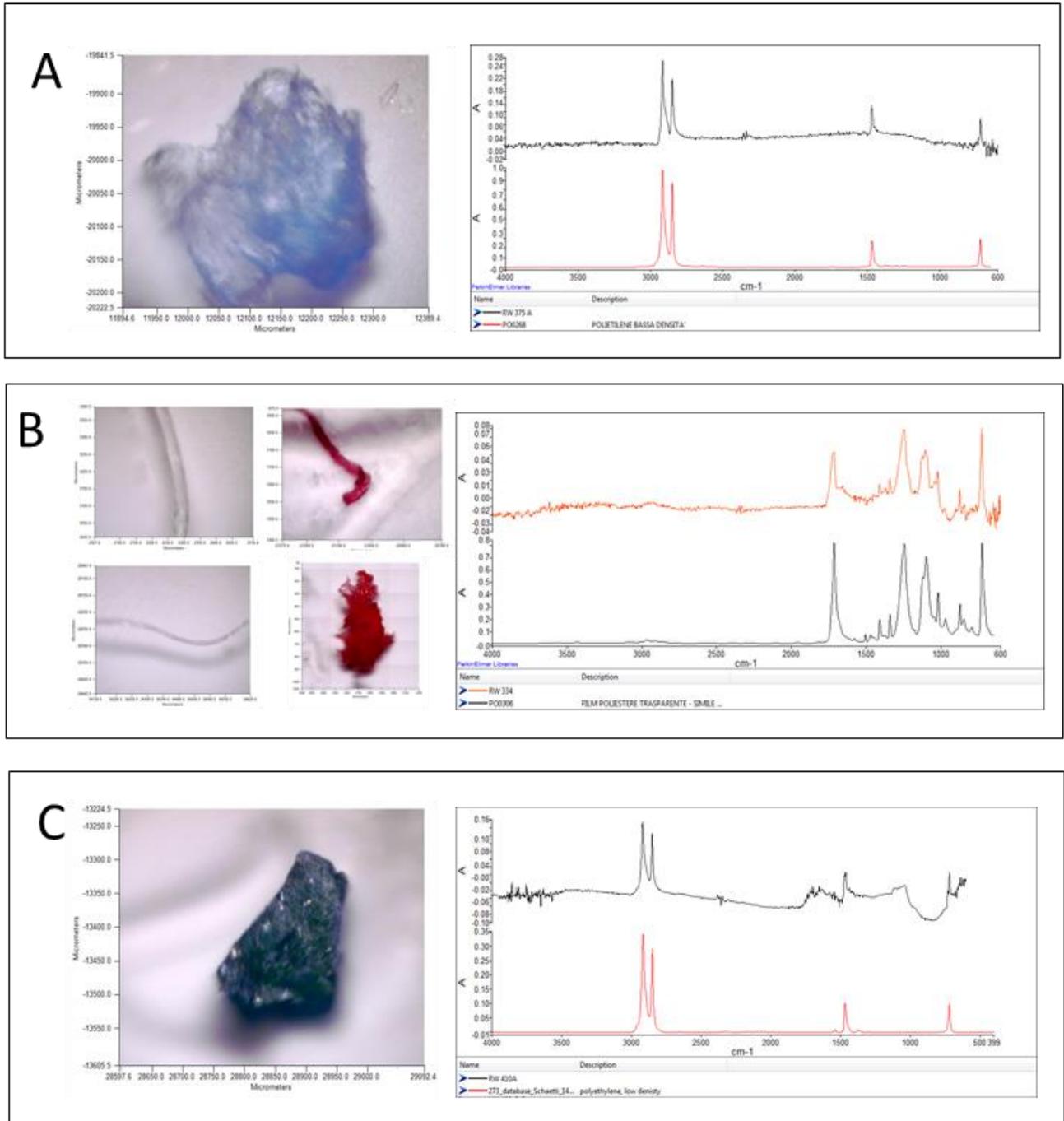


Figure 11. Examples of FT-IR spectra of PE (A and C) and polyester (B) found in organisms from Napoli. The red spectra show the reference spectra.

GENERAL CONCLUSIONS

- MPs ingestion is a widespread phenomenon in the Tyrrhenian sea with almost 25-30% of positive organisms on 201 analysed. These values are rather comparable to those of Adriatic organisms.
- The number of microplastics extracted in positive fish was rather similar in different sites and species, typically ranging between 1 and 2 particles. Frequency is a more appropriate index than number of ingested particles to discriminate geographical or biological differences.
- Slight geographical differences occurred in terms of size and typology of particles, suggesting the role of local activities and hydrographic characteristics.
- Three years after the Costa Concordia wreck was towed away to Genova, a significant recovery of microplastic pollution in fish of Giglio Island has been observed. This is the first microplastic monitoring study related to a specific case-study.
- No clear relationships could be observed between microplastic ingestion in different species and trophic position, feeding strategy or habitat preference. No biomagnification occurs for microplastics along food webs.
- The overall results on microplastics ingestion in biota from the Tyrrhenian Sea reveal a high ecological relevance of this problem but exclude human health risk from consumption of fish/shellfish.

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