Plastic litter transfer from sediments towards marine trophic webs: A case study on holothurians

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A B S T R A C T

This study estimates for the very first time plastic litter levels in sea cucumbers (Echinodermata, Holothuroidea) sampled in situ and their intakes from sediments in three different rocky bottom habitats (slides, cliff, banks) settled in Salina Island (Aeolian Archipelago). Macroplastic were never recorded while meso- and microplastics were identified in all sediment (81–438 items/kg d.w.) and animal samples (1.8–22 items/ind.). Plastic intakes by sea cucumbers resulted frequently associated to the size range included within 100–2000 μm. Over than 70% of ingested plastic litter is represented by the size fraction > 500 μm. Sediment/animals ratios % are included 2.7 ± 2.0% in studied habitats with a selective intake of fragments occurring in slides. Furthermore, results support the occurrence of selective ingestion of plastic litter by holothurians in natural environments underlining the role of these species in microplastic transfer from abiotic towards biotic compartments of the marine trophic web.

1. Introduction

Litter transfer from the environment towards marine trophic webs represent an important task that should be better described and clarified by the literature to achieve Horizon 2020 targets concerning the Marine Strategy Framework Directive principal purposes (2008/56/EC). Plastic litter represent the larger part of the marine litter recovered along Mediterranean coasts (over than 50% in Baleari Islands, Martinez-Ribes et al., 2007). According to the literature (Galgani et al., 2013) maximum size of plastic marine litter determine the classification as macroplastic (> 25,000 μm); mesoplastic (5001–25,000 μm), and microplastic (MPs; 63–5000 μm).

Recent studies evidence that plastic litter affects different marine species (Avio et al., 2015; Collard et al., 2017; Dehaut et al., 2016; Fossi et al., 2016) and that it penetrates the marine trophic web (Ivar Do Sul and Costa, 2014; Setala et al., 2014). Cole et al. (2011) in a recent review, reported many different researches documenting microplastic ingestion by marine species such as seabirds, crustaceans and fish. Plastic ingestion could affect the feeding habits, reproductive success, and breathing of many marine organisms (Cole et al., 2014), which ingest plastic litter, such as cetaceans (Tonay et al., 2007), and large pelagic fish species (i.e. *Xiphias gladius*, *Thunnus thynnus* and *Thunnus alalunga*) (Romeo et al., 2015). Also, small pelagic planktivorous fishes, such as sardines, could actively ingest microplastic directly from the water column (Avio et al., 2015) nevertheless, in a laboratory study, only 19% of tested sardines predate floating microplastics and the principal route of ingestion is related to the feeding. On the contrary a large number of tested sardine and anchovy evidenced microplastics in stomach contents almost during the whole year with a clear difference related to their colours (Renzi et al., unpublished data). According to the literature, pelagic species ingested more particles than benthic ones, which, on the contrary, ingested more fibres (Neves et al., 2015). Recently, the presence of MPs in 45% of biota from the Adriatic Sea (Dehaut et al., 2016), and in particular in 95% of the benthic flatfish *Solea solea* (Pellini et al., 2018) were recorded supporting the hypothesis that benthic species could be highly impacted by litter stored in sediments.

Species belonging to the lower trophic level, that are indiscriminate feeders, are not able to differentiate between plastics and prey ingesting high levels of plastic. In vitro studies reported by Cole et al. (2011) evidenced the occurrence of microplastic ingestion by gastropods (7–70 μm), echinoderm larvae (10–20 μm), trochophore larvae (3–10 μm), scallop (16–18 μm), amphipods (20–2000 μm), and mussel (2–16 μm). Also sea cucumbers in vitro ingested microplastic in a wide dimensional range (Graham and Thompson, 2009).

Some authors reported that many marine animals are able to remove ingested materials that could cause harm (Thompson, 2006; Andrady, 2011), including microplastics (Graham and Thompson,

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2009). Nevertheless, ingestion could represent a possible risk as well as a recent research performed in mussels recorded translocation of polystyrene microspheres in mussels' haemolymph up to 48 days after the exposure (Browne et al., 2008). Another research evidenced that in blue mussel, the exposure to microplastic is able to induce immune response and granuloma formation in the digestive glands (Köhler, 2010).

In spite of recent studies, relationship between microplastic content in organisms and environments still remains unclear (Qu et al., 2018). In particular, relationships among plastic litter settled in sediments and benthic species are not jet clarified by the literature.

This research was performed to evaluate plastic litter transfer from sediments towards benthic species. Sea cucumber (Holothurians) was selected as benthic species of specific interest in this study representing a first step from the detritus net towards the trophic web in shallow marine ecosystems. This taxonomic group was selected as it is abundant in different marine habitats, it is represented by sedentary deposit feeding or suspension-feeding species that are indiscriminate feeders, it evidenced ingestion of plastics (Graham and Thompson, 2009) and it is reported to be a key benthic taxonomic group in marine ecosystems (Purcell et al., 2016). Furthermore, it is highly exploited by fishery for human food purposes allowing evaluations related to human exposure levels (Purcell et al., 2016).

2. Materials and methods

2.1. Study area features

The entire Aeolian Islands are inhabited even if islands are subjected to different degree levels of human pressure. This research focuses on Salina Island as it was designated for the institution of a Marine Protected Area (MPA) for the Aeolian Archipelago according to the Italian Law 979/82. At this moment Salina Island is not jet insert in the MPA Italian list.

Salina Island (Aeolian Archipelago) was the study area as this research represents an advancement of a previous published study developed in the Aeolian Archipelago islands by Fastelli et al. (2016). Authors focused on levels of plastic litter in sediments from different Islands of the Aeolian Archipelago (Alicudi, Filicudi, Lipari, Panarea, Stromboli, Vulcano, and Salina); and evaluated levels according to size, shape, and colour differences to define levels in sediments from Islands subjected to different direct local human pressure (Lipari; Panarea ≫ Alicudi; Filicudi; Salina). Fastelli et al. (2016) did not evidenced significant difference concerning plastic litter levels in sediments due to known local pollution sources. The lower MPs levels were found in sediments from Stromboli and Salina Islands while Lipari showed the highest level.

We selected Salina Island as study area on the basis of low pollution levels observed by Fastelli et al. (2016) and the future institution of MPA. According to the geomorphological structure of bottoms closed to Salina Island (Bosman et al., 2013), three different rocky bottom habitats (slides, cliff, banks) were sampled as reported in Fig. 1.

2.2. Principal features of selected species

Sea cucumber (Holothurians) was selected as benthic species of specific interest in this study due to different reasons. First of all holothurians are abundant in selected sampling sites and in all of the selected rocky bottom habitats (sampled species *H. tubulosa*). These animals are wide representative of marine benthic species (Purcell et al., 2016) and evidence a benthic deposit-feeding or suspension-feeding behaviour that is easy to be compared to MPs in sediments. Furthermore, holothurians are key benthic taxonomic group in marine ecosystems to preserve ecosystem integrity (Purcell et al., 2016). A recent in vitro study evidences a specific preference for plastic feeding (Graham and Thompson, 2009). Last but not least, holothurians are linked with the marine trophic web through predation by stars; crustaceans, gastropods, fishes (Francour, 1997; Dance et al., 2003) and they are highly exploited by humans for food purposes (Purcell et al., 2016).

On the basis of the ecological roles of these species in marine ecosystems, the Italian Ministry of Agriculture and Forestry, implemented regulatory measures centred on the Ecosystem-Based Fisheries Management to safeguard sea cucumbers from overexploited. At this date, holothurians are protected species in Italy and target fishes or bycatch are not allowed starting from 21/02/2018 till 31/12/2019 for conservation purposes and for the defence of national resource from overexploitation by fishing (Italian Ministry of Agriculture and Forestry, Law n. 156; 27/02/2018).

2.3. Logic model applied to samplings

Three different rocky bottom habitats (slides, cliff, banks) settled in Salina Island were selected for the evaluation of plastic litter features in sediments. Contextually, sea cucumbers were collected in statistical replicates in each sampling sites closed to sediment collection points to determine plastic levels in animals and their intakes in natural conditions and to perform comparisons to sediment levels and to different rocky bottom habitats considered. The sampling strategy included fixed and random factors a priori selected. Considered factors were: Rocky bottom habitats (three levels, fixed), sampling sites (R1–R2; two levels, fixed), sampling replicates (five levels, random). Overall, 30 samples were collected ($n = 30$) per each considered matrix.

2.4. Sampling activities

Starting from June to September 2017, samplings were performed from different rocky bottom habitats as reported in Fig. 1. In particular were sampled: i) Franata Tre Pietre, and Franata Punta Vallespina, Slides habitat (big blocks/boulders); ii) Secca del Capo, and Secca di Pollara, Banks habitats (open sea conditions); iii) Parete dei Gamberi, and Parete Scoglio Piramide, Cliffs habitats (vertical substrata).

Undisturbed surface sediments (5 cm depth) from Salina Island were collected at −30 m of depth by scientific scuba divers using wide mouth glass jars in statistical replicates following the same sampling strategy and methods of previous research performed in the same area (Fastelli et al., 2016) to favour comparisons even if, in this study, only Salina Island was considered. Every replicates were taken approximately within a radius of 1 m and collected samples were stored frozen at −15 °C until analyses (Galgani et al., 2013; adapted).

H. tubulosa species were collected contextually to sediment sampling in significant replicates per each sampling site and stored separately in glass jars submerged in ethylene to preserve animals until laboratory dissection and analyses. Body size (max length) of animals was standardized during collection to fix animal age and to perform comparison among sampling sites.

2.5. Laboratory analyses

2.5.1. Sediment pretreatment & extraction

Sediment samples were analysed following the extraction and classification method reported by Fastelli et al. (2016) for the Aeolian Archipelago Islands to a better comparison of collected data with data reported previously by the literature. In brief, sediments were dried at 40 °C and sieved throughout 4000–63 μm standard test sieves to determine grain-size. Fractions retrained on 4 mm, 2 mm, and 1 mm test sieves were collected and directly analysed by stereomicroscopy performing the manual sorting of plastic litter. Sediments retained by the 63 μm sieve were extracted by the exposure of saturated NaCl solution following the extraction method reported by the literature (Blašković et al., 2017). The extraction of this sediment fraction was necessary to reduce interferences during microscope analyses.

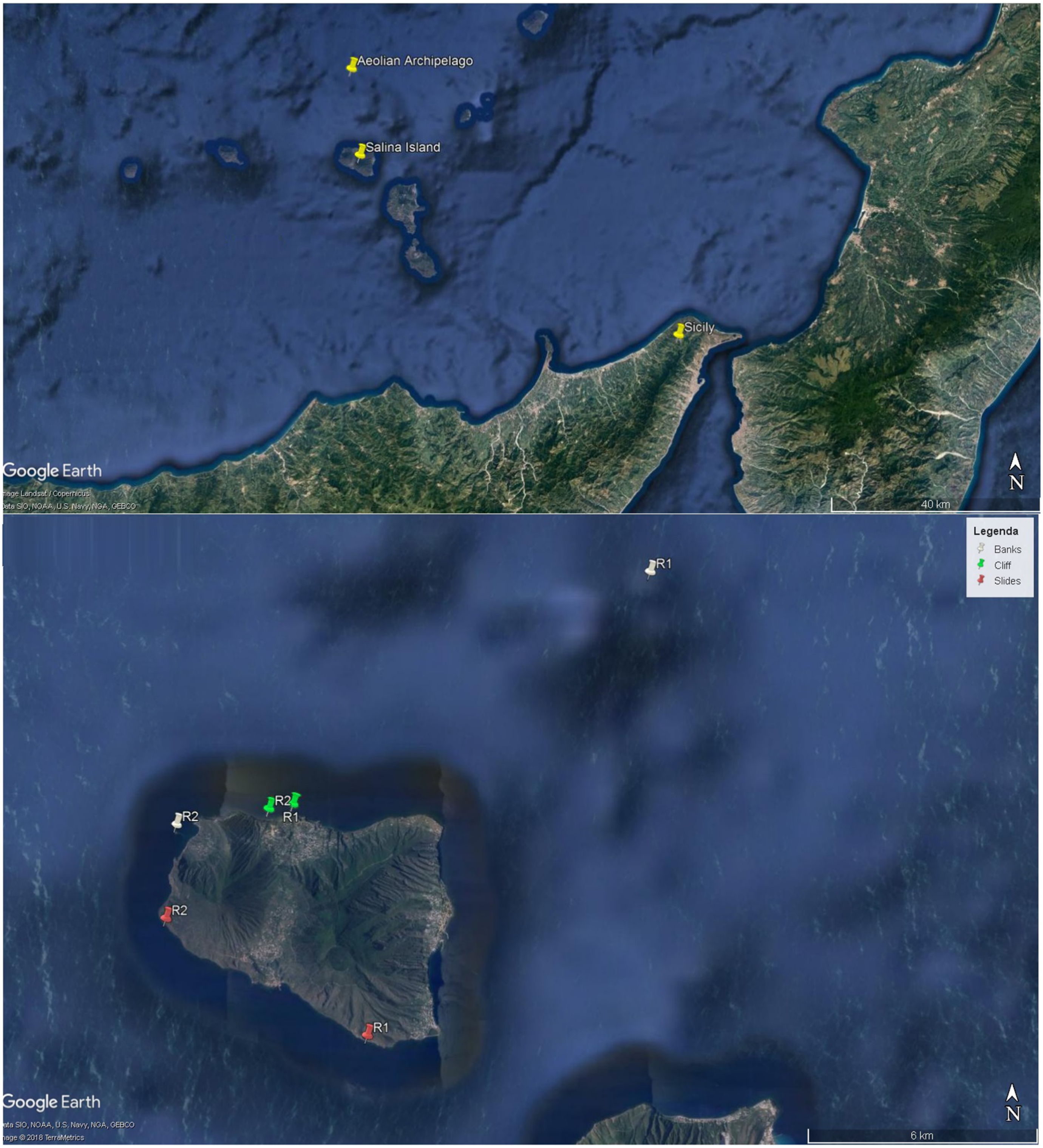

Fig. 1. Representation of the study area and sampling sites. Location of the Salina Island within the Aeolian Archipelago (Sicily, Italy), and georeferenced position of sampling sites around the Salina Island's coast. Slides (red), Banks (withe), Cliffs (green). R1 and R2 means sampling station replicates for the same rocky bottom habitat. Random replicates were performed for each matrix (sediment, animals) in each sampling station replicates. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.5.2. *Sea cucumber extraction*

Animal tissues and recovered ingested sediments were extracted after evisceration. Extraction was performed in glass beaker with 20 mL of H_2O_2 30% per gram of tissue, beakers were put in a Bain-marie at 50 °C till the complete digestion of tissues - 7 days (adapted, Nuelle et al., 2014).

2.5.3. *Plastic litter analyses & classification*

In both cases, extracted sample were completely recovered onto paper fibres (0.45 μm filter disks) by the use of a filtrating system, consisting in decontaminated filtration glass set, vacuum pump, manifold and funnel. Glassware was accurately rinsed during the filtration of each sample to increase recovery efficiency of litter. Filters were stored in glass Petri dish and dried in oven at 40 °C till constant weight. Collected litter were analysed by stereomicroscopy (Nikon SMZ-800 N) with micro tweezers and stored in Eppendorf tubes filled with distilled water for μFT-IR confirmations. All identified items were divided in shape, colour and dimensional classes reported by the literature (JRC EU, 2013; Galgani et al., 2013; Alomar et al., 2016; Blašković et al., 2017; Fastelli et al., 2016). The Nikon's software for the imaging analysis was applied to the litter dimensional measurements (Nikon ACT-1).

Litter was divided by shape (fibres, fragments, pellet, film, foam), size (macropalstics, > 25,000 μm; microplastic, MPs, 63–5000 μm; mesoplastic, 5001–25,000 μm), and colour according to Galgani et al. (2013).

2.6. Data standardization, post-processing & quality assurance

Data on sediments were normalized as items/kg d.w. sediment. Collected holothurians were extracted as pools of 5 individuals and results were normalized as items/individuals. Body size of collected animals (max. body length) rather than weight was standardized during animal collection to fix animal age and to perform comparisons among sampling sites. Weight standardization was avoided, as animals tend to eviscerate after collection and weight resulted affected by variability. Confirmation of targeted plastic litter was performed by μFT-IR (Nicolet iN10 MX, Thermo Scientific) technique on a fraction of collected items significant on a statistical basis to ensure data quality. Experimental blanks on sediment matrix were performed as detailed reported by Blašković et al. (2017), blanks were also performed on the digestion process by the exposure of blank beaker filled in with the solution used for the organic matter digestion. Average results on blanks were subtracted to values recorded in samples. Results were statistically analysed by classical univariate approach to evaluate significant differences among average recorded values (Pearson's correlation matrix, $p < 0.05$).

3. Results

Sea cucumbers were easily collected in all sampling sites confirming the attended wide abundance of selected species in all of the tested rocky bottom habitats. Sampled species was *Holothuria tubulosa* (Fig. 2) that represents the most abundant sea cucumber species recorded in sampling areas. Endocommensal fish species was occasionally recorded inside sampled animals. In Fig. 2a the dissection of a normal animal is compared to *H. tubulosa* affected by a sexual pair of symbionts species recovered inside sea cucumber's intestinal tract (Fig. 2b). As evidenced, fish species occupy a large amount of the intestinal tract of the hosting animal reducing the internal space available for the ingestion of sediments. Their presence was documented only occasionally in animals collected in banks sampling sites. The scarce solidity of such random records does not allow us to correlate the presence of symbionts neither to levels of ingested plastic litter nor to the rocky bottom habitat type. Nevertheless, their presence into sea cucumbers' intestinal tract reduces significantly total volume of sediment/day ingested by hosting animals.

On a general basis, plastic items are recorded in all collected sediments and in all collected animals form each tested rocky bottom habitats. Microplastics (size included within 100–5000 μm) are the principal component concerning the recorded number of items in both matrices. In Fig. 3 some plastic litter (different shape, colours) collected during this study from the holothurians' stomach are represented.

3.1. Sediments

3.1.1. General features

The average amount of sand is always over than 75% d.w. in almost all sediment samples with no differences related to the habitat. The other part of sampled sediments is represented by gravel (23% d.w.).

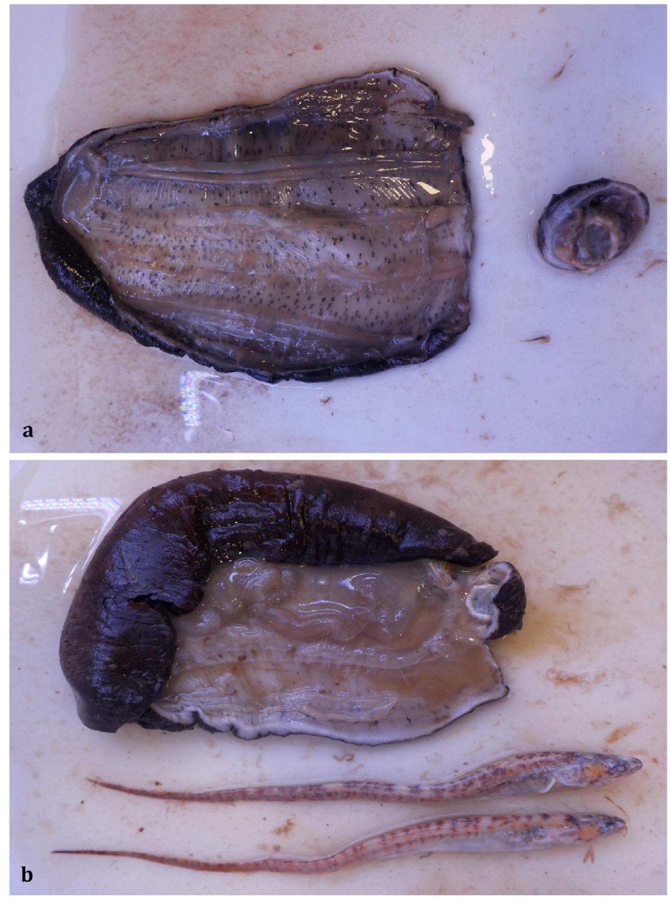

Fig. 2. Images of dissection of sampled animals. Dissection of a normal holothurian species is reported (a) and compared to animal affected by endocommensal fish species (b). Sexual pairs were represented with the sea cucumber into which they were recovered.

Silt (mud and clay) is represented lower than 1% in all tested samples. Any statistical relationships were recorded by grain-size structure of sediment and plastic litter amounts (Pearson's correlation matrix, $p > 0.05$).

3.1.2. Plastic features

Macroplastic were never recorded in sediments; microplastic dominated Salina sediments while mesoplastics were frequently recorded but at low percentages. Total amounts ranged between 99 and 431 items/kg d.w. (cliff - banks). Fibres are dominants on average (244 items/kg d.w.) even if films (60% items recorded) were the principal litter component only in slides (Fig. 4). Concerning fibres distribution in different rocky bottom habitats, cliffs and slides recorded average similar levels (respectively 85 and 72 items/kg d.w.) while banks showed the highest average levels of fibre in sediments (403 items/kg d.w.). Differences among rocky bottom habitats resulted significant concerning fibres in banks and films in slides (weakly significant, $p < 0.05$). As regards as colours, Black (BK), green (GN), white (W), Clear (C) fibres dominates in banks, while W, C, Pink (P) Films and GN Fibres in slides, as well as GN fibres are dominant in cliffs (Fig. 5). Almost all identified GN microplastics in sediments were fibres. Observed differences concerning colour features resulted significant according to rocky bottom habitats (weakly significant, $p < 0.05$).

3.2. Holothurian levels

Concerning animals, any macroplastics were recorded in sea cucumbers from any rocky bottom habitats. Microplastics were dominant

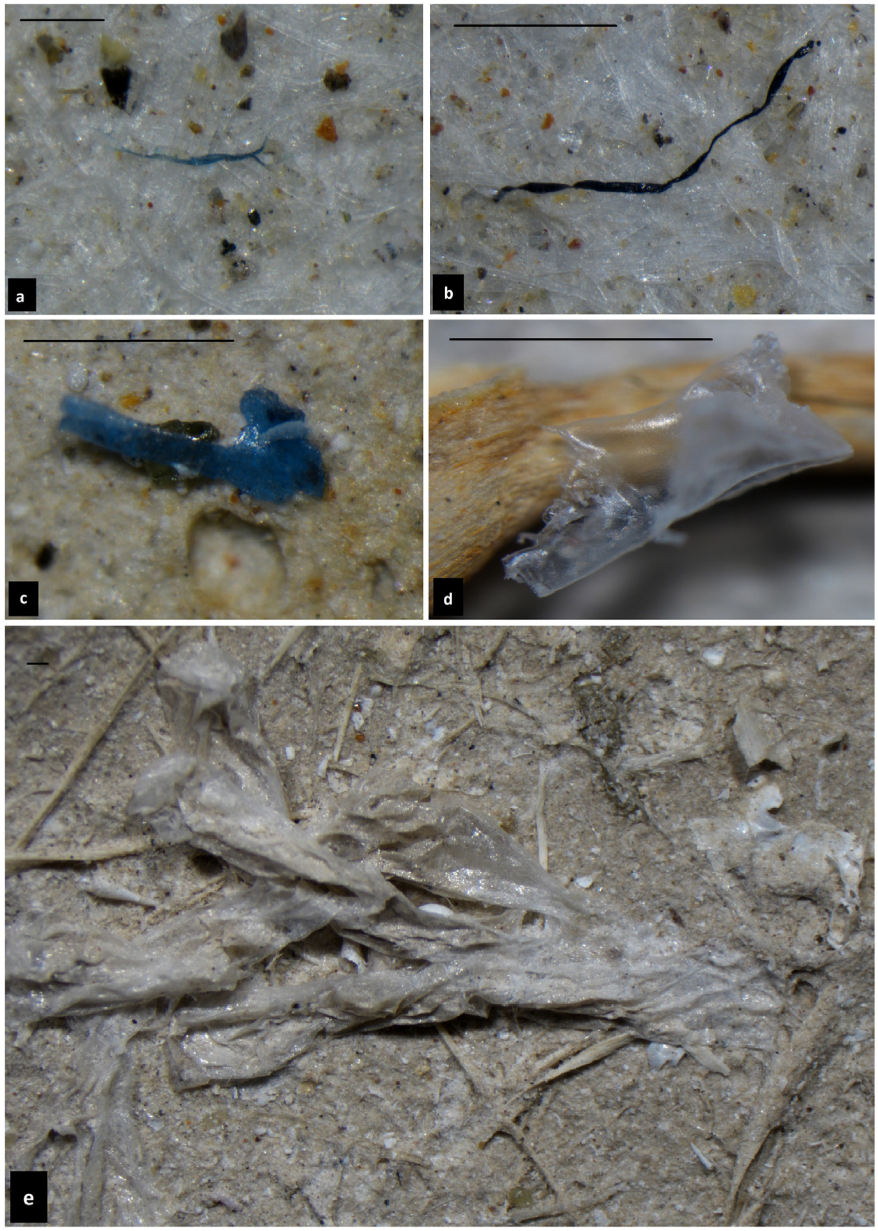

Fig. 3. Images of plastic litter recorded in holothurian species. Fibres and films are reported. Images numbered from a-e are representations of microplastic litter ingested by sea cucumbers in the study area. Black lines are referred to 100 µm. In particular, fibres (a,b), fragment (c), film (d), digested animals with a mesoplastic film (e).

even if mesoplastic were also recorded in sea cucumbers from all rocky bottom habitats. Mesoplastics average levels per individuals were similar in cliff, slides, and banks (not significant differences, $p > 0.05$).

Average levels of plastic litter (items/ind., SD) in different rocky bottom habitats are reported in Table 1. In particular, Table 1a reports data aggregated concerning size-class (macro, meso and microplastic) of litter and the average total amount per rocky bottom habitats; Table 1b details averages recorded concerning microplastics in each habitat.

Concerning MPs, over than 70% of ingested microplastic is represented by a size-class higher than 500 µm. Ingested microplastics within 63–500 µm size were on average 23.0% in cliff, 13.6% in slide, 28% in bank. Furthermore, animals coming from slides recorded the highest numbers of items/ind within 500–4000 µm compared to animals from cliff rocky bottom habitats even if a wide standard deviation is recorded.

In Fig. 4 average normalized items recorded in sediments and holothurians from different rocky bottom habitats are compared grouping data concerning shape of plastic litter. Fibre is the principal shape recorded in both sediments and holothurians followed by film (in both cases) and fragments. Some granules are recorded in holothurians from bank rocky bottom habitats. As reported, the highest average number of fibres than other rocky bottom habitats characterizes sediments from banks. On the contrary, the highest fibre average number in holothurians is recorded in slides.

Fig. 5 reports colour average fingerprints (level distribution per colour) recorded in both sediments and holothurians grouping data by rocky bottom habitats. Results evidence a strong correlation among colour recorded in sediments and colour fingerprint recorded in holothurians for each considered rocky bottom habitats.

3.3. Holothurian intakes

A comparative analysis of recorded plastic litter features in both

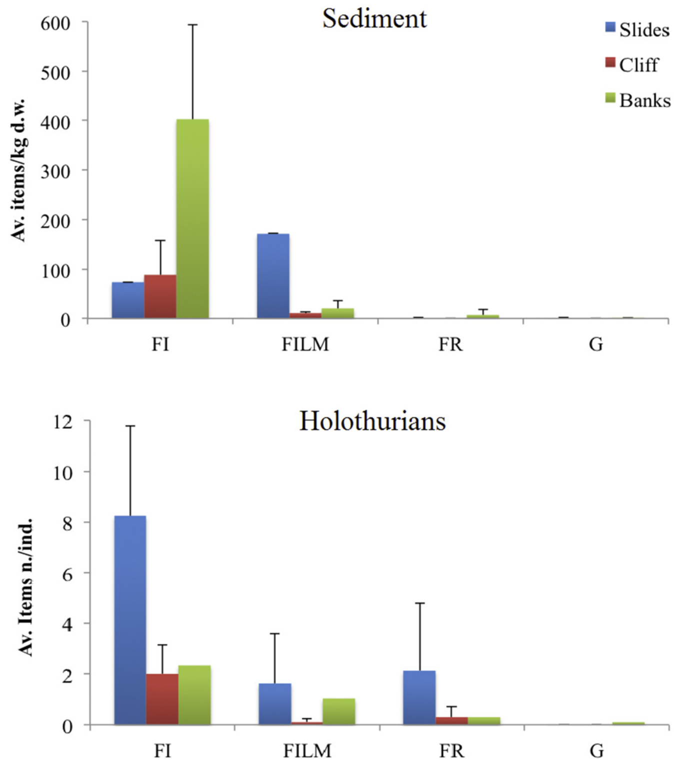

Fig. 4. Plastic litter shape classes in different rocky bottom habitats. Data recorded (average + SD) are reported in sediments (average number of items/kg d.w.) and holothurians (average number of items/animal). Data are grouped per shape classes (fibres, FI; films, FILM; fragments, FR; granule, G) and per rocky bottom habitat (slide, cliff, bank). Shape classes related to Pellet (P), foam (FO), and undetermined (U) are not represented as recorded at levels closed to zero.

sediments and sea cucumbers is reported in Table 2 grouping data per rocky bottom habitat types.

On average, sea cucumbers plastic litter intakes/individual from sediments is the 2.74 ± 2.0% of the plastic litter amount recorded in sediments as mg/kg d.w. Intakes are different comparing different rocky bottom habitats and are 4.9 ± 7.3% in slides, 2.4 ± 3.9% in cliff and 0.88 ± 1.0% in banks. The selective intake of fragments occurring in slides (Fig. 4) is associated to the highest intake value recorded.

4. Discussion

This study evidenced a widespread pollution in bottoms from shallow marine ecosystem by plastic litter as well as meso- and microplastics were recovered in all tested sediment and animal samples. Any tested rocky bottom habitat considered in this research resulted affected by plastic litter. These results confirm previous researches performed in the Northern Adriatic Sea by Renzi et al. (2018a) where all tested marine habitat resulted polluted by plastic litter.

4.1. Sediment features & plastic litter levels linked to bottom types

Sands as attended in shallow and not impacted marine ecosystems are the principal fraction of sediments. This occurrence is also confirmed by previous studies performed in the Aeolian Archipelago Islands (Renzi et al., 2011; Andaloro et al., 2012; Fastelli et al., 2016). Furthermore, previous researches evidenced the absence of correlations among plastic litter levels in sediments and grain-size structure (Martins and Sobral, 2011; Romeo et al., 2015; Fastelli et al., 2016; Blašković et al., 2017) as also confirmed by this study. These results support the fact that plastic litter does not follow typical silt dynamics.

Concerning average amount of plastic litter recorded in sediments, results obtained in this study are consistent to values previously recorded in Salina Island (Fastelli et al., 2016). As regards as numbers of items recorded per kg of dried sediment, this study confirms that microplastic is the principal size-class of plastic litter in both sediment and holothurian from the Salina Island.

Compared to plastic litter recorded in other Mediterranean areas, this study evidenced average levels similar to values recorded in bottoms from the Northern Adriatic Sea (137–703 items/kg d.w.) even if Salina's values are significantly lower to those recorded closed to the beach of Caorle (703 items/kg d.w.) by Renzi et al. (2018a). Results obtained in Slovenia (Laglbauer et al., 2014), and Croatia (Blašković et al., 2017) are comparable to values reported in this study. Furthermore, data are similar to values recorded in southern Tuscany (Cannas et al., 2017) but higher than values recorded in sediments from Cecina collected closed to the terrestrial protected area (Blašković et al., 2018).

Concerning shape of plastic litter, Fastelli et al. (2016) evidenced that microplastic fibres represent over than 85% of the recorded plastic litter in Salina Island. This study also supports previous researches as fibres resulted, on average, the dominant shape. In spite of that, this study also evidences a clear and different shape dominance correlated to the rocky bottom habitat considered. A different plastic litter fingerprint was observed in different rocky bottom habitats studied. In fact, in this study, over than 60% of the items recorded in slides were films. These results are quite different to results obtained in different marine bottom ecosystems from the Northern Adriatic Sea. Renzi et al. (2018a) recorded plastic litter levels in sediments from different marine habitats (shoreline, *Cymodocea nodosa* bottoms, Amphioxus sands, and Mäerl bed habitats) in Northern Adriatic Sea. In the cited study, even if films and fragments were recorded in almost all tested habitats, fibres resulted the principal size-class in all of them (74–100% of the total). Results obtained by this study evidenced that in marine ecosystems plastic litter measurements should be performed in each habitat type separately. Observed differences in plastic litter according to the rocky bottom habitat type (cliff, bank, and slide) that are reported in this study should be better explored by further researches to weight possible factors (i.e. local scale variability, different deposition processes linked to shape, etc.) which could be able to affect observed differences among rocky bottom habitats concerning levels and features of plastic litter. Different rocky bottom habitat types also resulted in a different dominant size-class (1000–2000 μm slides; 100–500 banks μm) supporting the occurrence to better explore relationships among settlement and resuspension rates of plastic litter in different habitats types related to litter size-class and shape. Difference related to the geographical location of studied habitats could have determined observed differences of size-class in sediments. In fact, banks are settled far from the coast in open sea cliff and slides are settled closed to Salina Island. These spatial differences could have determined size segregation due to local marine currents during the sedimentation processes of microplastics (Fastelli et al., 2016). Unluckily, to the best of our knowledge, there are very few studies exploring plastic litter amounts in different marine habitat types.

As regards as colours, results obtained by previous researches evidenced that in sediments from Salina Island green and black colours resulted highly represented within 20–28%; white, clear, red, blue and pink were found approximately in the same percentage (about 10% of the total) (Fastelli et al., 2016). Results obtained in this study confirm previously observed distribution of colours as evidenced by the average fingerprint reported for sediments in this study.

4.2. Holothurians: levels & intakes

The volume of sediments ingested and defecated per year by sea cucumbers is remarkable (9–82 kg/ind. per year) (Purcell et al., 2016 and citation therein). As consequence they can remobilize large

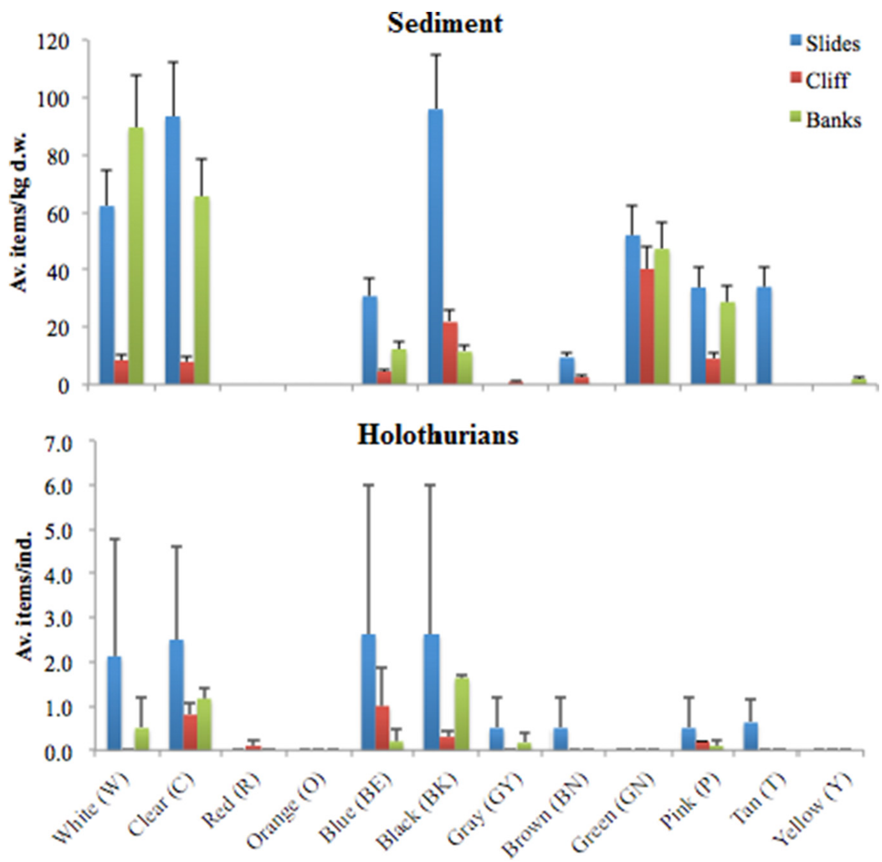

Fig. 5. Colour classes of plastic litter in different rocky bottom habitats. Data recorded (average + SD) are reported in sediments (number of items/kg d.w.) and holothurians (number of items/animal). Data are grouped per colour classes as reported in abscissa and per type of rocky bottom habitat (slide, cliff, bank).

Table 1
Principal plastic litter average levels (SD) recorded in different rocky bottoms types. a: average recorded amount of MesoPs and MPs are reported as number of items/animal (SD) in all tested rocky bottom habitats. b: average levels of MPs are further detailed per each of considered size-class (n. items/animal). Macroplastic (> 25,000 µm) were never founded and are not included in this table.

	Slides		Cliff		Banks	
	Average	SD	Average	SD	Average	SD
a						
MesoPs (5001–25,000 µm)	0.4	0.5	0.2	0.3	0.1	0.1
MPs (63–5000 µm)	11.6	14.7	2.2	0.6	3.7	0.9
Total	12.0	6.6	2.4	1.2	3.8	2.1
b						
5000–4000 µm	0.1	0.2	0.0	0.0	0.6	0.6
4000–2000 µm	3.1	4.1	0.5	0.1	0.6	0.6
2000–1000 µm	3.3	3.9	0.6	0.0	0.6	0.0
1000–500 µm	1.6	1.9	0.4	0.0	0.7	0.5
500–100 µm	3.0	4.2	0.6	0.6	0.9	0.1
< 100 µm	0.5	0.7	0.1	0.1	0.3	0.1

quantities of buried plastic litter in sediments. Furthermore, their direct action on sediment bioturbation could be significant as well as sea cucumbers are reported by the literature to mix sediments tens of centimetres deep displacing at least their own body volume in sediments (Purcell, 2004). Over long time scales, bioturbation effects are likely to be substantial in ecosystems where they are abundant representing the major bioturbators of sediments on tropical reefs (Reise, 2002).

Their action could produce plastic litter resuspension from sediments towards the water column representing a secondary source of plastic from bottom sediments towards the marine trophic web. Even if the species considered in this study was not related to significant bioturbation activity on sediments (Purcell et al., 2016), this occurrence could represent a focus point for other marine ecosystems inhabited by other species of the same Genera.

On the average, Holothurians evidenced the same plastic litter fingerprint observed in sediments with the following prevalence fibres > films > others. This is consistent with the major availability of this shape in sediments supporting the occurrence of the direct ingestion of plastic litter that is contained into the ingested sediment. Data are consistent with a previous research evidencing that benthic species are much more exposed to fibres than pelagic ones (Neves et al., 2015). The positive correlation per habitat type observed in this study between levels recorded in sediments and levels measured in sea cucumbers evidences that amount of plastic litter leaving sediments towards the detritus trophic web is habitat dependent.

Concerning the reported relationships among plastic litter recorded in sediments and animals, some important exceptions are to be highlighted. The presence of larger quantities of fibres in holothurians from slides were films prevails in sediments is probably related to a better removal efficiency from the intestinal tract of the animals of some other shapes (fragment, film, granules) rather than fibres. Fibres could be easily trapped by the intestinal tract of animals and could be more efficiently retained. This occurrence was jet documented for other species such as *M. galloprovincialis* in which fibres are the only one plastic shape recorded (Renzi et al., 2018b). Another possible explanation to the fact that holothurians ingested more fibres in slides rather than in banks and cliff habitats could be due to a major availability of this plastic shape on holothurian feeding substratum in slides rather than in other habitats. Nevertheless, further studies are necessary to better clarify these aspects. On the contrary, the presence of fragments and granule in sea

Table 2
Comparative analysis of principal features recorded in sediments and animals. Data are grouped per matrix and per rocky bottom habitat type. Average data are reported respectively as items/kg d.w. (SD) in sediments and as items/individual (SD) in holothurian species.

		Averages	Dominant size-class (μm)	Dominant shape	Most represented colours
Sediment	Slide	244 (2)	1000–2000	Film	W, C, Bk, Blue, Gr, P, T
	Cliff	99 (69)	100–2000	Fibre	W, C, Bk, Blue, Gr, P
	Bank	431 (191)	100–500	Fibre	W, C, Bk, Blue, Gr, P
Holoturians	Slide	12.0 (6.6)	1000–2000	Fibre	W, C, Bk, Blue, Gy, BN, P, T
	Cliff	2.4 (1.2)	100–2000	Fibre	C, Bk, Blue, P
	Bank	3.8 (2.1)	100–500	Fibre	W, C, Bk, Blue, Gy, P

cucumbers is significantly higher than values attended on the basis of levels recorded in sediments. This could be probably due to an active intake of these shapes of plastic litter from sediments by holothurians. This occurrence was also observed by the literature during in vitro experiments: authors observed that sea cucumbers ingested significant more plastic fragment than predicted values on the basis of levels in sediments. This behaviour seems to be due to an active predation of plastic fragments by tentacles (Graham and Thompson, 2009).

Concerning average size of ingested plastics; this study evidenced that the major frequencies were associated to 100–2000 μm for all tested rocky bottom habitats. Macroplastics were excluded and the principal size class reported for banks is significantly lower compared to the size class recorded in animals from slides and cliffs. These results are also consistent with literature as well as Graham and Thompson (2009) recorded a clear preference for small-sized fragments rather than to macroplastic fragments during in vitro tests. Higher numbers of small-sized plastics are recorded in banks. Dominant size-classes recorded in sediments are similar to those reported in holothurians. This fact supports the possibility that the observed difference among size-classes recorded in holothurians sampled in different habitat types could be due to the a different size-class present in sediments rather than to a selective feeding behaviour by animals. Nevertheless, further studies should be performed to evaluate if holothurians are selectively prefer a specific size-class of microplastic.

Holothurians showed the same colour fingerprint compared to sediments. The only significant exception observed is represented by a lower presence of GN (green) litter in holothurians compared to GN (green) litter levels recorded in sediments. This occurrence is probably due to the selective ingestion of plastic fragments by holothurians that determined the exclusion of fibres that are the only shape identified in sediments for the green plastic litter. Furthermore, some colour changes from green to grey (that increases in holothurians compared to sediment levels) could be due to the digestion process performed on animal tissues.

4.3. Ecological remarks

Holothurians play an important ecological role in benthic marine ecosystems actively digest refractory organic matter in sediments improving the efficiency of bacterial digestion (Purcell et al., 2016). Species living buried into sand are important for sediment bioturbation, sediment health, nutrient recycling and the capacity of ecosystems to buffer against ocean acidification, biodiversity of associated symbionts and transfer of organic matter from detritus to higher trophic levels (Purcell et al., 2016). For the exposed reasons, conservation of sea cucumbers represents a key aspect for the integrity marine ecosystems. To preserve this threatened species, the Italian government ruled specific protection actions aimed to reduce direct fishing and by-catch. Nevertheless, other threats rather than fishing could endanger holothurians.

Translocation of ingested plastic could produce significant damages to these animals affecting reproductive success and reducing population abundances in marine ecosystems. Previous researches did not found evidences of strong correlations among levels of plastic litter in sediments and the abundance of *A. lanceolatum* in Amphioxus sands marine habitat (Renzi et al., 2018a).

It is well known by the literature that plastic litter could absorb pathogens of ecological relevance (Zettler et al., 2013). Allochthonous microorganism could be spread in marine environment by absorption onto plastic litter and could successively affect species (Zettler et al., 2013; Rios et al., 2010; Wang et al., 2016). Absorbed bacteria on plastic litter could affect sea cucumbers directly representing important threats for holothurians.

Furthermore, many taxa actively prey sea cucumbers such as star, crustaceans, gastropods, fishes (Francour, 1997; Dance et al., 2003) and holothurian can represent a significant food source for multiple trophic levels (Purcell et al., 2016). As consequence pathogens absorbed onto plastic litter could be efficiently transfer towards other marine species along the trophic web. Bacteria absorbed onto plastic litter settled in bottom sediments could be resuspended by sea cucumber bioturbation activities or transferred towards the trophic web affecting predators or symbiotic species. Also plastic litter contained by holothurians could be transferred trough the trophic web by predation producing some negative effects on predators.

Biomarkers in vitro studies on holothurians after exposure to plastic litter could be performed to evaluate possible effects at molecular-scale level. Correlations among levels in sediments and population abundance could help to improve knowledge on ecological effects at the scale level of population dynamics. Further researches should be performed to better clarify these aspects. In particular, microbial characterization of recovered plastic litter could help to evaluate possible risks linked to pathogens.

4.4. Holothurians: endocommensalism & plastic litter

As regards as sea cucumber analysed in this study, only one sampling site (banks) showed the presence of endocommensal fish species. Endocommensals and parasites in sea cucumbers are a frequent occurrence as they host > 200 species of parasitic and commensal symbiotic species from seven phyla (Purcell et al., 2016). Frequently sexual pairs of pearlfish can be found in sea cucumbers, which are believed to also serve as breeding sites (González-Wangüemert et al., 2014) as also recorded in this study. *H. tubulosa*, is reported by the literature to be the symbiont of the Genera *Carapus*, *Encheliophis* (Purcella et al., 2016). Hosted species can feed on external sources while others can eat organs of their holothuroid hosts (Parmentier and Das, 2004).

Effects due to the presence of parasitic and commensal symbiotic species in the intestinal tract of sea cucumbers on the assumption and transfer towards the trophic web of plastic litter could not be evaluated in this study on a statistical basis due to the scarcity of records. Nevertheless, sea cucumbers hosting fishes showed a total amount of sediment ingested that were notably lower compared to sea cucumbers not affected. Symbionts of sea cucumbers are commonly present and could be endocommensals and parasites from at least nine phyla (Eeckhaut et al., 2004) and effects due to their presence should be taken into some account. To the best of our knowledge, effects due to the presence of internal symbionts on plastic litter intakes were never been explored by the literature and further research should be performed to improve specific knowledge on possible changes in plastic litter active ingestion related to this specific aspect.

4.5. Holothurians & plastic litter transfer towards trophic webs

As evidenced by this study, Holothurians can transfer on average 2.4–12 plastic items per animal towards higher trophic levels. Plastic litter ingested by each holothurian are on average similar to levels recorded in about 15 g of the sediments in which they're living with a particular enrichment inside animals of fragments and granules rather than fibres. Further studies will be important to confirm the observed sediment/animal ratios in different marine ecosystems. If constancy in measured ratios will be confirmed, solid data on enrichment ratios could allow performing case-by-case intake estimations only on the basis of plastic litter amounts recorded in sediments. It could be of particular importance to preserve animals' health avoiding direct and disruptive analyses on sea cucumbers. Results observed in this study concerning intakes evidenced that the highest recorded intake values were associated to an increase in fragment concentration in animals compared to sediment levels. This fact suggests the occurrence of an active ingestion of fragments by animals from slides as also recorded by in vitro studies (Graham and Thompson, 2009).

4.6. Human exposure risks

Holothurians are highly exploited by humans as food especially by the East populations. In spite of that, risks related to human health related to the ingestion of microplastic via feeding could be considered low as well as animals are eviscerated before consumption. Nevertheless indirect risks related to both translocation and absorption of chemicals associated to microplastic surfaces (Graham and Thompson, 2009; Cole et al., 2011; Fossi et al., 2016) could not be excluded without further researches. In spite of that, overexploitation by fishing of holothurian should be avoided to preserve from population declines inducing holothuroid species as vulnerable or endangered with extinction (Purcell et al., 2014).

5. Conclusions

This paper defines for the first time, macro-, meso-, and microplastic features (levels, shape, size, colour) in holothurians from different rocky bottoms habitats in Salina Island (Aeolian Archipelago), listed as a marine protected area of future institution. Furthermore our results evidence different plastic litter loads in sediments and holothurians from different rocky bottom types (cliff, banks, slide). This research provides relevant data on plastic litter on seafloor and associated levels and intakes in holothurians that could be considered representative of benthic and detritivore species. Different rocky bottom habitats showed different plastic litter levels, shapes and size-class. Further studies are needed to better clarify if observed differences in sediments according to the habitat are related to some ecological different aspects or if they are only of local origin. Results highlighted that in natural environments sea cucumbers show a selective ingestion of microplastic fragments within 100–2000 μm supporting an active role of these species in microplastic transfer from abiotic towards the biotic compartment. Further studies are needed to better clarify some aspects as extensively detailed above. A further study to evaluate, on a statistical basis, chemical composition of recovered plastic litter and to determine principal chemical types recovered in both sediments and holothurian is jet in progress.

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