BASELINE

TYPES, OCCURRENCE AND DISTRIBUTION OF MICROPLASTICS IN SEDIMENTS FROM THE NORTHERN TYRRHENIAN SEA

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Abstract

This is the first survey to investigate microplastic contamination in the Northern Tyrrhenian seafloor, along a 31 km-long transect from the port of Piombino (Tuscany) to the port of Portoferraio (Elba Island). Plastics extracted from 58 sediment samples were counted and identified by Fourier-transform infrared spectroscopy (FTIR). Plastic pollution occurred in sites closer to ports as well as in offshore stations. Microplastics (1–5 mm) accounted for over 80% of particles. For all samples, the dominant microplastic type was filaments, followed by fragments and films. Six polymers were identified: nylon, polyurethane, polyethylene, and polyethylene terephtalate were the most common. This part of the Tyrrhenian Sea is a busy shipping route connecting the mainland to the Elba Island, with thousands cargo and passenger ships passing by per year. Our data constitute a baseline for microplastic research in the Tyrrhenian seafloor.

The Marine Strategy Framework Directive (MSFD, 2008/56/EC; European Commission, 2008) identified a set of 11 Descriptors representing the state and functioning of the whole marine system (Borja et al., 2010). Descriptor 10 (D10) was identified as "Properties and quantities of marine litter do not cause harm to the coastal and marine environment" (European Commission, 2008).

One of the most incriminated materials for the pollution of seas and oceans is plastic (Avio et al., 2017). In the last 50 years, world plastic production has increased from 1.7 to 348 million tons, with a proportional increase in the production of plastic waste (PlasticsEurope, 2018). Part of this waste get discharged into the environment, a problem exacerbated by the common use of throw-away "user" plastic products that ultimately reach the sea.

Approximately 14 million tonnes of plastic litter are dumped in the oceans each year (Jambeck et al., 2015). An estimated 40% of that falls into the "single-use" category, which means it winds up in the ocean within the same year it was produced (UNEP, 2018). When at sea, plastic litters degrade slowly through a combination of photo-oxidation and mechanical abrasion, and result in production of small fragments and microplastics. Microplastics are considered specifically in descriptor 10 of the MSFD (10.1.3 "Trends in the amount, distribution and, where possible, composition of micro-particles (in particular micro-plastics)"), and implicitly in the indicator related with impacts of litter on marine life. According to the MSDF, microplastics should be categorized according to their physical characteristics including size and shape. It is also important to obtain information on polymer type (Gago et al., 2016). One of the primary environmental risks associated with microplastics is their bioavailability for marine organisms, since they mimic the appearance of food, thus obstructing and compromising the functionality of the digestive system (Gall and Thompson, 2015). Moreover microplastics can concentrate organic contaminants, thus acting as both source and vector for the contaminants (Mato et al., 2001), or even carry pathogens (Kirstein et al., 2016). Common types of microplastics identified at sea include pellets, irregular fragments, films and filaments (Wright et al., 2013). Primary microplastics are intentionally produced as precursors to other products, while secondary microplastics result from the degradation of larger items. The sources of primary microplastics are usually plastic pellet processing facilities at petrochemical plants, and specific trading activities such as oceanic shipping routes (Thompson et al., 2009). Small sized primary microplastics granules are also present in cosmetics products and used as abrasives in a wide range of applications (Browne, 2015).

The Mediterranean Sea is one of the busiest navigation crossroads and top touristic destinations in the world, surrounded by a heavily populated and industrialized coastline. The Mediterranean is one of the world areas characterized by the highest amount of man-generated solid waste (Galgani et al., 2014): despite plastics typically do not constitute the highest percentage of discarded waste, they are the most important part of marine litter, constituting over 80% of floating items (Galgani, 2014; Suaria and Aliani, 2014). The quantity of floating plastics recorded in recent studies (Cozar et al., 2015; Suaria et al., 2016) makes the Mediterranean one of the most impacted regions of the world. The Tyrrhenian Sea (Central Mediterranean) is a deep basin delimited by the Italian coast to the east, by Corsica and Sardinia to the west, and by Sicily to the south. It has a narrow and shallow northern opening toward the Ligurian Sea (the Corsica channel) and a much larger and deeper opening in the south. About at the center of the northern part of the basin is the Tuscan Archipelago, of which the Elba Island is the largest and most famous island both from a historical and tourist point of view. To date, there are few studies dealing with plastic contamination in the Tyrrhenian waters: Caldwell et al. (2019) found a meso and microplastic concentrations of over 28,300 particles km⁻² in the northern and western Tyrrhenian, while Baini et al. (2018) recorded over 69,100 particles km⁻² in the Tuscan coastal waters. Data on benthic macro-litter were recently given by Angiolillo et al. (2015) in 3 coastal areas of the southern Tyrrhenian through video transects, and by Cau et al. (2019) along the Sardinian continental margin through trawl surveys. Cannas et al. (2017) monitored the total load of plastic litter on submerged beaches of Tuscany, Guerranti et al. (2017) assessed plastic contamination in sediments at the mouth of the Ombrone river (southern Tuscany), and Fastelli et al. (2016) measured plastic contamination in coastal sediments in the Aeolian Archipelago. Studies dealing with benthic microplastics (>5 mm; Andrady, 2017) on the Tyrrhenian offshore seafloor are, to our knowledge, currently lacking.

Taking into account the global distribution and implications of plastic particles and the early stages of studies dealing with microplastics deposition in Mediterranean sediments (e.g. Vianello et al., 2013; Alomar et al., 2016; Filgueiras et al., 2019), and that microplastic is one of the descriptors of the MSFD, with the present study we wanted to assess, for the first time in the Northern Tyrrhenian Sea, the quality and quantity of plastic particles occurring in the seafloor to address the gap in knowledge and to serve as a baseline for future comparisons. We also wanted to assess whether plastic pollution is higher in the proximity of the coast than offshore.

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The survey was conducted in the framework of a project whose main objective was to provide information on benthic invertebrates in the Northern Tyrrhenian, however anthropogenic waste data were also gathered. The study area was a strecth of sea (named Piombino channel) comprised between the Gulf of Follonica (Tuscany) and Elba Island, in the northern Tyrrhenian Sea (Fig. 1). This channel is affected by the heavily impacted seaway connecting the port of Piombino (continent) to the port of Portoferraio (Elba Island), and by land-based pollution sources. The sampling campaign was carried out in November 2017: along a 31 km-long transect, duplicate sediment samples from 29 stations at depth varying between 1.0 and 71.5 m were collected with a Van Veen grab. Sediments were sieved on board on a 1 mm mesh, so the smallest size of gathered particles was 1 mm. A 400 g-subsample was used for determination of sediment dry weight and grain size distribution. The transect was divided into 3 sections. The section called "Piombino" includes the stations (from PB1 to PB10) included in the Gulf of Follonica. The section called "Portoferraio" includes the stations (from PF1 to PF14) included in the bay of Portoferraio, while the section called "Offshore" includes all the stations (from OFF15 to OFF25) in the stretch of open sea. In Table 1 the coordinates of the sampled stations are reported.

At our laboratories, the plastic debris in sediment samples were removed under a dissection microscope (Nikon SMZ45T, magnification 3.35-300x), counted, measured and classified into three groups (GESAMP, 2019): large microplastics (1-5 mm), mesoplastics (>5–25 mm), and macroplastics (>25 mm), and weighted to the nearest 0.0001 g. Plastic debris were also categorized according to shape, i.e. filament, film, and fragment. Filaments are cylindrical, pigmented or transparent; films tend to be flat, hard and do not tend to break or deform when pressed with a dissecting needle; fragments are particles of different colors that are hard or flexible but do not tear when pulled, nor do they shatter into many small pieces when pressed with a dissecting needle.

Plastic composition at the 29 stations was investigated by means of ordination analysis (nMDS) based on the Bray-Curtis similarity index calculated on quantity data. Plastic categories contributing to dissimilarity between stations were investigated using the similarity percentages (SIMPER) analysis (Clarke 1993).

Fourier-transform infrared spectroscopy (FT-IR) analysis of plastic debris was carried out with a CARY 600 FT-IR (Agilent Technologies) instrument. Measurements were carried out in attenuated total reflectance (ATR) configuration, with a Pike Miracle diamond cell. Tests were carried out at

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25°C in dry air. Particles were identified by comparing FT-IR absorbance spectra of the microplastics to those in a polymer reference library.

In Table 1, sedimentary composition at 29 stations is shown. Seafloor was characterized by particles ranging from gravels (diameter between 4-2 mm) to clay (diameter <0.0039 mm), according to the Wentworth grain-size classification. Sediments were generally more sandy in the off shore section (OFF17-25), and in the Gulf of Follonica (PB1-5), while the fraction of finer sediments was dominant elsewhere.

A total of 58 sediment samples were analyzed from the 29 stations. Some examples of plastic particles collected during the study are shown in Fig. 2. Only few sediment samples did not contain plastics, and several types of plastic particles were observed in all the 29 stations. Plastic particles ranged from 1 to 19 mm in length. The samples contained both filaments, film and fragments in a range of colors, implying that particles may have originated from multiple sources. Overall, 1483 plastic particles were gathered in our sediment samples: 593 in the Piombino section, 359 in the off shore section, and 531 in the Portoferraio section. In Fig. 3 the size distribution of particles through the sampling sites is shown. The plastic concentration resulted to be 2.21 ± 1.08 particles kg⁻¹ dry sediment (331.9 \pm 161.6 particles m⁻²) in the PF section, 1.09 \pm 0.52 particles kg⁻¹ dry sediment (163.2 ± 77.4 particles m⁻²) in OFF section, and 1.98 ± 0.83 particles kg⁻¹ dry sediment $(295.5 \pm 124.9 \text{ particles m}^{-2})$ in the PB section. The station with the highest concentration of plastics was PF2, with 4.0 particles kg⁻¹ dry sediment (600 particles m⁻²), while the lowest concentration was recorded at OFF20, with 0.43 particles kg⁻¹ dry sediment (65 particles m⁻²). The average total particle concentration was 1.70 ± 0.93 particles kg⁻¹ (244.5 ± 122.3 particles m⁻²). Due to their dimensions, particles were pooled by section for weight determination: we collected 0.247 g m⁻² in the PF section, 0.119 g m⁻² in the OFF section, and 0.069 g m⁻² in the PB section.

In terms of numerical abundance, large microplastics accounted for 84.5% of the total amount found, mesoplastics made up 13.2% of total amount, while macro debris accounted for 2.3%. In Fig. 4a the proportion of micro, meso and macroplastics in the 3 sections of the transect is shown. The primary shape types by number were filaments (73.8%), followed by fragments (17.2%), and films (9.0%). Fig. 4b shows their proportion in the sections of the transect.

Ordination analyses through nMDS showed a pattern in concentration of shape types (Fig. 5a) and size (Fig. 5b) of plastic particles, with stations from the PF and PB sections segregated together, and stations from the OFF section more apart. Similarity percentages analysis (SIMPER) revealed

that the concentration of fibers and microplastics mainly drove the differences between stations from the different sectors (data not shown for brevity).

Identification through FT-IR spectroscopy evidenced the presence of 6 polymer types: polyethylene terephthalate (PET), polyethylene (PE), thermoplastic polyurethane (TPU), acrylonitrile-butadiene-styrene copolymer (ABS), Nylon 6.6 (Nylon), epoxy resin (Epoxies). Considering abundance, the most common polymer was Nylon (44.7%), followed by TPU (34.1%), PE (11.1%), PET (5.8%), Epoxies (2.4%), and ABS (1.9%). In the PF section, the majority of plastic particles were TPU (49.6%), and Nylon (37.2%), while in the OFF section it was Nylon (62.7%), and again Nylon (38.9%) and PE (22.2%) in the PB section. The composition by abundance of polymer type of each section of the transect is shown in Fig. 6a. Conversely, considering weight, the most common polymer was PE (41%), followed by PET (24%), TPU (20.5%), Nylon (8.1%), Epoxies (4.9%), and ABS (1.5%). In the PF section, the most represented polymer were PE (38.2%), and TPU (25.5%). In the OFF and PB sections they were PE (45.1% and 40.9% respectively), and PET (16.8% and 38.5% respectively). The composition by weight of polymer type of each section of the transect is shown in Fig. 6b.

The monitoring of plastic particles on the seafloor may not be logistically feasible for all coastal areas because of limited resources, so that "opportunistic approaches" may be used to minimize costs (GESAMP, 2019). We took advantage of a macrobenthos monitoring campaign to study also microplastics contamination in the northern Tyrrhenian. Because of the high cost involved with sampling the seafloor in the open sea, very few data are available from Mediterranean seafloors for assessing trends in microplastics, neither specific monitoring programs have been developed up to date. Despite some limitations, such as the minimum size of plastics collected, this "opportunistic approach" is the first study to present an assessment of microplastics pollution in the northern Tyrrhenian offshore. Opportunistic approaches like this one may help to address the gap in knowledge and to make available otherwise unavailable data, for instance in the framework of the MSFD implementation, as shown by Ramirez-Llodra et al. (2013).

Recent studies seem to show how the seabed is a storage area for microplastics (Woodall et al., 2014), however, due to its remoteness, it remains a limited area of study compared, for example, to surface water. We found small plastics particles in 100% of the sediment samples collected in the Northern Tyrrhenian Sea. Plastic contamination appeared to be ubiquitous in the Gulf of Follonica, Piombino channel, and Portoferraio bay, although the concentration of plastic particles differed among samples. Higher plastic concentration was found at stations closer to the coast,

both in the Piombino and in the Portoferraio section, however, the offshore section was also polluted by plastics. The majority of our sample was composed of microplastic particles (~85%), with a smaller portion being comprised of mesoplastics (~13%), and macroplastics (~2%). Since the majority of the particles were within the microplastic size range, results of this study are in agreement with studies conducted in seas across the globe, i.e. that microplastics may account around 90% of the global plastic contamination in seas (Eriksen et al., 2014).

Published studies on plastic contamination of sediments have different unit of measure: items per volume of sediment (e.g. Woodall et al., 2014), items per weight of sediment (e.g. Claessens et al., 2011), items per surface area of sediment (e.g. Munari et al., 2017). Devices and methodologies with which the sediments are sampled are also different: Ekman grab (e.g. Thompson et al., 2004), Van Veen grab (e.g. Claessen et al., 2011), box corer and megacorer (e.g. Woodall et al., 2014), divers (e.g. Frias et al., 2016), remote operating vehicle (e.g. Woodall et al., 2015), hand-operated dredge (e.g. Zobkov and Esiukova, 2016), hand-operated "other tools" (e.g. Nor and Obbard, 2014; Cannas et al., 2017; Guerranti et al., 2017). This lack of harmonisation between sampling methods in published studies has hindered an inter-comparison of the relative abundance of microplastics in sediments worldwide (Hanvey et al., 2017). Table 2 shows the concentration of plastic particles in different areas of the Mediterranean seafloor. Considering the concentration of plastics particles as items per surface area of sediment, values reported in this study are higher than those reported for the central Adriatic Sea, where authors found, on average, 87.5 particles m⁻² (Mistri et al., 2017). Conversely, considering the concentration of plastics particles as items per weight of sediment, values reported in this study are among the lowest reported for the Mediterranean Sea. We believe that this discrepancy may derive (a) from the fact of having filtered, on board, on a 1 mm mesh, therefore any <1 mm-sized particles present were not retained, and (b) from the type of device used to sample sediments (Van Veen grab), which does not allow a stratification sediment sampling. In other studies (e.g. Vianello et al., 2013), box corers were used: with such a device the sediments deformation is minimal thus allowing stratification sampling (for plastic contamination, generally the first layer of sediments, 0-5 cm, is retained). Therefore our estimates as items per weight of sediment are clearly underestimates, having not been able to sample only the first centimeters of sediment, but rather the whole content of the grab.

The collected plastic particles were categorized in filaments (~74%), film (~17%), and fragments (~9%). The source of all our particles was the breaking down of larger items, and no primary microplastics (pellets or granules) were found. The proportion of different polymers found in this

study roughly corresponds to the global production stocks of plastic materials, with polyolefins accounting for the majority of the global plastic demand (PlasticEurope, 2018). The 6 different polymers identified in this study are used in a wide range of domestic and marine applications, including packaging and textiles, which indicates diverse sources. HDPE is one of the most popular thermoplastic materials and has a wide variety of applications: bottle caps, bottles, food storage containers, boats, water pipes for domestic water supply and agricultural processes, plastic surgery, 3D printer filaments, and many others. PET is used in containers for food and beverages, and fibres for clothing. Epoxies are utilized as repair resins for marine applications, paintings and adhesives. TPU has many applications including sporting goods, footwear, and outer cases of mobile phones. ABS find application in small kitchen appliances, automotive components, luggage and carrying cases, and toys including Lego. Nylon is a common polymer used for making ropes and fishing lines, draperies, etc.

Plastics found at stations farthest from the coast (stations OFF) were probably originated from marine-based sources including merchant and fishing vessels. The presence of epoxies for instance, seems to suggest an influence of ship-based pollution. Indeed, this part of Northern Tyrrhenian is characterized by the intense maritime traffic, first of all the connection by ferry of the Elba island with the mainland. These results agree with general finding that areas along shipping routes have high presence of microplastics (e.g. Claessens et al., 2011). Conversely, we suspect that plastics found in sections PF and PB could originate from the densely (especially in summer) populated coastline (with the towns of Piombino, Follonica, and Portoferraio), and consequent high anthropogenic pressures: plastics particles can be directly or indirectly discharged into the sea via wastewaters, sewage pipelines and terrestrial run-off. However, since sinking of the plastic particles is mostly governed by particle density and biofouling (Kaiser et al., 2017), during sinking they can be translocated by sea currents and, consequently, the source of the plastic particles cannot be accurately defined. In their study along the Spanish continental shelf, Filgueiras et al. (2019) found that the amount of microplastics in sediments were mainly associated with human activities, despite hydrodynamic and geographical conditions may have influenced their distribution and final sinks.

This study gives a first insight into microplastic pollution in the northern Tyrrhenian seafloor. It provides information on plastic particles pollution in the Follonica gulf, Piombino channel, and Portoferraio bay. We found that microplastics accounted for over 80% of particles on the seafloor, while filaments were the numerically dominant shape type (over 73%) of particles. Identification

through FT-IR spectroscopy evidenced the presence of 6 polymers, with nylon, polyurethane, polyethylene, and polyethylene terephtalate as the most common. These are useful baseline data to test the effectiveness of any reduction measures adopted to address the MSFD requirements. The knowledge of plastic particles pollution in the ocean seafloor is extremely limited, due to the high cost involved with sampling in the open sea. Opportunistic studies like ours supplement the role of routine benthic surveys performed by Environmental Agencies, and are a useful tool for the assessment of plastic particles pollution in the marine environment, at no additional cost.

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Figure legend

Fig. 1. Map of study site with transect sections: PB (Piombino), OFF (off shore), PF (Portoferraio)

- Fig. 2. Examples of the collected plastic particles. a: fragments and filaments; b: fragments and filaments; c: filaments; d: films.
- Fig. 3. Size distribution of particles through the sampling sites.
- Fig. 4. The ratios for particles size (a), and shape type (b) in samples from the Piombino (PB), off shore (OFF), and Portoferraio (PF) sections of the transect.
- Fig. 5. Ordination plots through nMDS of shape types (a), and dimension (b) data of plastic particles from the Piombino (PB), off shore (OFF), and Portoferraio (PF) sections of the transect.
- Fig. 6. Composition of plastic particles collected at the 3 transect sections according to the type of polymer by abundance (a), and by weight (b) (PET: polyethylene terephthalate, HDPE: high density polyethylene, TPU: thermoplastic polyurethane, ABS: acrylonitrile butadiene styrene copolymer, Epoxies: epoxy resin, Nylon: nylon 6.6).







Fig. 2a, b, c, d



Fig. 3



Fig. 4a, b



Fig. 5a, b



Fig.6a, b

Table 1.

Coordinates (WGS84 GMS), depth (m), and sediment characteristics (% gravel, sand, silt and clay) of the sampling stations.

		Longitude (E)	Latitude (N)	Depth	Gravel	Sand	Silt	Clay
Portoferraio	PF1	10°19'10.21"	42°48'13.33"	1	1.1	13	42.1	43.9
	PF2	10°19'15.95"	42°48'12.09"	2	0	12.5	46.6	40.9
	PF3	10°19'25.22"	42°48'11.11"	2.7	1.3	9.5	46.3	42.9
	PF4	10°19'34.19"	42°48'10.62"	2.9	14.5	40.4	27.3	17.9
	PF5	10°19'42.25"	42°48'12.92"	4.3	7	41.2	37.7	14.1
	PF8	10°20'32.81"	42°48'44.10"	31	6.2	31.3	44.8	17.7
	PF11	10°21'06.21"	42°49'21.71"	55	15.7	46	23.7	14.6
	PF14	10°21'41.72"	42°50'02.68"	65	4.7	43.8	32.8	18.7
	OFF15	10°22'14.01"	42°50'56.34"	71.5	4.1	36.4	31.3	28.1
Off-shore	OFF16	10°22'42.92"	42°51'50.07"	71.5	2.8	46	25	26.1
	OFF17	10°23'12.63"	42°52'43.59"	70.5	7.7	58.4	15.6	18.3
	OFF18	10°23'12.63"	42°52'43.59"	68.5	1.8	83.2	6.8	8.2
	OFF19	10°25'26.55"	42°53'35.76"	64.5	1.4	90.5	3.1	5
	OFF20	10°26'42.53"	42°53'49.65"	53	17.7	73.1	4.4	4.7
	OFF21	10°27'58.62"	42°54'03.76"	46	12	79.6	4	4.4
	OFF22	10°29'14.67"	42°54'19.76"	38.5	11.4	78.2	4.5	5.9
	OFF23	10°30'30.97"	42°54'29.46"	38	20.1	69.2	5.8	4.9
	OFF24	10°30'52.71"	42°54'29.14"	31.5	28.3	65.2	3.2	3.3
	OFF25	10°31'14.63"	42°54'29.24"	31	9.7	80.3	5.3	4.6
Piombino	PB10	10°33'59.84"	42°55'09.93"	26	0	18.9	46.4	34.7
	PB9	10°34'41.51"	42°55'52.54"	21.2	0	3.6	44	52.4
	PB8	10°35'39.73"	42°56'32.97"	14.5	0	1.4	48.1	50.5
	PB7	10°35'34.70"	42°56'41.53"	11.1	0	3.1	60.7	36.2
	PB6	10°35'32.08"	42°56'46.11"	10.5	0.4	43.9	30.5	25.2
	PB5	10°35'32.21"	42°56'46.11"	9	0	89.8	4.1	6.1
	PB4	10°35'32.43"	42°56'52.65"	7	4	86	5.6	4.5
	PB3	10°35'31.98"	42°56'55.87"	5.6	0.4	59.7	37.2	2.6
	PB2	10°35'31.59"	42°56'59.31"	4.2	11.8	83.6	1.7	2.9
	PB1	10°35'36.48"	42°57'02.61"	3.7	2.8	92.6	2.4	2.3

Table 2.

Quantity of plastic particles reported from the Mediterranean seafloor.

Area	No. surveyed stations	Depth range (m)	Min - Max	Reference	
Piombino channel (northern Tyrrhenian)	29	1 - 71.5	0.43 - 4.0 items kg ⁻¹ (65 - 600 items m ⁻²)	This study	
Talamone (northern Tyrrhenian)	3	0 - 1.5	88 items kg ⁻¹	Cannas et al., 2017	
Ombrone river mouth (northern Tyrrhenian)	14	0 - 0.5	45 - 1069 items kg ⁻¹	Guerranti et al., 2017	
Aeolian Archipelago (southern Tyrrhenian)	8	30	0 - 1037 items kg ⁻¹	Fastelli et al., 2016	
Lagoon of Venice	10	<1	672 - 2175 items kg ⁻¹	Vianello et al., 2013	
Central Adriatic (western sector)	16	7 - 142	2.5 - 87.5 items m ⁻²	Mistri et al., 2017	
Central Adriatic (eastern sector)	20	4.4 - 115	19 - 79 items 100g ⁻¹	Palatino et al., 2019	
Gulf of Trieste (northen Adriatic)	-	-	3 - 87 items 100g ⁻¹	Bajt et al., 2015	
Telaščica bay (eastern Adriatic)	10	3 - 15	3.4 - 84 items kg ⁻¹	Blaskovic et al., 2017	
Valletta harbour (central Mediterranean)	8	4 - 22	0 - 23 items kg ⁻¹	Romeo et al., 2015	
Balearic Islands (western Mediterranean)	6	10	0.10 - 0.90 items g ⁻¹	Alomar et al., 2016	
Spanish continental shelf (western Medit.)	10	43 - 154	45.9 - 280.3 items kg ⁻¹	Filgueiras et al., 2019	
Nile deep sea fan (eastern Mediterranean)	2	1176	0-1 items 25 cm ⁻²	Van Cauwenberghe et al., 2013	