



Benthic litter in the continental slope of the Gulf of Naples (central-western Mediterranean Sea) hosts limited fouling communities but facilitates molluscan spawning

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ABSTRACT

Seafloor pollution by benthic litter is an emerging phenomenon, although debris colonization by biota remains largely unexplored. We characterized the litter of the continental slope (~400–600 m) of the Gulf of Naples (Mediterranean) and investigated its fouling biota through integrative taxonomic approaches. Plastic pieces (82 %) with land-based origin (96 %) and limited sizes (10–20 cm) were the items most commonly encountered, suggesting a transfer to deep waters through floating and sinking. The majority of the items were not fouled, and the debris hosted an impoverished biota, leading to hypothesize that benthic litter supports wide communities only in shallow waters. Higher colonization rates were observed for gastropod and cephalopod eggs with no preference for materials and sizes, suggesting that even small pieces of soft plastic provide a spawning habitat for molluscs and affect species' connectivity in the deep-sea ecosystem. Holistic approaches are necessary to evaluate interactions between litter and biota.

1. Introduction

Marine litter is receiving a growing attention worldwide, with recent estimates accounting for a yearly discard of waste into the oceans of over 8 million tons (Jambeck et al., 2015). Such amount is generally composed of various materials, mostly plastic and derived polymers, but also metal, textiles, paper, and cigarette filters, that originates from a variety of sources, including fishing, maritime traffic, and coastal and sewage-related activities (Galgani et al., 2015; Jambeck et al., 2015). To further complicate the issue, composition, distribution, and accumulation of marine waste vary among areas and habitats depending on local anthropogenic activities, peculiar features of litter items, and intrinsic hydrographic and geomorphological factors of marine basins (Galgani et al., 2000, 2015; Barnes et al., 2009; Ramirez-Llodra et al., 2013). In fact, although low water circulation usually support higher rates of deposition and accumulation of marine litter (Galgani et al., 2000; Pham et al., 2014), which is in turn reflected in higher abun-

dances in surface waters and in shallow substrates along the coastlines, the longevity, transportability, and buoyancy of the items often depend on their shapes and materials (Barnes et al., 2009; Galgani et al., 2015). Thus, biofouling settlement and subsequent degradation may lead litter to sink even at long distances from the initial dumping site, thereby polluting deep-water seafloor (Galgani et al., 2015; Canals et al., 2021).

Impacts of marine litter on biodiversity are high and variable (de Carvalho-Souza et al., 2018; Galgani et al., 2019). They are generally considered as of high concern and range from the spread of toxic chemicals lethal to marine fauna (Engler, 2012) to the ingestion of microplastics and contamination of trophic web (Farrell and Nelson, 2013; de Oliveira Soares et al., 2020), the spread of non-native species (Gregory, 2009), and the alterations at species, community, and ecosystem levels (Ramirez-Llodra et al., 2013; Deudero and Alomar, 2015; Póvoa et al., 2021). However, seafloor waste also constitutes an additional hard substrate for native species, with larvae of several taxa that may even preferentially settle on anthropogenic substrates rather than on natural

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ones (Holst and Jarms, 2007; Chase et al., 2016; Li et al., 2016; Pinochet et al., 2020), and species that may use it to colonize incoherent and previously inaccessible bottoms, thus increasing local alpha diversity and enhancing connectivity between populations (Crocetta et al., 2020; Mancini et al., 2021). Some recent studies also highlighted the settlement of benthic organisms even on litter originating from fishing activities such as abandoned, derelict, or lost fishing gears, which rather constitute the specific target of removal campaigns (Cho, 2011; Hoeksema and Hermanto, 2018; Valderrama Ballesteros et al., 2018; Battaglia et al., 2019; Carugati et al., 2021).

The Mediterranean Sea is a relatively little and deep semi-enclosed basin that almost mimics a miniaturized ocean (Lejeusne et al., 2010). As such, it also suffers of widespread threats, including high pollution rates by plastics and derivatives (Deudero and Alomar, 2015) and impacts of marine litter on habitats and species (de Carvalho-Souza et al., 2018; Galgani et al., 2018). At the same time, it is also an important worldwide hotspot of biodiversity, hosting a large array of habitats and ecosystems from shallow to deep waters and ~7.5 % of the global biota, including endemisms and protected and endangered species (Bianchi and Morri, 2000; Coll et al., 2010). Although profound human-induced and climate-driven alterations of its biodiversity have been already widely reported in several research fields (Lejeusne et al., 2010; Coll et al., 2012; Micheli et al., 2013; Ramírez et al., 2018), the mechanisms of interaction between litter and native biota are still widely unexplored.

The same also holds true for the Gulf of Naples (GoN, Tyrrhenian Sea, western-central Mediterranean), with several researchers in the last decade using the GoN as a case study to tackle the issue of marine litter in shallow or coastal waters, providing baselines on its local distribution and abundance (Angiolillo et al., 2015; Crocetta et al., 2020; Ferrigno et al., 2021; Rizzo et al., 2021; Virgili et al., 2022), highlighting a broad support to fouling communities of native but even non-indigenous invertebrates (Crocetta et al., 2020; Virgili et al., 2022), entanglement of habitat forming species (Angiolillo et al., 2015; Ferrigno et al., 2021), and a low influence on the distribution of mega-benthic and mostly demersal fauna (Rizzo et al., 2021). On the other hand, the only study available thus far from the deep waters of the GoN focused on a local canyon [Dohrn Canyon (DC)] and evidenced litter coloniza-

tion by hard corals and a general co-occurrence between litter and biota until debris does not generate habitat destruction (Taviani et al., 2019). We hereby further widen the topic characterizing abundance, composition, size, weight, and most likely source of pollution of benthic litter in the continental slope of the GoN and assessing its relationship with local invertebrate communities. Results obtained here are finally discussed in the light of known geographical, geomorphological, biological, and anthropogenic factors and compared with similar researches conducted within and in other Mediterranean regions.

2. Materials and methods

2.1. Study area

The GoN is a peri-Tyrrhenian basin located in the eastern Tyrrhenian Sea margin and bounded on the northwest by the Gulf of Pozzuoli (GoP) and the Procida and Ischia Islands, on the east by the city of Naples and the Italian mainland, and on the southeast by the Sorrento Peninsula and the Capri Island (Fig. 1). Its continental shelf is characterized by an extensive system of volcanic banks surrounded by sandy and sandy-muddy substrates, whereas its continental slope hosts two major canyons that cut, and almost delimit, the GoN, namely the Magnaghi Canyon (MC), situated in its northern part, and the DC, situated in its southern part and flanked by a structural high known as Banco di Fuori (BdF) shoal (Aiello et al., 2020; Fig. 1). The continental slope between the two canyons (excluding the BdF shoal) has a depth of ~400–600 m and is characterized by muddy substrates exploited by the deepest local fishing activities (mostly targeting deep-water shrimps during summer), whereas the continental rise (situated where the GoN enters in the wider area of the Tyrrhenian Sea) fell down to depths almost inaccessible to the local fishing fleet (Aiello et al., 2020; Fig. 1).

2.2. Field work

Benthic litter was collected in the continental slope (~400–600 m) between the MC and DC canyons (excluding the BdF shoal) (~40.5758 N, 13.9665E; Fig. 1) during ~15 km long nonstraight tran-

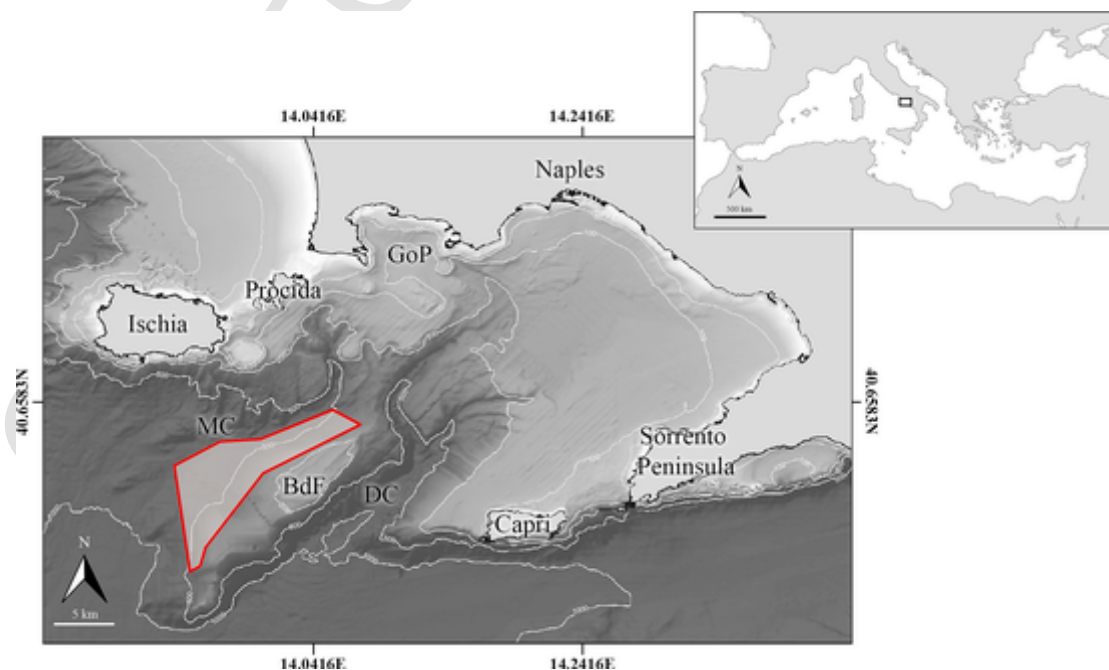


Fig. 1. The GoN and its position in the Mediterranean Sea (top right). Abbreviations used: BdF – Banco di Fuori shoal; DC – Dohrn canyon; GoP – Gulf of Pozzuoli; MC – Magnaghi canyon. Investigated area highlighted by a red outline. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

sects (according to the topography of the trawling ground), carried out in two sampling times (T1: August 2019; T2: August 2020) (six transects \times sampling time). Transects were held with a commercial fishing vessel operating with a bottom trawl net (mouth of 3×4 m in height and width, respectively; 40 mm mesh size) towed at ~ 2 – 2.5 knots. Benthic litter items collected onboard were soon placed into cold storage containers and transferred to the Laboratory of Benthos-Napoli (Stazione Zoologica Anton Dohrn, Naples, Italy) for subsequent laboratory work.

2.3. Litter composition

Litter items collected were counted, divided according to materials (soft, semi-hard, and hard plastic, cotton, glass, metal, pottery, and synthetic fiber), measured with a flexible ruler (in centimeters, size-classes based on their maximum length: $\times \leq 2$, $2 < \times \leq 5$, $5 < \times \leq 10$, $10 < \times \leq 20$, $20 < \times \leq 50$, and $\times > 50$ cm), weighted with a digital fishing scale (in grams), and finally assigned to a most likely source of pollution (land- or sea-based). In particular, land-based origin was assigned to waste deriving from the shoreline, tourism, and recreational activities, agriculture, medical related, but also mixed sources (sanitary and sewage related, fly-tipping, nonsourced items), whereas sea-based origin to waste deriving from fisheries and aquaculture (see Crocetta et al., 2020; Rizzo et al., 2021). It is worth noticing that, despite widely used, this approach often underestimates the sea-based source, as a considerable amount of litter items could not be attributed to a specific origin and items attributable to shoreline, tourism, and recreational related sources may also originate from fisheries and aquaculture or shipping (e.g., plastic bags) (discussions in Vlachogianni et al., 2018).

2.4. Fouling organisms and eggs: identification and colonization

During or soon after litter characterization, organisms (fully developed) and eggs (capsules and masses) fouling the litter items were searched in the laboratory with the help of magnifying glasses and a Zeiss Axio Zoom.V16 microscope (Carl Zeiss, Oberkochen, Germany), and subsequently counted *per* species/type. Organisms were identified to the highest possible taxonomic resolution through their external morphology. Eggs were first divided into different types based on their morphology (general shape and size, plug, suture, and ornamentation

pattern) and then, within each type, they were subdivided into intact (viable) or empty (hatched/predated). To identify the organisms that laid the capsules/masses, we initially proceeded through a DNA-barcoding approach, sequencing two eggs per type (from two different litter items, whenever possible). Basic Local Alignment Search Tool (BLAST; <https://blast.ncbi.nlm.nih.gov/Blast.cgi>) (Morgulis et al., 2008) was then used to assess the identity of each sequence against the GenBank database (last research: 15.III.2022) (see Table 1). Finally, the identification of the eggs was further validated (whenever possible) comparing BLASTn outcomes and (i) specialist literature; (ii) sequences obtained from putative “adult” specimens linked to each egg type, collected approximately in sympatry (GoN) with the eggs (Table 2).

Total genomic DNAs from egg and “adult” samples were extracted using the DNeasy® Blood & Tissue kit (Qiagen, Hilden, Germany), following the manufacturer's protocol. Partial sequences of the mitochondrial *cytochrome c oxidase subunit 1* gene locus (mtDNA *cox1*) were amplified using the primers reported in Table 1. Polymerase chain reactions (PCRs) were conducted in 25 μ L volume reaction, containing 2.5 μ L of Roche buffer (10 \times), 2.5 μ L (2 mM) of dNTPack Mixture (Roche), 1 μ L of each forward and reverse primers (10 μ M) (depending on the egg/specimen: see Tables 1 and 2), 0.25 μ L (5 U/ μ L) of Roche Taq DNA polymerase, 1 μ L of DNA (15 ng/ μ L), and distilled water up to 25 μ L. Amplifications were performed with the following conditions: initial denaturation at 95 $^{\circ}$ C (5 min), followed by 39 cycles of denaturation at 95 $^{\circ}$ C (1 min), annealing at 42–51 $^{\circ}$ C (1 min) (depending on the egg/specimen: see Tables 1 and 2), extension at 72 $^{\circ}$ C (1 min), with a final extension at 72 $^{\circ}$ C (5 min). The successful PCR products were purified and Sanger sequenced through an Automated Capillary Electrophoresis Sequencer 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA), using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Life Technologies, Renfrew, UK). Chromatograms for each sequence were then quality checked, assembled, and edited using Sequencher v.5.0.1 (GeneCodes, Ann Arbor, MI, USA).

2.5. Statistical analyses

The abundance and weight of benthic litter and the percentage of fouled litter were evaluated according to the length of the transect. Item/100 m² and grams/100 m² of benthic litter were calculated ac-

Table 1

Land-based, sea-based, and merged (land- and sea-based) litter abundance (number of items \pm standard error / 100 m²) and weight (grams \pm standard error/ 100 m²) in the two sampling times (T1 = 2019; T2 = 2020) and in total.

Source	Abundance			Weight			
	T1	T2	Total	T1	T2	Total	
Land-based	0.1119 \pm 0.0106	0.1069 \pm 0.0279	0.1094 \pm 0.0142	1.2161 \pm 0.3948	0.6714 \pm 0.2942	0.9438 \pm 0.2487	
Sea-based	0.0047 \pm 0.0012	0.0044 \pm 0.0013	0.0046 \pm 0.0008	0.0642 \pm 0.0360	0.0197 \pm 0.0079	0.0419 \pm 0.0189	
Merged	0.1167 \pm 0.0108	0.1114 \pm 0.0273	0.1140 \pm 0.0140	1.2803 \pm 0.3888	0.6911 \pm 0.2932	0.9857 \pm 0.2485	

Table 2

Litter abundance (number of items \pm standard error/100 m²) and weight (grams \pm standard error/100 m²) of materials in the two sampling times (T1 = 2019; T2 = 2020) and in total.

Material	Abundance			Weight			
	T1	T2	Total	T1	T2	Total	
Cigarette filters	0 \pm 0	0.0003 \pm 0.0003	0.0001 \pm 0.0001	0 \pm 0	0.0003 \pm 0.0003	0.0001 \pm 0.0001	
Cotton	0.0044 \pm 0.0007	0.0017 \pm 0.0009	0.0031 \pm 0.0007	0.0789 \pm 0.0201	0.0231 \pm 0.0217	0.0510 \pm 0.0164	
Glass	0.0008 \pm 0.0005	0.0003 \pm 0.0003	0.0006 \pm 0.0003	0.3875 \pm 0.2647	0.1300 \pm 0.1300	0.2588 \pm 0.1459	
Hard plastic	0.0106 \pm 0.0023	0.0103 \pm 0.0037	0.0104 \pm 0.0021	0.1211 \pm 0.0424	0.0700 \pm 0.0428	0.0956 \pm 0.0297	
Metal	0.0019 \pm 0.0008	0.0008 \pm 0.0006	0.0014 \pm 0.0005	0.0269 \pm 0.0123	0.0050 \pm 0.0044	0.0160 \pm 0.0071	
Pottery	0 \pm 0	0.0003 \pm 0.0003	0.0001 \pm 0.0001	0 \pm 0	0.0575 \pm 0.0575	0.0288 \pm 0.0288	
Semi-hard plastic	0.0117 \pm 0.0024	0.0114 \pm 0.0022	0.0115 \pm 0.0015	0.1669 \pm 0.0661	0.0983 \pm 0.0585	0.1326 \pm 0.0433	
Soft plastic	0.0742 \pm 0.0074	0.0690 \pm 0.0190	0.0718 \pm 0.0097	0.3550 \pm 0.0506	0.1431 \pm 0.0607	0.2490 \pm 0.0494	
Synthetic fiber	0.0131 \pm 0.0026	0.0169 \pm 0.0056	0.0150 \pm 0.0030	0.1439 \pm 0.0485	0.1639 \pm 0.0533	0.1539 \pm 0.0345	

ording to the length of the transect and the width of the fishing net. Data are reported as mean \pm standard error.

Univariate and multivariate analyses of variance (PERMANOVA) (Anderson, 2001; McArdle and Anderson, 2001) were performed to assess differences in: i) litter abundance and weight (divided in materials and size-classes) among sampling times; ii) sea-based and land-based litter among sampling times; and iii) percentage of litter fouled by organisms, intact, and empty eggs among sampling times and materials. PERMANOVA analyses were based on Euclidean distances on untransformed data, using 9.999 random permutations of the appropriate units (Anderson and Ter Braak, 2003). Because of the restricted number of unique permutations in the pairwise tests, *p*-values were obtained from Monte Carlo permutation test.

Data on biota fouling litter materials encountered in all transects in both sampling times were showed through a multidimensional scaling (MDS) plot. All analyses were performed using the software PRIMER v. 6 including the PERMANOVA β add-on package (Clarke and Gorley, 2006; Anderson, 2008).

3. Results

3.1. Litter

The sampling yielded in total 821 litter items (0.1140 ± 0.0140 items/100 m²). The putative source of pollution was land-based for 788 (96 %) items and sea-based for 33 (4 %) items, accounting for 0.1094 ± 0.0142 items/100 m² (land-based) and 0.0046 ± 0.0008 items/100 m² (sea-based) (Table 1).

Materials were similar between the two years investigated, with the sole exception of pottery and cigarette filters that were only found in 2020 in single items (Table 2). Soft plastic was by far the material most commonly encountered (517 items, 0.0718 ± 0.0097 items/100 m²), whereas all the other materials accounted for fewer items (Table 2; Fig. 2). The majority of the items encountered was between $10 < x \leq 20$ cm (280 items, 0.0389 ± 0.0061 items/100 m²), soon followed by $5 < x \leq 10$ (213 items, 0.0296 ± 0.0045 items/100 m²) and $20 < x \leq 50$ cm (198 items, 0.0275 ± 0.0047 items/100 m² trawl), whereas the other categories accounted for fewer items (Fig. 3).

The total weight of litter items was ~7 kg, accounting for 0.9857 ± 0.2485 g/100 m² (Table 1). Land-based items weighted 0.9438 ± 0.2487 g/100 m², whereas sea-based ones weighted 0.0419 ± 0.0189 g/100 m² (Table 1). Glass accounted for the highest

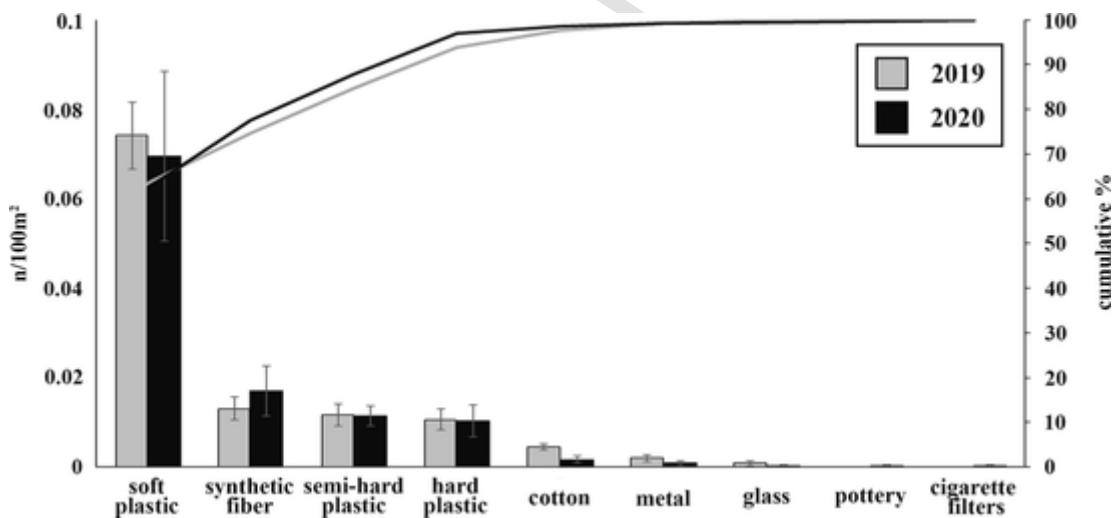


Fig. 2. Abundance of litter materials and cumulative percentage in the two sampling times.

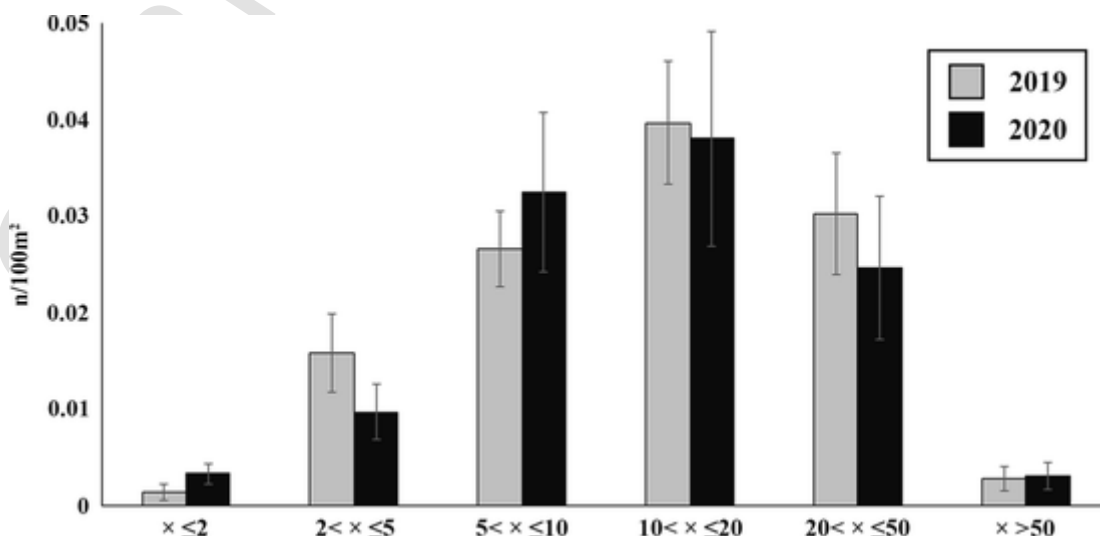


Fig. 3. Abundance and size-class (in cm) of litter items found in the two sampling times.

total weight despite the low number of items encountered (1863 g for 4 items, 0.2588 ± 0.1459 g/100 m²), soon followed by soft plastic (1793 g for 517 items, 0.3550 ± 0.0506 g/100 m² in 2019, and 0.1431 ± 0.0607 g/100 m² in 2020), whereas all the other materials accounted for lesser weights (Table 2; Fig. 4).

PERMANOVA analyses always revealed no significant differences among sampling times in all variables listed above (Tables S1–S4), with the sole exception of the weight of soft plastic (Table S5).

3.2. Fouling organisms and eggs: morphological and molecular identification

Fouling organisms accounted for six different taxa, four of which were identified to species level and two to family level. Among them, cnidarians were the most represented with three species, namely the soft coral *Alcyonium palmatum* Pallas, 1766, an actiniarian of the family Hormathiidae Carlgren, 1932 (Hormathiidae und.), and a zoantharian of the family Epizoanthidae Delage & Hérouard, 1901 (Epizoanthidae und.), whereas molluscs, arthropods, and tunicates accounted for one species each, namely the ostreoid bivalve *Neopycnodonte cochlear* (Poli, 1795), the lepadid cirriped *Scalpellum scalpellum* (Linnaeus, 1767), and the stolidobranch ascidian *Pyura microcosmus* (Savigny, 1816).

Eggs accounted for six different types, with partial sequences of the mtDNA cox1 (539–658 base pairs) successfully obtained from all types but one (Table S6). All BLASTn queries (97.96–100 %) suggested they belonged to Mollusca, with types 1–3 (capsules) being gastropods and types 4–5 (masses) being cephalopods (Table S6). Within gastropods, the two sequences each of types 1 and 2 (Fig. 5A–B, D–E) resulted identical *per* type and, respectively, showed high similarity (98.21–98.39 % and 99.36 %) with two sequences of *Pagodula echinata* (Kiener, 1839) and a single sequence of *Comarmondia gracilis* (Montagu, 1803) (Table S6). On the other hand, the two sequences of type 3 (Fig. 5G–H, N) gave an ambiguous result, yielding high similarity (~98 %) with the single sequences deposited for both *Gracilipurpura rostrata* (Olivi, 1792) and *Fusinus pulchellus* (Philippi, 1840) (Table S6). Within cephalopods, the two identical sequences of type 4 (Fig. 5J) only showed high similarity (99.07–100 %) with several sequences of *Rondeletiola minor* (Naef, 1912), whereas the two sequences of type 5 (Fig. 5K) again yielded an ambiguous result, showing high similarities (98.43–100 %) with a wide number of sequences ascribed in total to four different taxa, although the majority of them deposited as *Sepietta oweniana* (d'Orbigny, 1841) (Table S6).

Partial sequences of the mtDNA cox1 were also successfully obtained from five “adult” samples (out of five worked), yielding fragments of variable length (493–673 base pairs) (Table S7). Very high similarities (99.63 % and 98.99 %, respectively) were found between egg type 1 and *P. echinata* (Fig. 5C) and egg type 2 and *C. gracilis* (Fig. 5K) (Table S7), confirming BLASTn outcomes. Moreover, *P. echinata*

was among the few macro-gastropods found alive during the present trawling activities, whereas drawings of egg capsules of *C. gracilis* were already published by Lebour (1933) and well match our samples. Sequencing of *G. rostrata* (Fig. 5I) and *F. pulchellus* (Fig. 5O) confirmed the taxonomic assignment of the sequences reported in GenBank and thus strengthened uncertainties regarding the identification of egg type 3 (similarities of 98.94–100 % and 98.52–99.09 %, respectively). However, the taxonomic validity of *F. pulchellus* as a valid species or a mere variety of *G. rostrata* was debated since its original description, as the wide intraspecific variability of *G. rostrata* may also encompass “*F. pulchellus*” (Philippi, 1840, 1844; Buzzurro and Russo, 2007, and references therein). As this was never tested molecularly, we cautiously maintain both taxa as distinct, and assign the egg type 3 to *G. rostrata*. In fact, this latter species was the only fascioliid taxon found during present trawling activities and known to live up to bathyal depths, whereas *F. pulchellus* is usually confined to about 100 m depth (Buzzurro and Russo, 2007; Crocetta and Spanu, 2008). In addition to that, it is also worth a mention that an egg-capsule of *G. rostrata* was previously figured by Pelseener (1912) [redrawn by Lamy, 1928], and it roughly matches our samples, although the current material lacks the protrusion in the edge originally figured about a century ago. With regards the cephalopod egg-masses (types 4 and 5), no samples of *R. minor* were available for sequencing, and therefore the identification of the type 4 was only established on the basis of the results of the BLASTn hits. On the other hand, sequencing of *S. oweniana* (Fig. 5L) yielded similarities of 99.49–100 % with the type 5 (Table S7). Although also no specimens of *Sepietta obscura* Naef, 1916, *Sepietta neglecta* Naef, 1916, and *Euprymna stenodactyla* (Grant, 1833) were again available for comparison, a recent review of the family Sepiolidae Leach, 1817, in the Mediterranean Sea, also highlighted that the sequences AF036912, AY293722, and AF035704 were misidentified and belong to *S. oweniana* (Fernández-Álvarez et al., 2021). Moreover, although sepiolid egg-masses are somehow very similar among them, sizes of type 5 well match those reported in the literature for *S. oweniana* (Deickert and Bello, 2005; Cuccu et al., 2010). Finally, no intact eggs were found of the type 6 (Fig. 5M), and no sequences were obtained from the empty cases. Despite high similarities with egg-capsules laid by species of the families Muricidae and Nassariidae (e.g., D’Asaro, 1992, 1993), we were not able to assign it unequivocally to a putative species, and thus, type 6 remained as laid by an undetermined gastropod (Gastropoda und.).

3.3. Fouling organisms and eggs: litter colonization

Out of the 821 litter items found, 740 (~90.13 % of all litter items) were not fouled, whereas 61 (~7.43 %) showed colonization by organisms (15 items, 1.83 %), intact eggs (41 items, 4.99 %), or organisms and intact eggs (5 items, 0.61 %). Twenty additional items (2.44 %)

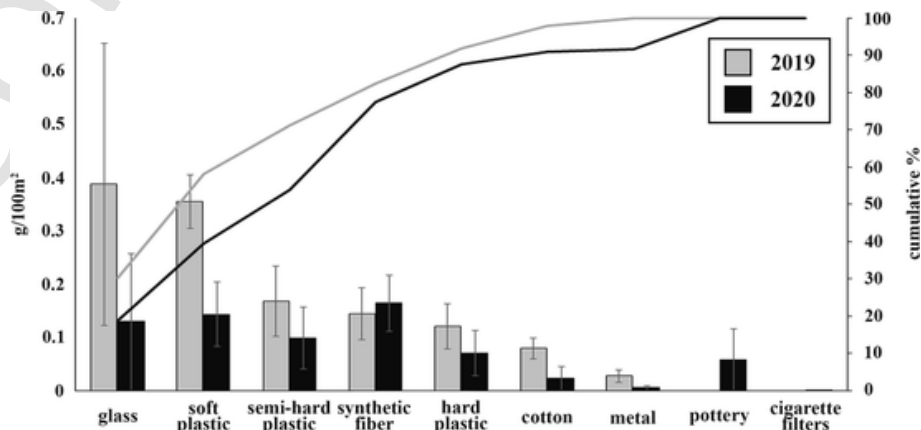


Fig. 4. Weight of litter materials and cumulative percentage in the two sampling times.

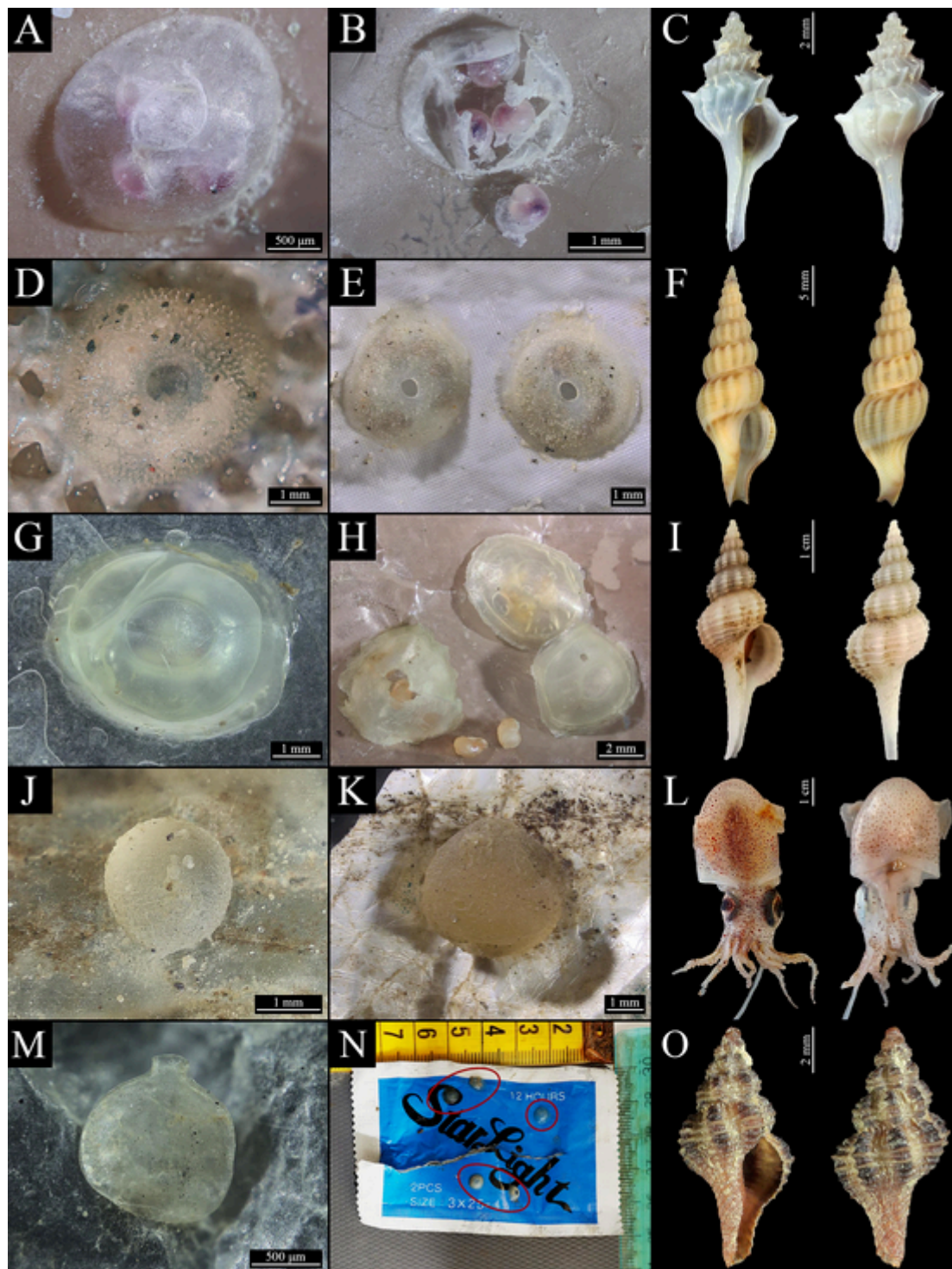


Fig. 5. Eggs and samples found/sequenced during the present study. A–C. Viable egg-capsules (type 1) and a sample of *Pagodula echinata* (SZN_B_3404ML234A). D–F. Viable and hatched egg-capsules (type 2) and a sample of *Comarmondia gracilis* (SZN_B_3390ML229A). G–I. Viable and predated egg-capsules (type 3) and a sample of *Gracilipurpura rostrata* (SZN_B_1306ML114E). J. Viable egg-mass (type 4) of *Rondeletiola minor*. K–L. Viable egg-mass (type 5) and adult of *Sepietta oweniana* (SZN_B_3241ML215A). M. Hatched egg-capsule of an unidentified gastropod (Gastropoda und.). N. Viable and hatched egg-capsules of *G. rostrata* on litter (red circles). O. The sample of *Fusinus pulchellus* (SZN_B_3386ML228D) used for comparison. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

were only fouled by empty eggs. However, empty eggs were also found in association with organisms (1 item, 0.12 %), organisms and intact eggs (2 items, 0.24 %), and intact eggs (16 items, 1.95 %). This resulted in 10.36 ± 1.68 % items fouled, of which 2.46 ± 0.55 % by organisms and 8.73 ± 1.57 % by eggs (6.14 ± 1.29 % by intact and 4.79 ± 0.92 % by empty eggs) (Table S8).

The analysis of the litter colonization by organisms, intact eggs, and empty eggs merged together revealed that soft plastic (55 items, 12.86 ± 3.02 % of all soft plastic items) was the material most commonly fouled among those encountered in all transects, followed by semi-hard plastic with a lower number of items but a similar total percentage (10 items, 10.87 ± 3.77 %). Noteworthy, metal showed the

highest percentage of fouled material (22.22 ± 10.24 %), although it was only found in four transects in T1 (5 not-fouled items and 2 fouled items) and in two transects in T2 (2 not-fouled items and 1 fouled item), whereas pottery, glass, and cigarette filters were only found in single items in a T2 transect, with all items being not fouled (Table S9). With regard to size-classes, $10 < \times \leq 20$ cm accounted for the highest number of fouled items (35 items, 13.49 ± 3.57 % of all the $10 < \times \leq 20$ cm items), followed by $20 < \times \leq 50$ (19 items, 9.24 ± 2.20 %) and $5 < \times \leq 10$ (19 items, 8.65 ± 2.19 %). However, the highest percentage of fouled items was again found in a size-class found in six transects only, namely $\times > 50$ (4 items, 19.44 ± 6.33 %). Sizes-classes of minor sizes (≤ 5) ranged from $\leq 5.27 \pm 3.04$ % to no colonization (Table S10).

The analysis of the litter colonization by organisms revealed that semi-hard plastic (6 items, 7.17 ± 2.38 % of all semi-hard plastic items) was the material most commonly fouled among those encountered in all transects, whereas the other items accounted for lower percentages ($\leq 2.50 \pm 0.67$ % to no colonization). Noteworthy, metal accounted again for the highest total percentage (8.33 ± 8.33 %), although only one item was found fouled in T2 (Table 3). With regard to size-classes, $20 < \times \leq 50$ accounted for the highest number of fouled items (10 items, 5.48 ± 1.90 % of all $20 < \times \leq 50$ cm items), followed by other size-classes with lower percentages ($\leq 2.06 \pm 0.74$ % to no colonization). However, the highest percentage of fouled items was again found in the size-class $\times > 50$ (3 items, 15.28 ± 6.94 %). Sizes-classes of minor sizes (≤ 20) ranged from 2.06 ± 0.74 % to no colonization (Table 3). The unidentified Epizoanthidae species was the most commonly encountered taxon (9 items, 11.37 ± 3.42 % of fouled items), found on semi-hard (3 items, 3.00 ± 1.78 % of all semi-hard plastic items) and soft (6 items, 1.23 ± 0.47 %) plastic, soon followed by *N. cochlear* (7 items, 10.55 ± 4.66 %), found on soft (3 items, 0.47 ± 0.27 %), semi-hard (2 items, 2.78 ± 1.87 %), and hard (1 item in T1) plastic, but also on metal (1 item in T2). The four remaining taxa were found on two to single items each (Table S11; Table 4).

The analysis of the litter colonization by intact eggs revealed that soft plastic (33 items, 8.04 ± 2.20 of soft plastic items) was the mater-

ial most commonly fouled among those encountered in all transects, whereas the other items accounted for lower percentages. Again, metal accounted for the highest total percentage (8.33 ± 8.33 %), although only one item was found fouled in T2 (Table 3). With regard to size-classes, $10 < \times \leq 20$ cm accounted for the highest number of fouled items (28 items, 11.16 ± 3.06 % of all $10 < \times \leq 20$ cm items), followed by other size-classes with lower percentages ($\leq 4.03 \pm 1.59$ % to no colonization) (Table 3). Intact eggs of *G. rostrata* were those most commonly encountered (32 items, 39.39 ± 7.60 % of fouled items) and were found on soft (21 items, 4.76 ± 1.33 % of soft plastic items) and semi-hard (4 items, 3.70 ± 2.08 %) plastic, on synthetic fiber (3 items, 3.25 ± 2.16 %), and on hard plastic (3 items, 2.08 ± 1.12 %). Egg-capsules of this taxon were also found on one metal item in T2. Egg-capsules of *C. gracilis* (6 items, 6.94 ± 3.11 %) soon follow, and were found on soft (3 items, 0.90 ± 0.58 %) and hard (1 item in T1) plastic, and on synthetic fiber (2 items in T2). Finally, egg-capsules of *P. echinata* (6 items, 6.29 ± 3.47 %) were only found on soft plastic (6 items, 1.89 ± 1.12 %). Egg-masses of *S. oweniana* and *R. minor* were found on a lower number of items and in lower percentages (Table S11; Table 4).

The analysis of the litter colonization by empty eggs revealed that soft plastic (26 items, 6.08 ± 1.72 % of all soft plastic items) was the material most commonly fouled among those encountered in all transects, followed by hard plastic (6 items, 7.36 ± 3.23 %), which accounted for a lower number of items but a higher percentage of colonization. All the other materials accounted for lower percentages ($\leq 2.31 \pm 1.73$ % to no colonization). Metal accounted again for the highest total percentage (22.22 ± 10.24 %), although only 3 items were found fouled in six trawls (Table 3). With regards size-classes, $10 < \times \leq 20$ cm accounted for the highest number of fouled items (17 items, 6.11 ± 2.26 % of all $10 < \times \leq 20$ cm items), followed by other size-classes with lower percentages ($\leq 4.84 \pm 1.53$ % to no colonization) (Table 3). Empty eggs of *G. rostrata* were those most commonly encountered (27 items, 33.77 ± 6.35 % of fouled items) and were found on soft (20 items, 5.00 ± 1.79 % of all soft plastic items), hard (3 items, 2.91 ± 1.77 %), and semi-hard (2 items, 2.31 ± 1.73 % of all

Table 3

Percentage of items (fouled material/total material and fouled size-class/total size-class) fouled by organisms, intact eggs, and empty eggs in the two sampling times [unless marked with asterisk(s): T1 = 2019, n = 6; T2 = 2020, n = 6] and in total.

Material	T1	T2	Total	Size-class (cm)	T1	T2	Total
Organisms				Organisms			
Hard plastic	2.78 ± 2.78	0 ± 0	1.39 ± 1.39	$\times \leq 2$	0 ± 0*	0 ± 0	0 ± 0
Metal	0 ± 0***	25.00 ± 25.00*	8.33 ± 8.33	$2 < \times \leq 5$	0 ± 0	0 ± 0****	0 ± 0
Semi-hard plastic	7.39 ± 3.69	6.94 ± 3.34	7.17 ± 2.38	$5 < \times \leq 10$	1.28 ± 1.28	0 ± 0	0.64 ± 0.64
Soft plastic	2.54 ± 0.87	2.47 ± 1.09	2.50 ± 0.67	$10 < \times \leq 20$	0.98 ± 0.63	3.14 ± 1.25	2.06 ± 0.74
Synthetic fiber	1.67 ± 1.67	0 ± 0	0.83 ± 0.83	$20 < \times \leq 50$	8.41 ± 2.87	2.56 ± 2.042	5.48 ± 1.90
				$\times > 50$	11.11 ± 11.11**	19.44 ± 10.02**	15.28 ± 6.94
Intact eggs				Intact eggs			
Hard plastic	5.96 ± 2.87	0.98 ± 0.98	3.47 ± 1.63	$\times \leq 2$	0 ± 0*	0 ± 0	0 ± 0
Metal	0 ± 0***	25.00 ± 25.00*	8.33 ± 8.33	$2 < \times \leq 5$	6.48 ± 5.45	0 ± 0****	3.54 ± 3.02
Semi-hard plastic	4.62 ± 3.32	2.78 ± 2.78	3.70 ± 2.08	$5 < \times \leq 10$	1.59 ± 1.59	6.47 ± 2.49	4.03 ± 1.59
Soft plastic	4.90 ± 1.72	11.18 ± 3.81	8.04 ± 2.20	$10 < \times \leq 20$	7.31 ± 2.50	15.02 ± 5.37	11.16 ± 3.06
Synthetic fiber	1.67 ± 1.67	5.5 ± 4.11	3.58 ± 2.19	$20 < \times \leq 50$	3.18 ± 1.68	2.33 ± 1.82	2.76 ± 1.19
				$\times > 50$	0 ± 0**	0 ± 0**	0 ± 0
Empty eggs				Empty eggs			
Hard plastic	7.07 ± 5.46	7.65 ± 4.02	7.36 ± 3.23	$\times \leq 2$	0 ± 0*	0 ± 0	0 ± 0
Metal	20.83 ± 12.50***	25.00 ± 25.00*	22.22 ± 10.24	$2 < \times \leq 5$	5.56 ± 5.56	3.82 ± 2.34****	4.77 ± 3.08
Semi-hard plastic	4.62 ± 3.32	0 ± 0	2.31 ± 1.73	$5 < \times \leq 10$	5.44 ± 2.207	4.23 ± 2.30	4.84 ± 1.53
Soft plastic	5.35 ± 1.48	6.81 ± 3.26	6.08 ± 1.72	$10 < \times \leq 20$	4.99 ± 2.70	7.23 ± 3.84	6.11 ± 2.26
Synthetic fiber	0 ± 0	1.33 ± 1.33	0.67 ± 0.67	$20 < \times \leq 50$	3.66 ± 1.45	1.52 ± 1.06	2.59 ± 0.91
				$\times > 50$	8.33 ± 8.33**	0 ± 0**	4.17 ± 4.17

* n = 2.

** n = 3.

*** n = 4.

**** n = 5.

Table 4

Percentage of materials fouled (fouled material/total material) by organisms, intact eggs, and empty eggs in the two different sampling times [unless marked with asterisk(s): T1 = 2019, n = 6; T2 = 2020, n = 6] and in total.

	Hard plastic			Metal			Semi-hard plastic			Soft plastic			Synthetic fiber		
	T1	T2	Total	T1	T2	Total	T1	T2	Total	T1	T2	Total	T1	T2	Total
Organisms															
<i>Alcyonium palmatum</i>	0 ± 0	0 ± 0	0 ± 0	0 ± 0*	0 ± 0*	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.49 ± 0.49	0 ± 0	0.25 ± 0.25	0 ± 0	0 ± 0	0 ± 0
Epizoanthidae und.	0 ± 0	0 ± 0	0 ± 0	0 ± 0*	0 ± 0*	0 ± 0	4.62 ± 3.32	1.39 ± 1.39	3.00 ± 1.78	1.28 ± 0.59	1.18 ± 0.78	1.23 ± 0.47	0 ± 0	0 ± 0	0 ± 0
Hormathiidae und.	0 ± 0	0 ± 0	0 ± 0	0 ± 0*	0 ± 0*	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.11 ± 1.11	0.56 ± 0.56	0 ± 0	0 ± 0	0 ± 0
<i>Neopycnodonte cochlear</i>	2.78 ± 2.78	0 ± 0	1.39 ± 1.39	0 ± 0*	25.00 ± 25.00*	8.33 ± 8.33	2.78 ± 2.78	2.78 ± 2.78	2.78 ± 1.87	0.77 ± 0.49	0.18 ± 0.18	0.47 ± 0.27	0 ± 0	0 ± 0	0 ± 0
<i>Pyura microcosmus</i>	0 ± 0	0 ± 0	0 ± 0	0 ± 0*	0 ± 0*	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.44 ± 0.44	0.22 ± 0.22	0 ± 0	0 ± 0	0 ± 0
<i>Scalpellum scalpellum</i>	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0*	0 ± 0	0 ± 0	2.78 ± 2.78	1.39 ± 1.39	0 ± 0	0 ± 0	0 ± 0	1.67 ± 1.67	0 ± 0	0.83 ± 0.83
Intact eggs															
<i>Comarmondia gracilis</i>	1.67 ± 1.67	0 ± 0	0.83 ± 0.83	0 ± 0*	0 ± 0*	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.51 ± 0.51	1.29 ± 1.09	0.90 ± 0.58	0 ± 0	1.33 ± 1.33	0.67 ± 0.67
<i>Gracilipurpura rostrata</i>	3.18 ± 2.02	0.98 ± 0.98	2.08 ± 1.12	0 ± 0*	25.00 ± 25.00*	8.33 ± 8.33	4.62 ± 3.32	2.78 ± 2.78	3.70 ± 2.08	2.77 ± 1.52	6.75 ± 1.97	4.76 ± 1.33	1.67 ± 1.67	4.83 ± 4.09	3.25 ± 3.25
<i>Pagodula echinata</i>	0 ± 0	0 ± 0	0 ± 0	0 ± 0*	0 ± 0*	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.82 ± 0.53	2.96 ± 2.20	1.89 ± 1.12	0 ± 0	0 ± 0	0 ± 0
<i>Rondeletiola minor</i>	0 ± 0	0 ± 0	0 ± 0	0 ± 0*	0 ± 0*	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.18 ± 0.18	0.09 ± 0.09	0 ± 0	0 ± 0	0 ± 0
<i>Sepietta oweniana</i>	0 ± 0	0 ± 0	0 ± 0	0 ± 0*	0 ± 0*	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.80 ± 0.53	1.11 ± 1.11	0.96 ± 0.59	0 ± 0	0 ± 0	0 ± 0
Empty eggs															
<i>Comarmondia gracilis</i>	2.78 ± 2.78	3.33 ± 3.33	3.06 ± 2.07	12.50 ± 12.50*	0 ± 0*	8.33 ± 8.33	0 ± 0	0 ± 0	0 ± 0	1.01 ± 1.01	0.18 ± 0.18	0.59 ± 0.50	0 ± 0	1.33 ± 1.33	0.67 ± 0.67
Gastropoda und.	0 ± 0	0 ± 0	0 ± 0	0 ± 0*	0 ± 0*	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.33 ± 0.33	0.64 ± 0.46	0.49 ± 0.28	0 ± 0	0 ± 0	0 ± 0
<i>Gracilipurpura rostrata</i>	1.52 ± 1.52	4.31 ± 3.28	2.91 ± 1.77	8.33 ± 8.33*	25.00 ± 25.00*	13.89 ± 9.04	4.62 ± 3.32	0 ± 0	2.31 ± 1.73	4.01 ± 1.15	5.99 ± 3.51	5.00 ± 1.79	0 ± 0	0 ± 0	0 ± 0
<i>Pagodula echinata</i>	0 ± 0	0 ± 0	0 ± 0	0 ± 0*	0 ± 0*	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1.11 ± 1.11	0.56 ± 0.56	0 ± 0	0 ± 0	0 ± 0

* n = 2.

** n = 4.

semi-hard items) plastics. Egg-capsules of this taxon were also found on two metal items. Egg-capsules of *C. gracilis* (8 items, 9.44 ± 4.95 % of all fouled items) soon follow, and were found on soft (3 items, 0.59 ± 0.50 %) and hard (2 items, 3.06 ± 2.07 %) plastics, and on synthetic fiber (2 items in T2). Empty egg-capsules of *C. gracilis* were also found on metal. Egg-capsules of the unidentified species (Gastropoda und.) and *P. echinata* were found on a lower number of items on soft plastic only (Table S11; Table 4).

Univariate (Tables S12–18) and multivariate (Table S19) analyses conducted on the abovementioned variables showed no significant differences among sampling times. The MDS plot did not show a clear colonization pattern of the different materials encountered (Fig. 6).

4. Discussion

4.1. Litter assessment

The Mediterranean basin is characterized by a densely populated and a highly exploited coastline and is widely considered as one of the seas most polluted by plastic and derivatives globally (Ramirez-Llodra et al., 2013; Deudero and Alomar, 2015). High litter abundances were already reported along the coast of many countries and in its deep waters (Ramirez-Llodra et al., 2013; Ioakeimidis et al., 2014; Pham et al., 2014; Crocetta et al., 2020), and the Mediterranean Sea also hosts an amount of floating particles comparable to the subtropical gyres of North Atlantic and North Pacific (Leichter, 2011). Such a large quantity of floating and benthic litter is particularly worrying when considering the high functional connectivity in the Mediterranean Sea and the limited water exchanges of the basin with the open ocean, thus potentially hampering a correct functioning of the basin in maintaining its native biodiversity (Harris and Whiteway, 2011).

The present study is perfectly encased in these statements and agrees with previous studies highlighting the presence of benthic litter in the GoN (Angiolillo et al., 2015; Crocetta et al., 2020; Ferrigno et al., 2021; Rizzo et al., 2021; Virgili et al., 2022), but also first investigates its occurrence in the local continental slope, accounting for 0.11 items/100 m². Although different methodologies and sampling techniques

were used so far in literature, the abundance reported here falls somehow in the range encountered at similar depths in European waters (Pham et al., 2014) and the Atlantic and Indian Oceans (Woodall et al., 2015). However, as expected, it is lower than the abundances reported in other Mediterranean coastal areas, including the GoN (Angiolillo et al., 2015; Crocetta et al., 2020; Rizzo et al., 2021), but higher with respect to remote locations already investigated in the basin (Barnes et al., 2009). Such result clearly reflects the intrinsic features of the GoN, which is characterized by 25 municipalities with high population densities, an important commercial port with >5200 employers, and several touristic services, resulting in a very urbanized and commercially active area chronically subjected to improper waste management and several multiple anthropogenic stressors, mostly acting synergistically on the coastal zone (Appolloni et al., 2018).

Sea-based source of marine litter only accounted for 4 % of the litter items found, although the area is commonly used as a trawling ground for the red shrimp *Aristaeomorpha foliacea* (Risso, 1827) and the red and blue shrimp *Aristeus antennatus* (Risso, 1816), and in a lesser extent for the rose shrimp *Parapenaeus longirostris* (H. Lucas, 1846). These data are in disagreement with recent studies conducted in the GoN up to 100 m of depths (Crocetta et al., 2020; Rizzo et al., 2021), where sea-based litter accounted for 16–18 % of the litter items found, and with the evidence emerged in the nearby DC, where Taviani et al. (2019) mentioned the presence of several lost fishing gears. Indeed, lost fishing gears are generally abundant on seamounts and other deep-sea floors in European waters (Pham et al., 2014), but also on seabeds in the southern Yellow Sea and the East China Sea (Lee et al., 2006), the Indian Ocean (Woodall et al., 2015), and the canyon shelf of the central California (Watters et al., 2010). However, sea-based litter mostly include longlines, set nets, ropes, or traps (Consoli et al., 2019), fishing devices that usually remain anchored to the rocky substrates where they were used. On the other hand, the investigated area is characterized by muddy substrates and is not exploited by such fishing devices, and this is presumably at the base of the result obtained here. Indeed, future litter assessments of the MC and DC canyons, as well of the BdF shoal, may reveal higher abundances of sea-based litter.

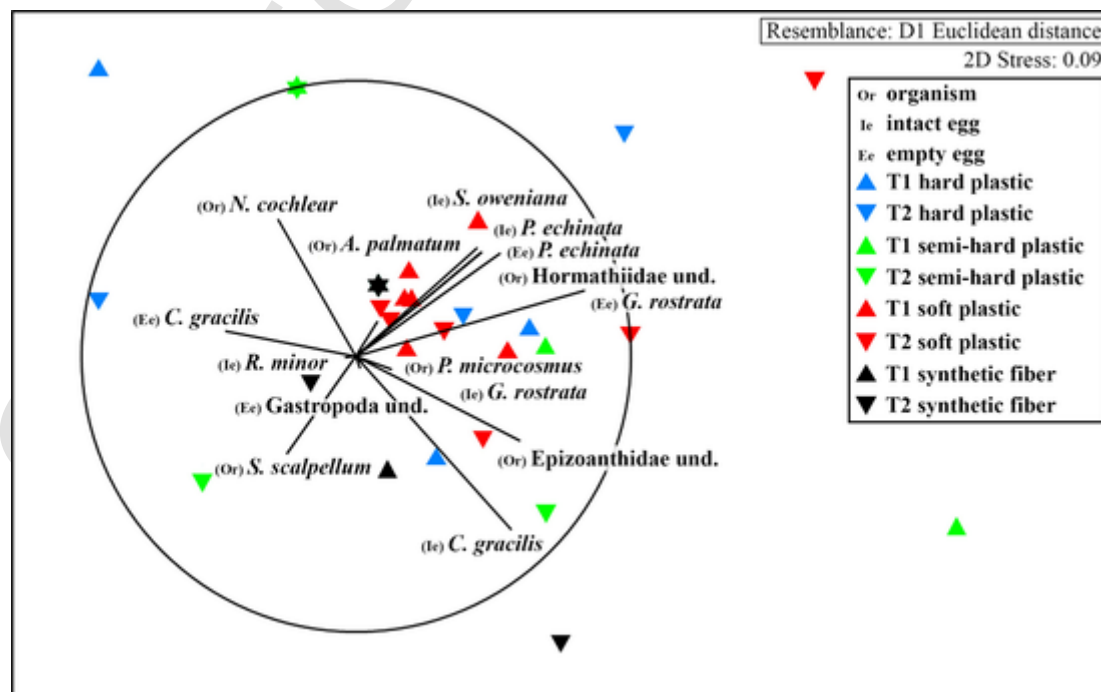


Fig. 6. MDS plot of the litter colonization by organisms and eggs. Vectors represent the correlation between the taxa encountered (both as organisms and as intact/empty eggs) and the different materials found fouled in all transects in the two sampling times.

Finally, plastic accounted for the material most frequently encountered in all transects, in agreement with studies carried out in other locations of the Mediterranean basin and worldwide (Galgani et al., 1996; Barnes et al., 2009; Schlining et al., 2013; Pham et al., 2014). In the present case, pieces of soft plastic of limited sizes (mostly composed by bags and wrappings of $10 < x \leq 20$) constituted the majority of the litter items. Such land-based source of pollution is similar to the one found by Woodall et al. (2015) in the Atlantic Ocean. On the other hand, the total weight of litter items (~7 kg, accounting for 1 g/100 m²) is generally lower than the one reported in the Mediterranean Sea not only in shallower waters (Crocetta et al., 2020) but also in deeper ones (Pham et al., 2014). Thus, present results do not provide evidence of illegal dumping in the investigated area despite Taviani et al. (2019) mentioned the usage of the nearby DC as a provider of waste disposal sink from land and vessel sources, but rather suggest a transfer of marine litter from land and coastal sources in deeper waters (presumably through floating and subsequent sinking), in agreement with the presence of floating litter in the GoN till the '80s (Cuomo et al., 1988) and as also already supposed in other research studies worldwide (Galgani et al., 1996; Ramirez-Llodra et al., 2013; Schlining et al., 2013; Pham et al., 2014).

4.2. Litter colonization by organisms and eggs

Litter colonization by fouling organisms is a widely acknowledged phenomenon worldwide and even in the Mediterranean Sea, with several studies reporting in the last decade the occurrence of variegated communities (Gündoğdu et al., 2017; Crocetta et al., 2020; Mancini et al., 2021; Subías-Barata et al., 2022; Virgili et al., 2022). In addition, specific communications also recorded rare, protected, or even habitat-forming species colonizing sea-based litter items, such as the sponge *Lycopodina hypogea* (Vacelet & Boury-Esnault, 1996) or the corals *Coralium rubrum* (Linnaeus, 1758), *Desmophyllum dianthus* (Esper, 1794) and *Desmophyllum pertusum* (Linnaeus, 1758), *Errina aspera* (Linnaeus, 1767), and *Madrepora oculata* Linnaeus, 1758 (Battaglia et al., 2019; Santín et al., 2020; Bergami et al., 2021; Carugati et al., 2021). All this generally led to the idea that benthic litter has a boosting effect on benthic communities, supplying additional tridimensional habitats for invertebrate settlement and colonization especially in flat and homogeneous bottoms (Goldberg, 1994; Williams et al., 2005; Crocetta et al., 2020; Song et al., 2021).

Results of the present study somehow disagree with previous researches and report an overall impoverished biota, composed by few individuals ascribed to six different taxa only, and mostly accounting for common and generalist species widely encountered on muddy and trawlable bottoms in the entire Mediterranean Sea (Moya-Urbano et al., 2016; Bastari et al., 2017; Arroyo et al., 2021; Tiralongo et al., 2021). Indeed, previous research efforts mostly focused on coastal environments (usually up to 200 m and barely up to 350 m), whereas we investigated the communities fouling marine debris in the continental slope (~400–600 m). Thus, the absence of similar researches held at the same depths makes it somehow difficult to discuss differences in the fouling assemblage found. However, the Mediterranean Sea is known to present a strong biodiversity pattern along depth gradients, with sessile invertebrates generally rarefying when depth increases (Ramirez-Llodra et al., 2008; Tecchio et al., 2011). In addition, chronic and intensive bottom trawling is known to affect negatively seabed habitats and biota, resulting in the impoverishment of the local communities (de Juan et al., 2007; Pusceddu et al., 2014), and, finally, harder and larger items are known to facilitate bioadhesion of invertebrate larvae (Crocetta et al., 2020; Mancini et al., 2021), as also confirmed here by the higher percentages of organisms found on metal and semi-hard plastic and on items > 20 cm with respect to the other materials and size-classes. Depth, exploitation of seabed through commercial trawling, or paucity of appropriate litter items, or even a combination of

these factors, may therefore have played a major role in the results obtained here. Notwithstanding such uncertainties, our results seem to suggest more favorable conditions for litter colonization in shallower waters, at odd with literature reporting biofouling being mostly influenced by abiotic factors not linked with depth (Crocetta et al., 2020).

On the other hand, the present results highlight a wider colonization of litter items by molluscan eggs, laid by both benthic (gastropods) and demersal (cephalopods) species. Although this phenomenon has received scarce attention thus far, with few studies both at a Mediterranean level and worldwide (Katsanevakis et al., 2007; Weślowski and Kotwicki, 2018; Crocetta et al., 2020; Song et al., 2021), the role of benthic litter as facilitator of molluscan spawning appears to be unequivocal, also considering that the investigated area does not host hard substrates or cliffs, and thus the local biota mostly rely on litter to spawn, or alternatively on biogenic substrates such as living or empty shells; however, these are particularly rare in the investigated area. In addition, no apparent preference for soft or hard materials, and for specific size-classes, was noticed, thus suggesting that even soft plastic items of few centimeters in length may constitute appropriate substrates for spawning, contrary to what usually observed in organisms. Despite the majority of the eggs found here were laid by gastropods, whereas only few items were found hosting cephalopod eggs, it worth a notice that egg-capsules laid by gastropods are somehow thick and durable, whereas egg-masses laid by cephalopods are softer. This is presumably why also empty egg-capsules were found by us, whereas egg-masses presumably easily detach during trawling activities and once cephalopod paralarvae and larvae start hatching. Alternatively, egg-masses by cephalopods may be more common in other seasons. Therefore, such a phenomenon may also be wider than expected, and the evaluation of its correct magnitude may presumably require less invasive sampling methods or whole-year samplings.

Finally, the integrative approach used here also allowed to strengthen or widen ecologic and taxonomic knowledge on the species that laid the eggs. Egg-capsules of *P. echinata* and egg-masses of *R. minor* were first figured here, whereas the finding of an egg clutch of *S. oweniana* at 544 m depth was considered unusual by Cuccu et al. (2010), but our samples come from a similar depth. *Comarmondia gracilis* was a species generally considered to live until the continental shelf (Gofas et al., 2011), but it presumably occupies a wider bathymetric range or migrates to the continental slope to spawn, at least during spring or summer. Although we did not find any live specimen during the current sampling activities, empty shells were present in the substrate. Moreover, the high similarity (> 98 %) between our sequences from the GoN and the single sequence deposited in GenBank from the North Sea (KR084515: Barco et al., 2016) confirm conspecificity between Atlantic and Mediterranean samples. Lastly, the sequences newly obtained here of *G. rostrata* and *F. pulchellus* confirm GenBank entries and highlight uncertainties in the taxonomy of these two species, with multilocus approaches required to test congenerity but even conspecificity between them. Indeed, the usage of the genus *Fusinus* Rafinesque, 1815 should be restricted to a group of Indo-West Pacific species and is therefore erroneous for Mediterranean ones (Fasulo et al., 2019). Also, the wide material encountered led us to suspect that the only egg-capsule of *G. rostrata* figured so far by Pelseener (1912) resulted to be abnormal in the edge protrusion. Egg-capsules of unidentified Mediterranean species of the subfamily Fusininae Wrigley, 1927 were already figured in the past by several authors (Bobretzky, 1877; Portmann, 1955; Fioroni and Portmann, 1968; Von Diehl, 1970) and always showed a regular outline. Based on present results, they may have been laid by *G. rostrata*, too.

5. Conclusions

Pollution by floating and benthic litter in world's beaches and coastal habitats is a well-known phenomenon that raised the attention

of the scientific community and of the general public, getting wide media and press coverage. However, deeper habitats are still poorly known as logistically challenging and expensive to survey, which is often reflected in an underestimate of the magnitude of litter pollution at such bathymetries. Although the present investigation did not reveal high litter abundances in the deep waters of the GoN, at least with respect to its coastal zones, the limited sizes of plastic debris strongly suggest ongoing fragmentation processes of land-based litter, ultimately leading to a large spread of microplastics. In addition, a growing amount of deep litter is expected in the next future, especially if improper waste operations will continue in the local coastal zone. The GoN, with its complex seabed topography characterized by two major submarine canyons, has a high socioeconomic and ecological value and is already known to host a wide biodiversity. The effective management and conservation of its deep-sea habitats is therefore an important challenge and should require appropriate marine policies starting from the land and communication plans to stimulate citizen consciousness.

On the other hand, colonization of benthic litter by fouling organisms is still an understudied issue, with research mostly focusing on communities living on floating debris. Noteworthy, the present data highlight that benthic litter in deep waters may host a limited fouling community, but that at the same time may provide support to molluscan spawning, with even small pieces of soft plastic constituting an appropriate substrate for gastropods and cephalopods egg deposition. Therefore, if on one side benthic litter can be perceived as a facilitator for invertebrates' survival, on the other, it can also somehow affect connectivity in deep-sea ecosystems, influencing species' behavioural traits and dispersal. As our understanding of the effect of benthic litter on the resident invertebrate biota is still limited, future studies should tackle this issue through holistic approaches.

CRediT authorship contribution statement

Lucia Rizzo: Conceptualization, Methodology, Investigation, Funding acquisition, Writing - original draft, Writing - review & editing. **Riccardo Minichino:** Investigation, Writing - review & editing. **Riccardo Virgili:** Investigation, Writing - review & editing. **Valentina Tanduo:** Investigation, Writing - review & editing. **David Ossa:** Investigation, Writing - review & editing. **Alessandro Manfredonia:** Investigation, Writing - review & editing. **Pierpaolo Consoli:** Funding acquisition, Writing - review & editing. **Francesco Colloca:** Funding acquisition, Writing - review & editing. **Fabio Crocetta:** Conceptualization, Methodology, Investigation, Supervision, Funding acquisition, Writing - original draft, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marpolbul.2022.113915>.

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