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Title: Distribution and assessment of marine debris in the deep Tyrrhenian Sea (NW Mediterranean Sea, Italy)

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Abstract: Marine debris is a recognized global ecological concern. Little is known about the extent of the problem in the Mediterranean Sea regarding litter distribution and its influence on deep rocky habitats. A quantitative assessment of debris present in the deep seafloor (30-300 m depth) was carried out in 26 areas off the coast of three Italian regions in the Tyrrhenian Sea, using a Remotely Operated Vehicle (ROV). The dominant type of debris (89%) was represented by fishing gears, mainly lines, while plastic objects were recorded only occasionally. Abundant quantities of gears were found on rocky banks in Sicily and Campania (0.09-0.12 debris m-2), proving intense fishing activity. Fifty-four percent of the recorded debris directly impacted benthic organisms, primarily gorgonians, followed by black corals and sponges. This work provides a first insight on the impact of marine debris in Mediterranean deep ecosystems and a valuable baseline for future comparisons.

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Lorenzo Bramanti Observatoire Océanologique, CNRS philebo@gmail.com **Highlights**

DISTRIBUTION AND ASSESSMENT OF MARINE DEBRIS IN THE DEEP TYRRHENIAN SEA (NW MEDITERRANEAN SEA, ITALY)

Michela Angiolillo, Bianca di Lorenzo, Alessio Farcomeni, Marzia Bo, Giorgio Bavestrello, Giovanni Santangelo, Angelo Cau, Vincenza Mastascusa, Alessandro Cau, Flavio Sacco, Simonepietro Canese.

HIGHLIGHTS

- Deep rocky grounds of Tyrrhenian Sea were explored by means of ROV
- Occurrence and abundance of submerged marine debris were described and quantified
- Different degree of debris impact on benthic fauna were assessed
- Fishing gears were the dominant source of debris in the deep Tyrrhenian Sea
- Gorgonians and corals resulted to be the most impacted organisms by debris

Running title: Mediterranean benthic marine debris

Distribution and assessment of marine debris in the deep Tyrrhenian Sea (NW Mediterranean Sea, Italy)

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ABSTRACT

Marine debris is a recognized global ecological concern. Little is known about the extent of the problem in the Mediterranean Sea regarding litter distribution and its influence on deep rocky habitats. A quantitative assessment of debris present in the deep seafloor (30-300 m depth) was carried out in 26 areas off the coast of three Italian regions in the Tyrrhenian Sea, using a Remotely Operated Vehicle (ROV). The dominant type of debris (89%) was represented by fishing gears, mainly lines, while plastic objects were recorded only occasionally. Abundant quantities of gears were found on rocky banks in Sicily and Campania (0.09-0.12 debris m⁻²), proving intense fishing activity. Fifty-four percent of the recorded debris directly impacted benthic organisms, primarily gorgonians, followed by black corals and sponges. This work provides a first insight on the impact of marine debris in Mediterranean deep ecosystems and a valuable baseline for future comparisons.

KEYWORDS: Submerged marine debris; Lost fishing gears; Fishing impact; Deep rocky bottom; Mediterranean Sea; ROV survey.

INTRODUCTION

In the past, deep-sea ecosystems were among the least studied and explored marine regions of the world, due to logistical difficulties in sampling deeper waters (Menza et al., 2008; Danovaro et al., 2010; Ramirez-Llodra et al., 2011). Recently, thanks to the availability of several technical devices (mainly Remote Operating Vehicles - ROVs), the interest of the marine scientific community has increasingly focused on these particular environments, which support high levels of habitat diversity, species longevity and provide a wealth of resources (Buhl-Mortensen et al., 2010; Ramirez-Llodra et al., 2011; Fabri et al., 2013).

The Mediterranean Sea, intensely studied over the past centuries, is considered a marine biodiversity hotspot, characterized by high levels of endemism (Bianchi and Morri, 2000). Most of the investigations carried out in this basin were conducted above 50 m depth. Only in recent times, several studies have focused on deeper assemblages characterized by a great variety and abundance of habitat-forming taxa, such as sponges and corals, providing high biomasses and structural complexity (e.g. Aguiliar et al., 2009; Bo et al., 2009, 2012 a, b; Freiwald et al., 2009; Bongiorni et al., 2010; Cerrano et al., 2010; Salvati et al., 2010; Gori et al., 2011; Fabri et al., 2013).

A common assumption is that deep sea areas are less impacted by anthropogenic disturbances (e.g., trawling, human litter, pollution, mining, oil drilling) (Hinderstein et al., 2010), whereas recent research has shown that this environment is more subjected to and affected by human and natural impacts than previously thought (Davies et al., 2007; Jones et al., 2007; Bongaerts et al., 2010).

The Mediterranean Sea is located among some of the most densely populated and highly industrialized regions of the world and it is affected by intense shipping activity. The pollution of this sea has been recognized internationally as a serious problem (Galil et al., 1995), raising concerns regarding threats to the conservation of the rich Mediterranean biodiversity (Coll et al., 2010).

Among the sources of marine pollution, litter is an ecological and social concern and an increasing issue worldwide (Galil et al., 1995; Galgani et al., 2000; Bauer et al., 2008; UNEP, 2009). Marine debris is defined as a solid or persistent material of human origin either discarded or abandoned in the marine and coastal environment (National Academy of Sciences, 1975). It represents a significant and persistent threat to wildlife due to its low biodegradability and its potential to be ingested by or to entangle marine organisms (Laist, 1987, 1997; Bavestrello et al., 1997; Yoshikawa and Asoh, 2004; Lee et al., 2006; Bo et al., 2014). Moreover, it can serve as

means of transport and habitat for alien species, altering the natural community composition (Katsanevakis et al., 2007)

Since the 1970s, several studies have faced the problem of debris in marine environments (Galgani et al., 1996; Matsuoka et al., 2005; Katsanevakis et al., 2007; Spengler and Costa, 2008; UNEP, 2009; Keller et al., 2010). In particular, beach and floating litter has been recognized as an important social problem due to its esthetic impact and its influence on public health (Hess et al., 1999; UNEP, 2009). Whereas, little information is available regarding the composition and distribution of submerged marine debris and its influence on the benthic organisms (Galgani et al., 2000; Spengler and Costa, 2008; Watters et al., 2010; Miyake et al., 2011). Once settled on the seabed, marine debris alters the habitat by providing a previously absent hard substrate that organisms can eventually cover. Moreover, the debris covering the sediment prevents gas exchange and interferes with life on the seabed (UNEP, 2009). Finally, lost fishing gears, such as lines and nets, and anchors may cause direct physical damage to benthic organisms (Donohue et al., 2001; Yoshikawa and Asoh, 2004; Bauer et al., 2008; Heifetz et al., 2009; Bo et al., 2013, 2014), since abrasive actions cause the progressive removal of tissues from sessile organisms (Bavestrello et al., 1997).

Various methods have been employed to quantify marine debris on the sea floor and the ones currently used in deep sea environments include: bottom trawlers, sonar, submersibles and ROVs (Spengler and Costa, 2008). In particular, submersibles and ROVs have been used to investigate benthic litter on the continental slope and the abyssal plain (Galgani et al., 1996, 2000; Freese, 2001; Fosså et al., 2002; Heifetz et al., 2009; Watters et al., 2010; Miyake et al., 2011; Mordecai et al., 2011; Bergmann and Klages, 2012; Fabri et al., 2013). Visual data, in form of videos and pictures, have been demonstrated to be useful in obtaining quantitative data on deep-sea litter; even if debris cannot be directly inspected and measured (Spengler and Costa, 2008; Watters et al., 2010). The most important feature of these methods is that they can be effectively applied to all sea bottom types, including complex rocky habitats, where some debris (especially fishing gears) may be found in abundance (Watters et al., 2010). Moreover, these methods do not cause any impact on the explored environments; whereas the bottom trawling gear method can affect the seafloor (Gage et al., 2005).

In the last decade, there has been an increased interest from the scientific communities on how commercial fisheries and the presence of debris have affected the sea bottom. However, the majority of studies have investigated the impact of mobile gears, such as trawls and dredges, on soft bottom community structure (e.g. Kaiser et al., 2000; Freese, 2001; Koslow et al., 2001; Cryer et al., 2002; Fosså et al., 2002; Maynou and Cartes, 2011; Mangano et al., 2013) or the effect of ghost

fishing (e.g. Matsuoka et al., 2005; Ayaz et al., 2006; Baeta et al., 2009). While, the impact of lost fishing gears on sessile organisms are less documented (Bavestrello et al., 1997; Eno et al., 2001; Freese, 2001; Chiappone et al., 2002, 2005; Asoh et al., 2004; Yoshikawa and Asoh, 2004; Heifetz et al., 2009; Bo et al., 2013, 2014), and little research has been focused on the impact of debris on rocky environments (e.g. Watters et al., 2010; Mordecai et al., 2011; Fabri et al., 2013).

Although, the Mediterranean basin is considered a particularly sensitive ecosystem (Bianchi and Morri, 2000; Coll et al., 2010), at present little is known about the extent of litter, especially in rocky areas deeper than 100 m (Galgani et al., 1996, 2000; Orejas et al., 2009; Madurell et al., 2012; Watremez, 2012; Bo et al., 2013, 2014; Fabri et al., 2013).

The aim of this study is to draw a baseline quantitative picture, by means of ROV, on the marine debris in three Italian regions (Tyrrhenian Sea, NW Mediterranean). This work attempts to evaluate, through a large number of observations, the occurrence and abundance of different types of debris and their potential impacts on benthic fauna. The study has been carried out in a marine area where there is a high level of tourism, commercial fishing and coastal urban population with respect to other areas of the Mediterranean basin.

MATERIAL AND METHODS

Study areas

The data on marine debris were collected during three different surveys financed respectively by the Italian Ministry for Environment, Land and Sea (MATTM) and by Sardinian Regional Council aimed to explore rocky coral assemblages and to study red coral (*Corallium rubrum*) deep-dwelling populations. The cruises were carried out on-board the R/V *Astrea* of ISPRA along the south Tyrrhenian coast (NW Mediterranean Sea, Italy), respectively in June-July 2010 in Campania and September-October 2011 in Sicily and Sardinia (Fig. 1).

Along the north coast of Campania six areas were explored (Fig. 1A), located in the Gulf of Naples and in the Sorrentine Peninsula. The Gulf of Naples is a SW oriented coastal embayment with an average depth of 170 m and a continental shelf with variable width ranging between 2.5 and 10–15 km offshore (Ribera d'Alcalà et al., 2004). The seafloor is characterized by a rough morphology, influencing hydrological features of the gulf, characterized by both oligotrophic and eutrophic systems and exhibiting a strong seasonal variability. The outer part of the Gulf of Naples is more directly influenced by offshore Tyrrhenian oligotrophic waters (Cianelli et al., 2011), that when move inside the gulf, creates a basin-scale cyclonic gyre transporting offshore the land runoff.

On the contrary, when the Tyrrhenian current moves South-eastward, the inner part remains separated forming anticyclonic gyres (De Maio et al., 1985; Cianelli et al., 2011). This condition prevents the renovation of the coastal waters, thus favoring stagnation and consequently pollution and high sedimentation rate (De Maio et al., 1985; Cianelli et al., 2011). The Gulf of Naples is among the most densely inhabited Italian areas and it is heavily influenced by the land runoff. Along its coasts approximately 30 ports and more than 300 maritime constructions are located (Uttieri et al., 2011). The intense anthropic pressures determine a strong impact on the marine ecosystem and its waters present hydrographic and biological properties reflecting anthropic stress (Ribera d'Alcalà et al., 1989; Zingone et al 2010).

In Sardinia, eleven areas located in the southern part of the island (Fig. 1B), and distributed through the Sardinia Channel and the Tyrrhenian Sea were explored. The seafloor morphology is different from the south eastern coast westwards; on the western side, a wide shelf area characterized by volcanic outcrops extends from the San Pietro Island to the Gulf of Cagliari, while a smaller shelf area (2km of extension on average) with several canyon heads occurs along the south eastern shelf margin (Sulli, 2000; Mascle et al., 2001). The Sardinia channel is a wide opening between Tunisia and Sardinia with a sill at about 1900 m that allows exchanges of deep waters between the western Mediterranean and the Tyrrhenian Sea. The surface layer is occupied by Modified Atlantic Water (MAW) directly coming from Gibraltar. On the opposite side, a significant flow of Levantine Intermediate Water (LIW) coming from Tyrrhenian Sea and extending from Sardinia slope to the centre of the channel, leaves this region to the west (Send et al., 1999). Along south Sardinian coast, the westward Algerian current is reported to be dominant: this current merges with the southward currents forming the quasi permanent South Eastern Sardinian gyre (Sorgente et al., 2011). South Sardinia hosts a wide range of ports, from small touristic (mostly located in the east/south eastern coast) and industrialized areas as Cagliari (southern coast) and Porto Scuso (south western coast). Moreover, the South western coast is also renewed for intense tuna fishing activities (Addis et al., 2013).

The eight studied areas in Sicily were located in the NW portion of the island and around the Egadi Archipelago (Fig. 1C), positioned between the strait of Sicily and the Tyrrhenian Sea. This zone represents the shallowest part of a wide submarine canyon that connects the Sicilian continental shelf to the Tyrrhenian Sea abyssal plane (Colantoni et al., 1993). The Sicilian continental shelf is very broad in front of Trapani coastlines and narrower between Levanzo and Marettimo Islands (Brugnano et al., 2008). The strait of Sicily can be considerate a small intermediate basin that subdivides the Mediterranean into western and eastern sub-basins and it is a topographically complex region. The surface circulation is mainly characterized by the flow of

Atlantic Water (AW) flowing eastward from the Sardinia Channel into the Tyrrhenian Sea (Astraldi et al., 1999) and a deeper Levantine Intermediate Water (LIW), coming from the eastern Mediterranean to west, forming a two-layer system with a 100 m thick transitional layer. The topographic constrains lead the LIW flow to turn eastwards and enter the Tyrrhenian Sea across a section offshore the Egadi Islands (Brugnano et al., 2008). The strait plays an important role in hydrodynamic and biological exchanges between the two principal sub-basins, acting as a transitional basin and as reservoir for deep water (Millot, 2005). This Sicilian zone hosts several touristic and commercial ports and the professional fishery represents one of the most important activities. Moreover, an rising number of recreational boats increases the fishing pressure on these areas.

Data acquisition

Surveys were conducted first mapping the seabed of the study areas using Multibeam echo sounders (RESON 8125 and Kongsberg Em 3002), and then carrying out exploratory video transect along the seafloor by means of a ROV 'Pollux' (Global Electric Italiana). The ROV was equipped with a digital camera (Nikon D80, 10 megapixels), an underwater strobe (Nikon SB 400), and 3 jaw grabbers (SeaBotix) to take samples. In addition, three laser beams, at a distance of 10 cm each other, provided a scale of the photos.

The ROV had a depth sensor, a compass and an underwater acoustic tracking position system (Tracklink 1500 MA, LinkQuest), providing detailed records of the tracks along the seabed. Geographical positions and depth were registered from the beginning to the end of each transect, every 1 second. The ROV moved ~1.5 m above the seabed, at constant speed (approximately 0.5 knots).

Along each transect, high resolution photo-sampling units were acquired every ≈ 30 seconds in order to identify and quantify the marine benthic litter. Litter was divided into 5 categories: fishing lines; nets; pots; plastic (bags, bottles, other objects, etc.); other items (glass, metal, etc.). The presence of debris was evaluated both by occurrence (frequency of debris types) and relative abundance (debris items m⁻²), assessed by counting the number of debris respect to the photo area.

Photos were analyzed by means of Image J software (http://rsbweb.nih.gov/nih-image), using laser beams as a scale. In order to assess the differences among the three studied regions, relative abundance (with a not normal distribution) was verified by the non-parametric Kruskal-Wallis H test and the post hoc Nemenyi-Damico-Wolfe-Dunn test.

Although, the distribution of debris is highly variable in space, abundance was converted to km⁻² to enable a comparison with other published results on litter from elsewhere.

The distance of each area from the nearest coast was also measured.

The impact of benthic debris was assessed and classified in 4 levels: i) covering, when debris covered the organisms; ii) abrasion, when the tissues of the organisms were injured; iii) hanging, when debris was under tension between rocky obstacles but apparently did not injure any organism; iv) lying, when debris was sitting on the bottom and did not impact the organisms.

The eventual fouling on marine debris by macro-benthic organisms was examined and the most common colonizing taxa were recognized. Thus, based on the number of taxa growing on debris items, three degrees of colonization were identified: none (0 taxa), moderate colonization (1–3 taxa), heavy colonization (>3 taxa).

The number of dead sessile organisms, not directly impacted by debris, was also recorded.

In order to evaluate the impact of debris, logistic models with mixed effects were used. The outcome was the presence or absence of a certain impact. Therefore, the probability of observing an impact of some kind was modeled. The logarithm of the odds of this probability was assumed to be a linear function of fixed effects associated with debris and other predictors, with a dive-specific intercept. The latter was used to take into account the effects of unobserved factors, and capture the dependence among the observations realized within the same dive. The dive-specific intercept was assumed to arise from a zero-centered Gaussian distribution, as in usual generalized linear mixed models. Bivariate association models were estimated at first, and then the final linear multivariate model with mixed effects was obtained through forward stepwise selection. Bivariate logistic models were also used to analyze the region specific effects of marine debris impacts. All logistic models are used to estimate predictor's effects. These are summarized as odds-ratios. The odds ratio is a measure of effect which can be interpreted as the fold change in probability of a presence when estimated with and without the predictor of interest. Consequently, an OR > 1 indicates that the presence of the predictor of interest increases the probability of observing a certain damage; while an OR < 1 indicates that the presence of the predictor decreases the probability of observing a certain damage.

The effect of depth and distance from the coast on the number of marine debris items was also analyzed using a Poisson regression model with mixed effects and with adjusted coefficients. The number of items observed can be naturally assumed to arise from a Poisson distribution. The logarithm of the expectation of this Poisson distribution was once again assumed to be a linear function of predictors (i.e., depth and distance from the coast), associated with fixed effects, and to have a Gaussian distributed dive-specific intercept.

The software R version 2.14 was used to carry out this analysis, in combination with package lme4, which is an R package for fitting and analyzing linear, nonlinear and generalized linear mixed models.

RESULTS

Abundance and distribution of debris

About 6.03 km² of rocky bottom were explored by means of 69 video transects in 26 areas of the three regions (Table 1). The initial and final depth of each transect varied between 30 and 300 m, depending on the geographical characteristic of each location. The length of each transect varied between 100 and 900 m.

A total of 3018 photos were analyzed and 368 benthic marine debris items were recorded. The number of areas without any sign of debris was negligible.

Overall, fishing gears were the dominant source of debris (89%): lines made up the most significant portion (62.5%), followed by nets (24.4%) and pots (2.1%). Plastic and other debris were occasionally found, representing respectively 5% and 6% of the samples. In particular, Sicily showed a higher occurrence of fishing lines (79% of analyzed debris), while in Campania and Sardinia this value was 57% and 55% respectively (Fig. 2).

The highest median value of debris abundance was found in Campania (0.12 debris m⁻², ranging from 0.02 to 0.16 debris m⁻²), while the lowest value was recorded in Sardinia (0.03 debris m⁻², 0.01–0.09 debris m⁻²). Sicily showed the widest range of debris abundance (0–0.3 debris m⁻²) and the median value of abundance was 0.09 debris m⁻² (Fig. 3A, 4).

Significant differences of debris abundance were recorded between Sardinia and the other two regions (Kruskal-Wallis H=8.9487, p<0.05; post-hoc Nemenyi test p<0.05; Fig. 3A). Similar results were obtained considering only the lines (post-hoc Nemenyi test p<0.01) (Fig. 3B).

Overall, debris abundance converted to km⁻² reached an average value of 60,967.5 debris km⁻².

The distance of the surveyed sites from the coast ranged from 0.065 to 16 NM. Bivariate Poisson regression models showed that only the presence of lines was function of the distance from the coast (p<0.001 and O.R.=1.2), while the presence of plastic was related to the depth (p<0.001 and O.R.=2.48).

Impact of debris

More than half of debris (54.5%) was observed in contact with sessile invertebrates, either covering them (17.2%) or causing abrasion (37.3%). The remaining portion of debris (45.5%) was recorded lying on the bottom (26.2%) or hanging from the rocks (19.3%) without producing any apparent injury to sessile organisms.

The results of logistic model showed that lines and nets carried out the most significant abrasive action (Table 2; Fig. 5A, B, D-F), producing the progressive removal of the tissues of entangled organisms. Nets and plastic carried out mainly a covering action (odds ratio value was 1200). Nets, for example, were found snagged on rocks, entangling or covering benthic organisms (Fig. 5A, B), while plastic bags were observed enveloping some organisms. Hanging debris was represented only by nets and lines (odds ratio value 444) (Fig. 5I), while lying debris was made up mainly of glass bottles, cans, tires or rigid sacks (odds ratio value 3156) that generally do not impact the organisms (Fig. 5G, H), but provide a secondary substrate or a refuge for others (e.g. Fig. 5J, K).

The effects of the models estimated for Campania and Sicily were not significantly different, while both were significantly different from the ones found in Sardinia for all the impact typologies, in particular for hanging debris (Table 3).

Ischia site (c5) in Campania and other three banks (sc3, sc5, sc7) in Sicily were the areas with the greatest relative abundance of debris determining a heavy impact (covering and abrasion action) on the organisms, as showed in Fig. 4.

Gorgonians were the most commonly affected organisms (53.0%), followed by red coral (23.5%), antipatharians (14.3%), sponges (6.0%) and other invertebrates (3.2%). This pattern was similar, but with a specific composition for the three regions (Table 4). The areas explored in Campania were characterized by coralligenous outcrops. Here, the large amount of debris, mainly lines and nets, abraded and covered especially the common gorgonians *Paramuricea clavata* (Risso, 1826) and *Eunicella cavolinii* (Koch, 1887) (\approx 73%) (Fig. 5A). In Sardinia, a greater number of different taxa were affected (Table 4). In Sicily, the most impacted species was the black coral *Antipathella subpinnata* (Ellis & Solander, 1896) (52%) (Fig. 5F), such as wide meadows of this species were recorded in some areas.

Several epibionts settled on the bare branches of impacted corals, forming large aggregates. Hydroids were the most common taxa (29%), followed by serpulids (17%), sponges (16%), other anthozoans (13%) and bryozoans (7%). The anthozoan *Alcyonium coralloides* (Pallas, 1766) was often observed forming large colonies mainly on *E. cavolinii*.

Debris items also represented a substrate for other organisms. The majority of debris (80%), in fact, were covered by colonial organisms. Fishing lines showed predominantly moderate colonization (59%), while considerable amount of taxa were observed on nets (Table 5).

Sometimes, lost nets still standing in their operative position and potentially still fishing were observed covered by organisms (Fig. 5B). 40% of other debris types showed no colonization.

The most frequent recorded taxa on debris were hydroids (31%), sponges (22%) and polychaetes (16%), followed by bryozoans, anthozoans and ascidians. Polychaetes such as Serpulidae species (*Protula* sp., *Sabella* sp.) were often observed growing on metal cans, pots, or sacks (Fig. 5J, K). Among anthozoans, various alcyonaceans (*Alcyonium acaule* Marion, 1878, *Alcyonium palmatum* Pallas, 1766, *A. coralloides*), actiniarians, scleractinians were commonly observed in association to lost gears. Some gorgonians, as *P. macrospina*, and more rarely *P. clavata* or *E. cavolinii*, were also recorded growing as epibiotic organisms on abandoned waste (nets or hard debris) or on dead portions of other corals. The basket star *Astrospartus mediterraneus* (Risso, 1826) was also observed on lost nets (Fig. 5D). Differences among regions were observed in the percentage of occurrence of epibiont taxa and species composition.

Besides sessile species, debris was utilized as habitat by numerous fish (e.g. Anthias anthias (Linnaeus, 1758), Callanthias ruber (Rafinesque, 1810), Muraena helena Linnaeus, 1758, Scorpaena elongata Cadenat, 1943, Scorpaena scrofa Linnaeus, 1758) and vagile benthic species (such as crustaceans, sea-urchins, octopuses etc.) which used their cavities as a refuge or dug in the sediment underneath them.

The logistic model revealed a significant relationship between dead specimens and fishing gears as nets (p=0.019 and O.R.=3.13) and lines (p<0.001 and O.R.=2.76), while no significant relationship was found with other types of debris (Table 6). Dead specimens were mainly corals, found broken, detached or buried in the sediments. Others were still anchored to the substratum but deprived of branches. Others were observed bare, with patches of living tissue or completely covered by epibiont species. Very few dead specimens were recorded in Sicily. In Campania, dead specimens mainly belonging to *P. clavata* and *E. cavolinii*, were recorded in 67% of the explored areas, particularly in Ischia (c5) and Amalfi (c1). Sardinia showed the highest incidence of dead specimens, belonging to several species such as *P. clavata*, *E. cavolinii*, *C. rubrum*, *V. flagellum* and *C. verticillata*, and recorded in 90% of the explored areas.

DISCUSSION

This study highlights a massive and widespread occurrence of anthropogenic debris in the deep rocky environment of the Tyrrhenian Sea, with a substantial inter-site variability. The occurrence of debris is mainly caused by fishing gears, particularly lost lines, which represent about a half of all

debris found in Campania and Sardinia. While in Sicily, this value reaches around 79% of all debris.

This pattern of debris composition is comparable with ones determined by other authors. For example, Chiappone et al. (2002, 2004, 2005) studied shallow areas of Florida, where fishing gears, mainly lines, contributed to up to 90% of the total quantified litter. In deep areas, similar results were obtained by Watters et al. (2010) off the coasts of California, and by Bo et al. (2014) in five Tyrrhenian banks.

The explored off-shore deep rocky banks in Sicily host numerous commercially relevant fishing stocks, that attract local recreational and professional fishing boats (Bo et al., 2013), likely responsible for the great abundance of lines found in this area.

The debris abundance estimated in this study (60,967.5 debris km⁻²) could be comparable to the ones determined by Galgani et al. (2000) (101,000 items km⁻²) and Watters et al. (2010) (76,000 items km⁻²) through submersible surveys, and by Mordecai et al. (2011) through ROV surveys (0–6,616 items km⁻²). However, these results are not strictly comparable as the authors used transect length and not area coverage and analyses video footage instead of still photos.

The lowest abundance of marine debris, recorded in Sardinia is likely related to the small human population living in this region. Therefore, Sardinia could represents minor potential source of litter in comparison with more populated regions, such as Campania. In fact, some authors have highlighted that the occurrence of high concentrations of debris in proximity to coastal urban areas is correlated to the size of the surrounding human population (Galgani et al., 1996, 2000; Hess et al., 1999; Mordecai et al., 2011), while a pattern of decreasing debris density is not always correlated with distance from the coast (Watters et al., 2010).

A significant positive correlation between debris and distance from the coast was found only for lines, highlighting how the presence of commercial target species may influence fishing efforts and justify the exploitation of banks far from the coastline (max 16NM). Moreover, the structurally complex rocky habitat of the explored areas increases the probability to find snagged lines (Chiappone et al., 2005; Watters et al., 2010).

The observed plastic items show a significant positive relation to depth. In most of the investigated areas, high depths are reached very rapidly, suggesting that plastic debris could originate mainly from land-based sources rather than maritime activities. However, the extensive spatial variation of waste may be related to hydrographical factors as well as to geomorphologic factors (Galgani et al., 2000; Mordecai et al., 2011), which are likely responsible for the transportation and accumulation of marine debris at greater depths (Galgani et al., 1996).

Most studies show that debris on the seafloor is mainly composed of plastic items (Galil et al., 1995; Galgani et al., 1996, 2000; Miyake et al., 2011), even though this result could possibly be due to the use of different sampling methodologies (Watters et al., 2010) such as the selective exploration of bottoms exploited by trawling nets (Galgani et al., 1996).

Even if plastic items represent a major component of the marine debris (Law et al., 2010), fishing litter may be predominant in areas characterized by intense fishing activities, such as the western Mediterranean Sea (Mordecai et al., 2011). Therefore, the use of alternative investigative methods (e.g. submersibles, ROVs), allow researchers to assess the abundance and type of debris also found on rocky banks (Keller et al., 2010; Watters et al., 2010). These rocky environment are attractive to fish because of their great topographic complexity (Chiappone et al., 2005; Watters et al., 2010), which therefore increases their chance of being subjected to recreational and professional fishery pressure (Bo et al., 2013).

It could take six hundred years to decompose a nylon line (Bianchi et al., 2004). Consequently, the most dangerous synthetic debris appears to be fishing gears, which can cause entanglement and break down over time into dangerous fragments that can be ingested by organisms (Laist, 1987).

Fishing litter in the studied areas had a major impact on the benthic communities, mainly on the arborescent large coral colonies that are easily snagged by derelict gears. Almost half of the recorded debris items, 54.5%, seriously impacted the benthic organisms by covering and abrading their tissues (Fig. 4). These results are consistent with Bo et al. (2013, 2014) findings indicating that 30% of arborescent corals found in the deep Tyrrhenian rocky banks were affected by lost lines. Similarly, Chiappone et al. (2005) have found that lost lines were responsible for damaging 84% of all sponges and benthic cnidarians in the Florida Keys National Marine Sanctuary.

Yoshikawa and Asoh (2004) have demonstrated that older cauliflower coral colonies, with large surface areas have a greater risk of entanglement compared to smaller younger colonies. Some of the most damaged specimens in this study were erect filter feeders composed of tall and branched colonies (as *P. clavata*, *E. cavolinii*, *C. verticillata*, *C. rubrum* and antipatharians). Coral skeletal characteristics, such as stiffness and flexibility of a colony, are known to play an important role in the resistance of friction, which explains the different responses of protein-based gorgonians or chitinous-based antipatharians to mechanical impacts (Bo et al., 2013).

Due to their elastic skeleton, *E. cavolinii* gorgonian colonies were rarely broken but were found to be covered in epibionts (mainly by the fast-growing parasitic alcyonacean *A. coralloides*). Colonies of *P. clavata* were recorded as being detached and buried in the sediment; thus, showing the typical arborescent morphology, and were rarely found with broken branches. The resistant skeleton of black coral allows this taxa to hold out against mechanical friction that normally only

scrapes the soft tissue of the branches. As a consequence, these corals were frequently observed covered in epibionts, but were rarely found having broken branches or being completely overgrown. On the contrary, numerous branching fragments of *C. verticillata*, known as being the most breakable gorgonian (Bo et al., 2013), were often observed lying on sea bottom, as also reported by Fabri et al. (2013).

The high impact of lost gear recorded on colonies of valuable red coral (*C. rubrum*) was observed in Sardinia and Campania populations. This finding suggests that other factors may be involved, depending on the target species. Indeed, these precious coral populations, are among the most seriously exploited and harvested in the Mediterranean basin for commercial purpose since ancient times (Cicogna et al., 1999; Santangelo and Abbiati, 2001; Tsounis et al., 2010). Therefore, it is plausible that prolonged and direct pressure placed on these banks, as well as secondary resuspension of sediments, may strongly damage these populations.

In this study, 30% of the observed coral colonies in contact with debris, showed traces of colonization of up to nine different taxa of sessile invertebrates, all with varying coverage. Opportunistic fast growing species, such as hydroids, polychaetes, sponges and bryozoans were vastly found on dead coral branches. Coral is capable of rapidly healing small lesions, but in cases of more extensive damage or frequent occurrence of physical stress, recovery may be difficult (Bavestrello et al., 1997; Yoshikawa and Asoh, 2004). In fact, the development of aggregates epibionts can in fact lead to the death of colony portions (Mistri, 1994).

Then, once caught by a fishing gear, the probability of death of a colony can be very high (Yoshikawa and Asoh, 2004). The positive correlation between the number of dead colonies, not directly entangled, and the presence of lost gears indicated the destructive effects of fishing activities, especially considering the longevity and slow growing rate of these species, which make them more susceptible and vulnerable.

Despite debris is harmful to biota altering the seafloor, these anthropogenic materials can also provide artificial habitat to sessile organisms (Watters et al., 2010; Miyake et al., 2011). In this study, the majority of debris (80%) was colonized quite heavily by encrusting invertebrates. Nets showed the highest frequency of epibiosis (66%), probably due to their greater surface (usually occurring as large agglomerates) with respect to single strands of nylon or other debris.

Hydroids, as well as encrusting sponges, observed growing on all types of debris were the most frequent fouling taxa, as already recorded by De Palma (1983) and Montanari et al. (1990). It is known that, due to their fast growth rates, they are among the pioneering species, capable to completely colonize artificial substrates and enhance the settlement of other organisms (Ardizzone et al., 1989), incorporating debris items into the habitat matrix.

The minor part of debris which did not show any sign of colonization may have been discarded or lost relatively recently. Fouling extent, in fact, may give indication of the age of lost gear (Saldanha et al., 2003), considering the most heavily encrusted debris presumably of older origin (Donohue et al., 2001). However, debris may show different species-specific variations in abundance of fouling organisms, depending also on geography, depth and/or season (Saldanha et al., 2003).

The presence of marine litter, used as substratum or as refuge by organisms, can increase both the number of species and the total abundance of individuals (Katsanevakis et al., 2007). However, it may alter the natural community structure, modifying the spatial heterogeneity (Saldanha et al., 2003) and in some cases enhancing the replacement of indigenous species (Mordecai et al., 2011). Therefore, although apparently its presence can increase diversity, these alterations contrast with the principle of habitat and biodiversity conservation and sustainability (Katsanevakis et al., 2007).

The results here presented can provide an important baseline for future monitoring efforts, as well as a quantitative assessment, useful to motivate adequate managerial actions. Further studies are necessary to reach a more comprehensive and precise understanding of litter distributions. Moreover, mapping the abundance of coral communities remains a necessary step in order to quantify the threats to which these characteristic environments are exposed.

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Figure legends

- **Fig. 1.** Distribution of the study areas along the three explored Tyrrhenian regions (solid black borders) off southern Italy: A) Campania, B) Sardinia and C) Sicily. The black dots denoted the dive sampling areas and the codes (ID) correspond to the list provided in Table 1.
- **Fig. 2.** Frequency of occurrence of marine debris items found in each region by debris category. "n" refers to the total number of debris items recorded in each region.
- **Fig. 3.** A) Debris relative abundance (debris items m⁻²) and B) Line relative abundance (lines m⁻²) in the three investigated regions. Black rectangles indicate median values, boxes indicate first and third quartiles and lines indicate the range between minimum and maximum values.
- **Fig. 4.** Relative abundance (debris items m⁻²) of total debris items (grey) and relative abundance of covering and abrading items (dark), in each investigated area of the three regions.
- Fig. 5. Impacts of debris and fishing litter on the rocky bottom of the Tyrrhenian Sea. A) An old lost net entangled on a colony of *Paramuricea clavata* and stretching over a mixed assemblage of *Eunicella cavolinii* and *Corallium rubrum* (Ischia c5, 118m). B) Lost net, hosting the basket star *Astrospartus mediterraneus*, entirely wrapping a rocky boulder and stretched over a population of *Paramuricea macrospina* (Banco Scuso sc3, 120m). C) Ghost net covered in various epibionts such as sertulariid hydroids, *Paramuricea macrospina*, *Alcyonium palmatum* and Cidaridae sea urchins (Banco Scuso sc3, 140m). D) Colonies of *Viminella flagellum* and *Callogorgia verticillata*, hosting the crinoids *Leptometra phalangium*, entangled and pulled by lines (Banco Marco sc7, 270m). E) Old long line entangling a colony of *Savalia savaglia*, peeling off the tissue of the gold coral. *Filograna implexa*, *Poecillastra compressa* and gastropods occurring on its dead parts (Porto corallo s4, 90m). F) Fishing lines entangled on the black coral *Antipathella subpinnata* (Banco Scuso sc3, 140m). G) Encrusted glass bottle (Amalfi c1, 94m). H) Discarded tire and net (Amalfi c1, 84m). I) A hanging rope hosting some specimens of Cidaridae (Banco

Marco - sc7, 270m). J) A sack laid on the seafloor attracting *Anthias anthias* and other benthic species (Nisida - c3, 77m). K) Epibionted net and other litter snagged on a rock (Isola del Toro - s12, 97m).

Scale bar: 10cm. For interpretation of the references to the areas, the reader is referred to Table 1 and Fig. 1.

Table(s)

Table 1Areas assessed for the presence and effects of debris to rocky benthic communities of the Tyrrhenian Sea. Location, number of transect, geographical position and depth range.

Region (year)	Area	ID	No.	Lat	Long	Depth
<i>2 3 7</i>			transects		Ü	range (m)
Campania (2010)	Amalfi	c1	4	40° 36.4' N	14° 31.5' E	30-180
• , , ,	Li Galli	c2	2	40° 34.6' N	14° 25.7' E	50-115
	Nisida	c3	2	40° 45.3' N	14° 08.3' E	40-110
	Ischia AMP	c4	2	40° 41.5' N	13° 53.6' E	40-120
	Ischia	c5	3	40° 41.3' N	13° 53.6' E	60-150
	Procida	c6	2	40° 41.8' N	14° 02.2' E	200-300
Sardinia (2011)	Capo Boi	s1	3	39° 06.6' N	9° 25.7' E	70-290
	SW Cavoli	s2	4	39° 04.1' N	9° 29.4' E	90-150
	Villasimius	s3	2	39° 05.4' N	9° 28.3' E	50-150
	Porto corallo	s4	6	39° 22.7' N	9° 41.2' E	70-275
	Capo Palmeri	s5	2	39° 37.4' N	9° 40.8' E	90-150
	Torre delle Stelle	s6	2	39° 07.3' N	9° 23.9' E	40-130
	Carloforte	s7	5	39° 07.1' N	8° 08.2' E	70-130
	Stella Maris	s8	2	38° 59.8' N	8° 07.7' E	150-200
	Punta delle Oche	s9	2	39° 14.3 N	8° 16.7' E	60-90
	Secca di Capo Teulada	s10	1	39° 44.3' N	9° 06.1' E	80-100
	Bancotto	s11	3	38° 41.3' N	8° 29.5' E	130-140
	Isola del Toro	s12	5	38° 50.9' N	8° 21.6' E	70-130
Sicily (2011)	Favignana	sc1	1	37° 53.1' N	12° 18.2' E	50-60
	Banco dei Pesci	sc2	2	38° 06.2' N	12° 13.3' E	80-120
	BancoScuso	sc3	2	38° 13.2' N	12° 33.3' E	70-140
	Zingaro	sc4	3	38° 11.4' N	12° 46.2' E	30-140
	Porcelli	sc5	2	38° 02.8' N	12° 25.3' E	80-120
	P.ta San Vito lo Capo	sc6	4	38° 10.6' N	12° 42.4' E	100-250
	Banco Marco	sc7	2	38° 16.9' N	12° 21.9' E	200-270
	Banco Marettimo	sc8	1	38° 01.3' N	12° 00.9' E	70-110

Table 2 Results of mixed logistic models for presence/absence of damages in each sampling unit. Results are grouped by damage (outcomes) and the relationship with debris types is investigated. The larger the Odd Ratio (OR), the stronger the impact of the debris type on the specific damage. For all tests, p<0.0001.

Damage	Debris types	OR	C.I. 9	95%	
Abrasion	(Intercept)	0,0011	0,0004	0,0030	
	Lines	846,6	303,3	2362,8	
	Nets	38,7	10,5	142,9	
Covering	(Intercept)	0,0008	0,0003	0,0027	
	Nets	1756,1	505,7	6097,5	
	Plastic	128,8	22,3	743,9	
Hanging	(Intercept)	0,0002	1,68E-05	0,0017	
	Lines	1200	120	12043	
	Nets	444	42	4717	
Lying	(Intercept)	0,0011	0,0004	0,0030	
	Lines	126,4	48,4	329,9	
	Nets	98,0	31,3	307,1	
	Plastic	393,4	77,3	2002,0	
-	Others	3156	556	17911	

Table 3Results of mixed logistic models for presence/absence of damages in each sampling unit. Results are grouped by damage (outcomes) and the relationship with region of sampling is investigated. For each outcome, regions with larger Odd Ratio (OR) are at higher risk of showing the specific kind of damage with respect to regions with a smaller OR.

Damage	Region	OR	C.I.	95%	P value
Abrasion	Campania	0,053	0,025	0,112	< 0.001
	Sardinia	0,228	0,087	0,600	0,003
	Sicily	0,845	0,281	2,540	0,765
Covering	Campania	0,029	0,015	0,058	< 0.001
	Sardinia	0,289	0,115	0,726	0,008
	Sicily	0,576	0,191	1,740	0,328
Hanging	Campania	0,038	0,019	0,076	< 0.001
	Sardinia	0,111	0,039	0,318	< 0.001
	Sicily	0,613	0,211	1,782	0,368
Lying	Campania	0,035	0,017	0,070	< 0.001
	Sardinia	0,299	0,119	0,750	0,010
	Sicily	1,510	0,565	4,039	0,412

Table 4Percent frequency (%) of taxa impacted by debris (T) and taxa occurrence (frequency of taxa respect to the total regional frames, F) in each investigated region.

Taxa	Campa	nia (%)	Sardin	ia (%)	Sicily (%)		
	T	F	T	F	T	F	
Porifera							
Aplysina cavernicola	4.7	6	-	-	-	-	
Poecillastra compressa	-		14.5	7.9	-	-	
ANTHOZOA							
Alcyonium n.i.	-		-	0.6	3.6	0.8	
Acanthogorgia hirsuta	-		-	1.3	-	5.2	
Corallium rubrum	22.7	56	47.3	38	1.8	5.6	
Eunicella cavolinii	41.5	64	5.4	16	3.6	11	
Eunicella verrucosa	-	0.4	-	1.9	1.8	0.1	
Bebryce mollis	-	-	3.6	1.8	-	2	
Paramuricea clavata	30.2	24	7.3	1.9	12.5	11	
Paramuricea macrospina	-	4	-	0.8	7.1	14	
Callogorgia verticillata	-	0.3	9.1	12	14.3	7.5	
Viminella flagellum	-	-	5.5	4	-	0.4	
Antipathes dichotoma	-	-	1.8	1.5	-	-	
Leiopathes glaberrima	-	-	1.8	6.1	-	-	
Antipathella subpinnata	-	0.1	-	3.7	51.8	13	
Ceriantharia	0.9	0.3	-	-	3.5	3	
Dendrophyllia cornigera	-	0.4	1.8	2.6	-	-	
Savalia savaglia	-	0.1	1.9	0.3	-	-	

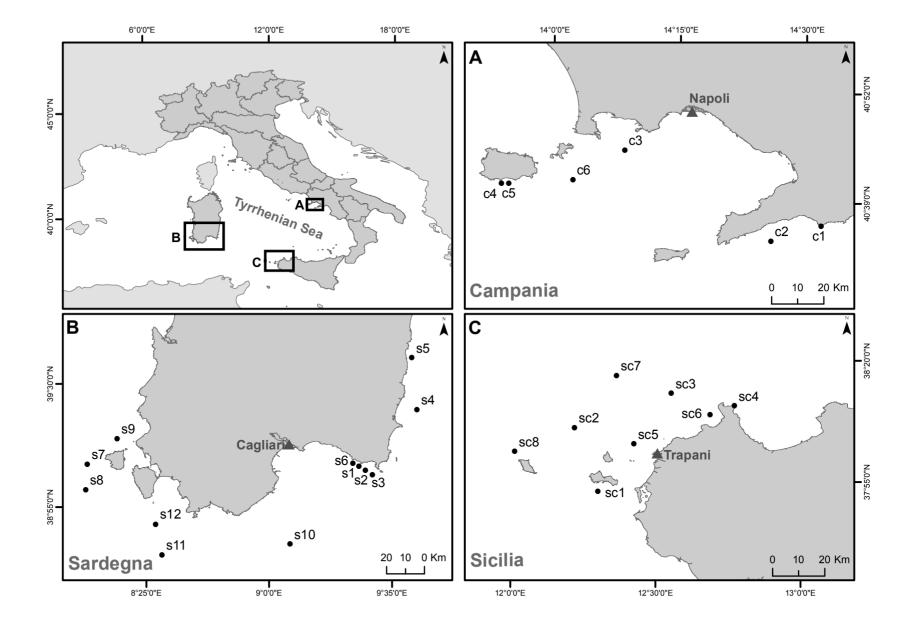
Table 5Percent frequency (%) of colonization degree on different debris types (lines, nets and other debris) and on all debris (tot) by macro-benthic invertebrates in each surveyed region.

Fishing gear	Campania (%)				Sardinia (%)				Sicily (%)			
colonization	Line	Net	Other	Tot	Line	Net	Other	Tot	Line	Net	Other	Tot
None	9.5	0.0	35.0	9.7	33.3	3.8	46.7	27.4	34.6	0.0	50.0	30.7
Moderate	44.2	34.0	25.0	38.8	42.6	30.8	46.7	40.0	44.4	50.0	50.0	45.5
Heavy	46.3	66.0	40.0	51.5	24.1	65.4	6.6	32.6	21.0	50.0	0.0	23.8

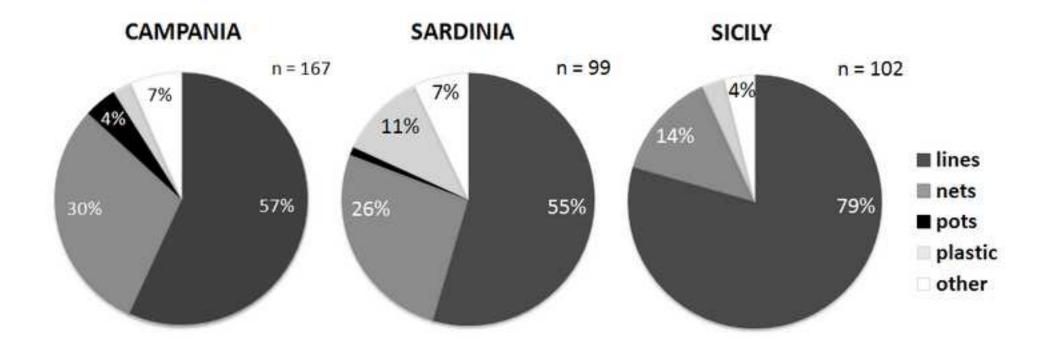
Table 6Results of mixed Poisson regression models for the number of dead specimens in each sampling unit as a function of the debris type detected. The coefficients can be interpreted as the expected additional number of dead specimens that are due to each debris type. For all tests, p<0.0001.

Debris types	Coefficient	C.I. 95%			
(Intercept)	0,016	0,011	0,024		
Line	3,13	1,72	5,69		
Net	2,76	1,18	6,46		
Plastic	2,75	0,28	26,86		

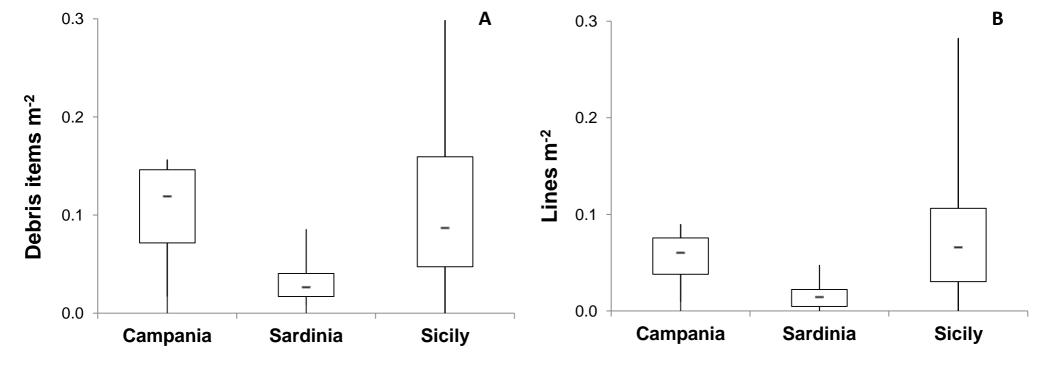
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