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Integrated approach for the assessment of microplastics pollution and its impacts in under-explored areas of the southern Italy (Central Mediterranean Sea)

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Introduction

1.1 Marine litter pollution

Marine litter (ML) is defined as any persistent, manufactured or processed solid material discarded, disposed or abandoned in the marine and coastal environment (UNEP 2009). ML consists of a wide range of materials produced and used by human beings including plastic, processed wood, glass, metal, rubber, paper, and textile materials, which accumulate in different compartments of the marine environment worldwide from the beaches to the seabed (Consoli et al., 2020; Galgani et al., 2022) including remote regions (Bergmann et al., 2019). Debris can enter the sea from both land-based and sea-based sources, from point and diffuse sources, and can be transported over long distances (Galgani et al., 2015). Land-based sources contribute up to 80% of total MPs pollution (Li et al., 2016) and include recreational and industrial activities (Lee et al., 2013), public waste, harbours, and uncontrolled landfills located near the shore (Galgani et al., 2015). Waste from land-based sources may be transported to the sea by wind, rivers (Rech et al., 2014; Moss et al., 2021), road runoff, storm water runoff, sewage discharges or drains (Galgani et al., 2015). Likewise, marine activities such as commercial shipping, ferries and liners, ships, both commercial and recreational fishing vessels, offshore installations and aquaculture sites can be significant sources of ML (Galgani et al., 2015).

ML distribution, composition and abundance in the marine environment depends on several drivers: ocean current patterns, climate and tides, proximity to urban centres but also the presence of industrial and tourist areas, shipping routes and fishing grounds (Barnes et al., 2009; Li et al., 2016). Once ML reaches the marine environment, it can float on the sea surface (floating litter), may be stranded along shorelines (beach litter), or move to the bottom until it sinks on the seabed (seafloor litter) (Claessens et al., 2011; Baini et al., 2018; Consoli et al., 2020; Galli et al., 2023). There are the presence of accumulation areas in oceanic convergence zones, such as the Pacific Garbage Patch, and on the seafloor, especially in coastal canyons, where ML accumulates in large quantities because the degradation process is much slow (Galgani et al., 2000; Mordecai et al., 2011; Harris et al., 2023).

ML can be classified by size into macrolitter (items larger than 25 mm), mesolitter (items ranging between 5 mm and 25 mm) and microlitter (items smaller than 5 mm). The microlitter size class is further categorised into large microlitter (between 5 mm to 1 mm) and small microlitter (between 1 mm and 0.1 mm (Hanke et al., 2013).

Among ML, plastics represent the most common debris found in the marine environment due to their widespread use but also glass or metal materials are also frequently observed (Liebezeit, 2008). These materials pose a threat to the marine ecosystem with environmental, aesthetic, social and economic implications (Galgani et al., 2000, 2019; Lusher, 2015). Of particular concern is the potential impact of ML on marine biodiversity, mainly related to the entanglement (Kühn, 2015; Lusher et al., 2018) and the ingestion (Kühn, 2015; Romeo et al., 2016; Fossi et al., 2018; Schirinzi et al., 2020; Bottari et al., 2022; Pedà et al., 2022b).

In recent decades, there has been a growing need to understand the real threat of ML pollution on the marine environment and biodiversity as well as to adopt preventive measures, including the establishment and improvement of legislation and regulatory frameworks. In this regards, the Barcelona Convention, amended in 1995, is one of the regional-level instruments aimed at protecting the marine and costal environment and promoting sustainable development in the Mediterranean Sea. It consists of 7 protocols, among them two are related to ML (LBS Protocol and Dumping Protocol). The Marine Strategy Framework Directive 2008/56/EC (MSFD) represents the main pillar of governance addressing the litter issue in the marine environment at the European level and establishes a framework action in the field of European Union's (EU) integrated maritime policy. The MSFD aims at preserving biodiversity and the health of the oceans by contrasting the adverse impacts of human activities. It identifies 11 Descriptors to achieve and maintain a Good Environmental Status (GES) of the marine environment. In particular, MSFD Descriptor 10 refers to the "Marine litter" and the corresponding criteria: D10C1/C2 "The composition, amount and spatial distribution of litter and micro-litter on the coastline, in the surface layer of the water column, on the seabed, are at levels that do not cause harm to the coastal and marine environment" and D10C3 "The amount of litter and micro-litter ingested by marine organisms is at a level that does not adversely affect the health of the species concerned". These criteria highlight the importance of assessing the composition, amount and spatial distribution of litter, both micro and macro, on different marine compartments including marine biota. A Technical Group (TG) on ML was also set up to support the European Member States with recommendations and information needed to commence the monitoring of the MSFD Descriptor 10. Specifically, TG has provided harmonized protocols to collect and assess data on ML, in different environmental compartment, as well as litter and microlitter in biota (Hanke et al., 2013).

Over time, many Mediterranean countries have taken actions to contrast plastic pollution by promoting a circular economy, but despite this, more should be done to achieve this goal. In this view, the European Union has recently approved two important directives. The Single-Use Plastics Directive (2019/904), which bans the trade in certain single-use plastics (SUP) in order to prevent and reduce the impact of some plastic products on the environment and biodiversity, and to promote the transition to a circular economy with innovative and sustainable business models, products and materials. Another directive concerns the Port Reception Facilities (2019/883), that allow fishermen to retrieve and dispose of waste from fishing operations. In the European context, Italy has always taken actions against ML, adopting EU directives and introducing more specific regulations both at national (e.g., D.L. 20 June 2017 n.91 and Law 27 December 2017 n.205) and at regional level (EUSAIR, COM/2014/357) (Morseletto, 2020; Pedà et al., 2023).

1.2 Plastic litter

Plastics are synthetic organic polymers resulted from the polymerisation of monomers extracted from oil or natural gas (Derraik, 2002; Rios et al., 2007; Shah et al., 2008; Thompson et al., 2009). The versatility and chemical/physical properties have made synthetic polymers perfect alternatives for glass, metal and paper daily use objects. Plastic polymers are usually classified into thermoplastics (change shape when heated but retain their chemical composition) and thermosetting plastics (harder and stronger, they retain their shape even when subjected to high temperatures) (Lithner et al., 2011). The most widely used types of polymers are: Polyethylene (PE), Polypropylene (PP), Polystyrene (PS), Polyethylene terephthalate (PET), Polyvinyl chloride (PVC), Polyurethane (PUR), Polybutylene terephthalate (PBT) and Nylon (Ivar do Sul and Costa, 2014). Properties such as low density, durability, good mechanical properties, lightweight, high thermal and electrical insulation, additives and relatively low cost of these petroleum-based synthetics materials allow for their rapid expansion and use in industries and everyday life (Barnes et al., 2009).

Plastics which first entered the commercial industry in 1950 (Harris et al., 2023), account for approximately 87% of the total ML pollution (Barboza et al., 2019; Galgani et al., 2019). It is estimated, that more than 8300 million tons of plastic have been produced since the dawn of the "*plastic age*"; in the ‘50s global production was less than one million tons/ year (Yarsley and Couzens, 1945; Thompson et al., 2009). According to recent estimate, around 390 million tonnes of plastics will be produced globally from fossil fuels in 2021, with China accounting for a third (32%) of global production and Europe for 15% (Fig. 1).

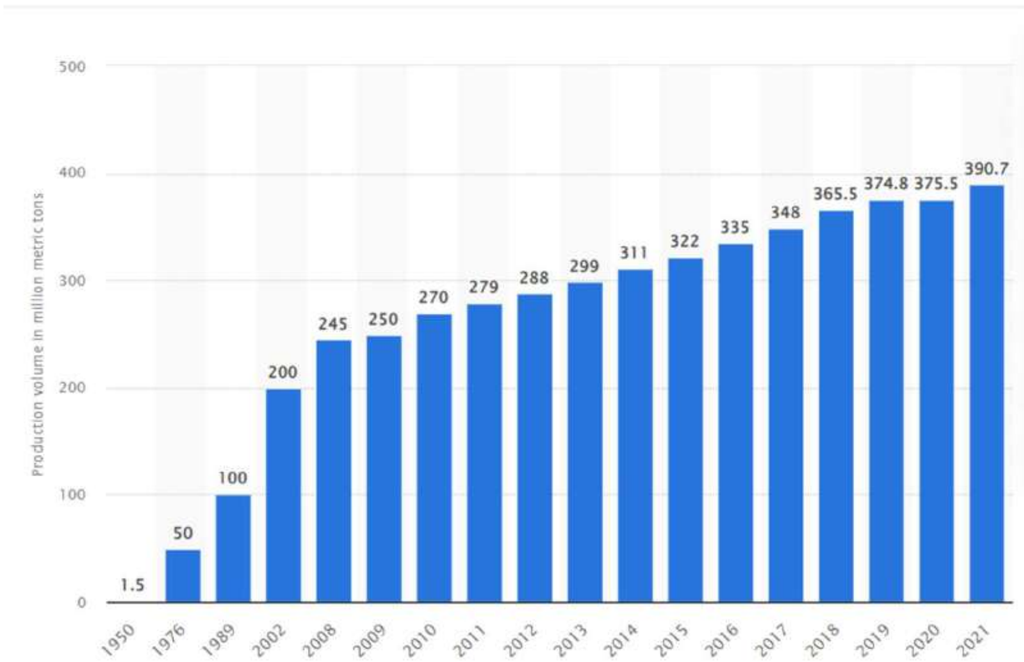


Figure 1. Global production of plastics from the 1950's to the 2021's (source: © Statista 2023).

In Europe, 57.2 Mt of plastics have been produced in 2021(Fig. 2), including mainly fossil-based plastics which represent the 87.6% and post-consumer recycled plastics (10.1%) and bioplastics (2.3%), which are considered good alternatives to conventional plastics (Plastic Europe, 2022).

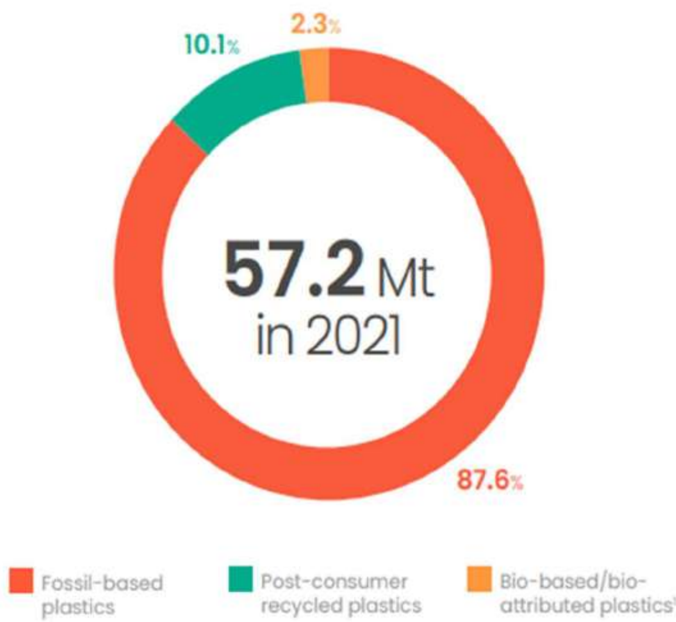


Figure 2. European plastics production in 2021 (source: Plastic Europe, 2022).

Plastics are considered ubiquitous pollutants even in the most remote areas of the world from the coast to the subtropical gyres (Barnes David K. A. et al., 2009; Hirai et al., 2011). The accumulation of plastic materials in the marine environment is linked to the successful use of this material in industries and everyday life as well as a poor system of anthropogenic waste management and disposal (Chen, 2015). In particular, the SUP products such as plastic bags, food containers, drinks cups, etc. are used for a short period of time and are generally discarded within a year of production; although some of these products are recycled, most are landfilled (Lea, 1996; Subramanian, 2000). Moreover, the high durability and resistance of plastic to degradation has resulted in its persistence in the marine environment for decades (Andrady, 2015; Worm et al., 2017).

The hazard of plastic materials in the marine environment is related to their chemical-physical properties. In particular, during the production stage, various chemical additives are added to the polymers to improve the natural properties of plastics (flexibility, strength and colour) and make them more durable. Sometimes, additives may be present in large amounts, accounting for more than 50% of the plastic product itself (Lithner et al., 2011). Since additives are not usually bound to the polymer matrix and are of low molecular weight, they can often leach out of plastics under favourable physical/chemical conditions (Lithner et al., 2011). Most additives, including stabilisers, flame retardants (PBDEs), plasticisers, antioxidants and phthalates, are hazardous to marine biota and human health (Lithner et al., 2011; Rochman, 2015). However, other hazardous substances can be emitted during all stages of a plastic product's life cycle (Lithner et al., 2011). Particularly, for some plastic materials (e.g. PUR foam, PVC and PS), monomers are also considered to be carcinogenic and toxic (Lithner et al., 2011).

At the same time, plastics due to the hydrophobic surface are able to sorb low-solubility lipophilic compounds in the environment, including persistent organic pollutants (POPs) (Teuten et al., 2009; Palmer and Herat, 2021). Thus, plastics ingestion by marine biota may induce toxicological harm due to the mixture of persistent, bioaccumulative and toxic compounds carried by plastics that can be released into animal fluids, chemically interact with important molecules, leading to long term toxic, endocrine-disruptive, carcinogenic or mutagenic effects including delayed maturity, morphological deformations, enzymatic and tissue alterations and reduced reproduction (Guillette et al., 1994; Lithner et al., 2011; Rochman et al., 2014; Pedà et al., 2022b). All these toxics substances are of particular concern for marine biodiversity and human health (Ivar do Sul and Costa, 2014; Kannan and Vimalkumar, 2021; Amelia et al., 2021). Indeed, some studies have shown that additives as phthalates and bisphenol A may cause adverse effects including endocrine disruption in humans

(Darbre, 2020, 2021) and cytotoxicity and genotoxicity harms in the marine organisms (Liu et al., 2019; Pedà et al., 2022b; Molino et al., 2023).

The distribution of plastics along the water column depends on several factors including their density. In fact, polymers such as PE, PP and the expanded polystyrene (EPS), characterised by a lower density than seawater, float on the surface, while polymers that are denser than seawater such as PVC and PET, sink to the bottom, generally where they have entered the environment (Ivar do Sul and Costa, 2014). Although plastic is a highly resistant material, it undergoes degradation processes that occur at different rates depending on the type, size and colour of the polymer, as well as the associated additive (Andrady, 2015, 2003). In addition, depending on different marine habitats, plastics may be exposed to different environmental conditions that increase or decrease the degradation processes. The fragmentation of plastics into small pieces occurs mainly through the action of the light (photodegradation) and the high temperatures (thermal degradation) (Andrady, 2011a, 2015). Indeed, plastics in the photic zone (sea surface and beaches) are subject to more photo-oxidation processes due to UV radiation (Andrady, 2015). In this regards, photo-oxidation will be faster in beached plastics than floating plastics, where UV light is rapidly absorbed by the water. In contrast, plastics found below the photic zone degrade much more slowly, due to the absence of light, limited oxygen supply and lower temperature, which will promote their persistence and accumulation in the seabed (Andrady, 2015). As mentioned before, the degradation rate is also colour-dependent. Darker plastics generally sorb more of the infrared energy, and therefore degrade faster, especially in stranded plastics due to the high temperature (Andrady, 2015). At a later stage, degradation continues by the action of microorganisms (biodegradation), which is a particularly slow process into the sea (Andrady, 2011a, 2015). The bacterial communities may use the plastic as a physical support for growth or use the polymer as a carbon source (Harshvardhan and Jha, 2013; Deepika and Jaya, 2015). Indeed, some microbial species are capable of secreting enzymes that can biodegrade common plastics such as PE (Sivan, 2011). In addition, the colonisation of plastics by algae and other marine organisms acts as a filtering barrier to solar radiation, slowing down the degradation processes. Several studies show that the development of biofouling leads to an increase in the density of plastics and subsequent their sinking (Ye and Andrady, 1991; Railkin, 2003). However, plastics may rise to the surface along the water column as a result of density changes. This cyclical process is known as *'bobbing'*. Indeed, underwater, the fouling formed at the surface is lost and the plastic having regained its normal density, returns to the surface (Andrady, 2011a).

1.3 Microplastics in the marine environment

Microplastics (MPs) refers to any plastic item lower than 5 mm in size (GESAMP, 2019). According to the MSFD this size class is further categorized into in large MPs (from 5 mm to 1 mm) and small MPs (from 1mm to 0.1 mm). The microscopic size of synthetic materials can result either directly from the production of tiny plastic particles (Cole et al., 2011) or indirectly from the fragmentation of larger plastics (Andrady, 2011a; Cole et al., 2011; Browne, 2015). Primary MPs are mostly small pellets or granules produced as precursors for other plastics, or used directly in the cosmetic industry (Thompson, 2015), as powders in stamping (Kershaw and Rochman, 2015), in abrasives (Gregory, 1996), in the clothing industry (Martellini et al., 2018) and also as drug carriers in the medical field (Ibrahim et al., 2022). Secondary MPs can result from the plastic fragmentation into smaller pieces due to a combination of abiotic (i.e., photodegradation and mechanical degradation) and biotic (biodegradation) factors (Derraik, 2002; Anastasopoulou and Fortibuoni, 2019). In the environment, primary MPs may be less abundant than secondary plastics (Ryan, 2015). Either primary or secondary, plastics reach the sea even by passing through the water channels of domestic and industrial drainage systems (Derraik, 2002; Sharma and Chatterjee, 2017) and due to the small size are not retained by wastewater treatment systems (Brown et al., 2010). MPs debris are ubiquitous in all marine environment compartments worldwide; from the coast to the deep sea (Galgani et al., 2022; Free et al., 2014; Consoli et al., 2021) where they accumulate for centuries (Cózar et al., 2014; Lusher, 2015; Xu et al., 2020). The hazardousness of these emerging pollutants is due to their features such as small size, composition, persistence and chemical/physical property. In particular, tiny particles have a higher surface area of exposure, which makes them more likely to accumulate toxic substances and organic contaminants (Andrady, 2011a; Pedà et al., 2022b). In addition, because of their small size and chemical/physical properties, MPs are easily available to marine organisms at different trophic levels and can be transferred along the food-web (Fossi et al., 2012; Panti et al., 2015; Romeo et al., 2016; Fossi et al., 2018).

1.4 Microplastics pollution in the Mediterranean Sea

The Mediterranean Sea has been considered as one of the most polluted areas by ML in the world (Cózar et al., 2015; UNEP/MAP, 2015). Morris (1980) reported the first observation of floating plastics in the Mediterranean in 1980, with around 1,300 plastics observed per square kilometre in a central area of the basin. Due to its geomorphological characteristics, the Mediterranean basin is considered a hotspot of MPs (Sharma et al., 2021). Indeed, the semi-enclosed configuration of the basin, with limited exchange of water with the Atlantic Ocean via the Strait of Gibraltar, leads to poor recirculation, resulting in waste accumulation (Cózar et al., 2015; Sharma et al., 2021). In particular, according to the distribution models of Lebreton et al. (2012) and Soto-Navarro et al. (2010) this exchange results in a potential accumulation of MPs from the Atlantic Ocean that implements the pollution rate of the Mediterranean basin. About 22 countries from three continents border the Mediterranean Sea. The presence of densely populated and exploited coastlines (tourism, fishing, intensive coastal traffic), together with the presence of rivers, torrents and low tidal currents, are the main contributors to MPs pollution in the basin. More than 1.000 tons of MPs accumulate in the basin (Sharma and Chatterjee, 2017) from beaches to the seabed with consequent impacts on the marine ecosystem. Land-based sources, as with ML, can be significant sources of MPs and include domestic activities, intensive tourism, industrial and commercial activities. In addition, millions of tourists populate the Mediterranean coast during summer contributing to an increase in plastic pollution (Galgani et al., 2013). As reported by Sharma et al. (2021), Italy is one of the main contributors to pollution in the Mediterranean Sea due to a high use of SUPs and a poor management system. Poor waste disposal also involves other Mediterranean countries such as France and Spain, where only half of the plastic products used are disposed of in landfills and only 6% are recycled (Alessi et al., 2018). The Mediterranean is considered an important global biodiversity hotspot, hosting more than 7,000 species and contributing more than 7% of the world's biodiversity (Coll et al., 2010). Despite this, the basin has been described as one of the most affected by plastic pollution worldwide, with a rate comparable to one of the largest plastic islands observed in the oceans (Cózar et al., 2015; UNEP/MAP, 2015). Moreover, MPs have been observed in different marine compartments of the Mediterranean Sea; lying on the beach (Lots et al., 2017; Martellini et al., 2018; Strafella et al., 2020), floating on the sea surface (Suaria et al., 2016; Baini et al., 2018; Galli et al., 2023), or sinking to the seafloor from the coast to the deep sea (Palatinus et al., 2019). Furthermore, due to their small size and high availability in the marine environment, ingestion of MPs have been documented both in Mediterranean vertebrates (Romeo et al., 2015, 2016; Fossi et al., 2018; Pedà et al., 2020; Schirinzi et al., 2020; Pedà et al., 2022a) and invertebrate (Setälä et al., 2016; Courtene-Jones et al., 2019; Lusher et al., 2020).

1.5 Microplastics impact

Due to their properties, plastic litter is ubiquity in the marine environment with several possible biological implications. In particular, the small size of MPs facilitates their dispersal in the marine ecosystem, and make them available within the food web. Marine biota can, thus easily ingest MPs, that may cause mechanical/physical damages in tissues (Kühn, 2015; Derraik, 2002) and induce toxicological harm in marine species (Pedà et al., 2022b). Particularly, the chemical properties of MPs facilitate the introduction of contaminants in marine organisms and into food web through the bioaccumulation and biomagnification processes (Rochman, 2015) (Fig.3). Furthermore, MPs may act as a dispersal vector for microorganisms including opportunistic pathogens such as specific members of the genus Vibrio and also alien species (Amaral-Zettler et al., 2015).

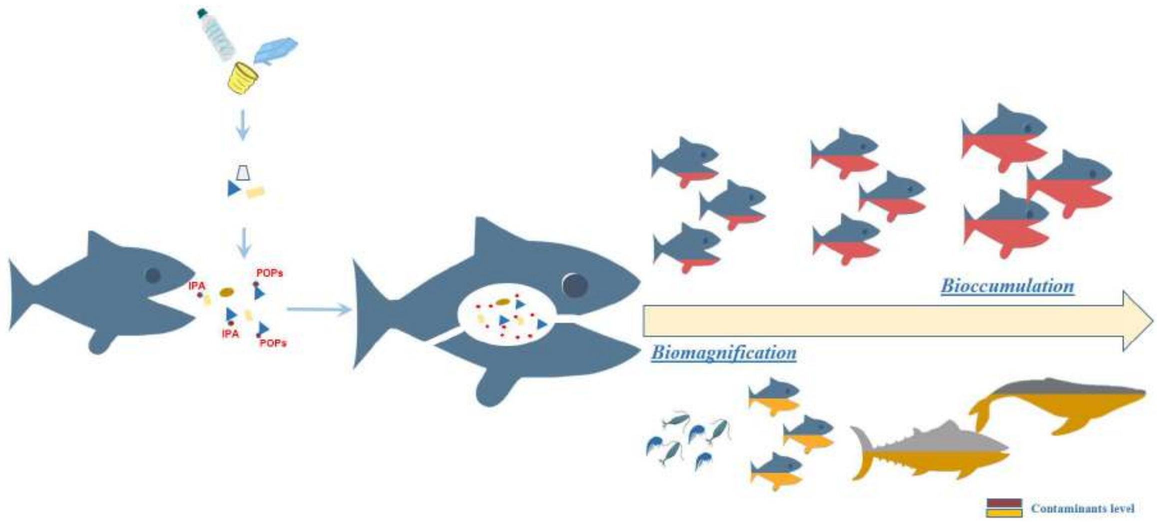


Figure 3. Diagram show MPs ingestion by marine organisms. Hazardous substances carried by plastics can accumulate in an organism's body over time through a bioaccumulation phenomenon. Biomagnification is the increased concentration of pollutants along the food web, up to the higher tropic level.

1.5.1 Microplastics ingestion

Ingestion of plastic litter represents one of the main threats for marine biodiversity (Fossi et al., 2018). In particular, over the past decade, plastics and especially MPs, are the most common litter found in the stomach contents of marine organisms (Pedà et al., 2020; Schirinzi et al., 2020; Tsangaris et al., 2021; Bottari et al., 2022). To date, MP ingestion in the Mediterranean Sea has been documented in organisms at different trophic levels, with the higher number of species affected in the Western Mediterranean Sea region (Anastasopoulou and Fortibuoni, 2019). MPs ingestion have been observed both in invertebrates such as zooplanktonic species (Frias et al., 2014), bivalves (Rochman, 2015; Tsangaris et al., 2021), cephalopods (Pedà et al., 2022a) and crustacean (D'Iglio et al., 2022) as well as in vertebrates including several fish and cartilaginous species (Anastasopoulou et al., 2013; Romeo et al., 2015; Bellas et al., 2016; Romeo et al., 2016; Giani et al., 2019; Pedà et al., 2020; Bottari et al., 2022), reptiles (Lazar and Gračan, 2011; Campani et al., 2013) and marine mammals (Fossi et al., 2012).

Marine organisms can ingest MPs either accidentally or intentionally. Indeed, plastic particles can be ingested accidentally during foraging activities as filter feeders. This behaviour has been reported for many marine organisms, including omnivorous predators as some species of seabirds (Provencher et al., 2010), filter feeders of different trophic levels (i.e., cetaceans and molluscs) that ingest MPs by filtering volumes of water (Fossi et al., 2016; Merrill et al., 2023) and in fish during hunting for shoal (Romeo et al., 2015; Battaglia et al., 2016). Accidental ingestion of MPs has also been documented in organisms that live in direct contact with the seabed, such as invertebrates and benthic fish (Anastasopoulou et al., 2013; Fanelli et al., 2009; Pedà et al., 2020; Valls et al., 2011). Sometimes some marine organisms may intentionally ingest MPs because of their resemblance in shape and colour to the natural prey (Fossi et al., 2018; Pedà et al., 2019). MPs can also be ingested indirectly through secondary ingestion, which occurs when a predator feeds on prey that has already ingested plastic (Eriksson and Burton, 2003; Chagnon et al., 2018; Fossi et al., 2018; Nelms et al., 2018; Pedà et al., 2020). Pedà et al. (2020) reported data on the ingestion of MPs by some elasmobranch species in the southern Tyrrhenian Sea. In the case of *Raja clavata*, the MPs ingestion is certainly related to its feeding behaviour. Indeed, this species find it food by excavating in soft sediments where prey is usually buried, increasing the risk of ingesting MPs. Instead, in the *Galeus melastomus*, the possibility of ingesting contaminated prey (secondary ingestion) cannot be ruled out.

MPs ingestion may cause physical/mechanic and chemical harms in marine organisms (Stamper et al., 2009; Wright et al., 2013; Jovanović, 2017). Physical damages may result in blockage of the gastrointestinal tract and functionality of feeding appendages, injuries, ulcerations, and/or pseudo-satiation that may lead to death of the organism (Galgani et al., 2010; Wright et al., 2013; Kühn, 2015). Moreover, chemical harm is due to the potential transfer of toxic substances from ingested plastic to marine fauna such as phthalates (Rochman et al., 2013, 2014; Baini et al., 2017; Liu et al., 2019; Amelia et al., 2021; Wang et al., 2021) and POPs (Lithner et al., 2011; Tanaka et al., 2013; Rochman, 2015; Koelmans et al., 2016) with long-term effects. As result of biomagnification and bioaccumulation phenomena, these substances could over time be transferred along the trophic web, causing damage to organisms at different levels of biological organization (Marsili and Focardi, 1997; Avio et al., 2015a; Trestrail et al., 2020; Pedà et al., 2022b). To date, some studies assessed the transfer of MPs in the trophic web. Trophic transfer is demonstrated mainly for low trophic level under laboratory conditions, while few studies have investigated the MPs transfer to higher trophic levels (Lusher et al., 2013; Farrell and Nelson, 2013; Watts et al., 2014; Chagnon et al., 2018; Nelms et al., 2018; Dool and Bosker, 2022; Justino et al., 2023).

1.5.2 Microplastics as vectors for microorganisms

Although a number of impacts of MPs have been widely documented, less attention has been paid to the hazardous potential of MPs as carriers of harmful microorganisms. MPs materials provide a persistent solid substrate for the adhesion, proliferation and spreading of microorganisms in the water column. Therefore, MPs could act also as hazard vectors for the diffusion of potential bacterial pathogenic species, antimicrobial resistant bacteria and harmful algal species, that are dangerous both to human health and marine organisms (Bowley et al., 2021). The microbial community associated with plastic debris, defined by Zettler et al. (2013) as the "plastisphere", appears to date to be an aspect worthy of attention for the scientific community due to several implications for the marine ecosystem. Another implication related to the distribution of MPs in the marine environment that could be influenced by biofouling growth. Moreover, the plastics colonization by marine microorganisms acts as a barrier filtering the solar radiation, slowing down the degradation processes and by changing the density of floating plastics. Indeed, the biofouling development can increase the plastic density by causing the subsequent plastic sink on the bottom (Andrady, 2011b) promoting even microbial spread in deep environments. The study of the relationships between MPs and the bacterial communities is important to assess the effective ecological impacts. The use of molecular methods such as high-throughput DNA sequencing has provided important information about the associated bacterial community characterization (Amaral-Zettler et al., 2020a). From the results of surveys based on culture-independent approaches, it has been observed that the community associated with plastic differs from the surrounding environment (Zettler et al., 2013; Oberbeckmann et al., 2014; Geyer et al., 2017) and depends on the type of substrate and polymer (Kirstein et al., 2018; Oberbeckmann et al., 2014). Seasonal and spatial conditions affect the community composition (Amaral-Zettler et al., 2015). Moreover, in general the microbial community appears less rich than the surrounding water (Amaral-Zettler et al., 2015) but more uniform (Dussud et al., 2018; Frère et al., 2018). So far, the widely used techniques for the determination of the microbial community associated with plastics is the use of scanning microscopy (SEM) techniques and molecular analyses such as next-generation sequencing (Zettler et al., 2013; Oberbeckmann et al., 2014; Dussud et al., 2018). Several studies showed that communities of prokaryotes and eukaryotes have been observed on MPs, including heterotrophs (i.e., *Candidatus pelagibacter* and *Pseudomonas spp*.), autotrophs (i.e., *Phormidium*, *Rivularia* and *Leptolyngbya*), predators (*Micromonas*) and symbionts Cyanobacteria (Amaral-Zettler et al., 2020a). Several studies on MPs samples from different areas worldwide including fresh and seawater, have been reported the presence of pathogenic organisms such as *Vibrio*, members of the Campylobacteraceae family and *Aeromonas salmonicida* and many others (Zettler et al., 2013; Oberbeckmann et al., 2014; Amaral-Zettler et al., 2020b).

1.6 Status of Microplastics investigation in Mediterranean region

MPs pollution in the marine environment and its associated impacts have raised serious global concern. In particular, MPs concentration in the Mediterranean basin is among the highest of the world, with important ecological and biological implications for ecosystems health and biodiversity (Lusher, 2015). In this regard, several studies have been carried out on the distribution of MPs debris (Galgani et al., 1995; Free et al., 2014; Suaria et al., 2016; Fossi et al., 2017; Lots et al., 2017; Galli et al., 2023) and its occurrence in organisms inhabiting marine ecosystems (Deudero and Alomar, 2015; Fossi et al., 2018) in the different regions of the Mediterranean basin.

Different methodological approaches have been applied to assess MPs pollution in Mediterranean beaches (Cesarano et al., 2023; Fossi et al., 2019), surface water (Cadiou et al., 2020; Galgani et al., 2013), sediments (Fossi et al., 2019; Hanke et al., 2013) and also to estimate its potential risk for marine fauna (Soto-Navarro et al., 2010; Avio et al., 2015b, 2015b; Karami et al., 2017; Lusher et al., 2017; Compa et al., 2019; Schirinzi et al., 2020; Galli et al., 2022; Laface et al., 2023; Tsangaris et al., 2021).

According to the monitoring guidelines provide by MSFD TG10, manta trawls are the main equipment required to assess the density of MPs floating in the surface waters (Pedrotti et al., 2016; Ruiz-Orejón et al., 2016; Baini et al., 2018; Compa et al., 2020; Fagiano et al., 2022b; Galli et al., 2023) whereas the collection and analysis of sediment samples are used to assess the density of MPs both in the seabed and the beaches (Galgani et al., 2013; Hanke et al., 2013).

Another important issue concerns the knowledge about the levels of MPs ingestion in marine biota, as reported by MSFD criteria D10C3 (Commission Decision EU 2017/845) states: "The amount of litter and micro-litter ingested by marine animals is at a level that does not adversely affect the health of the species concerned." In this regard, the best way to assess MP ingestion by marine organisms is to analyse the gastrointestinal tracts (GITs) using visual identification or chemical digestion protocol approaches (Lusher et al., 2017). Some studies suggested suitable organisms for plastic ingestion monitoring in the Mediterranean Sea (Fossi et al., 2018; Bray et al., 2019; Tsangaris et al., 2021; Multisanti et al., 2022; Reboa et al., 2022) in order to measure the occurrence of ML, especially MPs, within species and their environment and to assess the potential threat due to the exposure and accumulation of these contaminants in marine fauna (Fossi et al., 2018; Amelia et al., 2021; Palmer and Herat, 2021).

Different extraction methods including the visual inspection, the use of chemical and enzymatic digestion protocols and density separation have been applied to facilitate the isolation and identification of plastics litter by different environmental matrices (seawater, sediment and biota). However, there is a need to support validation and harmonization of methods to improve data reliability (Shim et al., 2017; Schirinzi et al., 2020; Tsangaris et al., 2021; Laface et al., 2023) as well as the application of quality control procedures aims to avoid the overestimation of MPs contamination (Pedà et al., 2020; Prata et al., 2021). Due to the difficulties in MPs visually identifying, the MSFD suggests to confirm the identity of potential plastic particles, especially in the smaller sizes ranges (Hanke et al., 2013; Shim et al., 2017; Hildebrandt et al., 2022) by using analytical techniques. The main analytical techniques for assessing the chemical identification of suspected particles are Fourier transform infrared (FTIR) and Raman spectroscopy (Hanke et al., 2013). These techniques make it possible not only to identify the nature of the particle materials, but also to gather information on the types of polymers present in environmental samples. This allows for understanding the polymers relative abundances, comparing the plastics composition in different environmental matrices and also assessing possible sources (Cowger et al., 2020; De Frond et al., 2023). Several techniques allow the detection and characterisation of polymers, some of which address the limitation of detecting smaller sizes (Shim et al., 2017; Tian et al., 2022). Among those, the laser direct infrared (LDIR) spectroscopy, is a new technique for chemical imaging, infrared (IR) microscopy, and IR spectral analysis in environmental MPs studies (Scircle et al., 2020; Cheng et al., 2022). LDIR is more advantageous than the FTIR technique for MPs quantification and identification because it uses a tunable quantum cascade laser (QCL) as the IR source and focuses only on particles (Tian et al., 2022; Cheng et al., 2022).

In this context, initiatives and transversal actions, including European project focusing on ML monitoring issues such as Plastic Buster MPAs (https://plasticbustersmpas.interreg-med.eu/), MEDSEALITTER (https://medsealitter.interreg-med.eu/), CleanSea (https://www.cleansea.co/en-eu/), Marelitt (https://www.marelittbaltic.eu/), Life projects and others contributed to support the MSFD to improve and provide relevant and applicable harmonized monitoring protocols to address the recent advances in this field (Fossi et al., 2019; MEDSEALITTER consortium, 2019).

Despite of the already mentioned, further researches in Mediterranean basin are needed because some areas are still under-explored, including the MPAs, and because there is still a need to ensure comparability of assessment approaches and methods within and across Mediterranean regions. Indeed, to date, the challenge for the scientific community is to identify standardized protocols and methods for plastics pollution assessing and monitoring in the marine compartments and to ensure comparability and reliability of data (Galgani et al., 2013; Fossi et al., 2018; Tsangaris et al., 2021).

2 Study aim

In accordance with the requirements of the MSFD, this research aims to fill the gaps on MPs pollution and its impacts in under-explored Mediterranean areas including sensitive and ecologically important areas such as Marine Protected Areas (MPAs).

In this view, coastline waters of the Calabria and north-eastern Sicily (southern Italy; central Mediterranean Sea) subjected to different levels of anthropogenic pressures and protection, were selected as pilot areas for assessing MPs pollution and its impacts. Two sampling sites including both an MPA and a tourist and/or urbanized settlement were chosen for each pilot area.

MPs pollution in the identified areas was assessed using an integrated approach based on the measurement of MPs load both in marine organisms as well as in the marine environment.

In both pilot study areas, bioindicator species were selected in accordance with the following ecological and biological criteria i) background, habitat and trophic information; ii) feeding behaviour and spatial distribution; iii) commercial value and conservation status; and iv) available data on microlitter ingestion as suggested by the study of Fossi et al. (2018).

In order to ensure comparability of assessment approaches and methods within and between Mediterranean regions, MSFD guidelines and protocols were used during field and laboratory activities (Hanke et al., 2013; Fossi et al., 2019).

The results of this research will provide new information and standardized data on MPs pollution at a local and regional scale of the Mediterranean basin. The obtained data will help to meet the MSFD's requirements for achieving the GES. In addition, insight related to the potential hazard of MPs as vector of pathogens and the human consumption of commercial species contaminated with MPs will be provided.

2.1 Main and specific aims

The main aim of this study is to assess MPs pollution and its impacts in under-explored Mediterranean areas with different levels of human impacts analysing the MPs abundance and composition in sea surface and bioindicator species.

The integrated approach included the following specific aims:

- Selection of under-explored sites in the coastline waters of Calabria and north-eastern Sicily considering different levels of anthropogenic pressure and protection;
- Identification and sampling of marine bioindicator species for the assessment of MPs ingestion in the study areas;
- Assessment of the MPs abundance, shape, size and polymer in the sea surface of the study areas;

- Assessment of the MPs abundance, shape, size and polymer in the gastrointestinal tract of bioindicator species in different study areas;
- First assessment of abundance and diversity of bacterial communities associated with plastics from floating MPs in coastline waters of the Calabria.

The thesis is divided into chapters, including an introductory chapter (Chapter 1) on the topic of the research with a focus on the Mediterranean Sea. Chapters 2 and 3 represent the core of the thesis and detail the specific objectives of the research. In particular, chapter 2 refers to the assessment of MPs in the coastal area of Calabria. In this area, analyses were carried out on seawater samples and fish species to assess the presence of MPs in surface waters and the impact of ingestion by marine organisms. In addition, microbiological analyses were carred out on plastics isolated from seawater samples to study the microbial community associated and assess their potential role as vectors for microorganisms. The other core chapter (Chapter 3) refers to the investigations conducted at the two selected survey sites in the Sicily study area. In particular, analyses were conducted to assess the presence of microplastics in surface seawater samples and fish species using different sampling and analysis strategies to the previous study area.

3 Microplastics assessment in the coastal area of Calabria
3.1 Materials and methods

In Calabrian coastal area, the MPs contamination of seawater and fish was investigated in sampling sites located both in the southern Ionian (Capo Rizzuto MPA) and in the Tyrrhenian Sea (Vibo Valentia) (Fig. 4). In this pilot study area, MPs sampling from both superficial water and bioindicator species was carried out during experimental surveys in winter 2021.

Figure 4. Survey areas. Capo Rizzuto MPA in souther Ionian Sea; Vibo Valentia in the Thyrrhenian Sea.

For each sites, sea surface samples were collected at 2 stations placed at 0.5 and 1.5 nautical miles (nm) from the coast along orthogonal transects according to the guidelines of the Monitoring Programs for the Marine Strategy (Art. 11, Legislative Decree 190/2010) of the European MSFD (Hanke et al., 2013). MPs were sampled using a manta trawl (335 µm mesh size, 60 × 16 cm mouth opening) towed at the sea surface, in the opposite direction to the current, at an average speed of 2 knots for 20 min and kept at a distance from ship's side to avoid wake turbulence. The manta net was equipped with a flowmeter to quantify the volume of water filtered (m^3). After the sampling, the net was thoroughly rinsed and the sample collected into the code-end was transferred and stored in glass jars with 70% ethanol solution for subsequent analyses (Fig 5). From each surface water sample collected, a subset of MPs were selected by eye, isolated directly from the code-end using sterile tweezers, and transferred to a sterile falcon for microbial community analysis. Samples were immediately stored at -20°C and transferred to the laboratory. Date and time, geographical coordinates, and weather conditions were recorded for each sampling activity.

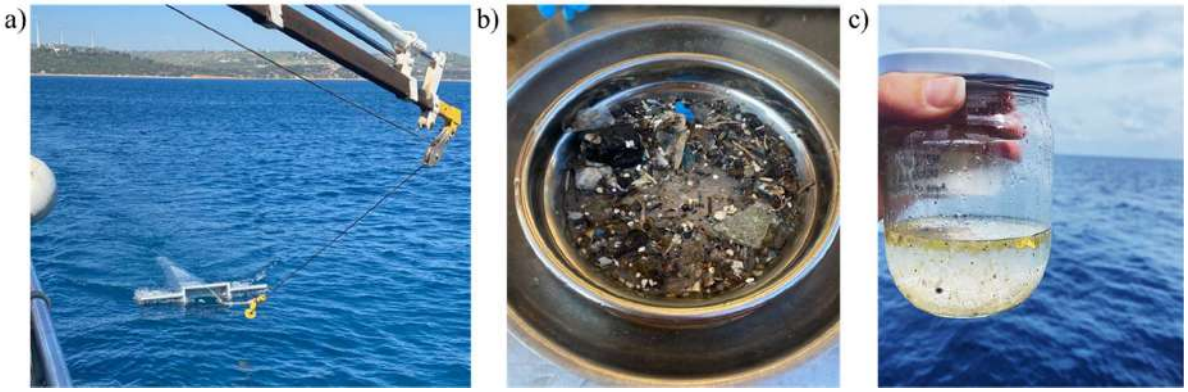


Figure 5. (a) Sea surface water sampling with manta net; (b) sample collection; (c) sample storage in glass jars.

Commercial fish were collected in the two sampling sites from the Calabrian coast area (Table 1).

The bioindicator species have been chosen according to the following criteria (Fossi et al., 2018):

1) knowledge on the occurrence of ingestion of MPs from literature;
2) commercial values;
3) habitat;
4) ecological compartment and feeding strategy.

Same bioindicator species were identified in both sites of Calabrian coasts. Fish specimens (6 *per* species) were collected both at the landing places, already dead, from local fishermen according to their availability in the main fisheries and during research survey, simultaneously to the surface waters sampling in the study area. Specimens of the same length class were selected for each species.

Table 1. Bioindicator species for MPs ingestion selected in the two site of Calabrian coastal area. Information on habitat, ecological compartments and feeding habits of bioindicator species.

Species	Common name	Habitat	Ecological compartment	Feading habits
 *Boops boops (Linnaeus, 1758)*	Bogue	Benthopelagic	Coastal water	Zooplanktivorous
 *Mullus barbatus Linnaeus, 1758*	Red mullet	Demersal	Coastal water	Benthivorous
 *Merluccius merluccius (*Linnaeus, 1758)	European hake	Benthopelagic	Coastal water	Piscivorous

3.1.1 Study area and sample collection

3.1.1.1 Sampling site in the southern Ionian Sea: Marine Protected Area of Capo Rizzuto

In the Calabria study area, the Capo Rizzuto MPA was chosen as sampling site because of its high ecological importance and biodiversity and also poor information on MPs pollution (Mezzelani et al., 2017). The Capo Rizzuto MPA has been established in 1991 by a Ministerial Decree of Dec. 27, 1991, and it extends 42 km of coastline involving two localities: Crotone and Capo Rizzuto (riservamarinacaporizzuto.it). It is the largest established MPA in Italy with an area of 14.721 ha (Fenner et al., 2013). The MPA seabed is characterized by Posidonia meadows, ciliates and banks, which provide shelter for many marine organisms. Indeed, it is an important habitat for several species, including the grouper *Epinephelus guaza*, the common octopus, *Octopus vulgaris,* and several species of echinoderms such as the urchin, *Stylocidaris affinis,* and starfish, *Echinaster sepositus* and *Ophidiaster ophidianus*. Some protected species such as dolphins and loggerhead turtles, *Caretta caretta* are also common in this MPA (riservamarinacaporizzuto.it).

In this site, the sampling of surface water was carried out on board the oceanographic vessel 'Astrea' of ISPRA. Fish specimens were collected by vessels operating near the studied site. In particular, Bogues were caught by local fishermen using trammel net at a depth ranging from 25 to 30 m, Red mullet were collected using bottom trawl at ~ 60 m of depth and European hake using a bottom longlines at 200 m.

3.1.1.2 Sampling site in the Tyrrhenian Sea: urbanized settlement of Vibo Valentia

Vibo Valentia represent the most densely centre of the "*Costa degli Dei*" tourist area with more than 30.000 inhabitants (Trecozzi et al., 2022). It is located on a raised Pleistocene terrace at about 500 m above sea level (Stanley and Bernasconi, 2012) and several torrents flow though this area on their way into the sea with estuarine mouths (Lena, 1989). This site is characterized by an important commercial and tourist harbour. In particular, due to the beautiful and relevance landscape, touristic activities increase especially during the summer season (https://www.portodigioiatauro.it/i-porti/vibo-valentia/). To the best of our knowledge, the only information regarding MPs pollution in the study area is reported by Marrone et al. (2021). In particular, this study investigated the distribution and composition of MPs in the surface waters in Tyrrhenian and Ionic Calabrian coasts including Vibo Valentia.

In this site, surface water and commercial fish species sampling were conducted on board the "Papà Carmelo" fishing boat. Bogues, Red mullet and European hake were caught by bottom trawl at a depth between 50 and 95 m. Once on board, specimens were stored at - 20°C for the subsequent laboratory analysis.

3.1.2 Laboratory analysis

3.1.2.1 Plastic litter isolation and characterization from sea surface

In the laboratory, the samples were filtered through a metal sieve (mesh size: 300 µm) and sorted under a Zeiss Discovery V.8 stereomicroscope. Plastics were manually isolated from the sample and photographed with the Axiocam 208 colour camera using ZEN 3.1 blue Edition software coupled to the microscope. The isolated plastic particles were then measured and classified according to the MSFD guidelines (Hanke et al., 2013) by shape (fragment, film, filament, pellet, foam), size class (0.3-0.5 mm; 0.5-1 mm; 1-2.5 mm; 2.5-5 mm; >5 mm) and colour (transparent, white, black, red, blue, green, other colour). Due to possible contamination during sampling activities (i.e., worn clothing and/or vessel equipment), fibres detected in the samples were not included in the analysis.

3.1.2.2 Detection and quantification of plastic in fish samples

In the laboratory, each individual was weighed (total weight, TW in g) and measured (total length, TL in cm) before dissection. GIT was removed from each specimen, weighed and transferred to a glass beaker for MPs extraction. Each GIT was subjected to chemical digestion protocol by Pedà et al. (2022a) to isolate ingested plastics. Specifically, 10% potassium hydroxide (KOH) solution was added to each GIT at a ratio of 1:5 (w/v). The solution was incubated in stove at 50°C for 6 hours and then filtered through fiberglass membranes (pore size 1.6 µm, GF/A Whatman) using a vacuum pump system (Fig. 6a). Membranes were observed under stereomicroscope and the sorted particles were counted, measured (length and width in mm) and photographed with a stereomicroscope Zeiss Discovery V.8. coupled with Axiocam 208 color microscope camera, using ZEN 3.1 blue Edition software. Plastic items isolated were classified according to MSFD guidelines (Hanke et al., 2013; Schirinzi et al., 2020) by size in macroplastics (> 25 mm; MAPs) mesoplastics (25 - 5 mm; MEPs), large MPs (5 - 1 mm; LMPs) and small MPs (1 - 0.1 mm; SMPs), shape (fragment, film, pellet, filament, fiber and foam) and colour(Fig. 6b).

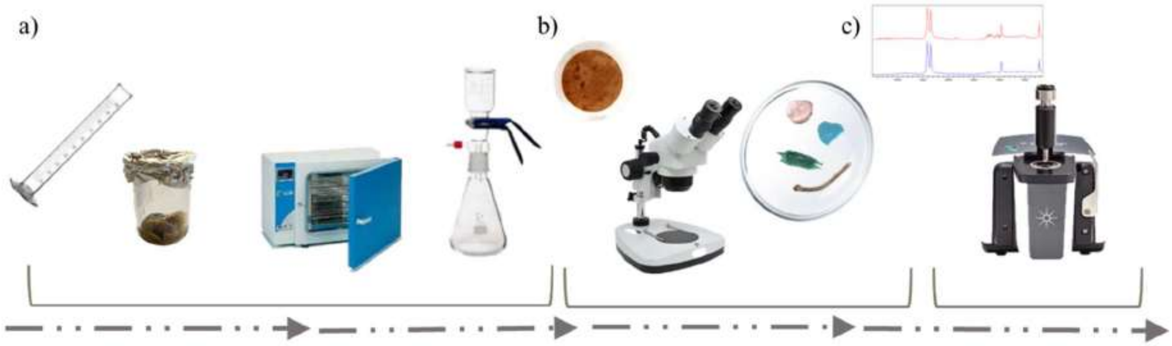


Figure 6. Analysis steps for the detection and quantification of plastics in fish samples. a) Chemical digestion of GITs; b) Membranes observation under stereomicroscope; c) Polymer identification by FT-IR spectroscopy.

During the laboratory activities, several mitigation measures were adopted to reduce sample contamination, especially from the risk of contamination by airborne fibres. All equipment used was cleaned and washed with Milli-Q water. A blank sample was processed simultaneously with each battery of samples. In addition, a membrane moistened with Milli-Q water was placed in a Petri dish and exposed during each digestion step, from sample preparation under the fume hood to observation near the stereomicroscope (Pedà et al., 2020; Schirinzi et al., 2020). Particles and fibers observed in the sample membranes with similar characteristics (i.e., structure or colour) to the airborne contamination found in the blank sample were not included in the analysis (Pedà et al., 2020).

3.1.2.3 DNA extraction, amplification of 16S rDNA and sequencing

For microbial community analysis, total DNA was extracted by MPs samples by Power Soil DNA extraction kit (MoBio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. Because of the small dimension of plastic, to optimize the success of the extraction procedure, two pools were created, including all fragments from the same study site. Three experimental extraction replicates have been performed. A BioSpec Nano (Shimadzu Corporation) was used to check the quality and concentration of all DNA extracts. Te 16S rDNA region V3-V4 with was amplified with two locus specific primers with Illumina overhangs 16S-341 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNBGCASCAG -3' and 16S-805R 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACNVGGGTATCTAATCC -3'following the standard Illumina protocol (https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf). Thirty cycles were used in the first PCR amplification with locus-specific PCR primers, while subsequent amplification that integrates relevant flow-cell binding domains and unique indices (NexteraXT Index Kit, FC-131-1001/FC-131-1002) was performed according to the protocol. Sequencing was performed using the Illumina MiSeq platforms, following the standard protocols of the company IGA Technology Services Srl (Udine, Italy).

3.1.2.4 Polymers identification

Polymer identification of both floating and ingested MPs was achieved by Fourier Transform Infrared (FT-IR) spectroscopy technique (Fig. 6c). The analysis was carried out using the Agilent Cary 630 spectrometer in attenuated total reflectance (ATR), supplied with polymer-specific libraries and the level of certainty to match the sample spectrum with reference spectra was set up to > 70% (Pedà et al., 2022a; Schirinzi et al., 2020). Polymer identification of floating litter was performed on a sub-sample (20%) of the total isolated particles according to the different percentage of abundance of shape category for each sample. Identification of too small or too thin (i.e., fibres) plastics isolated from GITs sample could not always be analysed due to instrumental limitations. In this case, the plastic nature was confirmed by the "hot needle test" (Hanke et al., 2013; Pedà et al., 2022a), and the particle was classified as an undefined polymer (UP).

3.1.3 Data and statistical analysis

Surface water data were expressed as items per cubic metre (items/m^3) and items per square metre (items/m^2). Differences in size class, shape and polymer MPs abundance between 0.5 and 1.5 nm from the coast were assessed using the Wilcoxon rank sum test (Bauer, 1972; Hollander et al., 2013). To assess the general well-being of bioindicator species and their feeding intensity the Fulton's condition index (K) and the Gastro Somatic Index (GaSI) were calculated as follows:

K= (total weight in g/ total length in cm^3) × 100 (Lloret et al., 2013)

GaSI= Stomach weight in g / (total weight in g - stomach weight in g) × 100 (Desai, 1970)

Plastic abundance indices were calculated for each bioindicator species as follows:

1) Litter and plastic percentage of occurrence (O%) was estimated as the proportion, on the total sample, of the individuals which ingested plastics: (%O= N. individuals which ingested litter and plastics/N. total samples × 100);

2) Mean number of plastic items found in the GITs, calculated on the total number of individuals (N. plastic items/N. all examined individuals);

3) Mean number of plastic items found in the GITs, calculated on the total number of individuals that ingested plastics (N. plastic items/N. individuals that ingested plastics).

Kendall's Tau correlation has been performed to assess the correlation between MPs abundance *vs.* K index and MPs abundance *vs*. GaSI. Results were interpreted as follow: Tau = 0: no correlation, 0

< Tau ≤ 0.25: extremely weak, 0.25 < Tau ≤ 0.34: weak, 0.35 < Tau ≤0.39: moderate, Tau ≥ 0.40: strong.

All statistical analyses were performed using R and R-studio software (R Core Team, 2022).

3.1.4 Bioinformatic analysis

For bioinformatic analysis, sequence quality was verified by using FastQC. Quality filtering, trimming, de-noising and merging were carried out by following the R package DADA2 to infer amplicon sequence variants (ASVs), i.e., biologically relevant variants. During the analysis, filters for reducing replicate, length, and chimera errors were also applied. Bacterial taxonomy annotation was performed using Silva database formatted for DADA2, offering an updated framework for annotating microbial taxonomy (silva_nr99_v138.1_wSpecies_train_set.fa.gz and silva_species_assignment_v138.1.fa.gz). A taxa filter was used for the decontamination from eukaryotic, chloroplast, and mitochondrial sequences. The analysis was carried out with the support of IGA Technology Services Srl (Udine, Italy).

3.2 Results and Discussion

3.2.1 Marine Protected Area of Capo Rizzuto

A total of 924 plastic items was isolated from the 4 samples of the Capo Rizzuto MPA surface water with a mean concentration of 0.29 ± 0.27 items/m^2 corresponding to 1.81 ± 1.68 items/m^3 (Table 2).

Table 2. MPs number, abundance expressed as items/m^3 and items/m^2, in water surface samples collected in the two transects (TR1; TR2) at 0.5 nm; 1.5 nm distance from the coast of Capo Rizzuto MPA; mean ± standard deviation (SD) per each transect and total.

Sample ID	Distance from the coast (nm)	Latitude	Longitude	N items	items/m^3	items/m^2
TR1_0.5	0.5	35°57'934"	17°10'381"	95	0.70	0.11
TR1_1.5	1.5	38°57'2961"	17°11'0608"	52	0.50	0.08
TR1 (mean ± SD)				77.50 ± 36.06	0.6 ± 0.14	0.10 ± 0.02
TR2_0.5	0.5	38°53'5575"	17°01'2132"	537	4.16	0.66
TR2_1.5	1.5	38°53'1239"	17°00'3926"	240	1.89	0.30
TR2 (mean ± SD)				395.50 ± 204.35	3.02 ± 1.6	0.48 ± 0.26
Total				942		
(mean ± SD)				235.5 ± 218.26	1.81 ± 1.68	0.29 ± 0.27

These results are consistent with the concentrations of other Mediterranean areas (Table 3) as well as with other Mediterranean MPAs: The Balearic Islands and Columbretes MPAs (3.52 ± 8.81 items/m^3; 0.04 ±0.03 particles/m^2) (Fagiano et al., 2022a, 2022b) and the Pelagos Sanctuary (0.082 ± 0.079 items/m^2) (Fossi et al., 2017). On the contrary, at local level, higher concentrations of floating MPs were detected compared to Mezzelani et al. (2017) in the same survey site (0.06 items/m^3) and lowest values were observed by Marrone et al. (2021) in the Ionian Sea with a range 0.01-0.10 items/m^2 both to the north (Neto) and south (Cosenza) of the Capo Rizzuto MPA at the same distance from the coast. This difference could be linked to multiple factors influencing the distribution of plastic particles in the marine environment, such as the combined effect induced by wind and water coastal currents, as shown in studies on the spatial distribution of plastic (Cózar et al., 2015; Mansui et al., 2015), and the surface circulation of the Ionian sea, characterized by strong interannual variability (Kalimeris and Kassis, 2020).

Table 3. Literature data on microplastic abundance in Mediterranean surface waters.

Mediterranean Sea sub-region	Sampling area	Net mesh (µm)	Abundance	Abundance units	References
Ionian Sea	Italian coastlines (Capo Rizzuto MPA)	335	0.29 ± 0.27	items/m^2 ± SD	Present study
Western Mediterranean Sea	Tyrrhenian sea (Vibo valentia)	335	0.34 ± 0.24	items/m^2 ± SD	Present study
Western Mediterranean Sea	Tyrrhenian sea (Capo Milazzo MPA)	335	0.20±0.13	items/m^2 ± SD	Present study
Western Mediterranean Sea	Tyrrhenian sea (Aeolian Islands Archipelago)	335	0.34±0.20	items/m^2 ± SD	Present study
Western Mediterranean Sea	Balearic sea (Balearic Islands)	330	3.52 ± 8.81	items/m^3 ± SD	Fagiano et al., 2022a
Western Mediterranean Sea	Balearic sea (Cabrera MPA)	330	0.04 ±0.03	items/m^2 ± SD	Fagiano et al., 2022b
Western Mediterranean Sea	North Tyrrhenian and Ligurian Sea(Pelagos Sanctuary)	330	0.082 ± 0.079	items/m^2 ± SD	Fossi et al., 2017
Ionian Sea and Western Mediterranean Sea	Italian coastlines (Calabria)	333	0.01-0.10	items/m^2 (range)	Marrone et al., 2021
Ionian Sea and Western Mediterranean Sea	Italian coastlines (Capo Rizzuto MPA)	330	0.06	items/m^3	Mezzelani et al. (2017)
Ionian Sea and Western Mediterranean Sea	Italian coastlines (Lipari)	330	0.3	items/m^3	Mezzelani et al. (2017)
Western Mediterranean Sea	Tyrrhenian Sea(Pelagos Sanctuary)	330	0.26 ± 0.33	items/m^3 ± SD	Baini et al., 2018
Ionian basin	Italian coastlines	330	0.10 ± 0.06	items/m^2 ± SD	Galli et al., 2022
Western Mediterranean Sea	Pelagos Sanctuary and Tuscan Archipelago National Park	330	259,490 ± 586,477	items/km^2 ± SD	Galli et al., 2023
Western Mediterranean Sea	Ligurian Sea (Pelagos Sanctuary)	330	28,376 ± 28,917	items/km^2 ± SD	Caldwell et al., 2019
Western Mediterranean Sea		200	1.00 ± 1.84	items/m^3 ± SD	Suaria., 2016
Western Mediterranean Sea	Ligurian Sea (Pelagos Sanctuary) and Sardinian Sea	200	0.62 ± 2.00	items/m^3 ± SD	Fossi et al., 2012
Western Mediterranean Sea	Tyrrhenian Sea (Ventotene MPA)	333	0.20 ± 0.09	items/m^3 ± SD	De Lucia et al., 2018
Western Mediterranean Sea	Tyrrhenian Sea (Aeolian Island)	333	0.27 ± 0.08	items/m^3 ± SD	De Lucia et al., 2018
Western Mediterranean Sea	Ligurian Sea (Pelagos Sanctuary)	200	125,930 ± 132,485	items/km^2 ± SD	Pedrotti et al., 2016

Western Mediterranean Sea	Balearic island and Tyrrhenian Sea	333	101,408 ± 148,114	items/km^2 ± SD	Ruiz-Orejón et al., 2016
Western Mediterranean Sea	Balearic islands	335	0.86 ± 4.08	items/m^2 ± SD	Compa et al., 2020
Adriatic basin	Croatian coastline	308	0.13 ± 0.29	items/m^2 ± SD	Palatinus et al., 2019
Aegean-Levantine Sea		333	7.68 ± 2.38	items/m^3 ± SD	van der Hal et al., 2017
Adriatic basin	Italian, Slovenian, Croatian and Greek coastlines	330	0.32 ± 0.57	items/m^2 ± SD	Zeri et al., 2018
Whole Mediterranean Sea		200	243853	items/km^2	Cózar et al., 2015
Western Mediterranean Sea	Ligurian Sea	200	0.17 ± 0.32	items/m^3 ± SD	Panti et al., 2015

The estimated concentrations for each net tow ranged from 0.08 items/m^2 at the offshore station (1.5 nm) to a maximum concentration of 0.66 items/m^2 recorded near the shore station(0.5 nm) (table 2). The highest MPs abundance in the study site was recorded in shore area (0.5 nm) with a mean ± SD of 0.39 ± 0.39 items/m^2 (Fig. 7). Consistently with previous studies in other Mediterranean waters (Pedrotti et al., 2016; Pini et al., 2018; Marrone et al., 2021; Sbrana et al., 2023) the concentration of MPs in the investigated site decrease with distance from the coast. Nevertheless, the concentrations of floating MPs in the Ionian Sea appear to be unaffected by distance from the coast (Sbrana et al., 2023) or even show an inverse gradient (Marrone et al., 2021). It is possible that the coastal morphology features of MPA, particularly the presence of bays, may affected the MPs retention in MPA shore area while the ocean currents promoted their decrease offshore. In addition, although it is a MPA, this result may still suggest a potential relationship between the presence of plastic particles and the anthropic pressures insisting near this site including the presence of gas platforms in Crotone and the intense fishing activities from Le Castella and Crotone.

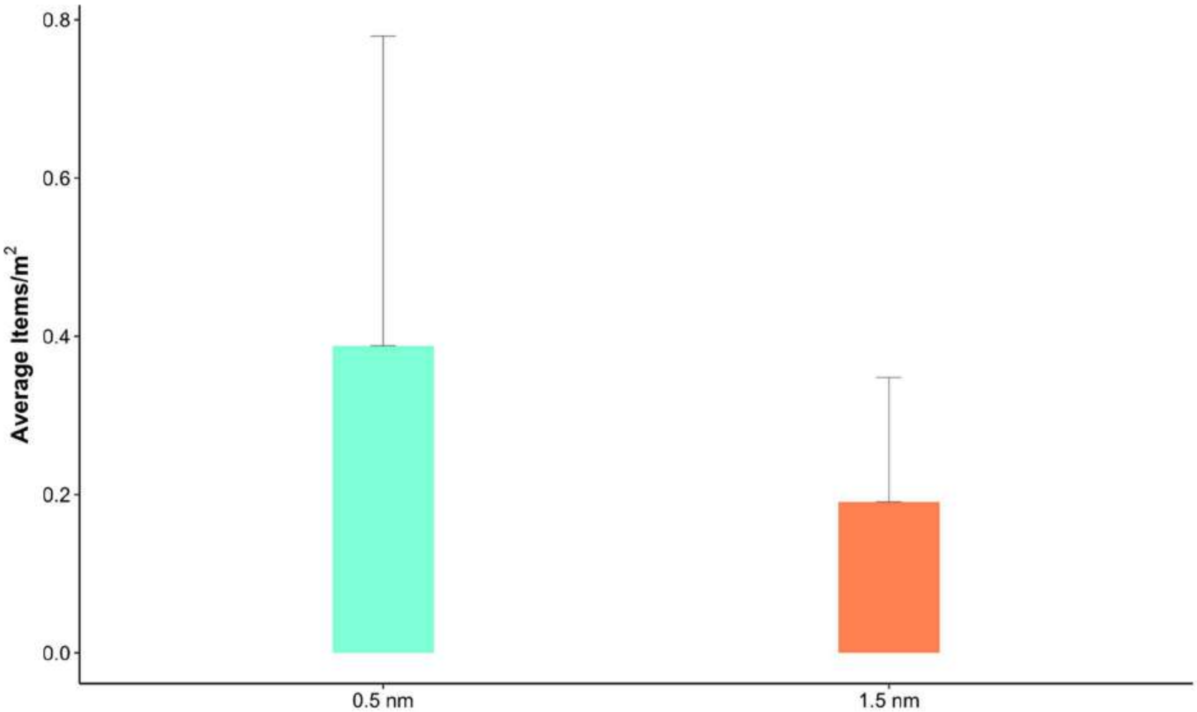


Figure 7. Representation of the total mean MPs collected in MPA Capo Rizzuto at 0.5 and 1.5 nm from the coast.

It is known that the MPs features including size, density and chemical/physical properties as well as the interaction with other particles and living organisms (biofouling) could influence MPs behaviour and regulate their distribution in the marine environment (Tsiaras et al., 2022). The most abundant plastics size class ranged between 1-2.5 mm in length (49.32% at 1.5 nm; 49.53% at 0.5 nm; Fig. 8), while the less abundant size class was 0.3-0.5 nm (4.45% at 1.5 nm; 3.17% at 0.5 nm). Similar result was observed by Baini et al. (2018) in the Tuscany's coastal waters, by Compa et al. (2020) in the Balearic Islands, Galli et al. (2023) in the Western Ionian Sea and Ruiz-Orejón et al.(2016) in the Western and Central Mediterranean Sea. Plastics abundance per each size class was not affected by distance from the coast, indeed, no statistical differences were observed (p-value > 0.05;). The MPs fraction below 1 mm in size represented the minor portion of the total isolated plastics in accordance with other previous studies carried out in the Mediterranean basin (Baini et al., 2018; Galli et al., 2022).

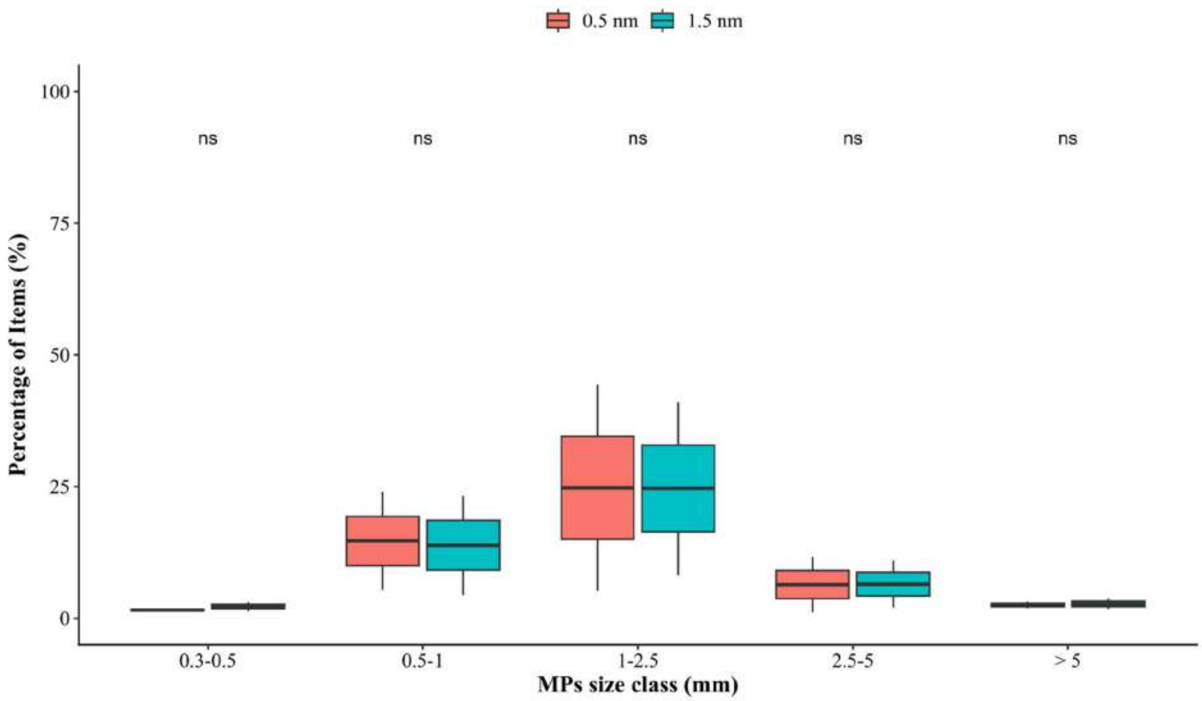


Figure 8. Results of the Wilcoxon rank sum test related to the size class of MPs in the MPA of Capo Rizzuto at different distance from the coast. Red = 0.5 nm; Green = 1.5 nm.

Fragment was the most common shape in both coast distances (n= 561 and 88.77% at 0.5 nm; n=242 and 82.88% at 1.5 nm) confirming worldwide literature (Baini et al., 2018; Galli et al., 2022; Kooi et al., 2016; Suaria et al., 2016). The presence of high number of MPs belonging to this shape, might suggest that it may be originate from the fragmentation of large plastic manufactured objects (Baini et al., 2018). Moreover, film (n=20 and 3.17% at 0.5 nm; n=13 and 4.45% at 1.5); filament (n=24 and 3.80% at 0.5 nm; n=20 and 6.85% at 1.5) and pellet (n=27 and 4.27% at 0.5 nm; n=11 and 3.77 % at 1.5) were also found and foam category was observed only at 1.5 nm distance from the coast (n= 6; 2.05%). Wilcoxon rank sum test showed no statistical variations of shape categories in relation to distance from the coast within the studied site (p-value > 0.05 Fig. 9).

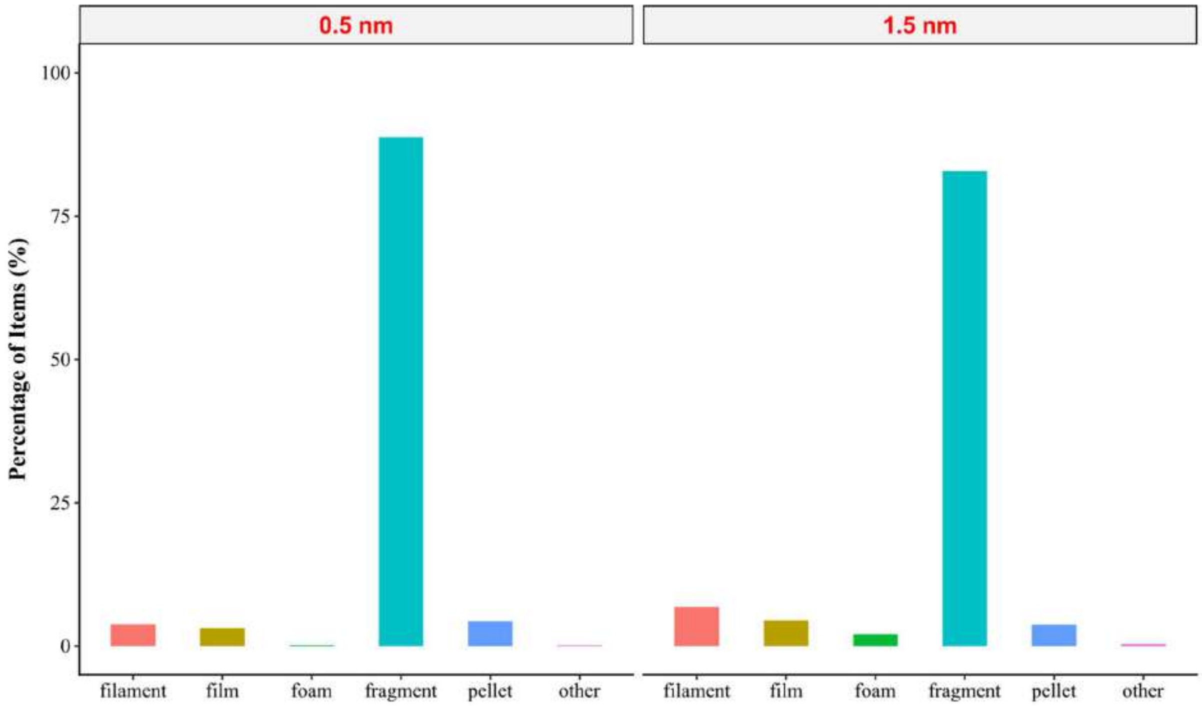


Figure 9. Percentage of MPs based on their shape in MPA of Capo Rizzuto.

Light-coloured plastics (white and transparent) were the most frequent observed in Capo Rizzuto MPA (Fig. 10) in consistent with the finding of Marrone et al. (2021) in the Calabrian area and elsewhere in the Mediterranean basin (Fossi et al., 2017; Van der Hal et al., 2017;Fagiano et al., 2022; Galli et al., 2022). It is possible to assume that they derive from SUP such as plastic bags, plastic bottles, packaging or lost fishing gear including lines and nets (Gao et al., 2023) as well as from aged plastics (Marrone et al., 2021).

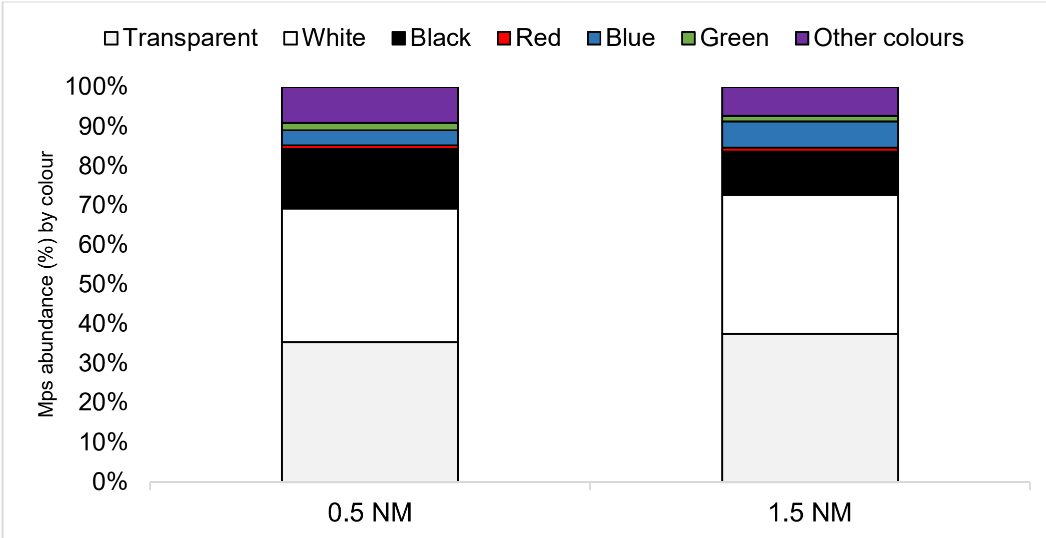


Figure 10. MPs classification by colour expressed as percentage of abundance. Within the "other colours" category: yellow, orange, light blue, brown, pink and grey.

This result seems to be confirmed by the polymer analysis. In fact, a total of 189 particles (~20% of the total sample) were analysed by FTIR spectroscopy. This investigation allowed to identifiy 12 polymer types from the Capo Rizzuto MPA water samples. The most frequent polymer observed in the investigated site was PE (70.08% at 0.5 nm; 70.97% at 1.5 nm) followed by PP (20.47% at 0.5 nm; 20.97% at 1.5 nm). Distance from the coast reveled the presence of polymers that have not been recovered in the 1.5 nm such as EPA, PA, PBT, PUR, PVC and SBR. On the contrary, EPDM and PS were only observed in samples from 1.5 nm from the coast. Despite this, was not observed any statistical difference between polymers across the two distance from the coast (p-value > 0.05; Fig. 11). This results could by related to the presence of several anthropic stressors both from sea and land including fishing activity and gas platforms as well as agriculture.

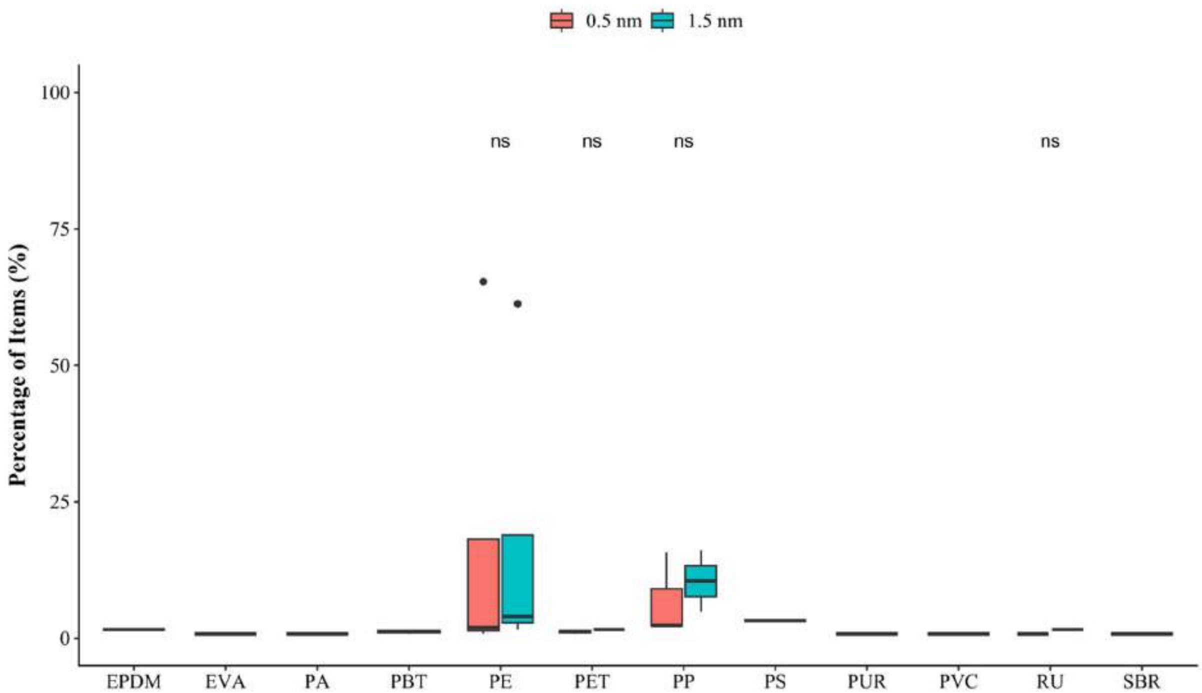


Figure 11. Results of the Wilcoxon rank sum test related to the abundance of polymers in the MPA of Capo Rizzuto at different distance from the coast. Red = 0.5 nm; Green = 1.5 nm.

Polymers were characterised by different shape, specifically, PE was mainly found in the form of fragment (65.35% and 61.29% at 0.5 nm and 1.5 nm, respectively) and film (2.36% at 0.5 nm; 4.84% at 1.5 nm) and minor percentage of filament and pellet, while the PP was present at 0.5 nm from the coast as fragment (15.74%), filament (2.36%), and pellet (2.36%) and at 1.5 nm from the coast as fragment (16.13%), filament (4.84%) (Fig.12). These polymers have positive buoyancy and are generally constituent material of both SUP and fishing gear, especially in the form of fragment and film, which is consistent with different anthropic activities conducted in this area.

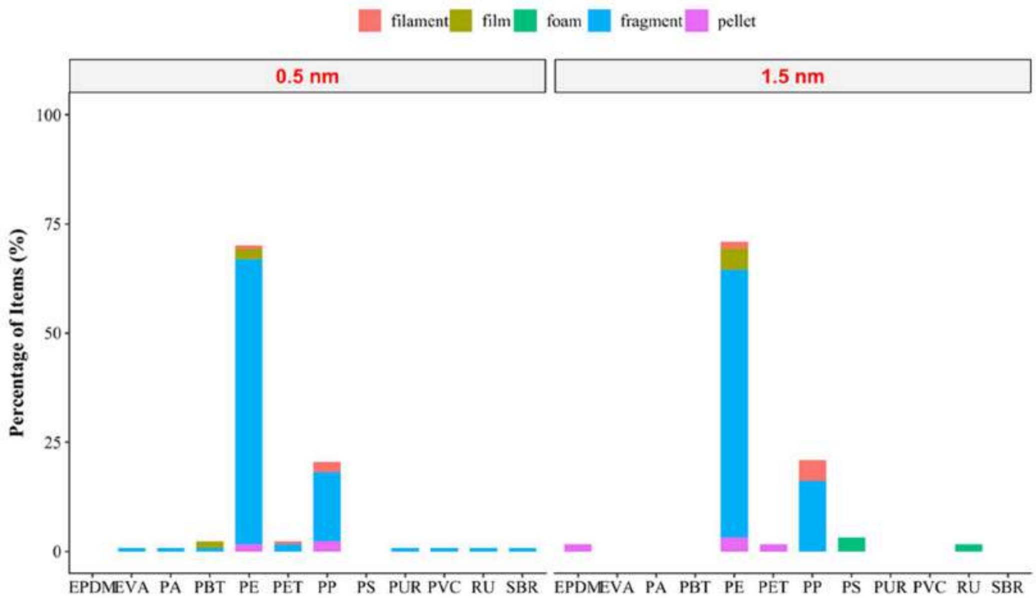


Figure 12. Percentage of polymer types in relation to the distance from the coast and shape of floating plastics from Capo Rizzuto MPA. PE= polyethylene; PP= polypropylene; PS=polystyrene; PET= polyethylene terephthalate; EVA= ethylene-vinyl acetate; RU= undefined rubber; PUR= polyurethane; PA= polyamide (nylon); PVC= polyvinyl chloride; EPDM= ethylene propylene diene monomer; SBR= styrene-butadiene rubber; PBT= Polybutylene Terephthalate.

The present study reports first data on the presence of MPs in the gastrointestinal content of bentopelagic (*B. boops* and *M. merluccius*) and demersal (*M. barbatus*) in the study site. A total of 18 specimens belonging to the 3 bioindicator species were examined. Size and weight of specimens and the corresponding mean values for each species are reported in Table 4.

Table 4. Biometric parameters (total length TL, cm, total weight TW, g), GITs weight (GITW), Fulton's condition factor (K) and Gastro Somatic Index (GaSI) for each species from Capo Rizzuto MPA. Plastics occurrence (% O) and abundance are also reported.

Data fish	*Boops boops*	*Mullus barbatus*	*Merluccius merluccius*	*Total*
N samples	6	6	6	**18**
TL (cm) mean ± SD	14.55 ± 0.85	12.42 ± 0.59	27.13 ± 5.07	
TW (g) mean ± SD	31.73 ± 6.43	19.51 ± 2.78	172.01 ± 100.20	
GITW (g)	1.04 ± 0.37	0.86 ± 0.23	2.83 ± 2.86	
K (g/cm^3)	1.02 ± 0.07	1.02 ± 0.12	0.77 ± 0.11	
GaSI	3.35 ± 0.81	4.57 ± 0.95	1.84 ± 0.71	
Data plastic				
N samples with plastics	4	3	0	**7**
N of plastic items	5	4	0	**9**

Plastics range	0-2	0-2	-	
Plastics occurrence (%O)	66.67	50	0	**38.89**
N. plastic items/N. all examined individuals	0.83	0.67	0	**0.5**
N. plastic items/N. individuals which ingested plastics	1.25	1.33	-	**1.29**

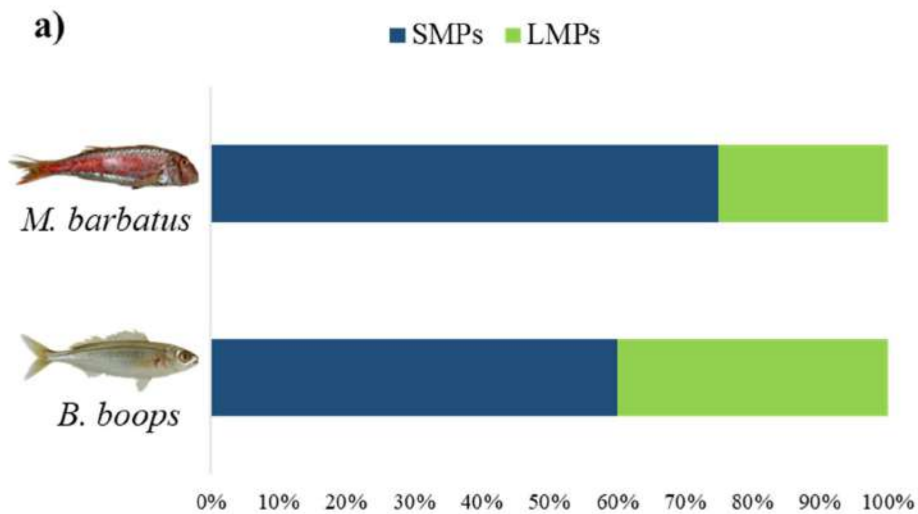
Overall, 9 plastics particles were isolated from 7 GITs (%O= 38.89): 5 items from *B. boops* (%O= 66.67) and 4 items from *M. barbatus* (%O= 50), while no plastics were found in the GITs of *M. merluccius*. Similar occurrence in *B. boops* was been reported by Capó et al. (2022) for the same species from the Mediterranean MPAs of Cabrera (%O= 64), while lower values have been reported by Giani et al. (2023) (%O= 37) from different geographical sub-areas of the Mediterranean Sea and Tsangaris et al. (2020) (%O= 46.8) from several coastal areas of the Western Mediterranean Sea. The percentage of MPs occurrence of *M. barbatus* in the investigated site was higher than value observed in Turkish (%O= 42), Greek (%O= 32) and Western Mediterranean Sea (%O= 19) as well as in southern Tyrrhenian Sea (14.28%) (Bellas et al., 2016; Capillo et al., 2020; Digka et al., 2018a; Giani et al., 2023). At the same time values was lower than data previously observed on the same species from the Adriatic Sea (%O= 64%) and Marine Protected Area of Porto Cesareo along the Ionian Sea coast (%O= 75)(Avio et al., 2015a; Felline et al., 2022). In this study, no plastic items were found in the GITs of *M. merluccius*, although other authors had already observed plastics ingestion in this commercial species. Indeed, in the Mediterranean Sea, Bellas et al. (2016); Giani et al. (2023) and Mancuso et al. (2019) reported %O values of 16.7, 26.8, and 46.3 %, respectively.

The observed within-species variability between different areas of the Mediterranean basin, could be attributed to a different diet related to prey availability in the survey area (Capó et al., 2022).

The influence of ingested MPs on the well-being of commercial fish was evaluated considering the Fulton's condition index (K). Bogue and Red mullet showed a K of 1.02 (±0.07) and 1.02 (±0.12), respectively, whereas in European Hake the K value was of 0.77 (±0.11) (Table 3). These results fall within the range of data reported by previous studies investigating MPs ingestion on these bioindicator species from Mediterranean Sea (Capó et al., 2022; Garcia-Garin et al., 2019; Trani et al., 2023). Negative moderate correlation was observed between K index and the MPs abundance in *B. boops* (Tau = -0.36) whereas a positive weak correlation (Tau= 0.08) was found in *M. barbatus*. According to some studies on MPs ingestion in fish (Rummel et al., 2016; Güven et al., 2017; Digka et al., 2018b; Giani et al., 2019; Lefebvre et al., 2019; Tsangaris et al., 2021; Bottari et al., 2022) MPs abundance was not related to K.

The gastro-somatic index (GasI) was used to determine the feeding intensity of fish. The highest GaSI index values were found for both Red mullet (4.57 ± 1.85) and Bogue (3.35 ± 0.81), whereas the lowest values were observed in European Hake (1.84 ± 0.71) (Tab.3). A positive weak correlation (Tau= 0.08) and a moderate correlation (Tau= 0.39) has been found between the MPs abundance and the GaSI index in *B. boops* and in *M. barbatus*, respectively.

This studies provided information regarding the size, shape and colour of the ingested particles. All the items belonged to the size class of MPs (67% SMPs and 33% LMPs) ranged from 0.02 to 1.60 mm (Fig.13a). In particular, both bogue and red mullet ingested mainly SMPs (Fig. 13a) with a mean length of 0.38 (± 0.27) and of 0.43 (±0.47), respectively. This results could be related to the feeding strategy and habits of the investigated species. *B. boops* is a benthopelagic species that feeds both near the bottom and in the water column while *M. barbatus* feeds on the seabed usually by swallowing sediment together with its prey through the gills (Giani et al., 2019; Rodrigues et al., 2023). It is therefore possible for both species accidentally ingest MPs during feeding (Bottari et al., 2022; Giani et al., 2019, 2023; Rodrigues et al., 2023) since they are both non-selective in predation (Linde et al., 2004). As previously observed by Bottari et al. (2022) *B. boops* can ingest plastics by feeding on contaminated prey (secondary ingestion), by mistaking habitual prey for plastics or by feeding on the organisms attached to plastics thus indirectly ingesting MPs. Fragment and fiber were the only shape categories observed in both bioindicator species (Fig. 13b). Fiber was the most common shape in GITs of bogues (60%), mainly blue (66%) and grey (20%), while fragment (40%) were orange and blue (all having 50%). Plastics from red mullet were fragment and fiber (with a proportion of 50%), transparent and orange, respectively (fig.13b). The presence of fiber in GITs has already been observed in other studies for the same species (Rodríguez-Romeu et al., 2020; Bottari et al., 2022; Giani et al., 2019, 2023; Rodrigues et al., 2023) as well as for others (Lusher et al., 2013; Rochman, 2015; Bellas et al., 2016) and could be related to the non-selective predation of the surveyed species or to the inability to discriminate (Rodrigues et al., 2023) and passively intake of fiber. Some studies do not consider the presence of fibres in the analysis leading to an underestimation of the ingested MPs. Conversely, failure to apply control measures to avoid contamination of the sample during analysis can lead to an overestimation of real ingested plastics concentrations (Pedà et al., 2020).



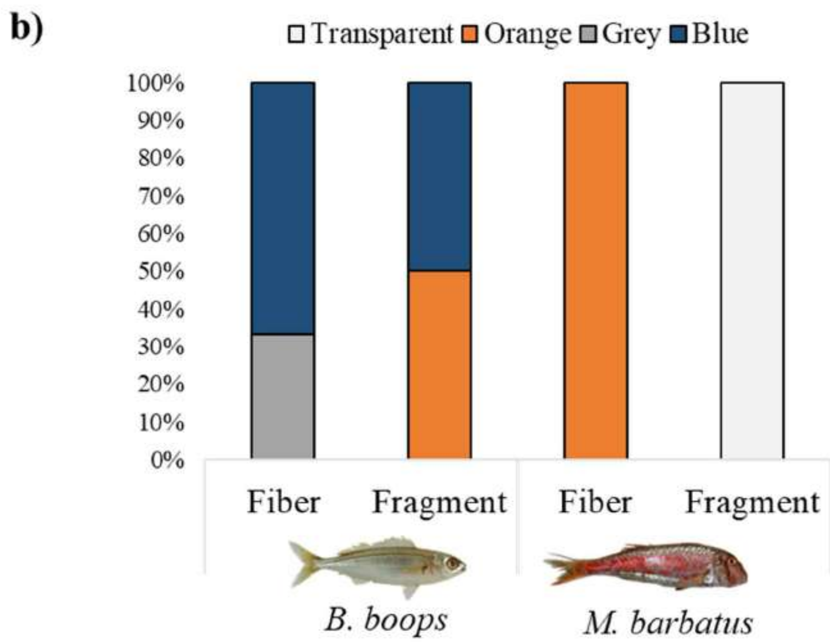


Figure 13. Percentage in relation to size (a), shape (b) colour (c) of plastics ingested by bioindicator species from Capo Rizzuto MPA.

All the isolated items were identified by FT-IR spectroscopic analysis. A total of 7 out of 9 extracted samples was successfully identified. The identification did not show a wide variety of polymers (Fig 14). PET and PE were the only polymers found in both species. In bogue, fragments were identifing as RU (20%), PE (20%) and PET (20%) while the two fibers were not identified by FTIR (UP; 40%). PE and PET were ingested to a equal extent in the red mullet (50%). Fig. 15 reports some images of plastic samples found in the bioindicator species GITs together with the corresponding FT-IR spectra. Polyethylene is the most commonly used plastic globally, especially in packaging (Suaria et al., 2016), and it is also the most abundant polymer type observed in the Mediterranean Sea (Andrady, 2011; Cózar et al., 2014; Suaria et al., 2016; Geyer et al., 2017; Digka et al., 2018b). Furthermore, the PE ingestion by the fish species is consistent with the data on surface waters observed in the Capo Rizzuto MPA during this study. The ingestion of low-density polymers by bottom-feeding organisms showed that these particles can move along the water column after density modifications due to abiotic (e.g., currents and water circulation) and biotic (biofouling) factors, becaming available in the seabed (Pedà et al., 2020; Andrady, 2017). In addition, the use of *B. boops* and *M barbatus* as small-scale bioindicators of MPs in coastal waters and seafloor allowed us to gather further information on MP pollution in the study area.

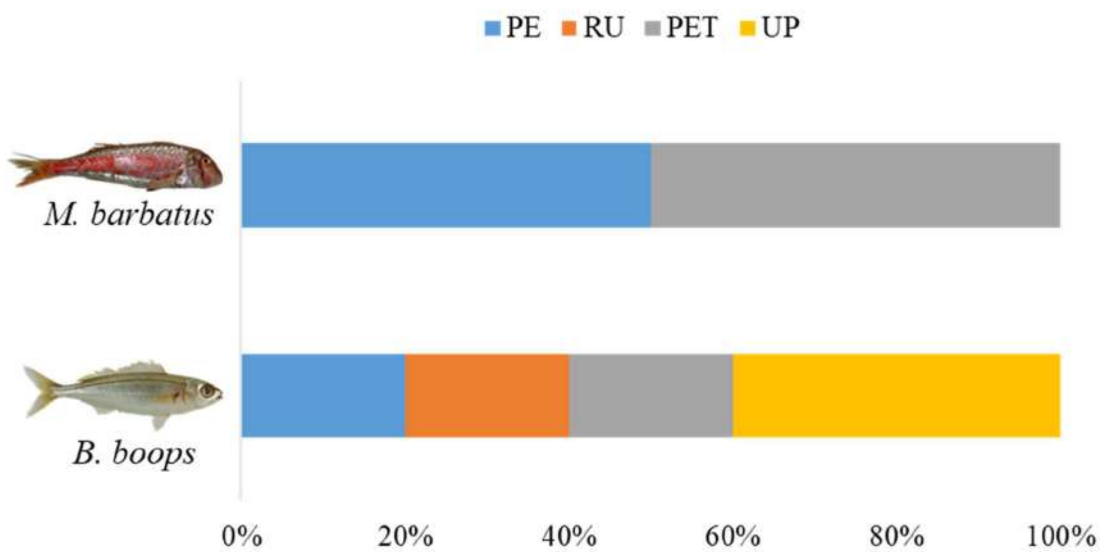


Figure 14. Polymers abundance (%) in bioindicator species of Capo Rizzuto MPA.

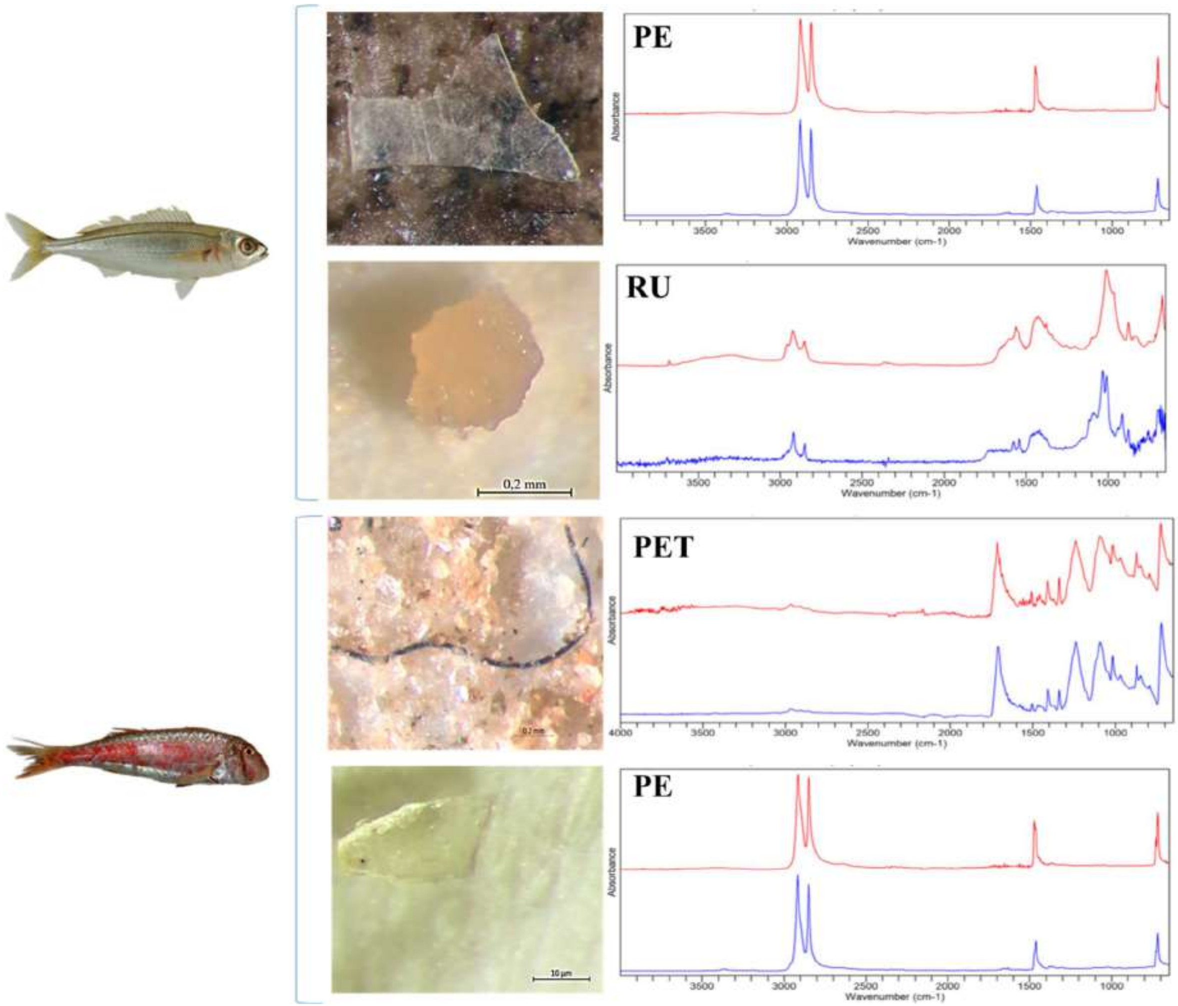


Figure 15. Images of some plastics ingested by *B. boops* and *M. barbatus.* PE fragment and RU fragment in *B. boops;* PET filament and PE fragment in *M. barbatus.*

A total of 12 items have been isolated from surface water sample, used for the DNA extraction. Figure 16 shows the obtained plastic pool collected, with related classification of items.

n MPs	Shape	Colour	Size class
2	fragment	black	small
2	fragment	blue	small
3	fragment	white	small
1	fragment	transparent	small
1	filament	green	small
1	filament	blue	large
2	fragment	yellow	small

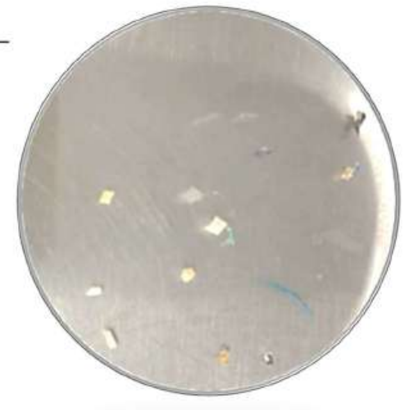


Figure 16. MPs pool used for the DNA extraction collected in the study site of Capo Rizzuto MPA. MPs number classified by shape, colour and class size (large MPs = from 5 mm to 1 mm; small MPs = from 1mm to 0.1 mm).

The taxonomic composition of bacterial community in terms of phyla abundance is reported in Figure 17. The bacterial community was mainly represented by Proteobacteria members, with an mean percentage of 46.8% of the total bacterial community, of which the 35% were constituted by Alphaproteobacteria and the 65% by Gammaproteobacteria. Firmicutes accounted for the 17.8% of the total bacterial community as the second most abundant phylum, followed by Halanaerobiaeota with an mean relative abundance of 14.4%. The phyla Verrucomicrobiota and Bacteroidota were present with mean abundances of 5.8 and 5.3%, respectively. Globally, our results are thus in line with the bacterial colonization succession previously reported, e.g. by Yang et al. (2021) investigating the bacterial biofilm colonizing plastic particles (size <5 mm) collected in the riverine waters of the Pearl River Delta, China, and by De Tender et al. (2015), which profiled the taxonomic composition of bacterial communities associated with seafloor marine plastic litter sampled across the Belgian part of the North Sea.

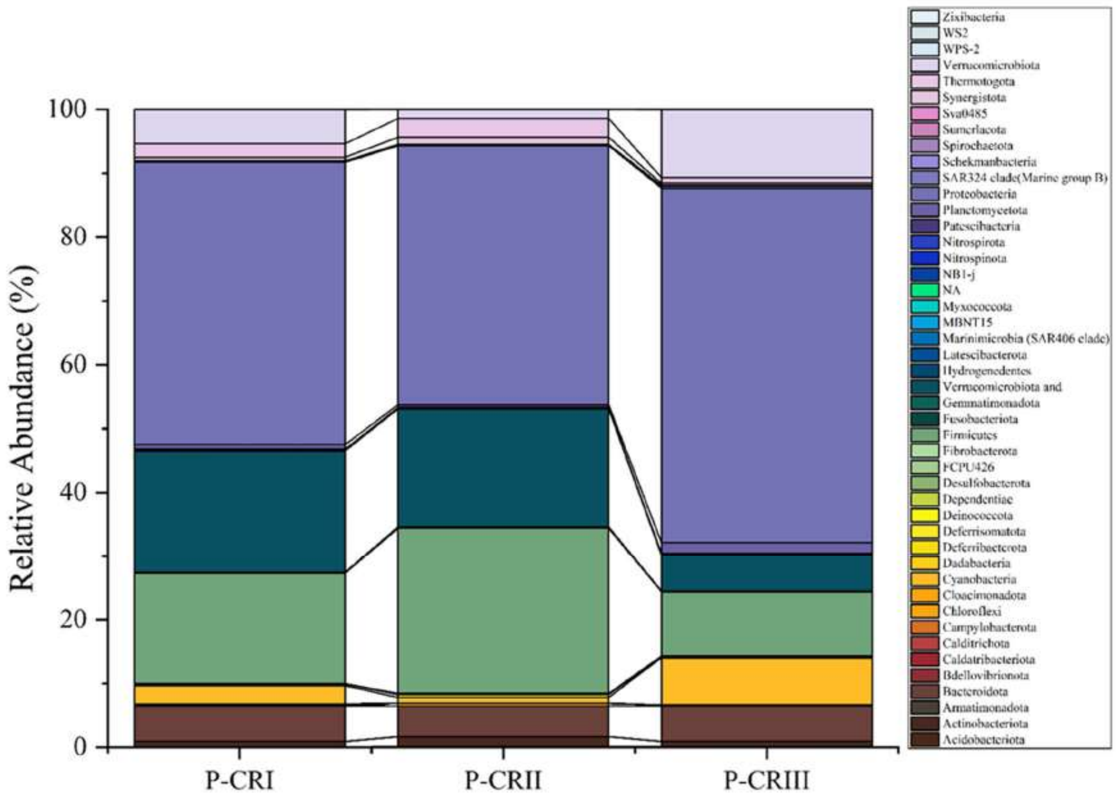
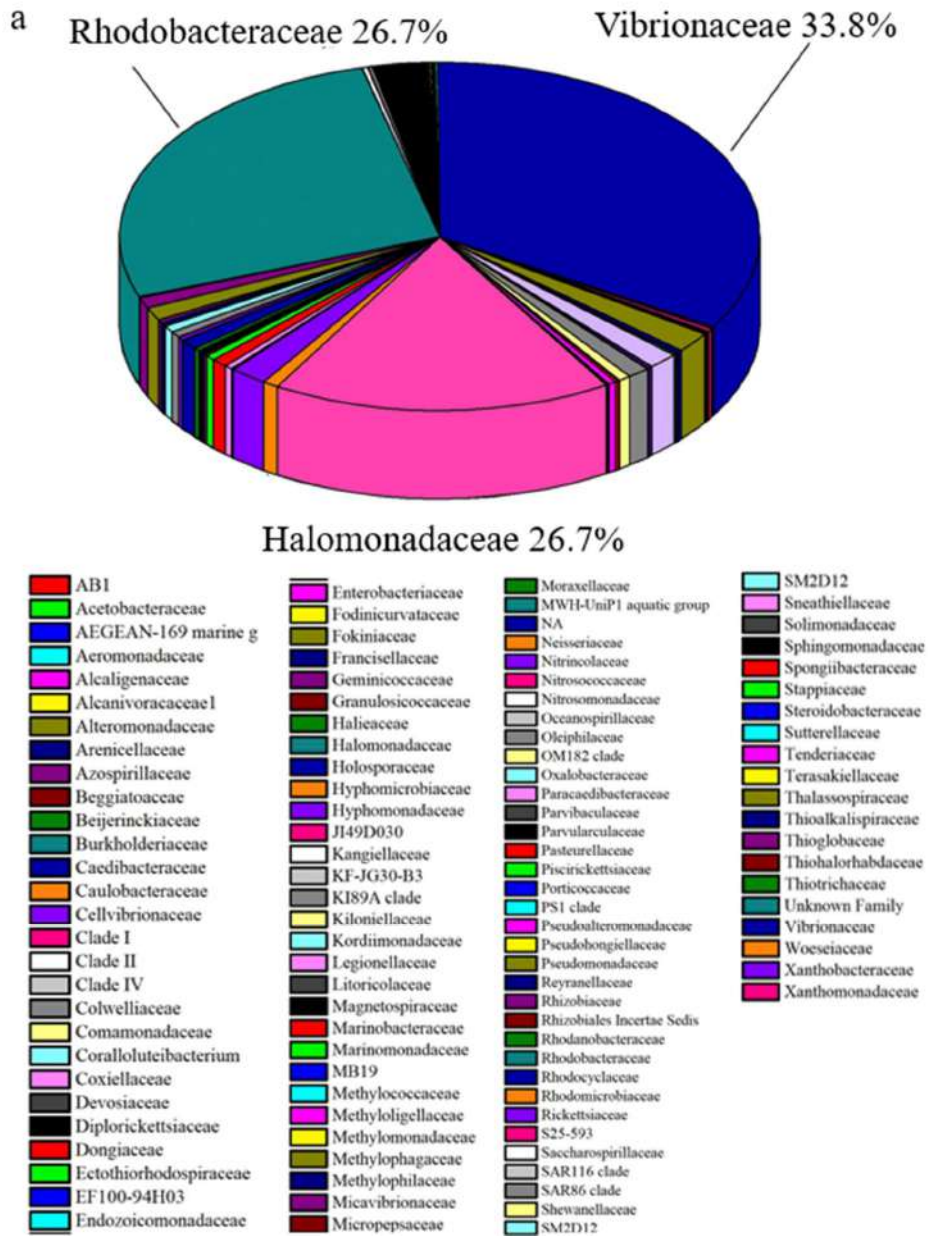


Figure 17. Bacterial phyla abundance in MPs pools from Capo Rizzuto.

Proteobacteria members were mainly constituted at family levels by the groups of Vibrionaceae (33.8% of total Proteobacteria), Halomonadaceae (17.2% of total Proteobacteria) and Rhodobacteraceae (26.7% of total Proteobacteria). Differently, Firmicutes phylum was predominated by members of Bacillaceae (34% of total Firmicutes) and Carnobacteriaceae (13.8% of total Firmicutes), followed by Enterococcaceae (9% of total Firmicutes). The phylum Halanaerobiota was represented by two families, namely Halanaerobiaceae (85.4% of total Halanaerobiota) and Halobacteroidaceae (14.6% of total Halanaerobiota). Finally, the phylum Verrucomicrobiota showed high relative abundance of Simkaniaceae (30% of total Verrucomicrobiota), Puniceicoccaceae (30.9% of total Verrucomicrobiota), and DEV007 (21.2% of total Verrucomicrobiota) (Figure 18). The composition of the MP-associated bacterial communities could provide interesting indication about the occurrence and stage of biofilm formation (Yang et al., 2021), as generally Proteobacteria and Bacteroidetes affiliates are initial colonizers (Dang et al., 2008; Lee and Cho, 2008). Proteobacteria are globally the most abundant phylum in marine environments and is reported among the most observed on marine plastic debris (De Tender et al., 2015; Oberbeckmann et al., 2014;

Ogonowski et al., 2018; Pollet et al., 2018; Syranidou et al., 2017) with several alphaproteobacterial and gammaproteobacterial members. Among these, Rhodobacteraceae were commonly reported for the colonization of plastic debris and are considered pioneer colonizers of solid substrates in marine environments (Dang et al., 2008). The group of Rhodobacteraceae was predominated by *Thalassobius* affiliates, recently detected in the early stages of biofilm formation (Zhang et al., 2022) and *Jannaschia* affiliates, including Gram-negative, halophilic, strictly aerobic, chemo-organotrophic bacteria already observed in marine MPs microbial communities (Marsay et al., 2023). Together with them, other halophilic phylotypes were also detected within Proteobacteria specifically in members of the genus *Halomonas*, family Halomonadaceae. Halophilic bacteria are considered optimal degraders of plastics due to their special extremophilic adaptations, often including the production of extremophilic enzymes (Atanasova et al., 2021). Halophilic phylotypes have been also detected within the group of Halanaerobiota, consisting of fermentative halotolerant/halophilic bacteria (Roush et al., 2014). The presence of Firmicutes as second most abundant phylum in our samples, generally typical of soil and sewage origin, suggested an influence from land in the colonization of MPs in marine environments. However, while the terrigenous contribution can be easily justified in the case of a riverine environment, it is more complex in the case of a marine environment, as in this study, suggesting that initial bacterial colonisation on individual MPs fragments may then develop once in marine waters.



a
Rhodobacteraceae 26.7%
Vibrionaceae 33.8%
Halomonadaceae 26.7%
AB1
Acetobacteraceae
AEGEAN-169 marine g
Aeromonadaceae
Alcaligenaceae
Alcanivoracaceae1
Alteromonadaceae
Arenicellaceae
Azospirillaceae
Beggiatoaceae
Beijerinckiaceae
Burkholderiaceae
Caedibacteraceae
Caulobacteraceae
Cellvibrionaceae
Clade I
Clade II
Clade IV
Colwelliaceae
Comamonadaceae
Coralloluteibacterium
Coxiellaceae
Devosiaceae
Diplorickettsiaceae
Dongiaceae
Ectothiorhodospiraceae
EF100-94H03
Endozoicomonadaceae
Enterobacteriaceae
Fodinicurvataceae
Fokiniaceae
Francisellaceae
Geminicoccaceae
Granulosicoccaceae
Halieaceae
Halomonadaceae
Holosporaceae
Hyphomicrobiaceae
Hyphomonadaceae
JI49D030
Kangiellaceae
KF-JG30-B3
KI89A clade
Kiloniellaceae
Kordiimonadaceae
Legionellaceae
Litoricolaceae
Magnetospiraceae
Marinobacteraceae
Marinomonadaceae
MB19
Methylococcaceae
Methyloligellaceae
Methylomonadaceae
Methylophagaceae
Methylophilaceae
Micavibrionaceae
Micropepsaceae
Moraxellaceae
MWH-UniP1 aquatic group
NA
Neisseriaceae
Nitrincolaceae
Nitrosococcaceae
Nitrosomonadaceae
Oceanospirillaceae
Oleiphilaceae
OM182 clade
Oxalobacteraceae
Paracaedibacteraceae
Parvibaculaceae
Parvularculaceae
Pasteurellaceae
Piscirickettsiaceae
Porticoccaceae
PS1 clade
Pseudoalteromonadaceae
Pseudohongiellaceae
Pseudomonadaceae
Reyranellaceae
Rhizobiaceae
Rhizobiales Incertae Sedis
Rhodanobacteraceae
Rhodobacteraceae
Rhodocyclaceae
Rhodomicrobiaceae
Rickettsiaceae
S25-593
Saccharospirillaceae
SAR116 clade
SAR86 clade
Shewanellaceae
SM2D12
SM2D12
Sneathiellaceae
Solimonadaceae
Sphingomonadaceae
Spongiibacteraceae
Stappiaceae
Steroidobacteraceae
Sutterellaceae
Tenderiaceae
Terasakiellaceae
Thalassospiraceae
Thioalkalispiraceae
Thioglobaceae
Thiohalorhabdaceae
Thiotrichaceae
Unknown Family
Vibrionaceae
Woeseiaceae
Xanthobacteraceae
Xanthomonadaceae

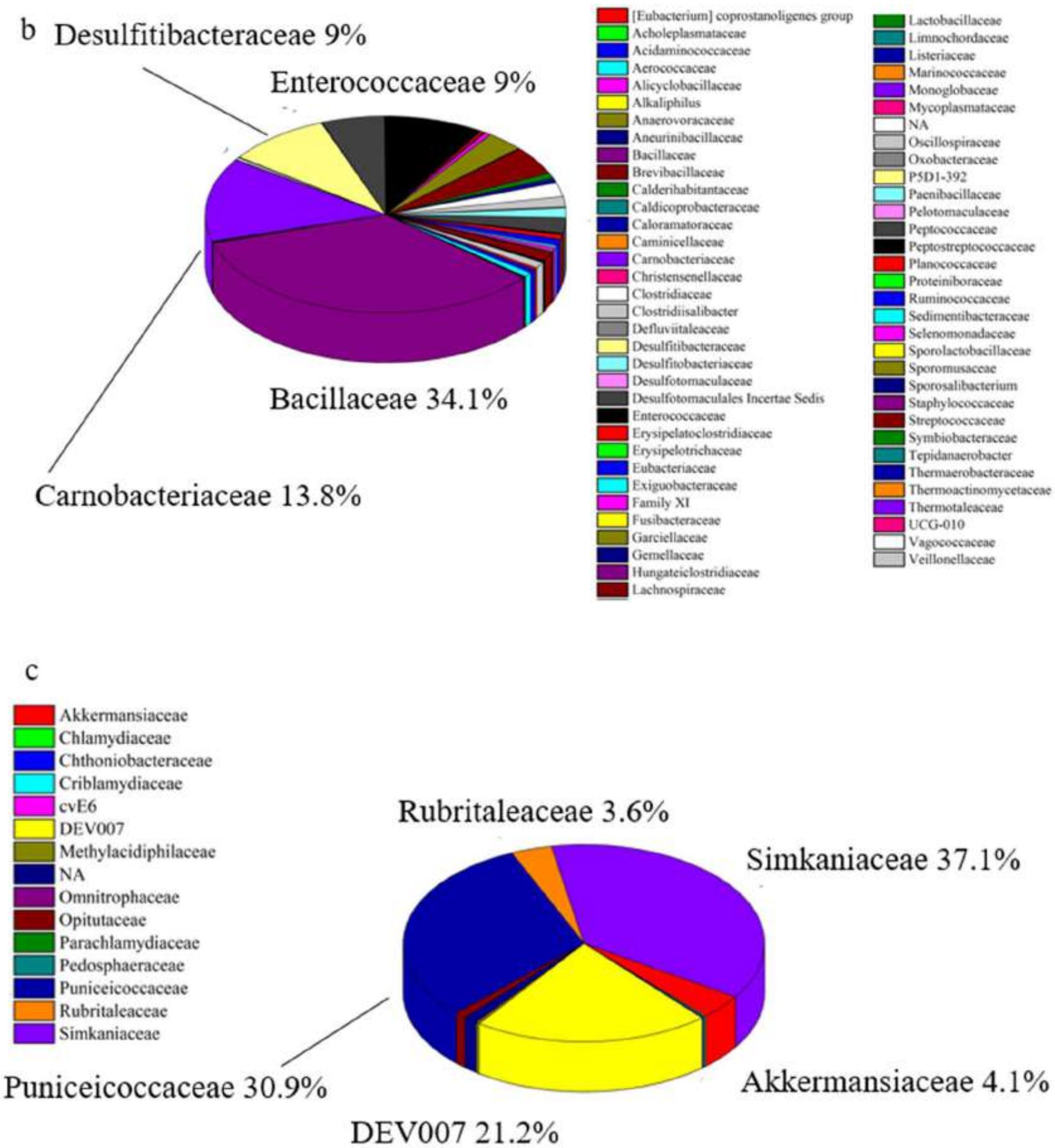


Figure 18. Mean bacterial abundance at family level of microbial community associated with MPs pool from Capo Rizzuto, within the phylum Proteobacteria (a), Firmicutes (b) and Verrucomicrobiota (c). Please note that mean values of three experimental replicates are reported.

At genus level, considering genera with mean relative abundance higher than 1% of the total bacterial community, the more represented groups were *Vibrio* (Proteobacteria), *Halanaerobium* (Halanaerobiota) and *Halomonas* (Proteobacteria) with 13.7%. 12% and 7.5% of abundance on the total bacterial community. The family of Vibrionaceae is composed of aquatic bacteria that often are naturally endemic to warm marine and estuarine waters, and generally provide a habitat for biofilm-forming bacteria. The potential of MPs as vector/enhancer of *Vibrio* spp. establishment has been widely discussed, since members of the genus were detected irregularly on MPs and plastic debris (Kirstein et al., 2016; Laverty et al., 2020; Silva et al., 2019). A recent work observed the MPs colonization by *Vibrio* spp. strains, and reported that their abundance on field-collected particles is very variable, strongly correlated to the proximity to urban centres. *Vibrio* spp. affiliates are considered as early components of plastic colonization, which is influenced by nutrients availability (Kesy et al., 2021).

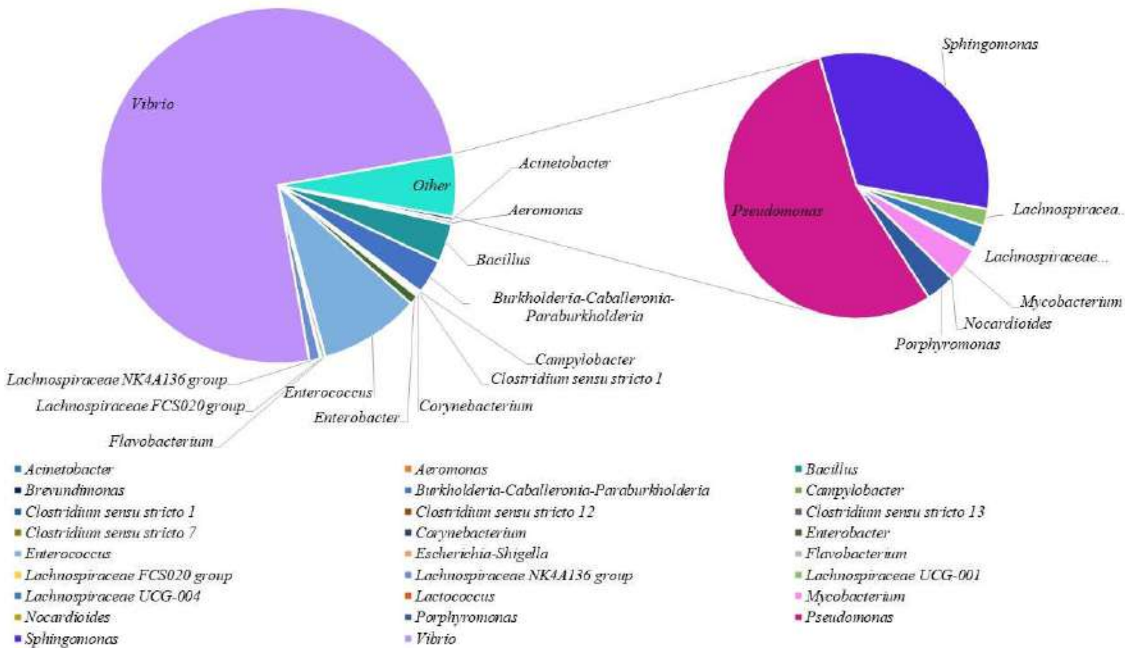


Figure 19. Representation of bacterial pathogens retrieved among ASVs detected in MPs from Capo Rizzuto.

The search for genera including strains hydrocarbon-degrading bacteria led to the detection of a fraction of 10.4% on the total of bacterial community. This fraction was mainly represented by *Halomonas* spp. affiliates, followed by *Geobacillus* spp. members, and at lower extent by the genera *Pseudomonas*, *Sphingomonas*, *Thalassospira* and *Oleiphilus.* This result suggest that a good percentage of bacterial community is represented by strains with affinity towards apolar matrices, and thus could be involved in the bacterial colonization processes.

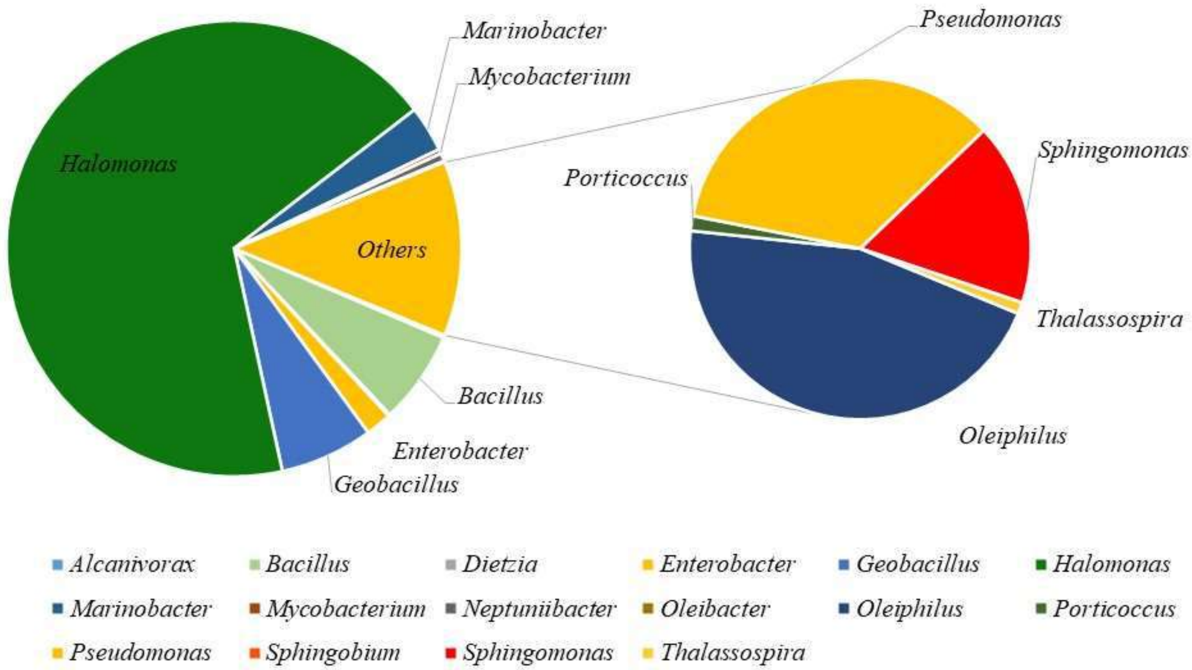


Figure 20. Representation of ASVs related to hydrocarbon-degrading bacteria retrieved in MPs from Capo Rizzuto.

The archaeal microbial fraction was represented by the three phyla Euryarchaeota, including only the taxonomic family Methanobacteriaceae (genus *Methanobrevibacter)*, and Halobacterota, including the families Methanomicrobiaceae (genus *Methanoculleus*).

3.2.2 Urbanized settlement of Vibo Valentia

Overall, a total of 1678 floationg plastics was isolated from the 4 water surface samples collected in the Vibo Valentia site with a mean ± SD concentration of 0.34 ± 0.24 items/m^2 corresponding to 2.13 ± 1.53 items/m^3 (Table 4). The results obtained are in line with the heterogeneity of data in Mediterranean basin (Table 3) but higher concentrations of floating MPs were detected compared to Marrone et al. (2021) in the same survey site (range 0.01-0.10 items/m^2).

Table 5. Plastics number, abundance reported as items/m3 and items/m2 in water surface samples collected in the two transects at 0.5 nm; 1.5 nm distance from the coast of Vibo Valentia mean ± standard deviation (SD) per each transect and station are also report.

Station ID	Distance from the coast (nm)	Latitude	Longitude	N items	items/m^3	items/m^2
TR1_0.5	0.5	38°43'835"	16°08'377"	203	1.28	0.20
TR1_1.5	1.5	38°44'372"	16°07'170"	339	1.61	0.26
TR1 (mean ± SD)				271 ± 96.17	1.44 ± 0.23	0.23 ± 0.04
TR2_0.5	0.5	38°45'905"	15°59'681"	887	4.42	0.71
TR2_1.5	1.5	38°44'872"	15°59'197"	250	1.22	0.19
TR2 (mean ± SD)				568.5 ± 450.4	2.81 ± 2.26	0.45 ± 0.36
Total (mean ± SD)				1678 419.5± 316.79	1.13 ± 1.56	0.34 ± 0.24

The estimated concentrations for each sample ranged from 0.19 items/m^2 at the offshore (1.5 nm) station to a maximum concentration of 0.71 items/m^2 recorded at near the shore (0.5 nm) station (table 4). The highest MPs abundance in the study site was recorded near the shore (0.5 nm) with a mean ± SD of 0.45 (± 0.04) items/m^2 (Fig. 21) decreasing with the distance from the coast. This is also confirmed by other Mediterranean studies (Pedrotti et al., 2016; Ruiz-Orejón et al., 2016; Zeri et al., 2018; Sathish et al., 2020; Tsiaras et al., 2022), in particular, Pedrotti et al. (2016) reported a trend in the distribution of plastics with the highest concentrations in the first kilometre near shore. The higher concentrations of MPs in shore area could suggest input from land-based sources as well as the environmental factors (wave current, tides, hydrodynamic conditions) inducing MPs retention in the coastal area (Zeri et al., 2018; Tsiaras et al., 2022). Particularly, in Vibo Valentia site the high touristic harbours and nautical activities especially in the summer months represent possible terrestrial inputs. Furthermore, the presence north of Vibo Valentia of the Mesima river (50 Km long) which coming from Mazzuccolo mount to the Tyrrhenian Sea, as well as several torrents and rivers outflows in the area could be considered an important input routes of synthetic materials.

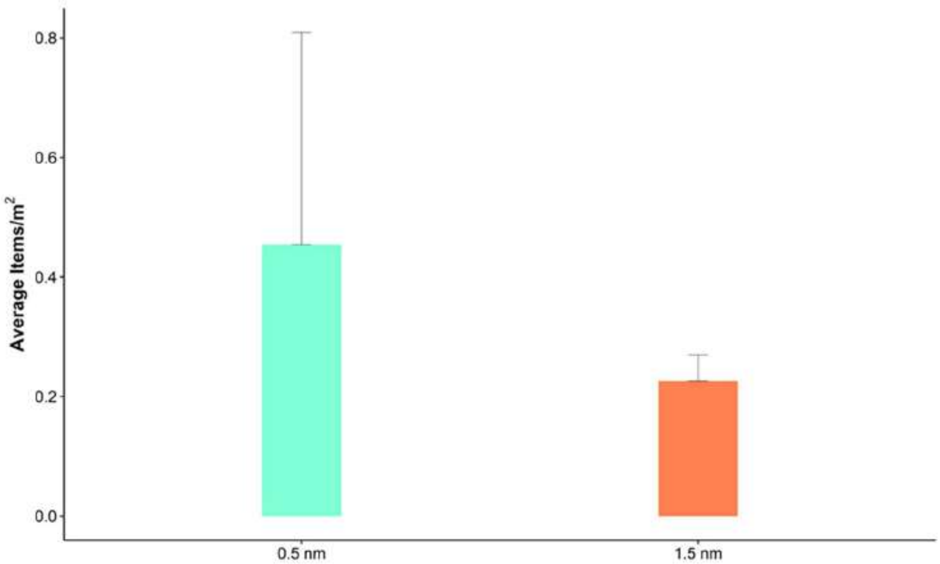


Figure 21. Rapresentation of the total mean MPs collected in Vibo Valentia at 0.5 and 1.5 nm from the coast.

Figure 22 shows size distribution of plastics in surface water samples of Vibo Valentia. The most abundance size class is 1-2.5 mm in the study site (52% at 1.5 nm; 49% at 0.5 nm) while items smaller than 1 mm in length accounted for less of the 20% (8% at 1.5 nm; 11% at 0.5 nm). Statistical analysis showed no difference in size class abundance in relation to distance from the coast (P-value > 0.05; Fig.22). This result is consistent with most of the published Mediterranean studies (Suaria et al., 2016; Ruiz-Orejón et al., 2016; Baini et al., 2018; Compa et al., 2020; Galli et al., 2023). In line with previous Mediterranean study (Baini et al., 2018; Giani et al., 2022) the small MPs represented the minor portion of the total isolated plastics (Fig. 22). The presence of smaller particles in coastal waters can be linked to several factors, including the physical properties of these materials, such as their density, which regulates their distribution and removal from the surface, but it is also linked to the fragmentation process, which is particularly evident on coastlines where UV degradation and wave abrasion are greater, making plastic objects more fragile and therefore more prone to fragmentation into micro particles (Barnes et al., 2009; Lusher, 2015; Pedrotti et al., 2016).

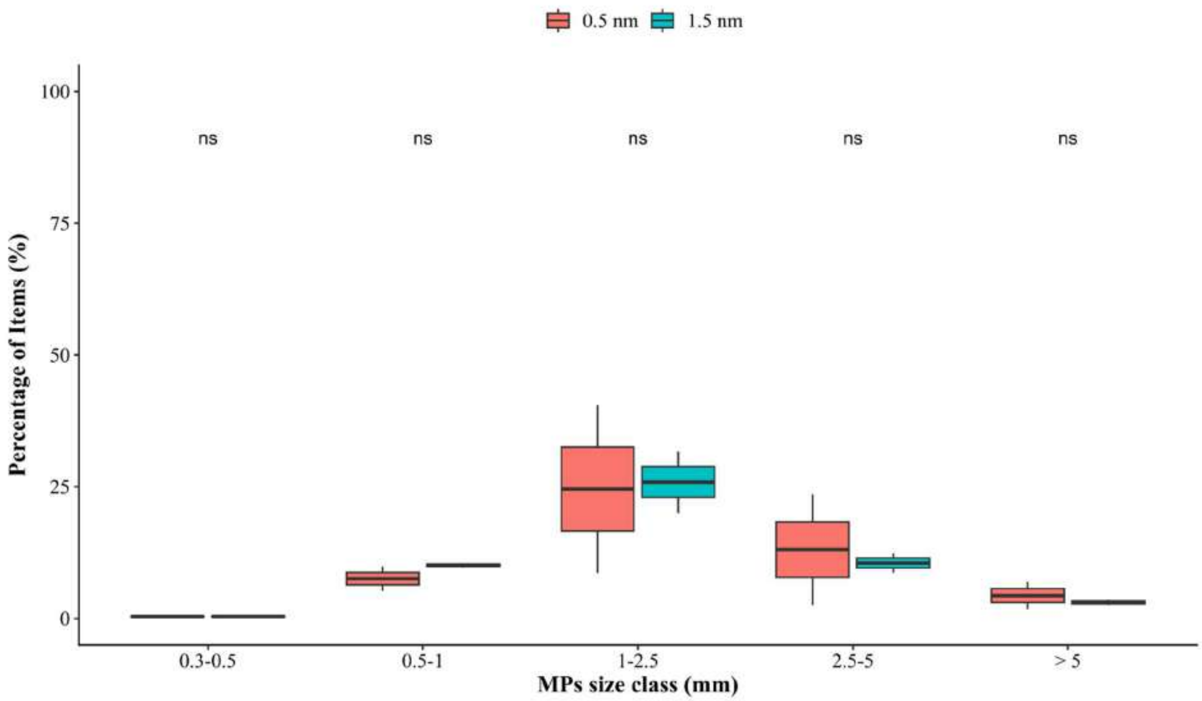


Figure 22. Results of the Wilcoxon rank sum test related to the size class of MPs in the Vibo Valentia area at different distance from the coast. Red = 0.5 nm; Green = 1.5 nm.

It is known that shape and colours of MPs are highly heterogeneous in Mediterranean waters (Sbrana et al., 2022). A total of 1288 particles (76.6% tot; 74.7% at 0.5 nm; 80.1% at 1.5 nm) was the majority of particles isolated in Vibo Valentia site followed by foam (n= 260, 15.5%), film (n= 57, 3.4%) and filament (n= 56, 3.3%). Pellets (n= 16) were also observed in the study site but accounted less than 1% (Fig. 23). Fragment is considered to be the most common shape observed worldwide and this is also due to their buoyancy, which makes them more easily detectable at the sea surface (Kooi et al., 2016; Suaria et al., 2016; Baini et al., 2018; Galli et al., 2022). In this site, the fragments suggesting that they may be aged and originate from distant sources. No variations of shape in relation to distance from the coast were highlighted within the studied site (Fig. 23).

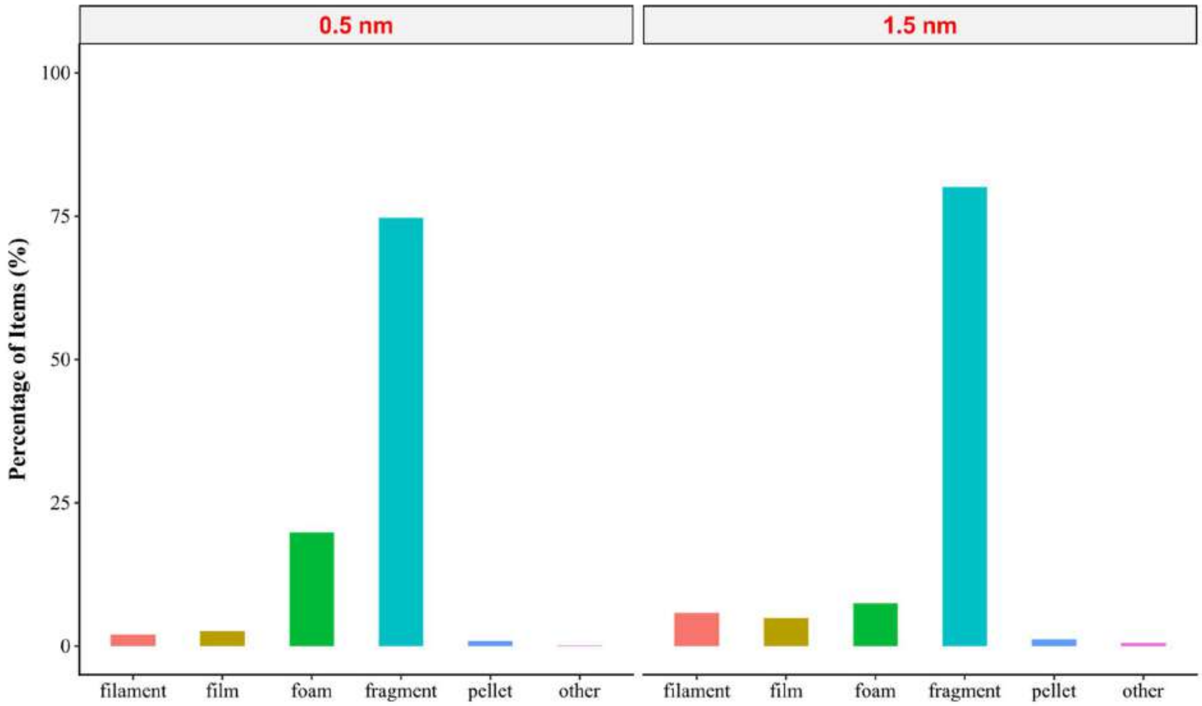


Figure 23. Percentage of MPs based on their shape in Vibo Valentia.

The colour of isolated plastics was mainly white (37% at 0.5 MN; 26% at 1.5 nm) and transparent (35% at 0.5 nm; 44% at 1.5 nm; Fig 24), which has been confirmed by previous studies in the same site (Marrone et al., 2021) as well as in other Mediterranean areas (Fossi et al., 2017; van der Hal et al., 2017; Fagiano et al., 2022b; Galli et al., 2022). The abundance of clear colour is probably associated to the fragmentation of single use plastics, generally disposed of within one year of production, and of lost or abandoned fishing gear (Gao et al., 2023). However, colour loss due to aging of plastic at marine environment cannot be ruled out (Marrone et al., 2021).

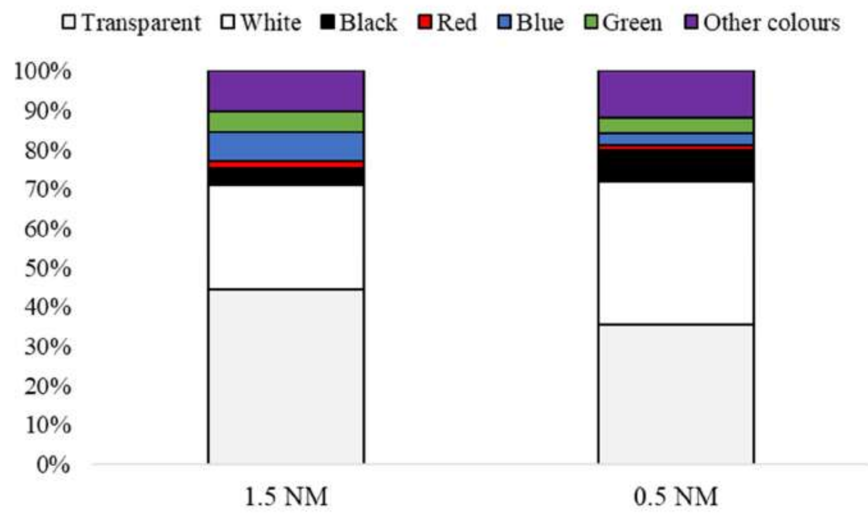


Figure 24. MPs classification by colour expressed as percentage of abundance. The other colour category includes yellow, light blue, brown, pink and grey.

Some information on possible sources of MPs and input pathways can be inferred from the type of polymer. For this purpose, a sub-sample from both sites was subjected to chemical identification. A total of 343 items (~ 20% of the total sample) was identified by FTIR analysis. This investigation allowed identifying 9 polymer types from the Vibo Valentia water samples (Fig.25). In particular, PE (46.35%), PP (34.69%), PS (15.45%) were the most common followed by PET, polyvinyl acetate (PVA) and several rubber polymers (SBR and PBT), which accounted for less than 1% of the total plastic items identified. Generally, PE, PET, PP, PS and WAX were present at both distance from the coast. However, near shore (0.5 nm), we observed the presence of PBT and RU, while at 1.5 nm SBR and PVA were detected. Despite this, was not observed any statistical difference between polymers across the two distance from the coast (p-value > 0.05). Furthermore, PE and PP were mainly observed as fragments and films in the study area while filaments were composed of PE, PP and PET (Fig. 25). It is not surprising that the polyolefins (PE and PP) account for the majority in this study, and the result is consistent with what has been observed in marine environments worldwide (Eriksen et al., 2014; Frias et al., 2014; Suaria et al., 2016; Baini et al., 2018). Indeed, PE and PP are thermoplastic polymers characterised by high versatility, strength and chemical stability, which make them widely used in the manufacture of various products, particularly packaging. In addition, PP in particular is particularly prone to photodegradation processes, which increases the probability of their occurrence in MPs size. These polymers are also characterised by a low density that facilitates their persistence in surface waters. In this urbanized settlement, concentrations of foam PS have been also identified. This polymer has a higher density than seawater, but its presence in surface sea water has been reported in the same site (Marrone et al., 2021) as well as in other o Mediterranean areas (Suaria et al., 2016; Fagiano et al., 2022) since its distribution also depends on hydrodynamic and wind conditions, salinity and temperature (Zhao et al., 2015). This finding suggests a possible connection with marine sources in this site and more specifically with fishing activities, given the extensive use of PS in the fishing industry for the production of containers and packaging materials (Cai et al., 2018).

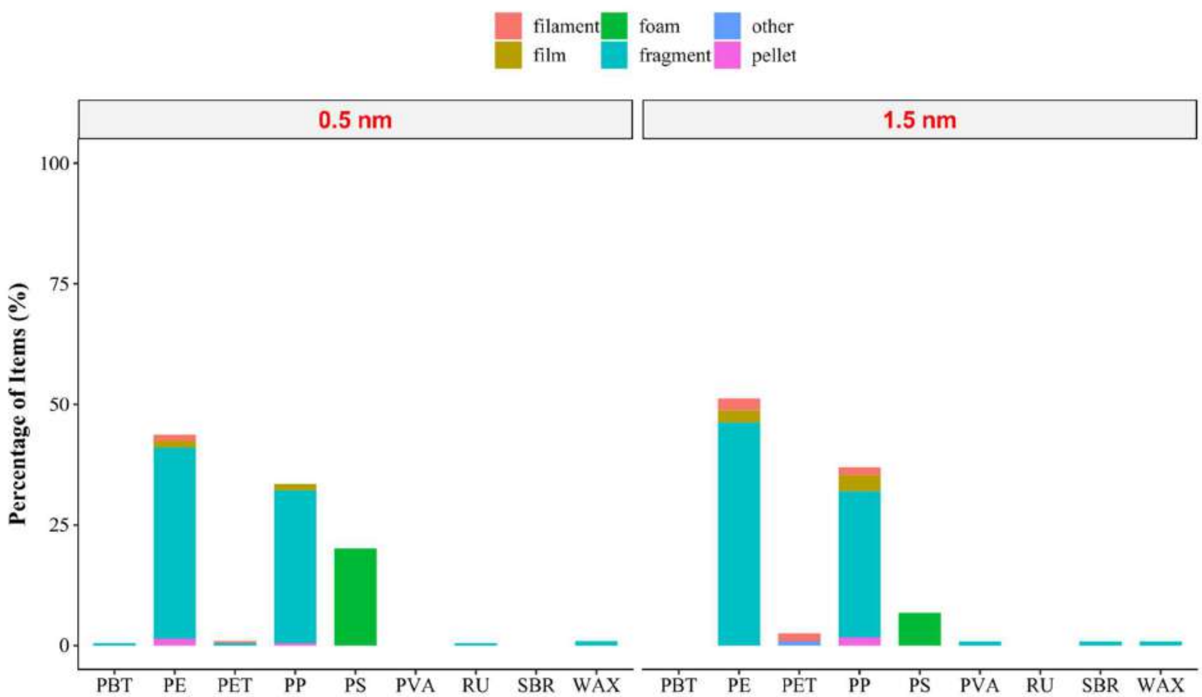


Figure 25. Percentage of polymer types in relation to the shape of floating plastics from Vibo Valentia. PE= polyethylene; PP= polypropylene; PS=polystyrene; PET= polyethylene terephthalate; PVA= polyvinyl acetate; RU= undefined rubber; SBR= styrene-butadiene rubber.

The present study provided, for the first time, information on the MPs pollution in the study site by investigating MPs ingestion in bentopelagic and demersal bioindicator species. A total of 18 specimens belonging to the 3 commercial species (*B. boops*, *M. barbatus* and *M. merluccius*) were examined. Size and weight of specimens and the corresponding mean values for each species are reported in Table 6. Overall, 29 plastics particles were isolated from 10 GITs (O%= 55.56): 9 items from *B. boops* (O%= 66.7) and 20 items from *M. barbatus* (O%= 100), while no plastics were found in the GITs of *M. merluccius* (Table 5).

Table 6. Biometric parameters (total length (TL, cm), total weight (TW, g), GITs weight (GITW), Fulton's condition factor (K) and Gastro Somatic Index (GaSI) for each species from Capo Rizzuto MPA. Plastics occurrence (% O) and abundance are also reported.

Data fish	*Boops boops*	*Mullus barbatus*	*Merluccius merluccius*	*Total*
N samples	6	6	6	**18**
TL (cm) mean ± SD	18.20 ± 2.86	14.83 ±1 .64	20.60 ± 3.87	
TW (g) mean ± SD	59.63 ± 23.83	40.99 ± 21.64	72.96 ± 47.65	
GITW (g)	2.90 ± 0.96	3.27 ± 1.66	1.86 ± 0.82	
K (g/cm3)	0.96 ± 0.06	1.33 ± 0.16	0.68 ± 0.03	
GaSI	5.08 ± 1.74	6.91 ± 1.85	3.36 ± 2.16	
Data plastic				
N samples with plastics	4	6	0	**10**
N of plastic items	9	20	0	**29**
Plastics range	0-3	0-9	-	
Plastics occurrence (%O)	66.67	100	0	**55.56**
N. plastic items/N. all examined individuals	1.5	3.33	0	**1.61**
N. plastic items/N. individuals which ingested plastics	2.25	3.33	-	**2.90**

Our results report higher occurrence than values previously observed by Giani et al. (2023); Rodrigues et al. (2023); Tsangaris et al.(2020) for *B. boops* by Bellas et al. (2016); Capillo et al. (2020), Digka et al. (2018), Giani et al (2019) and *M. barbatus* in Mediterranean Sea coastal areas. In particular, the occurrence of MPs (100%) in GITs of *M. barbatus* in the study area was higher than data observed on the same species from present observation in the Capo Rizzuto MPA (50%). The high MPs occurrence observed in demersal species respect to benthopelagic species might be related to the heterogeneous distribution of plastic items throughout the marine compartments in the investigated site as well as the sinking of floating MPs in the sediments due to abiotic and biotic factors (Andrady, 2017; Giani et al., 2023; Pedà et al., 2020).

In the same way as the site of Capo Rizzuto MPA, no plastic items were found in the GITs of *M. merluccius*. However, plastics ingestion in this important commercial species were documented in other Mediterranean area by Bellas et al. (2013), Giani et al. (2023) and Mancuso et al. (2019), Cocci et al. (2022).

Bogues and red mullet showed K values of 0.96 (±0.06) and 1.33 (±0.16), respectively, whereas the K of European Hake was 0.68 ± 0.03 (Table 6). These results fall within the range of values observed to date in studies investigating MPs ingestion on bogues, red mullet and european hake from Mediterranean Sea (Giani et al., 2023; Garcia-Garin et al., 2019; Trani et al., 2023). The correlation between K index and MPs abundance of showed a strong correlation for *B. boops* (Tau= 0.68) and *M. barbatus* (Tau= -0.65) respectively. Same finding has been reported by Compa et al.(2018) and Mizraji et al. (2017) but this parameter provides information for a first assessment of health status and does not necessarily imply a MPs-related effect on fish health (Tsangaris et al., 2020; Bottari et al., 2022). For this reason, further research is needed.
The highest GaSI index value was observed both in red mullet (6.91 ± 1.85) and bogues (5.08 ± 1.74), whereas the lowest value was found in European Hake (3.36 ± 2.16) (Tab.5). Kendall correlation showed positive weak correlation in *B. boops* (Tau= 0.21) and strong correlation in *M. barbatus* (Tau= 0.55) between the MPs ingested and GaSI index. This result may indicate that the presence of MPs in the GIT is not closely related to stomach fullness.
Bogues ingested mainly LMPs (89%) ranged from 1.08 to 2.63 mm while the other 11% were SMPs. Otherwise, in *M. barbatus* more than 50% of plastics belonged to MPs category (SMPs 21%; LMPs 47%) and MEPs (32%) were also found (Fig. 26a). The mean length of LMPs and SMPs from red mullet were 2.78 (±1.02) mm and 0.40 (±0.29) mm with a range from 1.82 - 4.82 mm and from 0.17 to 0.8 mm, respectively. In this species MEPs were also found. The mean length all MEPs recovered from red mullet were 13.46 (± 5.2) mm with a range from 5.87 to 20.32 mm range.
These findings could be linked to the feeding traits of the investigated species as also suggested by other authors (Nadal et al., 2016; Fossi et al., 2018; Felline et al., 2022). Indeed, *B. boops* are opportunistic predators that may accidentally or intentionally ingest the MPs but also eat plastics contaminated prey (secondary ingestion) (Bottari et al., 2022; Tsangaris et al., 2020). Red mullet is a benthivorous species that live in direct contact with the seafloor mainly feeds on crustaceans, worms and mollusks (Felline et al., 2022). Furthermore, *M. barbatus* swallows sediment with the prey and expels it through the gills (Labropoulou and Eleftheriou, 2005; Giani et al., 2019). This feeding behaviour may cause an increase of the risk to ingest plastics, but also small plastics can be indirectly ingested by feeding on contaminated prey(Trani et al., 2023).
Concerning the classification by plastic shape, film, fiber and fragment were observed in both species while filament was isolated only from the GITs of *M. barbatus* (Fig. 26b). In the bogue, plastics were equally distributed in the shape categories of film, fragment and fiber (with a proportion of ~33%). Fibres and fragments are the most frequently shape categories observed in previous studies for this species in the Mediterranean basin (Giani et al., 2019; Tsangaris et al., 2020; Bottari et al., 2022).

The uptake of fibres could be related to the non-selective predation of the species studied, which leads to passive ingestion of tiny particles (Rodrigues et al., 2023). The most common colour of fiber in bogue were black (66%) and red (33%), while fragment and film were transparent and white, respectively.

The most frequent colours of plastic ingested by the red mullet were transparent (n=15) and with (n=8), but items black and green were also observed. The presence of light-coloured could be related to the similarity with their potential prey, as suggested by Bottari et al. (2021) for bogue. Our result is consistent with other studies on MPs ingestion by the same species (Giani et al., 2019; Tsangaris et al., 2020; Bottari et al., 2022; Giani et al., 2023).

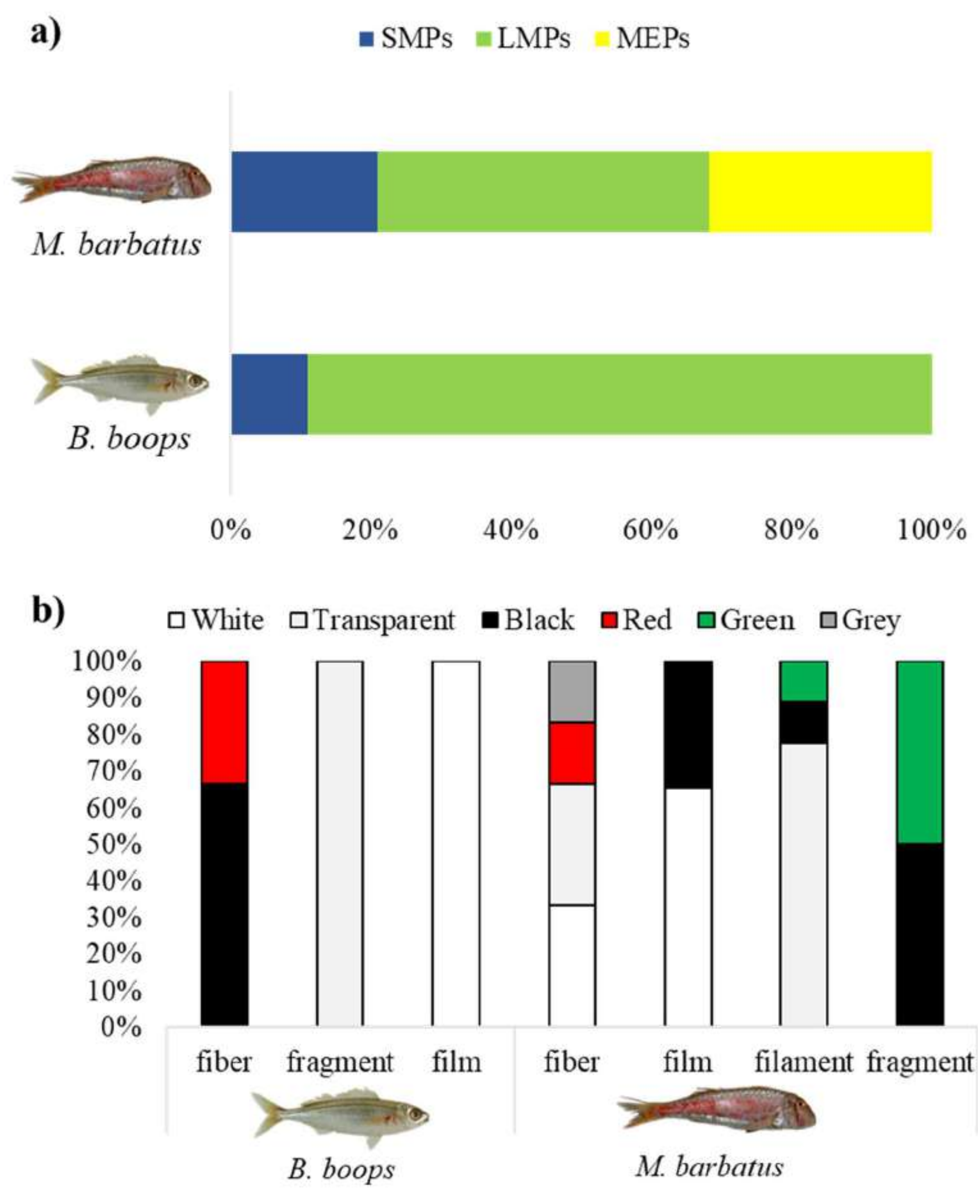


Figure 26. MPs percentage according to size-classes (a), shape and colour (b) classification in *B. boops* and *M. barbatus* from Vibo Valentia.

Chemical characterization by FTIR was performed on 23 out items of 29 extracted MPs from the GITs of bioindicator species (Fig. 27). Due to the limitations of the instrument, the 6 remaining particles were tested by the hot needle method in order to confirm their plastic polymers nature and then were recorded as undetermined polymers (UP). Overall, a total of 7 polymers were identified. *B. boops* ingested only PE (67%) and PET (22%) while the other 22% were UP. Six polymer types were instead identified in *M. barbatus*: PP (20%) nylon included in PA (15%), PE (5%), and several rubber polymers such us ethylene propylene rubber (5% EPR), undefined rubber (20% RU) and polyurethane (10% PUR). Images of some plastic samples found in the GITs of bioindicator species are reported in Figure 28, together with the corresponding FTIR spectra.

The ingestion of PE and PP was not surprising as considered to be the most common polymer types from different Mediterranean regions (Capillo et al., 2020; Tsangaris et al., 2020; Bottari et al., 2022; Giani et al., 2023). Furthermore, this result is consistent with the data on surface waters observed in the Vibo Valentia during this study. PET and PA (a family of polymers including nylon) can be derived from marine sources as they are widely used in fishing activities as constituent of several fishing net and equipment (Pedà et al., 2022a). The presence of several rubber polymers may rely on runoff processes of rivers and torrents with different flow rates as well as on agricultural activities that insisting in the study site. In addition, this site is characterized by an important harbour as well as fishing and nautical activities and thus same polymers as PE, PP, and nylon particles may derive by the degradation of lost or abandoned fishing gears. Finally, the variability in colours and shapes of MPs observed in the Vibo Valentia site suggest both land-based and marine sources.

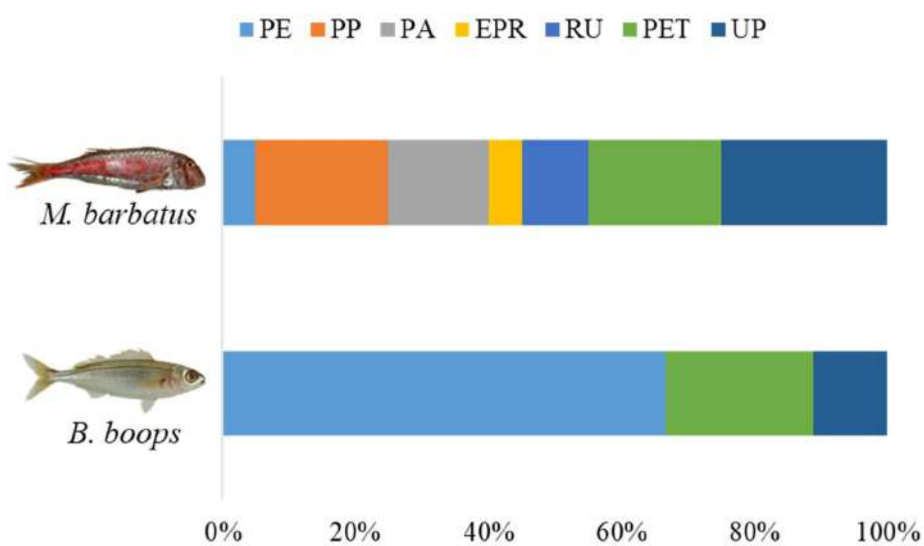


Figure 27. Polymers abundance (%) in fish samples of Vibo Valentia.

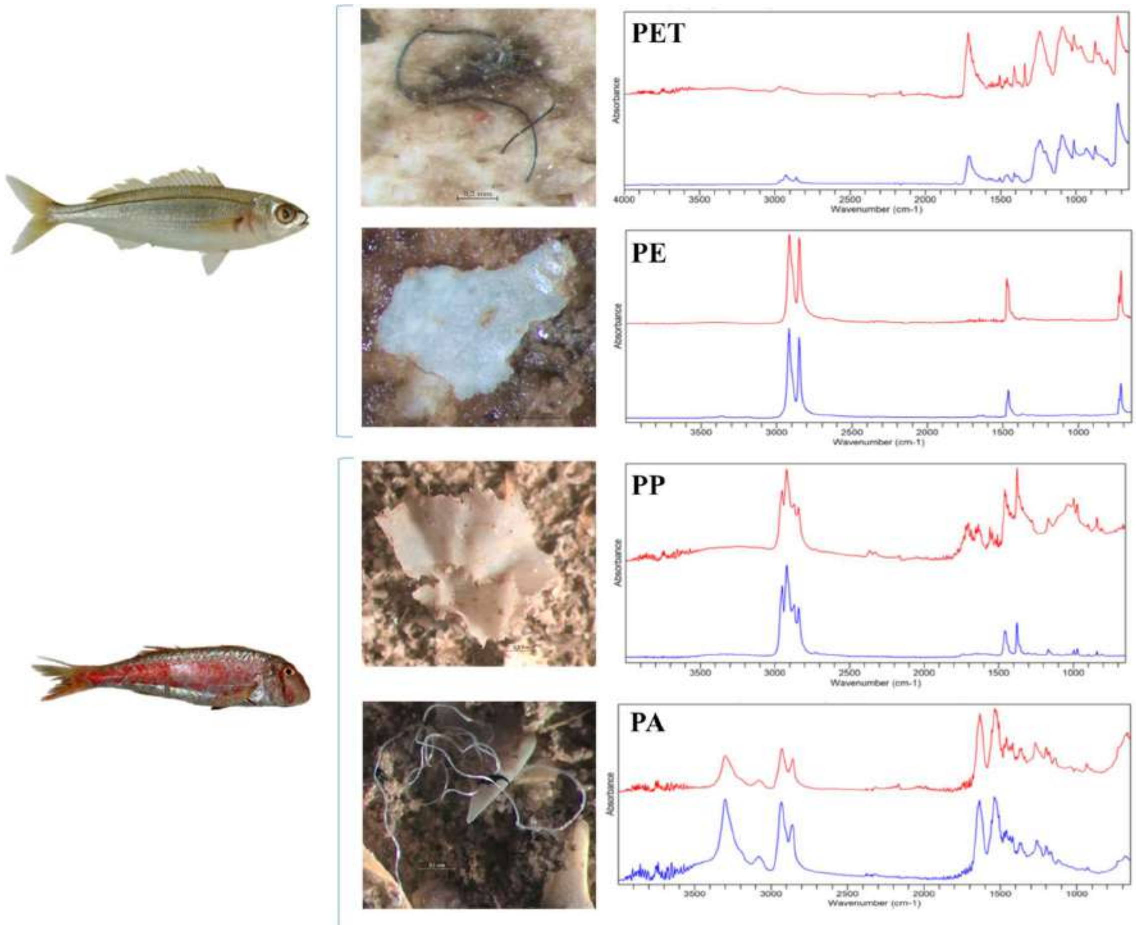


Figure 28. Images of some plastics samples found in the GITs of B. boops and M. barbatus. PET filament and PE film in *B. boops*; PP film and PA filament in *M. barbatus*.

The present study also investigated the bacterial community associated with the MPs collected at Vibo Valentia. A total of 12 items have been isolated from surface water sample, used for the DNA extraction. Figure 29 shows the obtained plastic pool collected, with related classification of items.

n MPs	*Shape*	*Colour*	*Size class*
1	fragment	white	small
2	fragment	transparent	large
6	fragment	white	small
1	pellet	transparent	large
1	sheet	white	large
1	sheet	transparent	large

Figure 29. MPs pool used for the DNA extraction collected in the study site of Vibo Valentia. MPs number classified by shape, colour and class size (large MPs = from 5 mm to 1 mm; small MPs = from 1mm to 0.1 mm).

The bacterial community associated with the MPs pool collected at Vibo Valentia was mainly represented by Proteobacteria with the 67.2% of mean relative abundance, of which the 43.6% was constituted by Alphaproteobacteria members, while Gammaprotobacteria accounted for the 56.3%. The second most abundant phylum was Firmicutes, with mean abundance of 19.1%, followed by Cyanobacteria and Bacteroidota accounting for the 4.1% and 3.7% of the total bacterial community, respectively (Figure 30). This result reflected the taxonomic structure retrieved for MPs from Capo Rizzuto, with predominance of Proteobacteria and Firmicutes. The only difference relies in the detection of Cyanobacteria as third most abundant group, instead of Halanaerobiaeota. The group of Cyanobacteria is ubiquitous in aquatic environments and its affiliates play a key role in the oxygen production, so studying and understanding their interactions with plastic particles is of paramount importance. Different studies observed the cyanobacteria as predominant fraction of plastic attached microorganisms, which probably find stability and protection on these surfaces (Oberbeckmann et al., 2014; Kaiser et al., 2017). Interestingly, the attachment process of cyanobacteria affiliates on different plastic polymers has been investigated, and it was found that the adhesion is mediated by the production of extracellular polymeric substances (de Oliveira et al., 2020). According to Silva de Oliveira et al., (2020) the adhesion of cyanobacteria on poly(methyl methacrylate) and polystyrene microparticles is specie specific. Indeed, the two investigated species, namely the seawater *Synechococcus elongatus* PCC 7002 and freshwater cyanobacteria *S. elongatus* Nageli PCC 7942 formed aggregates which in the case of marine system tended to settle, with interesting implications in the transport and fate of plastics in waters, as well as in the possible underestimation of plastics in natural samples. Moreover, our finding confirms previous scientific evidence of plastics as potential vectors of micropollutants, as cyanobacterial toxins, harmful to humans and wildlife (Pestana et al., 2021). Three MPs (PVC, PE, and PS) have been proved to be as mobile reservoirs of microcystin, and the exposure of aquatic organisms to MPs particle- assembled microcystin was also assessed (Pestana et al., 2021). Within the Cyanobacteria taxa, the Vibo Valentia plastic associated communities were enriched in families of filamentous *Phormidesmiaceae* and *Nostocaceae,* which are known for their ability to trap suspended particles, as previously reported (Vaksmaa et al., 2022).

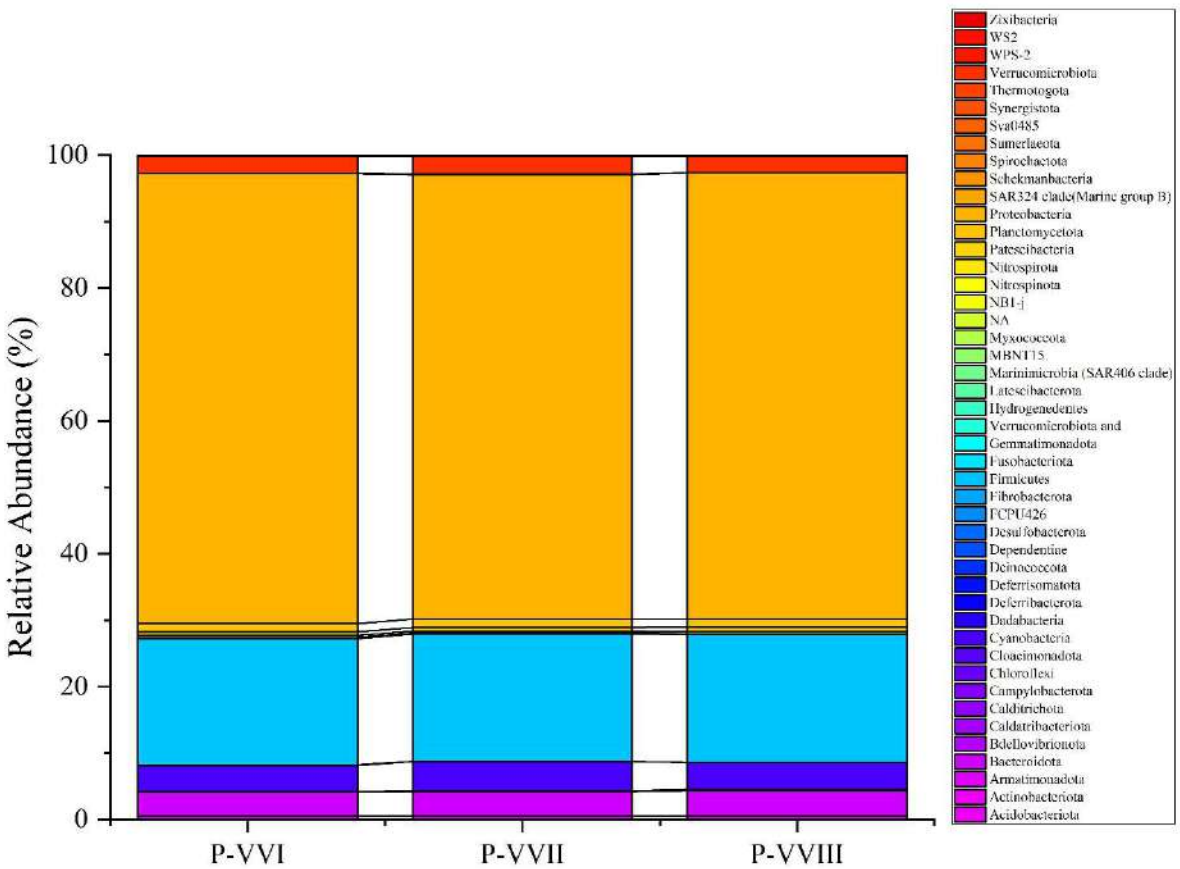
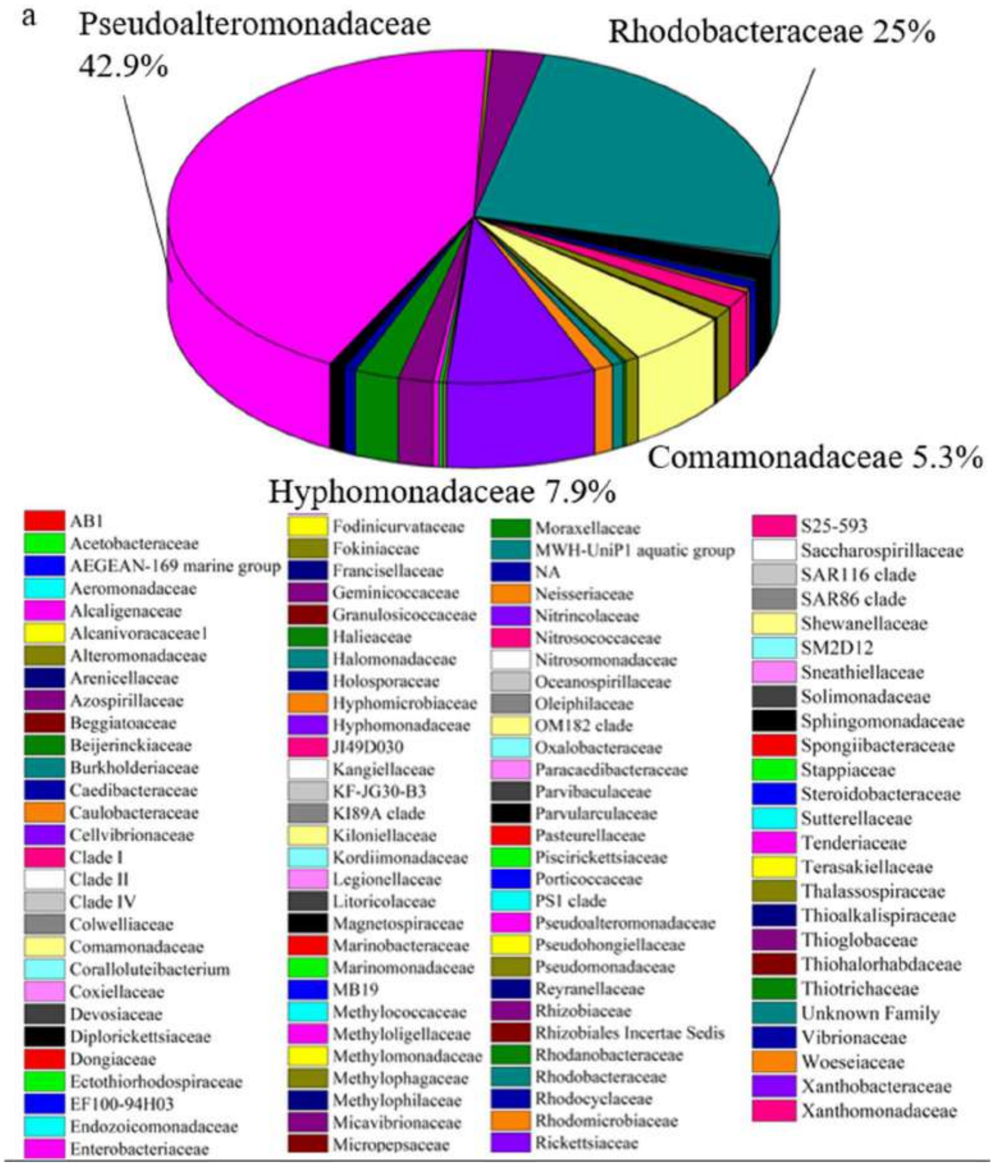


Figure 30. Bacterial phyla abundance in MPs pools from Vibo Valentia.

Within the Proteobacteria, the most abundant families were Pseudoalteromonadaceae (42.9% of total Proteobacteria), Rhodobacteraceae (25% of total Proteobacteria), Hyphomonadaceae (7.8% of total Proteobacteria) and Comamonadaceae (5.3% of total Proteobacteria). Firmicutes were mainly represented by Bacillaceae (87.9% of total Firmicutes), Exiguobacteraceae (5.8% of total Firmicutes), and Planococcaceae (5.7% of total Firmicutes). The details of families retrieved within the most predominant phyla is reported in Figure 31. The Pseudoalteromonadaceae includes many species involved in the production of secondary metabolites and hydrolytic enzymes, which are characterized by a great metabolic versatility and are so highly adaptable to several ecological conditions (Ivanova et al., 2014). The taxa was totally represented by *Pseudoalteromonas* members, previously detected on plastic particles (Zettler et al., 2013) and generally known ad optimal hydrocarbon degraders (Frère et al., 2018). Rhodobacteraceae members, mostly reported as colonizers of plastic debris (Dang et al., 2008) mainly included the genera *Roseovarius*, whose members have been reported in the biofilm from plastics collected in the Pacific (Pinto et al., 2020), and *Tateyamaria* whose members have been detected in marina plastic associated communities (Debroas et al., 2017; Briand et al., 2022). The phylum Firmicutes was predominated by members of Bacillaceae and specifically by *Bacillus* affiliates, known as resistant bacterial forms able to produce endospore and previously retrieved as colonizers of plastics and in plastic biodegradation processes (Dang et al., 2018).


a
Pseudoalteromonadaceae 42.9%
Rhodobacteraceae 25%
Comamonadaceae 5.3%
Hyphomonadaceae 7.9%
AB1
Acetobacteraceae
AEGEAN-169 marine group
Aeromonadaceae
Alcaligenaceae
Alcanivoracaceae1
Alteromonadaceae
Arenicellaceae
Azospirillaceae
Beggiatoaceae
Beijerinckiaceae
Burkholderiaceae
Caedibacteraceae
Caulobacteraceae
Cellvibrionaceae
Clade I
Clade II
Clade IV
Colwelliaceae
Comamonadaceae
Coralloluteibacterium
Coxiellaceae
Devosiaceae
Diplorickettsiaceae
Dongiaceae
Ectothiorhodospiraceae
EF100-94H03
Endozoicomonadaceae
Enterobacteriaceae
Fodinicurvataceae
Fokiniaceae
Francisellaceae
Geminicoccaceae
Granulosicoccaceae
Halieaceae
Halomonadaceae
Holosporaceae
Hyphomicrobiaceae
Hyphomonadaceae
JI49D030
Kangiellaceae
KF-JG30-B3
KI89A clade
Kiloniellaceae
Kordiimonadaceae
Legionellaceae
Litoricolaceae
Magnetospiraceae
Marinobacteraceae
Marinomonadaceae
MB19
Methylococcaceae
Methyloligellaceae
Methylomonadaceae
Methylophagaceae
Methylophilaceae
Micavibrionaceae
Micropepsaceae
Moraxellaceae
MWH-UniP1 aquatic group
NA
Neisseriaceae
Nitrincolaceae
Nitrosococcaceae
Nitrosomonadaceae
Oceanospirillaceae
Oleiphilaceae
OM182 clade
Oxalobacteraceae
Paracaedibacteraceae
Parvibaculaceae
Parvularculaceae
Pasteurellaceae
Piscirickettsiaceae
Porticoccaceae
PS1 clade
Pseudoalteromonadaceae
Pseudohongiellaceae
Pseudomonadaceae
Reyranellaceae
Rhizobiaceae
Rhizobiales Incertae Sedis
Rhodanobacteraceae
Rhodobacteraceae
Rhodocyclaceae
Rhodomicrobiaceae
Rickettsiaceae
S25-593
Saccharospirillaceae
SAR116 clade
SAR86 clade
Shewanellaceae
SM2D12
Sneathiellaceae
Solimonadaceae
Sphingomonadaceae
Spongiibacteraceae
Stappiaceae
Steroidobacteraceae
Sutterellaceae
Tenderiaceae
Terasakiellaceae
Thalassospiraceae
Thioalkalispiraceae
Thioglobaceae
Thiohalorhabdaceae
Thiotrichaceae
Unknown Family
Vibrionaceae
Woeseiaceae
Xanthobacteraceae
Xanthomonadaceae

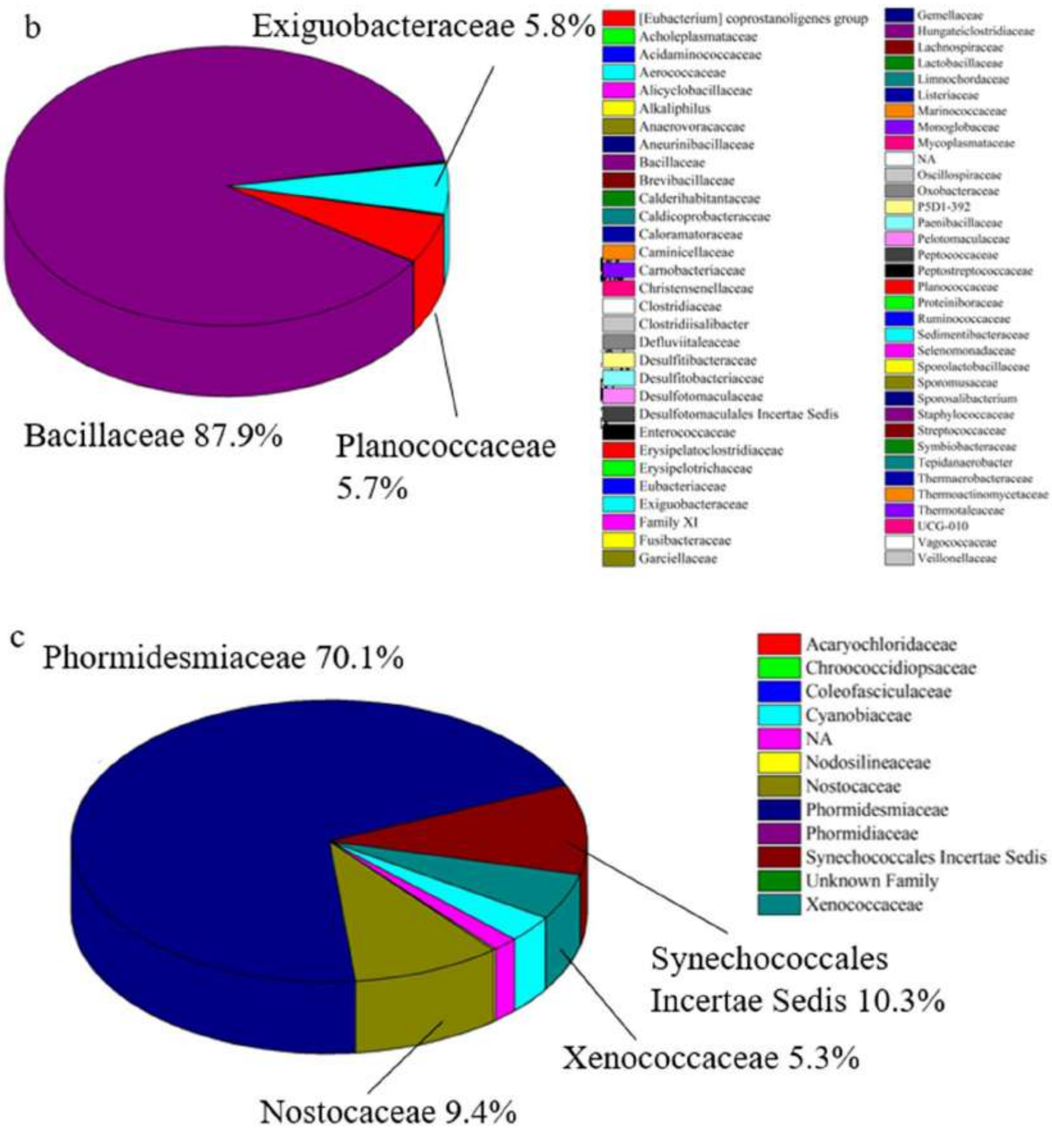


Figure 31. Mean bacterial abundance at family level of microbial community associated with MPs pool from Vibo Valentia, within the phylum Proteobacteria (a), Firmicutes (b) and Cyanobacteria (c). Please note that mean values of three experimental replicates are reported.

The most predominant genera in Vibo Valentia MPs were *Bacillus* and *Pseudoalteromonas*, with 16.7% and 28.9% of the total bacterial community.

The fraction of bacterial pathogens was estimated as an overall percentage of 17.8% on the total bacterial community (measured as fraction at genus level). The search for specific ASV affiliated with bacterial pathogens, revealed the absolute higher abundance of *Bacillus* spp. and *Acinetobacter* spp., but also the genera *Vibrio* and *Pseudomonas* were represented with high percentages. The search for potential bacterial pathogens evidenced a 17% fraction on the total bacterial communities, thus confirming that MPs could act as vector of dangerous species. This could represent an indirect effect towards other organisms, and for the human health. In this case, the most abundant groups that could be involved in potential pathogens are *Bacillus* spp. and *Acinetobacter* spp., both of which are well known for their capacity to produce extracellular polymeric compounds, and thus potentially contributing to the colonization processes and biofilm formation.

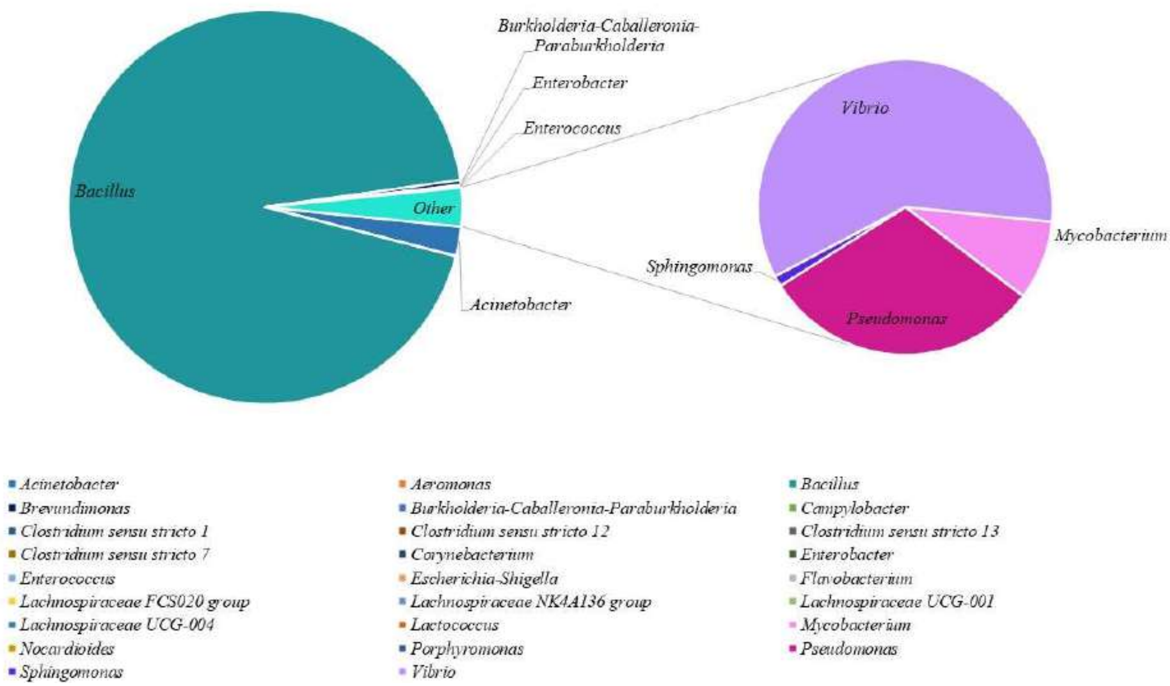


Figure 32. Representation of bacterial pathogens retrieved among ASVs detected in MPs from Vibo Valentia.

The search for best known genera including strains able to degrade hydrocarbon led to detect a hydrocarbon-degrading fraction of 17% on the total of bacterial community. This fraction was mainly represented by members of the genus *Bacillus*, *Pseudomonas*, *Sphingobium* and *Sphingomonas*. The hydrocarbon-degrading species detected in this study are not representatives of strains obligate to the use of hydrocarbon sources, but probably - due to their special affinity towards hydrophobic surfaces - could mediate the adhesion and proliferation of other species in the colonization process.

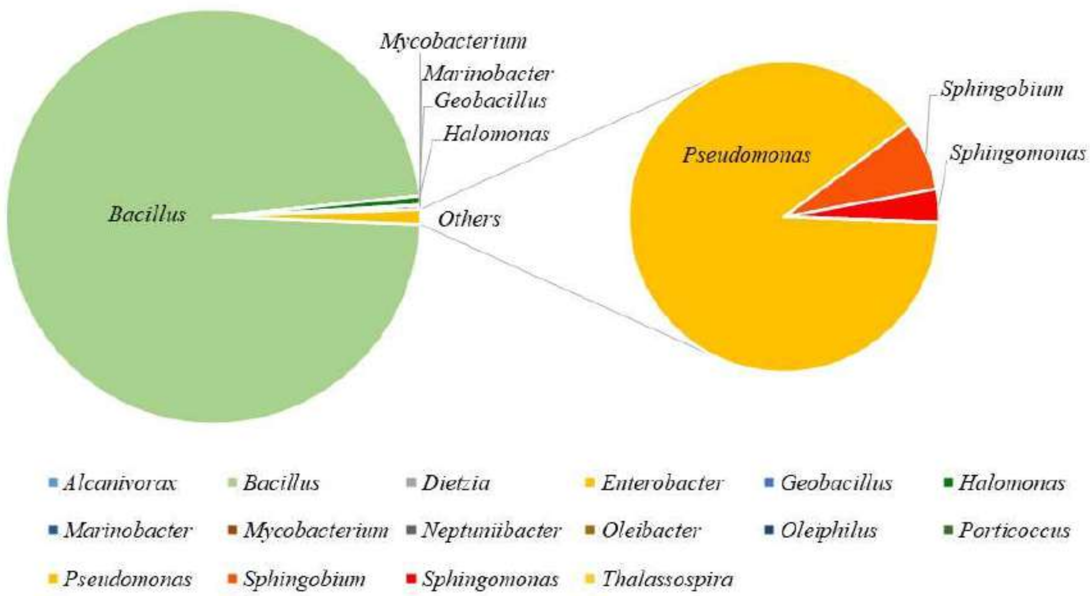


Figure 33. Representation of ASVs related to hydrocarbon-degrading bacteria retrieved in MPs from Vibo Valentia.

Archaeal community was represented exclusively from the Halobacterota group, with the family Haloferacaceae and *Halostagnicola* genus.

4 Microplastics assessment in the coastal area of Sicily

4.1 Materials and methods

Along the coasts of north-eastern Sicily, the Capo Milazzo Marine Protected Area and the Aeolian Islands Archipelago (Fig. 34) were selected as survey sites to investigate the abundance and composition of MPs in the sea surface and to assess MPs ingestion by selected bioindicator species. Sampling activities were carried out during the "*Sicily expedition*" by Sicily Marine Centre and Sail and Explore Association in September 2021.

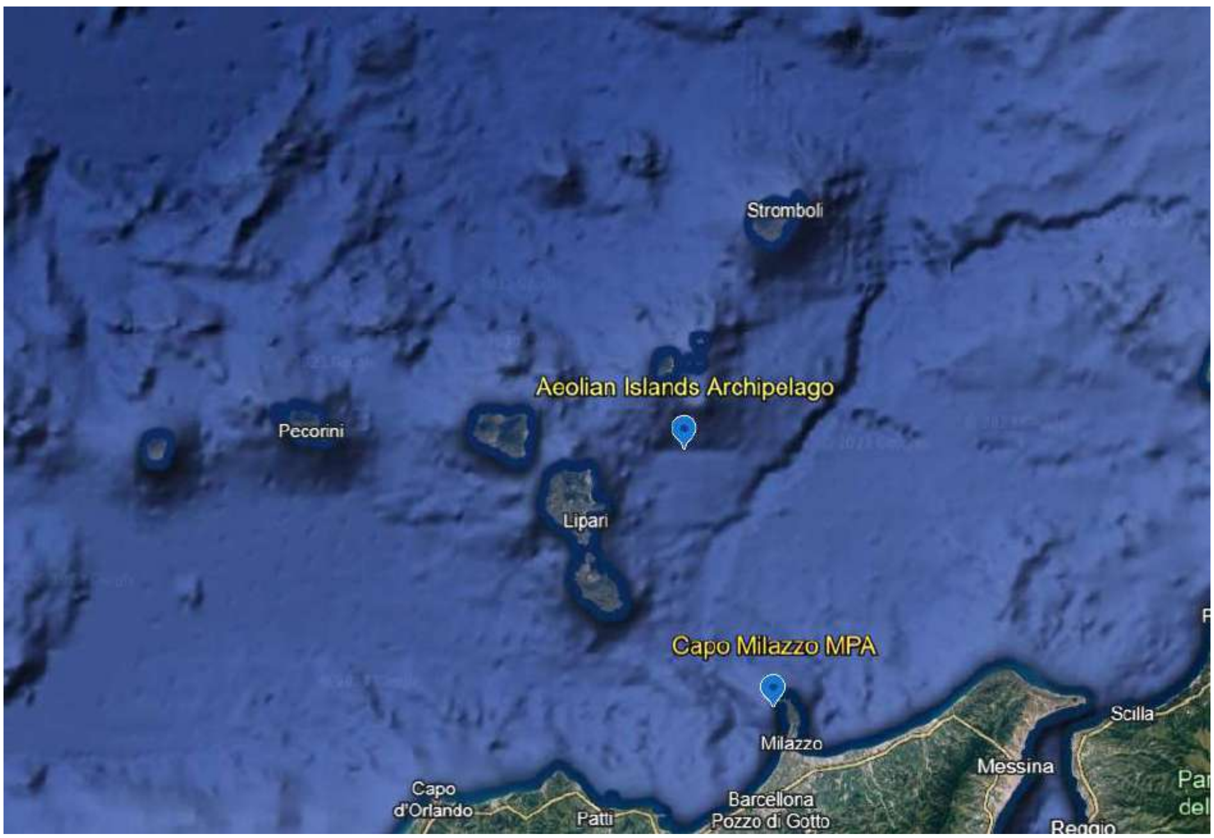


Figure 34. Survey areas. Capo Milazzo MPA; Aeolian Island Archipelago.

Surface water samples were collected on the eastern side Aeolian Islands Archipelago, from Lipari to Stromboli, along 6 transects located at 3 nautical miles from the coast. Two additional sample were collected in the surface water of Capo Milazzo MPs to provide, for the first time, preliminary information on plastic pollution in this young MPA.

Floating plastics were sampled using a manta trawl (335 µm mesh size, 60 × 16 cm mouth opening), towed at the sea surface, at a speed < 3 knots for 20 minutes and kept at a distance from ship's side to avoid wake turbulence on a side of the sailboat. Date, geographical coordinates weather conditions start-end geographical coordinates were recorded for each sampling activity. At the end of each sampling, the net was thoroughly washed with seawater and MPs samples were collected into the cod-end. Thus, samples were transferred to clean glass jars and stored in a 70% ethanol solution for further analysis. In order to estimate the water filtered (m^3), the manta net was coupled with a flowmeter.

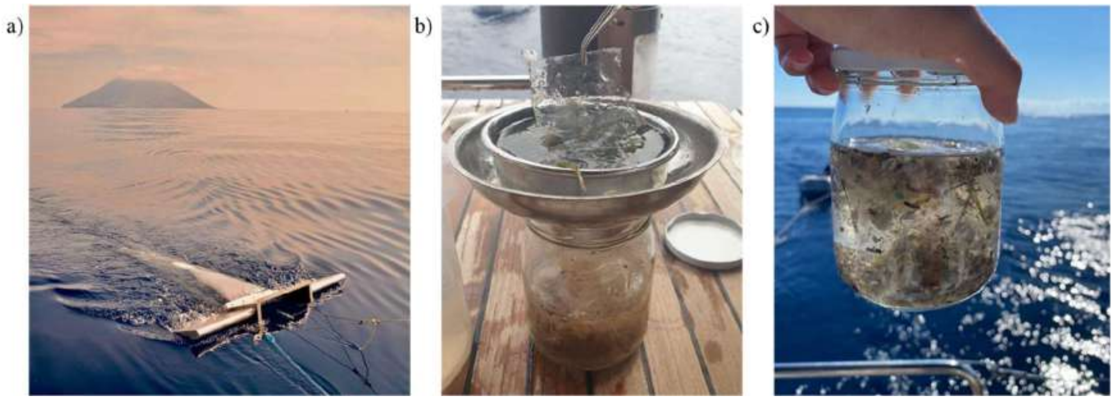


Figure 35. (a) Sea surface water sampling with manta net; (b) sample collection; (c) sample storage in glass jars.

Fish species were collected in the two sites from the coasts of north-eastern Sicily to assess the MP ingestion impact. Four bioindicator species were selected according to ecological and biological criteria reported by Fossi et al. (2018) as well as their availability in the main fisheries of the study area (Tab. 7). A total of 6 specimens of the same length class per each species were collected at the two survey site with the collaboration of fishermen.

Table 7. Bioindicator species for MPs ingestion selected in the two site of Sicilian coastal area. Information on habitat, ecological compartments and feeding habits of bioindicator species.

Species	Common name	Habitat	Ecological compartment	Feading habits
 *Boops boops* (Linnaeus,1758)	Bogue	Benthopelagic	Coastal water	Zooplanktivorous
 *Mullus surmuletus* Linnaeus, 1758	Striped red mullet	Demersal	Seafloor	Benthivorous
 *Auxis rochei* (Risso, 1810)	Bullet tuna	Pelagic	Open water	Zooplanktivorous
 *Diplodus spp.*	Sea bream	Demersal	Coastal water	Benthivorous

4.1.1 Study area and sample collection

4.1.1.1 Sampling site: Marine Protected Area of Capo Milazzo

The Capo Milazzo MPA is located on the north-eastern coast of Sicily and is the youngest Italian MPA (Scotti et al., 2023). The Capo Milazzo MPA has been established in 2019, and it hosts important coastal habitats such as posidonia meadows and vermetid reefs (Consoli et al., 2008; Battaglia et al., 2017). The rocky peninsula of Capo Milazzo is placed between a heavily anthropised and industrialised centre, the Gulf of Patti and the Gulf of Milazzo, respectively. In particular, the Gulf of Patti, extends about 50 km of coastline to the western site of MPA and it is characterized by the presence of different human activities such as tourism (seasonal), maritime traffic and professional and recreational fishing activities as well as several torrent inputs having a considerable flow during the winter (Battaglia et al., 2017; Pedà et al., 2022a). Furthermore, the Gulf of Milazzo is exposed to intense anthropogenic pressures including oil refinery, thermoelectric power plants, steel mills, marine traffic due to fishing, commercial and tourism activities (D'Alessandro et al., 2016; D'Agostino et al., 2020). For these reasons, the Milazzo industrial area has been considered a Contaminated Site of National Interest (SIN; Italian Directive 23 December 2005 n. 266, art. 1 com.

561) since 2005. Currently, MPs pollution in the Capo Milazzo MPA has been poorly investigated. In particular, to the best of our knowledge, the presence and distribution of MPs in the sea surface water of the MPA has not been assessed to date, while MPs ingestion has been studied in pelagic barnacles by Scotti et al (2023).

4.1.1.2 Sampling site: Archipelago of Aeolian Island

The Aeolian archipelago is located off the north-eastern coast of Sicily (southern Tyrrhenian Sea) and consists of seven inhabited islands (Vulcano, Lipari, Salina, Panarea, Stromboli, Filicudi and Alicudi) and many submerged islets (Dattilo, Lisca Nera, Lisca Bianca, Basiluzzo and Bottaro) of volcanic origin, covering an area of 112.6 km^2 (Battaglia et al., 2010). Stromboli and Vulcano are the only active volcanoes, but also submarine hydrothermal activity is present in some areas such as Panarea and Vulcano (Esposito et al., 2015; Consoli et al., 2021). Because of the high ecological, biological and landscape value, the archipelago has been declared a UNESCO Human Heritage site in 2001. In fact, the Aeolian marine ecosystem represents a reproduction and nursery area for pelagic fish such as the swordfish, *Xiphias gladius* and the bluefin tuna, *Thunnus thynnus* (De Metrio et al., 1995), but also other species such as *Thunnus alalunga*, *Tetrapturus belone* (Andaloro, 2006), *Coryphaena hippurus* and the amberjack *Seriola dumerili* (Potoschi et al., 1999) are important fishery resources in the area. Furthermore, the archipelago is an important habitat for cetaceans (Romeo et al., 2003; Blasi and Boitani, 2012), in particular bottlenose dolphins and striped dolphins (Fortuna et al., 2007; Blasi and Boitani, 2014; Blasi et al., 2020) as well as a foraging and / or wintering habitat for Mediterranean sea turtles, *Caretta caretta* (Blasi et al., 2022, 2016). The archipelago becomes a tourist spot for tourist from all over the world, particularly during the summer season, due to its natural beautiful landscape. Furthermore, this area is characterized by fishing and shipping activities (Battaglia et al., 2010) that can act as sources of plastic, threatening species and habitats (Blasi et al., 2016; Fastelli et al., 2016; Consoli et al., 2021). To the best of our knowledge, the impact of MPs ingestion in fish has never been assessed while the presence of MPs in surface waters was reported by Mezzelani et al. (2017) and De Lucia et al. (2018). To date, studies conducted in the Aeolian archipelago have assessed the presence of plastic in sediments and on the seafloor (Fastelli et al., 2016; Consoli et al., 2021), while the impact on marine biodiversity has only been assessed for benthic organisms (Renzi et al., 2020) and for the loggerhead sea turtle *Caretta caretta* (Blasi et al., 2016).

4.1.2 Laboratory analysis

4.1.2 1 Plastic litter isolation and characterization from sea surface

In the laboratory, samples were subjected several steps to minimise the amount of non-plastic material in the sample. First, the sample was filtered using 1 mm and 300 μm metal sieves to remove the ethanol solution and organic matter. Large plastics (≥ 5 mm) were directly isolated, placed in Petri dishes and identified by FTIR spectroscopy (seen details further in the text). Any large organic elements were rinsed over the sieve with ultrapure water (Milli Q) and then isolated by tweezers from the sample. In a second step, each sample was digested by applying a chemical digestion protocol including oxidizing (hydrogen peroxide) agent (Avio et al., 2015b) with some modifications. In detail, the sample was transferred to a conical flask with 200 ml of 10% H_2O_2 and placed in incubator at 60 °C for 72 h (fig. 36a). After digestion, the sample was filtered on Whatman, Nuclepore Track-Etch polycarbonate membrane (pore size 12 μm) under fume hood through a glass vacuum filtration system. MPs were then transferred from the filter to the Kevley reflective slide using a small amount of ethanol to move and transfer the items to the slide. Slides were placed in glass Petri dishes to allow evaporation of ethanol and stored for subsequent analysis (Fig. 36b). Plastic particles classification was performed according to the Marine Strategy Framework Directive MSFD guidelines (Hanke et al., 2013) by shape (fragment, film, filament, pellet, foam), size class (0.3-0.5 mm; 0.5-1 mm; 1-2.5 mm; 2.5-5 mm; >5mm) and colour (transparent, white, black, red, blue, green, other colour).

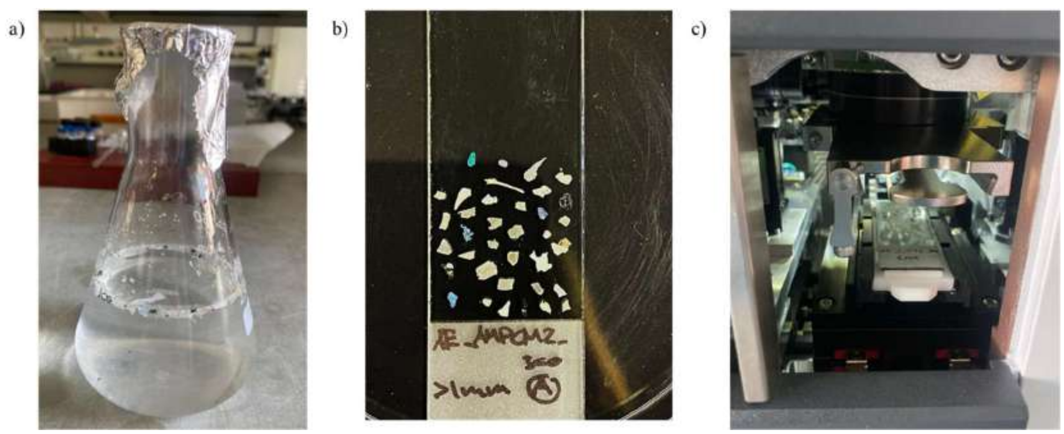


Figure 36. Analysis steps for the detection and quantification of plastics in sea surface water samples. a) Chemical digestion; b) Plastics on Kevley reflective slide; c) Polymer identification by LDIR.

4.1.2.2 Detection and quantification of plastic in fish samples

In the laboratory, the biometric parameters (total length, TL in cm and weight, TW in g) for each fish specimen were recorded before dissection. The GIT was removed from each specimen, weighed and placed in glass beakers for chemical digestion. According to the protocol of Pedà et al. (2022a), 10% KOH was added to the each GITs at a ratio of 1:5 (w/v) and incubated at 50°C for 6 h. The solution was then filtered on glass fibre membranes (1.6 μm, GF/A Whitman) using a vacuum pump system. The obtained membranes were examined under stereomicroscope Zeiss Discovery V.8 coupled with AxioVision digital image processing software. Possible synthetic particles were photographed and isolated onto slides by micro-tweezers (López-Rosales et al., 2022) for subsequent polymer identification analysis.

Plastic items were classified by size in macroplastics (> 25 mm; MAPs) mesoplastics (5 - 25 mm; MEPs), large MPs (5 - 1 mm; LMPs) and small MPs (1 - 0.1 mm; SMPs), shape (fragment, film, pellet, filament, fiber and foam) and colour according to MSFD guidelines (Hanke et al., 2013; Schirinzi et al., 2020). To reduce sample contamination (especially from airborne fibres) during the laboratory analysis, mitigation measures were applied according to (Schirinzi et al., 2020; Pedà et al., 2020). In particular, a blank sample was processed simultaneously battery of samples and a membrane moistened with Milli-Q water was placed in a Petri dish and exposed during each digestion step, from sample preparation under the fume hood to observation near the stereomicroscope. Particles and fiber observed in the sample membranes having similar structure and colour to the contamination found in blank samples were excluded from the analysis (Pedà et al., 2020).

4.1.2.3 Polymers identification

MPs identification was performed using the Laser Direct Infrared Imaging (LDIR, Agilent 8700), a new technique for chemical imaging, infrared (IR) microscopy and IR spectral analysis (Fig. 36c). LDIR uses quantum cascade laser (QCL) light and works in the mid-infrared (1800 cm-1 to 975 cm-1) region, targeting and focusing on particles without considering empty spaces. The system uses image analysis techniques to determine the particle boundary and thus size, and provides information on particle composition with spectral data (Ourgaud et al., 2022; Scircle et al., 2020). LDIR analyser scans the particles obtaining a spectrum compared with the polymer spectrum library provided by Agilent that coverage of most common polymer types.

Both a sub-sample of the total of items isolated from each water sample (~20%) and the possible synthetic particles detected in the GITs were analysed by the LDIR system. The polymer nature was accepted when the identification match was >70% and all data analysis and processing were done in real time using the Agilent Clarity Software. Polymer identification of plastics > 5mm was achieved by Fourier Transform Infrared (FT-IR) Spectroscopy technique. Only matches >70% between the sample spectra and spectra from the reference library were considered (Pedà et al., 2022a; Schirinzi et al., 2020).

4.1.2.4 Statistical analysis

Surface water data were expressed as items per cubic metre (items/m^3) and items per square metre (items/m^2).

Principal component analysis (PCA) was applied to the surface water MPs dataset of the Aeolian Archipelago, which include: abundance, size and shape. The results were visualized in biplots.

To assess the general well-being of bioindicator species and their feeding intensity the Fulton's condition index (K) and the Gastro Somatic Index (GaSI) were calculated as follows:

K= (total weight in g/ total length in cm^3) × 100 (Lloret et al., 2013)

GaSI = Stomach weight in g / (total weight in g - stomach weight in g) × 100 (Desai, 1970)

Plastic abundance indices were calculated for each bioindicator species as follows:

Litter and plastic percentage of occurrence (O%):

%O = N. individuals which ingested litter and plastics/N. total samples × 100

Average number of plastic items found in the GITs:

N. plastic items/N. all examined individuals

N. plastic items/N. individuals that ingested plastics

Kendall's Tau correlation has been performed to assess the correlation between MPs abundance *vs.* K index and MPs abundance *vs*. GaSI. Results were interpreted as follow: Tau = 0: no correlation, 0 < Tau ≤ 0.25: extremely weak, 0.25 < Tau ≤ 0.34: weak, 0.35 < Tau ≤0.39: moderate, Tau ≥ 0.40: strong.

All statistical analyses were performed using R and R-studio software (R Core Team, 2022).

4.2 Results and discussion

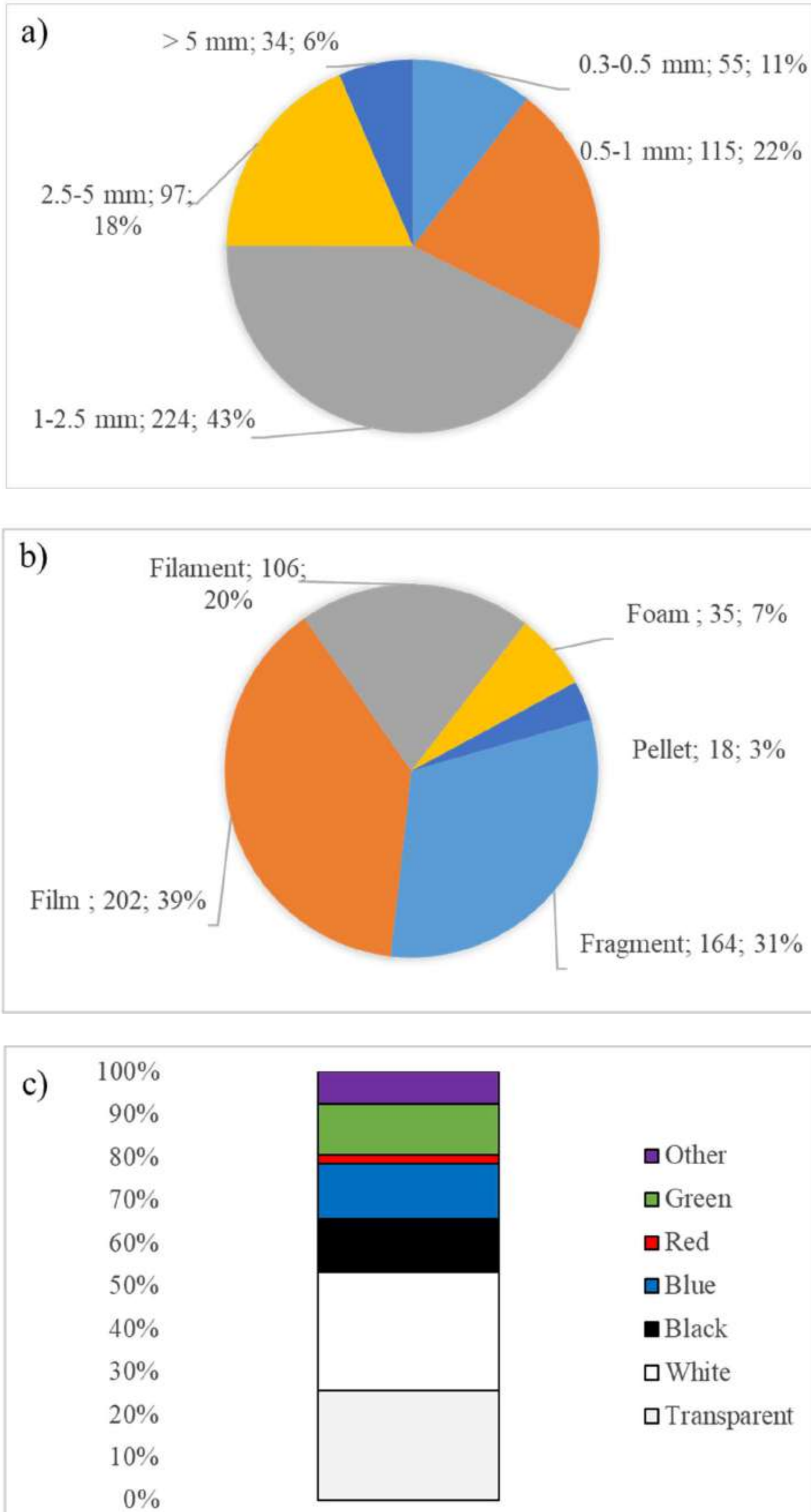
4.2.1 Marine Protected Area of Capo Milazzo

A total of 525 items were isolated from the 2 sea surface water samples of the Capo Milazzo MPA with a mean ± SD abundance of 0.20 ±0.13 items/m^2 corresponding to 1.24 ±0.84 items/m^3. Table 8 shows MPs abundance observed collected in the coastal waters of the Capo Milazzo MPA. This result is in agreement with the floating MPs abundances observed in other Mediterranean MPAs (De Lucia et al., 2018; Fagiano et al., 2022b, 2022a; Fossi et al., 2012; Galli et al., 2023) and consistent with the results of other studies conducted in Mediterranean basin (Table 3). The heterogeneity of MPs concentrations observed within Mediterranean basin could be attributed to different inputs source. Furthermore, methodological approaches used for sample collection such as the type and mesh size of the nets and the protocol used can affect the diversity of the data reported in the various studies (Baini et al., 2018).

Table 8. Plastic number, abundance expressed as items/m^3 and items/m^2 in water surface samples collected in the two transect (TR1; TR2) of the MPA of Capo Milazzo; mean ± standard deviation (SD) per each transect and total.

Station ID	Latitude	Longitude	N items	items/m^3	items/m^2
TR1	38°16'119"	15°13'473"	380	1.84	0.29
TR2	38°16'198"	15°13'240"	145	0.65	0.10
Total (mean±SD)			**525**	**1.24±0.84**	**0.20±0.13**

Analysis of the size distribution showed that the most abundant class size in the study site was 1 - 2.5 mm (n= 224; 43%). This finding is consistent with other studies conducted in the Tyrrhenian Sea (Baini et al., 2018; Galli et al.,2022; Galli et al., 2023; Marrone et al., 2021) following a trend observed in the Mediterranean bacin and in other oceans (Cózar et al. 2014; Eriksen et al., 2013; Lusher et al., 2014;Suaria et al., 2016; Compa et al., 2020; Fagiano et al., 2022b; 2022a).
The size classes of 0.5-1 mm (n= 102) and 2.5-5 mm (n= 97) accounted for the 19% and 18% of the total plastic while the size classes of 0.3–0.5 mm (n= 55; 11%) and > 5 mm (n= 34; 6%), accounted for the smaller portion of the total samples (Fig. 37a). The abundance of MPs plastics detected in surface waters poses a serious threat to a wide range of organisms with prey niches and feeding behaviours influencing potential transfer throughout the food web (Panti et al., 2015). The occurrence of small fragments in coastal water would suggest the presence of secondary plastics resulting from the fragmentation of meso and macro-plastics probably beached and/or transported by currents from distant sources.
Film (n=202; 39%) and fragment (n= 264; 31%) were the most abundant shape categories, followed by filament (n= 106; 20%), while foam (n= 35) and pellets (n= 18) accounted for less than 10% of the total (Fig. 37b). The greater abundance of films and fragments is not surprising as they are characterised by greater buoyancy, resulting in a longer residence time on the sea surface (Kooi et al., 2016; Galli et al., 2022). Furthermore, taking into consideration plastics size, shape and colour could help identify possible plastics sources and provide information on the potential factors regulating their distribution in the environment. Light coloured plastics (white and transparent) were the most frequent observed in the sea surface of Capo Milazzo MPA (Fig. 37c) as observed also in other regions (van der Hal et al., 2017; Fagiano et al., 2022a; Galli et al., 2022).


a)
> 5 mm; 34; 6%
0.3-0.5 mm; 55; 11%
0.5-1 mm; 115; 22%
2.5-5 mm; 97; 18%
1-2.5 mm; 224; 43%
b)
Filament; 106; 20%
Foam ; 35; 7%
Pellet; 18; 3%
Film ; 202; 39%
Fragment; 164; 31%
c)
100%
90%
80%
70%
60%
50%
40%
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Red
Blue
Black
White
Transparent

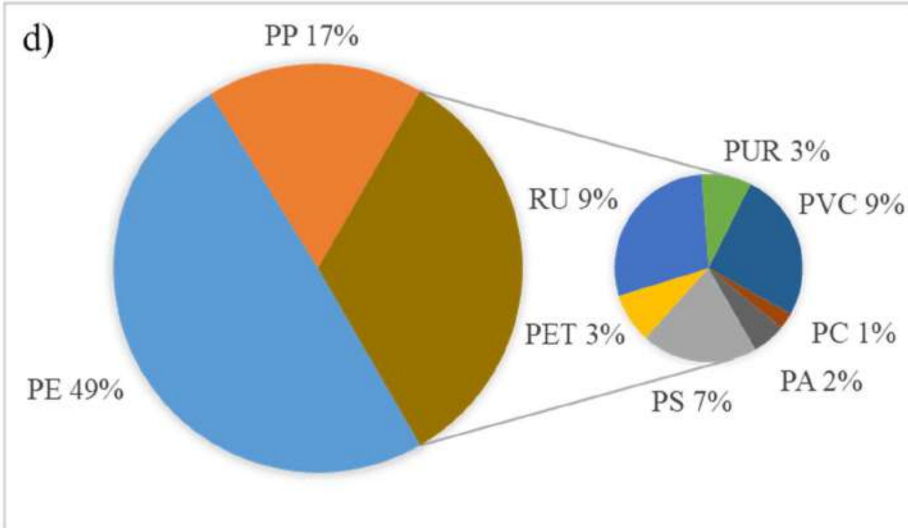


Figure 37. Classification of plastic from the surface water samples of Capo Milazzo MPA by size class (a) shape (b), colour (c) and percentage of polymer identified (d). Polymer types: PE= polyethylene; PP= polypropylene; PS=polystyrene; PET= polyethylene terephthalate; PC= polycarbonate; RU= undefined rubber; PUR= polyurethane; PA= polyamide (nylon); PVC= polyvinyl chloride.

A total of 105 items, correspondig to about the 20% of the total sample, were identified by LDIR (Fig.37d). This investigation allowed to identifiy 9 polymer types from the site of the Capo Milazzo MPA. The majority of plastic items identified were made of polyolefins, mainly PE (49%) and PP (17%) in agreement with findings in surface waters in other Mediterranean regions (Pedrotti et al., 2016; Suaria et al., 2016; Baini et al., 2018; Caldwell et al., 2019; Fagiano et al., 2022b; Galli et al., 2022) but also in surface waters worldwide (Eriksen et al., 2014; Enders et al., 2015; Suaria et al., 2016). The occurrence of polyolefins in marine surface waters depends mainly on their extensive use as packaging and SUP, density (0.917-0.965 g/cm^3 PE, 0.9-0.91 g/cm^3 PP) and susceptibility to degradation in the marine environment. In addition, 7 other polymers accounted for less than 10% of the total plastic articles identified. Seven other polymers were identified, representing less than 10% of total. The occurrence of these polymers in the MPA of Capo Milazzo could be related to several sources close to the study site, both marine, due to the degradation of fishing gear, and terrestrial, due to the presence of many productive activities in the Milazzo area, petroleum refineries, thermal power stations, shipyards, and a commercial and tourist ports (Battaglia et al., 2017).

This study report first data on MPs ingestion by investigating the abundance and composition of MPs in GITs of bentopelagic (*B. boops*), demersal (*M. surmuletus* and *Diplodus spp.*) and pelagic (*Auxis rochei*) fish in the study site.

A total of 24 specimens belonging to the 4 bioindicator species were examined. A summary of biological parameters (total length, total weight, gastrointestinal weigh) and abundance of MPs are reported in Table 9. In detail, 16 plastic items were found in 12 GITs (O%= 50): 3 plastic particles from 3 *B. boops* (%O = 50), 3 items from 3 *A. rochei* (%O = 50) and 7 items from 6 *M. surmuletus* (%O = 100). Similar occurrence for *B. boops* has been previously observed in the Tyrrhenian Sea by Sbrana et al. (2019) (56%) while lower values (37%) were reported by Giani et al. (2023). In addition, a study of Savoca et al. (2019) in the nearby Gulf of Patti assessing textile waste pollution reported a 67% occurrence for the same species. The MPs ingestion by bullet tuna was previously reported (Abiñon et al., 2021; Widyastuti et al., 2023; Chen et al., 2021) The occurrence of MP in this species could be linked to the tuna's tendency to feed on almost all available resources. No plastic ingestion was observed in *Diplodus spp.* GITs. It can be inferred that the absence of MPs in sea bream is linked to the ability of this species to discriminate natural prey from non-edible material as observed by Muller et al. (2020) in a study simulating natural conditions in the laboratory. *M. surmuletus* shows the highest occurrence in comparison with the other species of our study but consistent with previously reported ingestion values in the Northern Adriatic Sea (Neves et al., 2015). In agreement with some authors, the higher abundance of MPs in species that feed on the seafloor is not surprising since these habitats are considered sinking areas of MPs (Bellas et al., 2016). Furthermore, the highest ingestion of MPs observed in bottom-dwelling species, among those analysed could indicate a higher presence of plastics in the seafloor of the study site.

Table 9. Biometric parameters (total length (TL, cm), total weight (TW, g), GITs weight (GITW), Fulton's condition factor (K) and Gastro Somatic Index (GaSI) for each species from Capo Milazzo MPA. Plastics occurrence (% O) and abundance are also reported.

Data fish	*Boops boops*	*Diplodus sargus*	*Auxis rochei*	*Mullus surmuletus*	*Total*
N samples	6	6	6	6	**24**
TL (cm) mean ± SD	24.02±1.05	20.58±0.90	37.93±3.21	20.92±1.14	
TW (g) mean ± SD	235.82±218.66	128.93±13.26	621.05±256.14	137.08±47.30	
GITW (g)	8.34 ± 3.11	2.33 ±0.99	27.02 ±11.43	3.91 ±1.11	
K (g/cm^3)	0.99 ± 0.04	1.48 ±0.11	1.49 ±0.11	1.46 ±0.34	
GaSI	5.5 ± 1.41	1.84 ±0.67	3.84 ±2.20	3.29 ±1.67	

Data plastic					
N samples with plastics	3	0	3	6	**12**
N of plastic items	3	-	6	7	**16**
Plastics range	0-1	-	0-3	0-2	
Plastics occurrence (%O)	50	0	50.00	100.00	**50.0**
N. plastic items/N. all examined individuals	0.5	-	1.00	1.17	**0.67**
N. plastic items/N. individuals which ingested plastics	1	0	2.00	1.17	**1.33**

Bogues and striped red mullet showed K values of 0.99 (± 0.04) and 1.46 (±0.34), respectively, whereas bullet tuna and sea bream of 1.49 (±0.11) and 1.48 (±0.11) (Table 9). Similar values were observed in MPs ingestion studies previously conducted in the Mediterranean Sea (Capó et al., 2022; Giani et al., 2023; Müller et al., 2020). A positive strong correlation (Tau = 0.39) was found between the K index and the MPs abundance in *A. rochei* whereas a negative moderate (Tau = -0.43) and weak (Tau = -0.12) correlation was found in *B. boops* and *M. surmuletus*, respectively. The abundance of MPs was not related to Fulton's condition factor (K), as also observed in the present study for the same and different species investigated in the Calabria study area. Considering the well-being of fish could be important when investigating the impact of MPs. Some studies revels influence of ingested MPs on fish condition (Compa et al., 2018; Sbrana et al., 2020) while others not (Rummel et al., 2016; Garcia-Garin et al., 2019). Furthermore, the feeding intensity index was calculated for each species and the values are shown in Tab 9. A positive moderate correlation (Tau = 0.09) and a negative weak (Tau = -0.15) and strong (Tau = -0.35) correlation has been found between the MPs abundance and the GaSI index in *B. boops*, in *A. rochei* and in *M. surmuletus*, respectively.

Figure 38a shows the classification of plastic based on size-classes. The 80% of the items belonged from the size class of MPs (75% SMPs and 25% LMPs) ranged from 0.09 to 9.41 mm. In the GITs of *A. rochei* only SMPs with a mean length of 0.28(±0.27) were observed, in *M. surmuletus* SMPs and LMPs with a mean length of 0.09±0.01 and 2.94 (±2.61) respectively, while in *B. boops* only MEPs with a mean length of 7.22 (±3.10) were observed. As suggested by other authors (Bernal et al., 2015; Bray et al., 2019), this result could be related to the diet and behavioural traits of the species that in addition to feeding behaviour can influence the ingestion rate of MPs. *B. boops* can accidentally or intentionally ingest the MPs but also indirectly by feeding on contaminated prey (secondary ingestion)(Bottari et al., 2022). The same observation could be applied to *A. rochei* since this species feeds on small fish, fish larvae, crustaceans, and also shrimp, for which ingestion of anthropogenic debris has already been reported (D'Iglio et al., 2022). Striped red mullet feeds on benthic preys, swallowing sediment together with the prey, after identifying them with their barbels (Bellas et al., 2016). This feeding behaviour could lead to the accidental ingestion of small MPs that might be lodged in the sediment (Neves et al., 2015; Rodrigues et al., 2023).

The shape and the colour of plastic items (Fig. 38b) was quite variable between the species; filament was the only shape observed in *B. boops* in the colours of blue (50%) and transparent (50%) while green foam (25%) and white (25%) and blue fragment (25%) are the two shapes found in *A. rochei* and thirdly, with a proportion of 25%, pink film, transparent filament, yellow foam, and green fragment were found in *M. surmuletus.* The present results highlight that different fish species can ingest items that are similar in shape, size and colour to their prey (Bellas et al., 2016; Boerger et al., 2010; Capó et al., 2022). No fiber were observed in the GITs of the fish species of the present study in contrast with previous observation for *B. boops* in the nearby Gulf of Patty by other authors (Bottari et al., 2022; Savoca et al., 2019) and in other marine organisms investigated in the MPA of Capo Milazzo (Scotti et al., 2023). On the other hand, our results are in agreement with the observation of Pedà et al. (2020) which observed almost exclusively filaments in the stomach of several demersal elasmobranchs sampled in the Gulf of Patti. This similar result might be due to the application of the same control measures from contamination that allowed excluding fibers derived from secondary contamination.

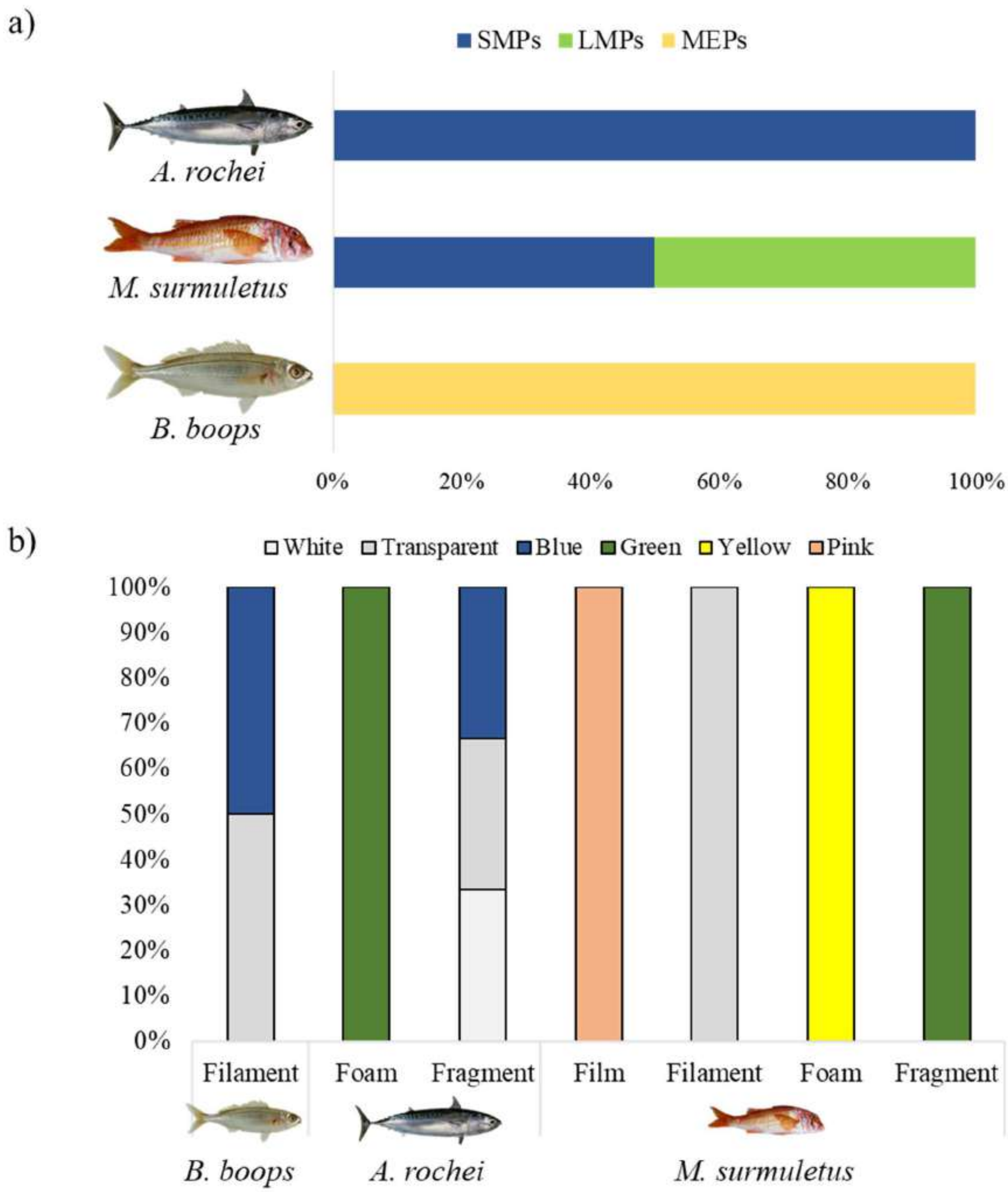


Figure 38. MPs percentage according to size-classes (a), shape and colour (b) classification in *B. boops, A. rochei* and *M. surmuletus* from Capo Milazzo MPA.

The LDIR analysis allowed the chemical identification of all the plastics (n= 16) isolated from fish GITs of the species studied (Fig 39). A total of six different polymers were identified, with PE (56% of the total) being the main polymer type found in all species followed by PA found in *B. boops* (33%) and *M. surmuletus* (29%). The other polymers identified in *M. surmuletus* were PS (14%) and RU (14%) while in *A. rochei* PUR (17%) and EPR (17%). This result is consistent with previous studies in the nearby Gulf of Patti, where in addition to the predominance of polyolefins, the ingestion of rubber was also observed in the stomach contents of demersal and benthopelagic fish species (Pedà et al., 2020; Bottari et al., 2022) and cephalopods (Pedà et al., 2022a). Similarly, the presence of rubbers was also observed in our study, which as reported by Bottari et al. (2022) could be related to the motorway-related pollution (rubbers from automotive manufacturers and component suppliers) or or the proximity of several industrial facilities. MPAs represent an important management tools aiming at the conservation of areas with high ecological value in terms of habitats and biodiversity. These sites are protected from direct anthropogenic impacts (anchoring of boats, maritime traffic or exploitation of marine resources) but as reported by several studies (Capó et al., 2022; Fagiano et al., 2022a; Fossi et al., 2017; Galli et al., 2023) these areas are not immune to plastic pollution. The results of the present study provide first data on the presence of MPs in the surface waters of the Capo Milazzo MPA and confirm that marine organisms in MPAs are exposed to MPs, highlighting the potential transfer of pollutants to MPAs from nearby anthropised areas.

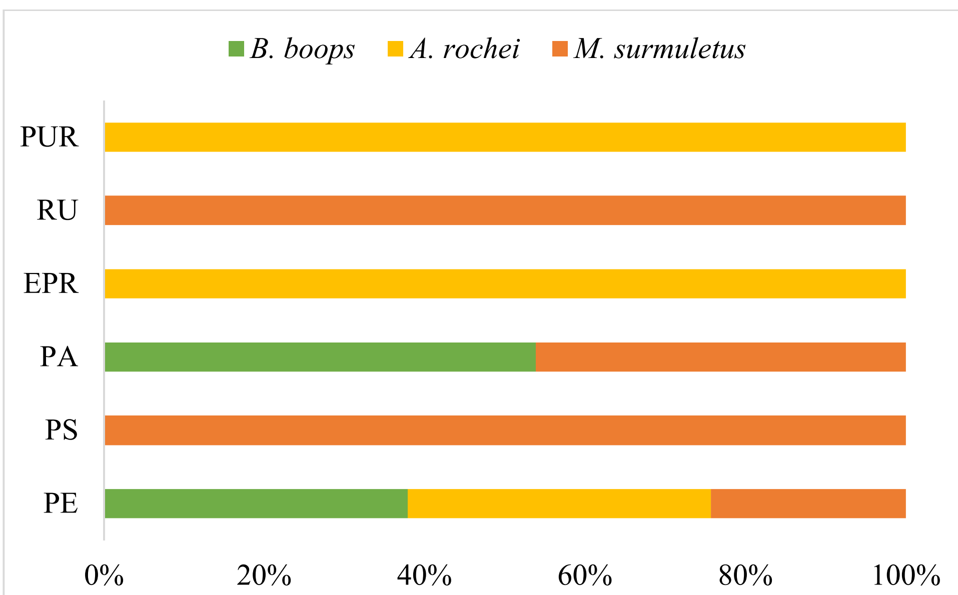


Figure 39. Polymers abundance (%) in fish samples of Capo Milazzo MPA. PE=polyethylene; PS=polystyrene; PA=polyamide; EPR= Ethylene propylene rubber; RU=undefined rubber; PUR= polyurethane.

4.2.2 Archipelago of Aeolian Island

Plastic particles were found in all 6 sea surface water samples collected at the study site of Aeolian Islands with a mean concentration of 0.34 ±0.20 items/m^2 (mean ± SD), corresponding to 1.83 ± 1.21 items/m^3.Table 10 shows data on plastics abbundance for each transect. Many investigations were carried out in the Tyrrhenian Sea to assess MPs pollution in waters (Baini et al., 2018; Caldwell et al., 2019; Suaria et al., 2016). At local scale, higher concentrations of floating MPs were detected during our survey in comparison to previous data for the island of Lipari (0.3 item/m^3) by Mezzelani et al. (2017) and by De lucia et al. (2018) (0.27 ± 0.08 item/m^3).

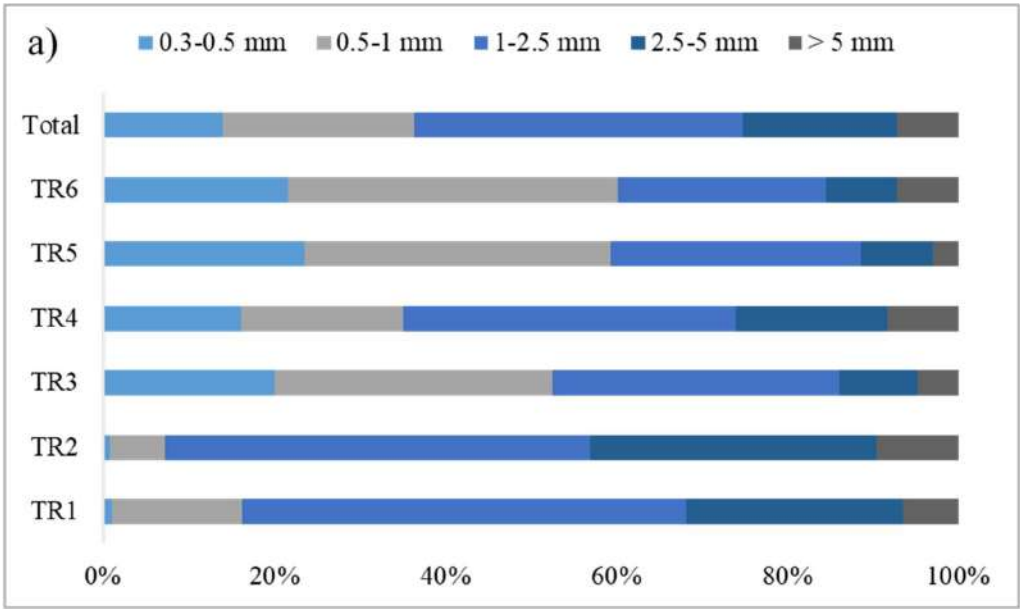
Table 10. MPs number, abundance expressed as items/m3 and items/m2 in sea surface water samples collected in the 6 transect (TR1; TR2, TR3, TR4, TR5 and TR6) at Aeolian Island study site; mean ± standard deviation (SD) per each transect and total.

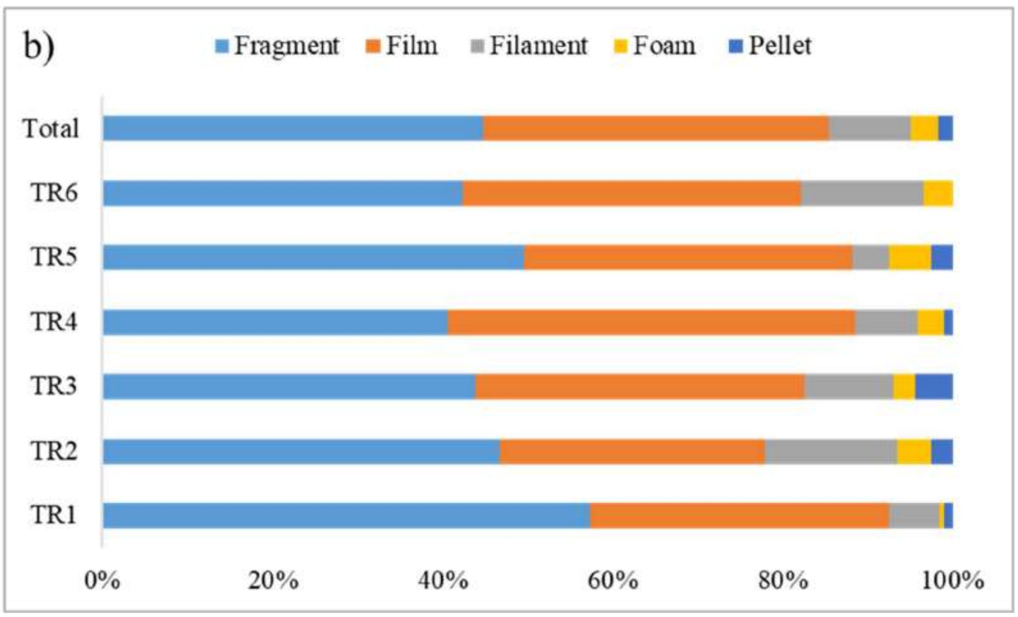
Station ID	Latitude	Longitude	N items	items/m^3	items/m^2
TR1	38°49'447"	15°13'298"	185	1.05	0.17
TR2	38°48'128"	15°08'606"	385	2.01	0.32
TR3	38°38’06”	15°00’46”	230	1.11	0.18
TR4	38°40’55”	15°04’07”	799	5.81	0.67
TR5	38°30'825"	14°58'428"	238	3.08	0.49
TR6	38°27'897"	15°00'317"	291	1.44	0.23
Total (mean±SD)				**1.83±1.20**	**0.34±0.20**

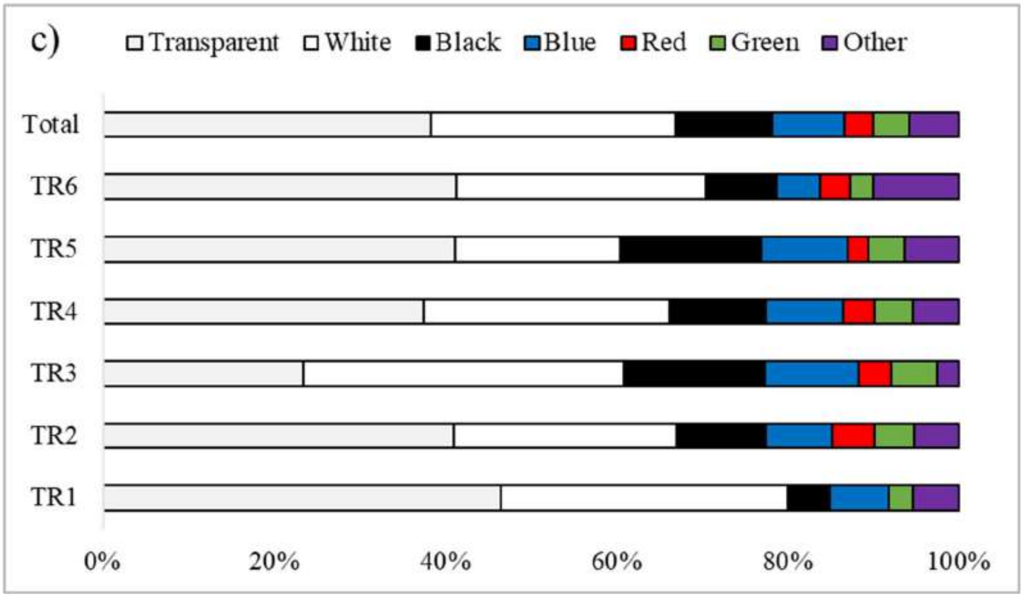
MPs characterization analysis showed that the most abundant MPs size class in the study area was 1-2.5 mm (n=816; 38%), followed by 0.5-1 mm (n= 476; 23%) and 2.5-5mm (n= 383; 18%). MPs belonging to this size classes were found to be the most abundant in several areas of the Mediterranean as reported by Baini et al. (2018); Galli et al. (2022); Pedrotti et al. (2016); Ruiz-Orejón et al. (2016); Trani et al. (2023); Zeri et al. (2018). Items within the size classes of 0.3-0.5 mm (n= 299; 14%) and >5 mm (n= 154; 7%) were also found, albeit with lower abundance (Fig. 40a). This dimensional pattern has also been previously observed in the Mediterranean Sea (Suaria et al., 2016; Baini et al., 2018; Galli et al., 2023). A study by Fastelli et al. (2016) assessing plastic litter in sediments of the Aeolian Archipelago, shows the predominance of plastics (40.35%) ranging from 1.0 mm to 63 μm in size. Comparing the size classes of the plastics observed on the sea surface and in the depths of the area, a greater presence of smaller MPs in the depths than in surface waters can be observed. This would confirm that smaller MPs are more susceptible to vertical transport (Reisser et al., 2015) also due to the rapid formation of fouling or aggregation with phytoplankton that facilitates the sinking of MPs along the water column (Andrady, 2017).

Fragment (n= 952; 45%) and film (n= 865; 40%) were the most abundant shape categories in the whole site (Fig. 40b) followed by filament (n= 206; 10%), while foam (n= 69; 3%) and pellet (n= 36;

2%) categories accounted for the smaller portion of the total. Figure 40c shows the categorization based on plastic colour, whit light-coloured plastics such as transparent (38%) and white (29%) dominating over the other colours. It seems that the fragmentation of large plastic manufactured was the main source of MPs. In addition, the MPs distribution along the water column is also driven by their buoyancy (Kooi et al., 2016). The greater presence of films and fragments observed in the sea surface during our analysis confirms their greater persistence over time at the surface than filaments, which, based on the data previously provided for the study area (Fastelli et al., 2016), were the most abundant shape found in the sediment.


a)
0.3-0.5 mm
0.5-1 mm
1-2.5 mm
2.5-5 mm
> 5 mm
Total
TR6
TR5
TR4
TR3
TR2
TR1
0%
20%
40%
60%
80%
100%


b)
Fragment
Film
Filament
Foam
Pellet
Total
TR6
TR5
TR4
TR3
TR2
TR1
0%
20%
40%
60%
80%
100%



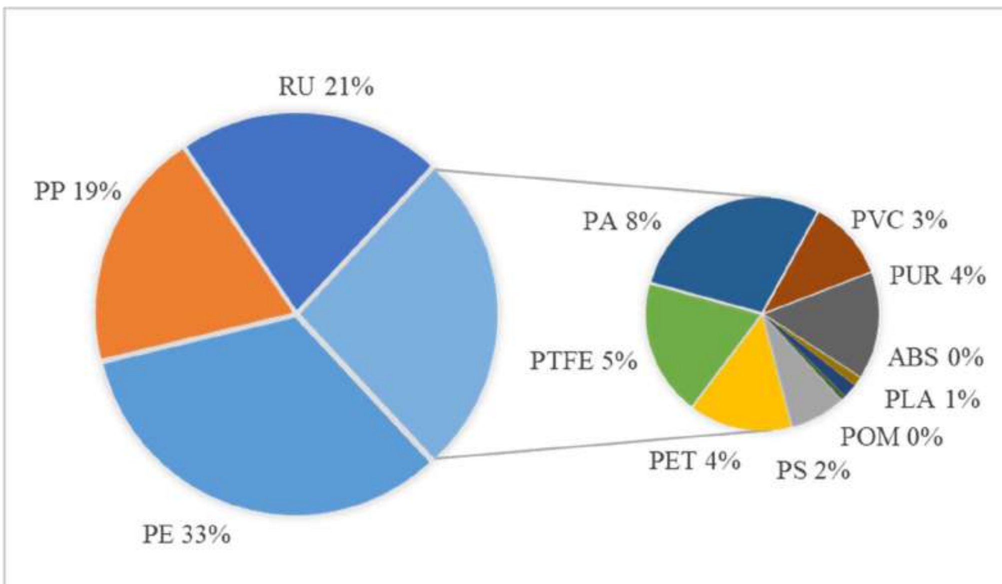


Figure 40. Classification of plastic from the surface water samples of the Aeolian Islands site by size class (a) shape (b), colour (c) and percentage of polymer identified (d). Polymer types: PE= polyethylene; PP= polypropylene; RU= undefined rubber; PS=polystyrene; PET= polyethylene terephthalate; PVC= polyvinyl chloride; PUR= polyurethane; PA= polyamide (nylon); ABS= acrylonitrile butadiene styrene; PLA= polylactic Acid; POM= polyoxymethylene; PTFE= polytetrafluoroethylene.

A Principal Component Analysis (PCA) has been performed as an exploratory data analysis to investigate sampling transect could influence abundance and characteristic of microplastics (shape, size class and colours) the results are represented in the Biplot (fig.41).

A total of 523 items (~20% of the total sample), were characterized using LDIR (Fig. 40d). According to the polymer characterization, plastics quantified within the study site were composed mainly of PE (33%), RU (21%) and PP (18%) while the 8% was PA. Finally, eight other polymers were found to be less than 5% of the total plastic items identified within the study site. Our results is in agreement with the observation reported by De Lucia et al., (2018) showing the predominance of polyolefins in the sea surface of the Aeolian Island. The polymeric composition of the floating MPs of the Aeolian Archipelago suggests the existence of an anthropic pressure insisting on the area mainly linked to fishing activity, considering the presence of an important artisanal fishing fleet in the area but also a pressure linked to seasonal tourism both from land and sea (Battaglia et al., 2017; Consoli et al., 2021). Finally, the possible transport of these pollutants from contiguous areas cannot be ruled out.

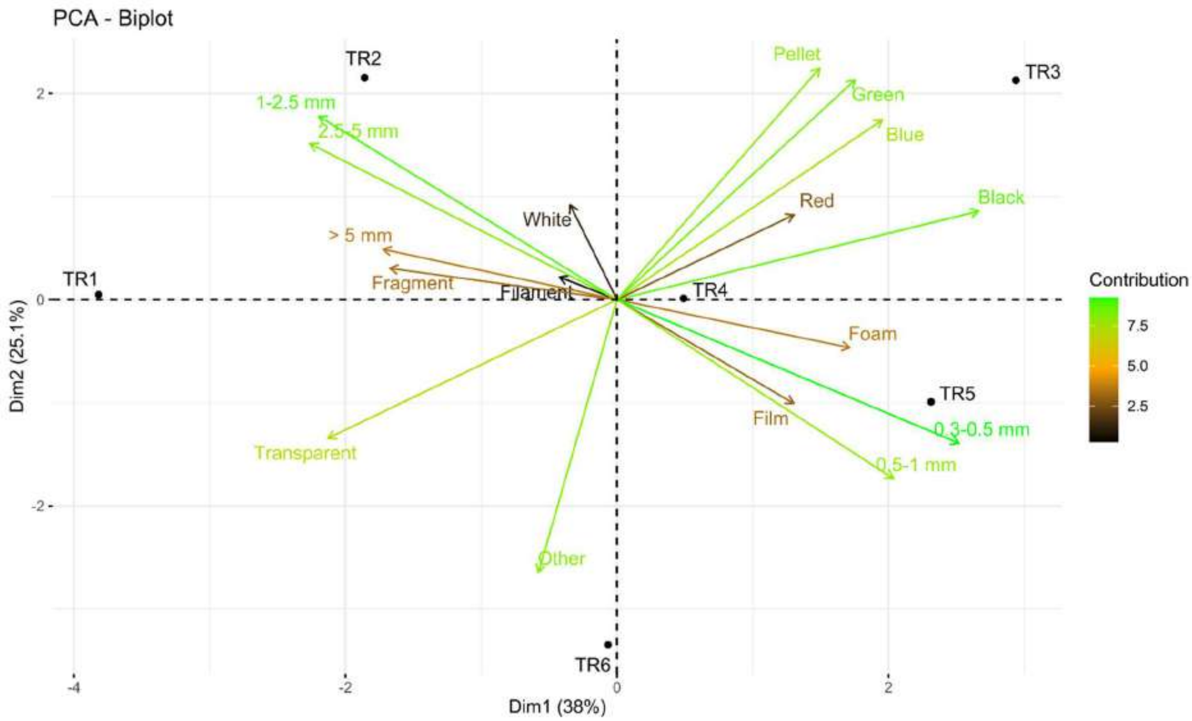


Figure 41. PCA of the percentage abundance of MPs observed in each transect in the Aeolian archipelago based on their shape, colour and size class.

The present study constitutes the first report of MPs ingestion by bentopelagic, demersal and pelagic fish species in the Aeolian Archipelago.

A total of 24 specimens belonging to the four bioindicator species were analysed. A total of 20 particles were isolated from 11 samples (O%= 45.83). The results for each species are presented in Table 11.

Table 11. List of species investigated in the Aeolian island site. Data are reported on the number of samples (n), main biometric data (total length (TL, cm), total weight (TW, g) expressed as average, standard deviation (SD) and ranges), number of individuals analysed who had ingested plastic, and data on the occurrence and abundance of ingested plastic particles.

Data fish	*Boops boops*	*Diplodus spp.*	*Auxis rochei*	*Mullus surmuletus*	*Total*
N samples	6	6	6	6	**24**
TL (cm) mean ± SD	24.97±1.84	24.50±1.07	24.60±1.37	19.83±2.81	
TW (g) mean ± SD	155.67±28.98	243.79±64.86	183.33±36.02	100.58±43.72	
GITW (g)	8.34 ± 3.11	6.79 ±2.37	2.15 ± 0.54	4.51 ± 2.04	
K (g/cm^3)	0.99 ± 0.04	1.64 ± 0.27	1.22 ± 0.09	1.21 ± 0.08	
GaSI	5.5 ± 1.41	2.99 ± 1.21	1.21 ± 0.4	4.65 ± 0.34	
Data plastic					
N samples with plastics	5	1	2	3	**11**
N of plastic items	12	2	2	4	**20**
Plastics range	0-7	0-2	0-1	0-1	
Plastics occurrence (%O)	83.33	16.66	33.33	50.00	**45.8**
N. plastic items/N. all examined individuals	2	0.33	0.33	0.67	**0.83**
N. plastic items/N. individuals which ingested plastics	2.40	2.00	1.00	1.33	**1.82**

Overall, 12 items were isolated from 5 *B. boops* GITs (O%= 83.33), 2 items from 1 *Diplodus spp.* (O%= 16.66), 2 items from 2 *A. rochei* (O%= 33.33) and 4 items from 3 *M. surmuletus* (O%= 50).

Our findings show that different species from the same study area may have different levels of MPs occurrence. Contrary to that observed at the Capo Milazzo MPA site, in the Aeolian archipelago bogue showed the highest ingestion rate. A higher abundance of MPs found in benthopelagic species than in demersal species has also been observed in other Mediterranean sites (Giani et al., 2023). This could be related to a series of factors including a heterogeneous distribution of particles along the water column that may influence the different MPs uptake (Reisser et al., 2015) but also the different diet of the species based on the types of prey available in the area. The fish body condition could also contribute to the different uptake of MPs. For this purpose, the condition factor K and the GaSI were calculated and the values are shown in table 11. In agreement with Giani et al. (2023), the species that had ingested the most plastics showed lower biological condition index. Kendall correlation showed no correlation between K index and the MPs abundance in *B. boops* (Tau = 0) and in *A. rochei* (Tau = 0) and a moderate correlation was observed in *Diplodus spp.* (Tau = 0.59) and *M. surmuletus* (Tau = 0.39) while correlation between GaSI and the number of ingested items showed a negative strong correlation in *A. rochei* (Tau=-0.73) and weak correlation in *B. boops* (Tau = 0.15), *Diplodus spp* (Tau = 0.12) and *M. surmuletus* (Tau = 0.7). The results of the statistical analysis suggest the development of further investigations to assess the effects of MPs on fish health.

All items belonged to the size class of MPs (83% SMPs and 17% LMPs). Figure 42a shows the percentage abundance of MPs according to their size. In *B. boops* and *A. rochei*, 100% of the plastics belonged to the size class of SMPs with a mean length of 0.58 ± 0.28 and of 0.56 ± 0.13 (mean± SD), respectively, whereas in *M. surmuletus* the plastics were SMPs (67%) and LMPs (33%) with a mean length of 1.04 ± 0.83 and also in *Diplodus spp.* both SMPs and LMPs with a mean length of 1.76 ± 2.2 were measured with a proportion of 50%. Among fish species, there are multiple feeding strategies, which influence MP ingestion. Considering the feeding habits and behaviour of the species studied, we assume that the ingestion of MPs at this site may have occurred accidentally, especially in species that feed by swallowing sediment, such as the striped mullet (Felline et al., 2022), or in species that are not selective in predation, such as the bullet tuna, which tends to feed on almost any available resource (Widyastuti et al., 2023). The same observation could also be applied for *B. boops* which, moreover, like other species, can also ingest MPs, by secondary ingestion, indirectly ingesting prey that already carry MPs (Neves et al., 2015; Romeo et al., 2015; Fossi et al., 2018; Nelms et al., 2018; Bottari et al., 2022).

Fragment was the only shape common to all the species from the site of Aeolian Islands (Figure 42b). *B.boops* ingested fragment (92%) and fiber (8%); *Diplodus spp.* fragment (50%) and fiber (50%); *A. rochei* ingested only fragment (100%); *M. surmuletus* ingested fragment (50%), foam (25%) and fiber

(25%). Transparent was the most common colour, observed in all the species, mainly in the shape of fragments (56%), and six other colours were observed in fibers, fragments and foam. According to Kühn et al. (2015), specific colours might attract predators, which may confuse ML for their prey. The prevalence of light-coloured MPs in the examined GITs could be linked to a possible resemblance to potential prey of the species, suggesting that fish are not able to discriminate MPs from fish, as the colours are not visibly different.

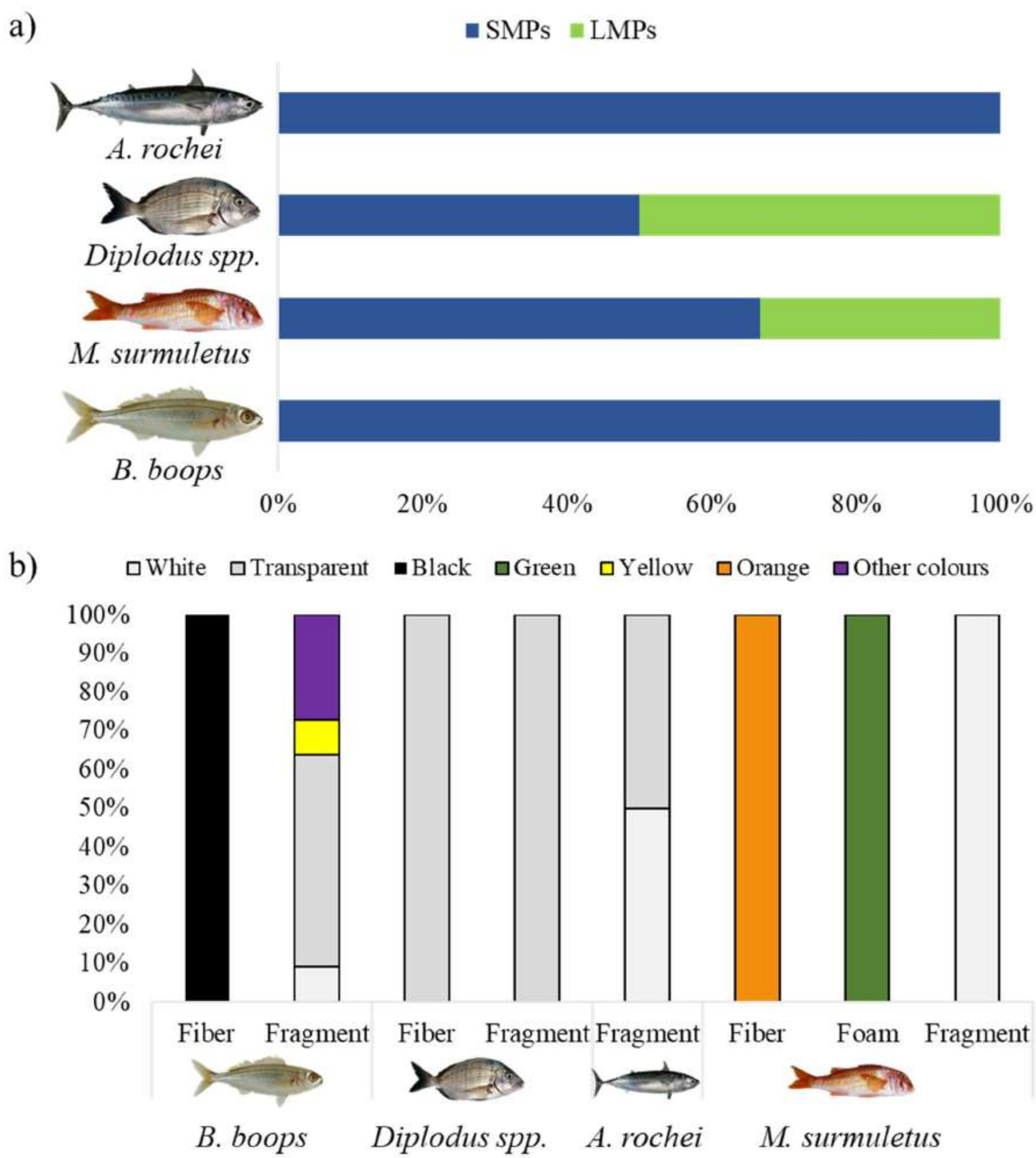


Figure 42. MPs percentage according to size-classes (a), shape and colour (b) classification in *B. boops, Diplodus spp., A. rochei* and *M. surmuletus* from Aeolian Islands site.

The polymeric nature of all the particles isolated from the fish GITs were identified by the LDIR. Figure 43 shows the polymer types identified. Six polymers were identified and only PP and PET were found in more than one of the species. Specifically, PP was identified in bogue and bullet tuna while PET in sea bream and striped mullet. The highest polymer diversity was observed in *B. boops* (33%PE; 50%PP; 17% PA). The greater number of polymers observed in *B. boops* could be related to feeding habit, since this species feeds on both pelagic and benthic prey, is exposed to both floating and settled MPs (Garcia-Garin et al., 2019; Rodrigues et al., 2023). Furthermore, this result is consistent with the distribution pattern of polymers according to density, with PET (1.37−1.45 g/cm^3) and PVC (1.16−1.58 g/cm^3), characterised by higher density than seawater (Hanke et al., 2013), being ingested by bottom dwelling species and PP and PE, more buoyant, in pelagic (*A. rochei*) and bentopelagic (*B. boops*) species. In particular, PE and PP are considered the more commonly polymers found in all the marine environments (Frias et al., 2014). Their ingestion by several demersal, benthopelagic and pelagic species has already been observed in the southern Tyrrhenian Sea by (Savoca et al., 2019; Capillo et al., 2020; Pedà et al., 2020; Schirinzi et al., 2020; Bottari et al., 2022). Overall, the polymers identified in the species studied were all used in fishing, suggesting a possible link to the degradation of lost or abandoned artisanal fishing gears in the study area.

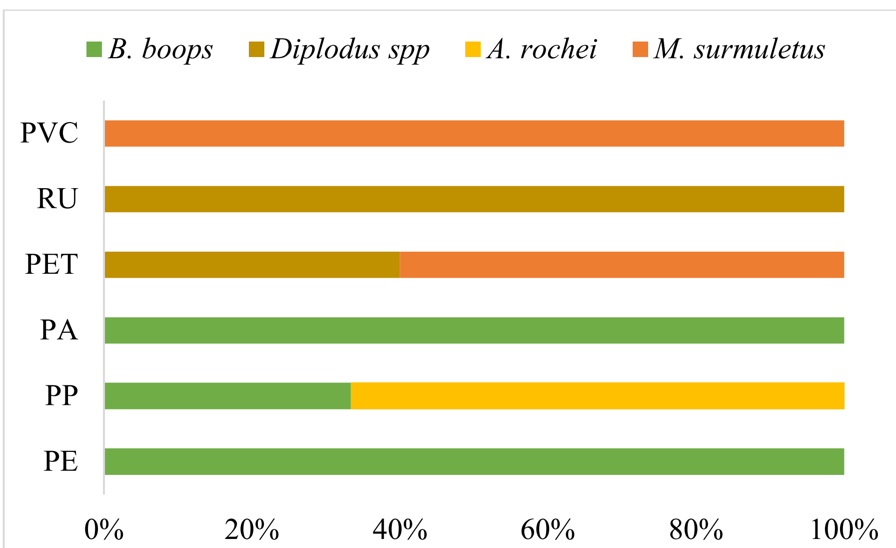


Figure 43. Polymers abundance (%) in fish samples from Aeolian Islands site. PE=polyethylene; PP= polypropylene; PA=polyamide; PET=polyethylene terephthalate; RU=undefined rubber; PVC= polyvinylchloride.

5 Conclusion

This study used an integrated approach that aimed to assess the presence of plastics both on the sea water surface and in the stomach contents of selected species considered to be bio-indicators of MPs ingestion. The use of this approach provided data on the status of plastic pollution in the study areas that could highlight the presence and distribution of MPs in different compartments of the marine environment.

Furthermore, to the best of our knowledge, this study provides first evidence of MPs ingestion by selected bio-indicator species never before investigated in the study areas. In addition, for the first time in the Calabria study area, the microbial community associated with plastics isolated from the sea surface was analysed, revealing the presence of various species, including pathogens and hydrocarbon degrading species.

The use of standardised protocols allowed the production of comparable data that will help meet the requirements of the MSFD for achieving good ecological status (GES).

The high heterogeneity between the results obtained from this study and the data in the literature for these same study areas and other Mediterranean sub-regions stresses the need to create and adopt standardised monitoring protocols to share comparable and consistent data. The challenge for the scientific community is to identify standardised protocols and methods for assessing and monitoring plastic pollution in marine compartments and to ensure comparability and reliability of data.

Finally, this investigation, which also included the assessment of MPs in two MPAs, highlighted how these sensitive areas are not immune to MP pollution and demonstrates how assessment studies applying standardised protocols at these areas are important for preserving these sites and planning possible management and mitigation measures.

6 Scientific contributions

Annex 1 – F. Laface, C. Pedà, F. Longo, F. De Domenico, R. Minichino, P. Consoli, P. Battaglia, S. Greco, T. Romeo 2021. Evidence of microplastics ingestion in two commercial cephalopod species: *Octopus vulgaris* and *Sepia officinalis. Accepted for XV. International Marine Pollution and Management Conference November 11-12, 2021 Rome, Italy. Vol:15, No:11, 2021.*

Annex 2 - T. Bottari, M. Mancuso, C. Pedà, F. De Domenico, **F. Laface**, G. F. Schirinzi, P. Battaglia, P. Consoli, N. Spanò, S. Greco, T. Romeo 2021. Microplastics in the bogue, *Boops boops*: A snapshot of the past from the southern Tyrrhenian Sea. Journal of Hazardous Materials, 424, 127669. https://doi.org/10.1016/j.jhazmat.2021.127669.

Annex 3- C. Pedà, F. Longo, C. Berti, **F. Laface**, F. De Domenico, P. Consoli, P. Battaglia, S. Greco, T. Romeo 2022. The waste collector: information from a pilot study on the interaction between the common octopus (*Octopus vulgaris*, Cuvier, 1797) and marine litter in bottom traps fishing and first evidence of plastic ingestion. Marine Pollution Bulletin, 174, 113185. https://doi.org/10.1016/j.marpolbul.2021.113185.

Annex 4- F. Laface, C. Pedà, M. Nannini, G. Cangemi, V. Sciutteri, P. Battaglia, T. Romeo. 2022. May mesopelagic fishes play an important role as vector of microplastics across the Mediterranean trophic web? A case of study in the Strait of Messina. Accepted for International Conference on Microplastic Pollution in the Mediterranean Sea – μMED 2022, September 25-28, 2022, Naples, Italy. https://doi.org/10.1007/978-3-031-34455-8.

Annex 5- F. Laface, F. Longo, C. Pedà, M.G. Stipa, P. Battaglia, C. Berti, P. Consoli, S. Greco, T. Romeo. 2022. The use of artificial baits in swordfish longline fishery: potential impacts on trophic web assessed from fishers' Local Ecological Knowledge and stomach content analysis- S.It.E SIENA 2022, September 13-15, Siena, Italy. Acts of Congress pag. 218.

Annex 6-F. Laface, C. Pedà, P. Battaglia, C. Berti, P. Consoli, F. De Domenico, F. Longo, S. Greco, T. Romeo. 2022. Use of Fourier transform infrared (FT-IR) spectroscopy for the polymeric composition assessment of microplastics ingested by Mediterranean fishery resource- Chimica Analitica 2022, September 11-15, Milazzo, Italy. ISBN: 978-88-94952-30-8, 444-445.

Annex 7- S. Scozzafava, **F. Laface**, C. Giommi, C. Pedà, D. Pica, N. Ruocco, T. Romeo, S. Greco. Bio-based materials for sustainable mussel production: preliminary results of a multidisciplinary study- ESCPB 2022, August 28-31, Naples, Italy. Book of abstract pag. 129-130.

Annex 8- R. Calogero, E. Arcadi, C. Giommi, S. Scozzafava, **F. Laface**, S. Greco, T. Romeo, C. Rizzo. 2022. Microbial colonization in mussel farming facilities made with Mater-Bi, an innovative biodegradable material- ESCPB 2022, 28-31 agosto, Napoli, Italia. Book of abstract pag. 115-116.

Annex 9- C. D'Iglio, Dario Di Fresco, N. Spanò, M. Albano, G. Panarello, **F. Laface**, C. Faggio, G. Capillo, S. Savoca 2022. Occurrence of anthropogenic debris in three commercial shrimp species from south-western Ionian Sea. Biology 11, 1616. https://doi.org/10.3390/biology11111616.

Annex 10- F. Laface, C. Pedà, c. Giommi, S. Scozzafava, C. Rizzo, D. Malara, S. Greco, T. Romeo 2023. A glimpse into the future: A suitable methodological approach for the detection and identification of micro-bioplastics in biota. Sci. Total Environ. 899, 165613. https://doi.org/10.1016/j.scitotenv.2023.165613.

Annex 11- M. Galli, M. Baini, C. Panti., D. Giani, I. Caliani, T. Campani, M. Rosso, P. Tepsich, V. Levati, **F. Laface**, T. Romeo, G. Scotti, F. Galgani, M.C. Fossi 2023. Oceanographic and anthropogenic variables driving marine litter distribution in Mediterranean protected areas: Extensive field data supported by forecasting modelling. Sci. Total Environ. 166266. https://doi.org/10.1016/j.scitotenv.2023.166266.

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Evidence of Microplastics Ingestion in Two Commercial Cephalopod Species: Octopus Vulgaris and Sepia Officinalis

Authors : Federica Laface, Cristina Pedà, Francesco Longo, Francesca de Domenico, Riccardo Minichino, Pierpaolo Consoli, Pietro Battaglia, Silvestro Greco, Teresa Romeo

Abstract : Plastics pollution represents one of the most important threats to marine biodiversity. In the last decades, different species are investigated to evaluate the extent of the plastic ingestion phenomenon. Even if the cephalopods play an important role in the food chain, they are still poorly studied. The aim of this research was to investigate the plastic ingestion in two commercial cephalopod species from the southern Tyrrhenian Sea: the common octopus, Octopus vulgaris (n=6; mean mantle length ML 10.7 ± 1.8) and the common cuttlefish, Sepia officinalis (n=13; mean ML 13.2 ± 1.7). Plastics were extracted from the filters obtained by the chemical digestion of cephalopods gastrointestinal tracts (GITs), using 10% potassium hydroxide (KOH) solution in a 1:5 (w/v) ratio. Once isolated, particles were photographed, measured, and their size class, shape and color were recorded. A total of 81 items was isolated from 16 of the 19 examined GITs, representing a total occurrence (%O) of 84.2% with a mean value of 4.3 ± 8.6 particles per individual. In particular, 62 plastics were found in 6 specimens of O. vulgaris (%O=100) and 19 particles in 10 S. officinalis (%O=94.7). In both species, the microplastics size class was the most abundant (93.8%). Plastic items found in O. vulgaris were mainly fibers (61%) while fragments were the most frequent in S. officinalis (53%). Transparent was the most common color in both species. The analysis will be completed by Fourier transform infrared (FT-IR) spectroscopy technique in order to identify polymers nature. This study reports preliminary data on plastic ingestion events in two cephalopods species and represents the first record of plastic ingestion by the common octopus. Microplastic items detected in both common octopus and common cuttlefish could derive from secondary and/or accidental ingestion events, probably due to their behavior, feeding habits and anatomical features. Further studies will be required to assess the effect of marine litter pollution in these ecologically and commercially important species.

Keywords : cephalopods, GIT analysis, marine pollution, Mediterranean sea, microplastics
Conference Title : ICMPM 2021 : International Conference on Marine Pollution and Management
Conference Location : Rome, Italy
Conference Dates : November 11-12, 2021

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Research Paper

Microplastics in the bogue, *Boops boops*: A snapshot of the past from the southern Tyrrhenian Sea

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ABSTRACT

The present investigation focuses on *Boops boops* specimens gathered in the Gulf of Patti in 2010. Providing a snapshot from the past, this paper represents, chronologically, the first record of microplastic ingestion in the Mediterranean bogue. The plastic abundance and composition in gastrointestinal tracts of the bogue was assess, in order to improve the knowledge on spatial-temporal variability of microplastics pollution in the Mediterranean basin and in particular, in the southern Tyrrhenian Sea. In a total of 65 specimens, 180 particles of plastic (2.8 items/specimens), mainly belonging to microplastics class, were found. Fragments (63%) and fibres (30%) were the predominant shape categories. Eleven polymers were identified: polypropylene and polyethylene were the most abundant. Several synthetic polymers belonging to the class of elastomers were also observed. The study area is strongly influenced by the absence of trawl fishing activities and a low mixing level of the seabed that, together with the confluence of different watercourses and the presence of different kind of anthropic impact, including motorway, could make it a 'waste disposal site'. Finally, our results suggest the usefulness to retrieve older samples to better understand spatial-temporal changes in marine litter pollution over time.

1. Introduction

Marine litter is considered one of the main issues of anthropogenic pollution that has affected the marine ecosystem in the last few decades (Galgani et al., 2015). In particular, plastic litter is the most abundant type of marine debris, and its impact represents a serious hazard affecting worldwide biodiversity. Because the Mediterranean Sea is one of the most impacted regions in the world (Suaria et al., 2016; Llorca et al., 2020; Tsangaris et al., 2020), the scientific effort, in accordance with the recommendations of the Marine Strategy Framework Directive (MSFD; EC 2017/848), has mainly focused on monitoring and assessing the amount and composition of litter and microlitter in marine ecosystems as well as their impacts on marine fauna, to understand and to mitigate the potential effects at different trophic levels. In particular, the MSFD considers the study of fish stomach contents important to define the trends of marine plastic ingestion from European waters, especially in selected and standardised bioindicator species.

In this context, some researchers have proposed the most suitable organisms for plastic ingestion monitoring in the Mediterranean basin. They have generally considered the following criteria to individuate the

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key species: (a) background, habitat and trophic information; (b) feeding behaviour and spatial distribution; (c) commercial value and conservation status; and (d) available data on microlitter ingestion (Fossi et al., 2018; Bray et al., 2019; Tsangaris et al., 2020). Regarding microplastics (MPs) pollution in the Mediterranean Sea, the bogue – *Boops boops* (Linnaeus, 1758) – has been identified as a suitable small-scale indicator for monitoring MPs in coastal waters (Fossi et al., 2018) because it meets the suggested criteria (Tsangaris et al., 2020).

B. boops is a benthopelagic and gregarious species, inhabiting a broad depth range distribution from 0 to 350 m, commonly between 0 and 100 m. It occurs in coastal and pelagic waters on different types of bottom including sand, mud, rocks and seagrass beds. *B. boops* is an omnivorous species, feeding on a wide variety of prey, preferring crustaceans and cnidaria. The bogue is included within the top 13 most landed demersal fish species in the Mediterranean Sea (Lleonart and Maynou, 2003) and it is a commercial species caught by several Mediterranean fisheries. To date, MPs ingestion in *B. boops* has been widely documented in the Mediterranean Sea (Nadal et al., 2016; Garcia-Garin et al., 2019; Rios-Fuster et al., 2019; Savoca et al., 2019; Sbrana et al., 2020; Tsangaris et al., 2020) and it has been chosen as bioindicator species for MPs ingestion in the MEDSEALITTER, PLASTIC BUSTERS MPAs Interreg Project and UNEP/MAP MED POL programme. In particular, the recent study of Tsangaris et al. (2020) investigated the MPs ingestion by *B. boops* on Mediterranean basin scale supporting their suitability as bioindicator species. However, in the southern Tyrrhenian Sea only the Savoca et al. (2019) revealed the occurrence of man-made cellulose fibres in specimens of *B. boops.* Although the MPs ingestion by fish species has been investigated since 1988 (Fossi et al., 2018; Anastasopoulou and Fortibuoni, 2019) through the application of different plastic extraction methods (Pedà et al., 2020), about *B. boops* the most recent study date back to 2014 (Nadal et al., 2016).

In the light of these considerations, the present study investigates the MPs abundance and composition in gastrointestinal tracts (GITs) of Mediterranean bogue from 2010 with the aim to take a picture of the MPs pollution condition ten years ago. Thus, these data represent, chronologically, the first record of MPs ingestion for this species. Because, the bogue is a good bioindicator of MPs ingestion, this study, also, aims to provide further information on MPs pollution in the southern Tyrrhenian Sea, giving us the opportunity to improve the knowledge in this Mediterranean area.

2. Material and methods

2.1. Study area and sampling

Sampling was carried out in October 2010 on board the Scientific Research Vessel *Maria Grazia*, during an experimental trawl survey in the Gulf of Patti, licenced by national and local authorities (southern Tyrrhenian Sea, GSA 10) (Fig. S-1).

The Gulf of Patti is a Fishery Exclusion Zone (FEZ) and trawling has been banned since 1990 to date; only small-scale fisheries are permitted (Battaglia et al., 2017). It is characterised by different kinds of anthropogenic and natural factors including touristic activities and run-off processes due to the presence of several torrents coming from the hinterland (e.g. Timeto, Longano and Mazzarà) (AA.VV, 2016).

The bogue specimens employed (N=65) in this study were collected by trawl net from a single sampling site at a depth ranging from 205 to 250 m (mean depth: 225 m). Once on board, the bogues were sorted from the haul catch and counted, the overall weight was recorded and then the specimens were stored at -20°C.

2.2. MPs isolation

The laboratory analysis was carried out in the autumn of 2020. Each sampled specimen was measured (TL: total length, cm) and weighed (TW: total weight, g), GITs from the oesophagus to the end of the intestine were removed.

For the following basic-acid digestion, GITs were pooled in four groups based on the specimens' total length (cm length class: LC_15; LC_20; LC_25; LC_30). The digestion was carried out following the protocol of Schirinzi et al. (2020). In brief, GITs were weighed and placed into glass beakers in a 1:3 (w/v) ratio with 10% potassium hydroxide (KOH). The solution was incubated in a stove at 60 ± 5°C for 6 h and left at room temperature overnight. Subsequently, the sample was filtered through fiberglass filters (pore size 1.6 µm, GF/A Whatman) using a vacuum system. After the filtration, 40 mL of 20% nitric acid (HNO_3) was added to the clogged filters. The solution remained in contact with the filter for 60 min at room temperature before being removed by filtration. Then, the filters were gently cleaned with ultrapure water and were placed in Petri dishes for subsequent observations under the stereomicroscope.

2.3. Preventing contamination

To avoid contamination during laboratory analysis, rigorous precautions were carried out according to Schirinzi et al. (2020). The samples were processed in a room with restricted access, to prevent any accidental external contamination. Glassware was used and all instruments and equipment (including tweezers and scissors) were rinsed thoroughly with ultrapure water. Additionally, cotton coats were worn by operators. All procedures were conducted under the fume hood and the beakers were covered with paper or aluminium foil between each step to limit airborne contamination. Procedural blanks without tissue were also run concurrently with samples in order to contamination control. Blank sample consisted of 20 mL of 10% KOH and of 20 mL of 20% NHO_3. To avoid fibre overestimation, moist filters in Petri dishes were exposed to the laboratory air and put under the fume hood and near the stereomicroscope. All particles fixed on these filters were recorded and photographed. All particles found in the samples presenting the same shape and colour of those in blank samples were excluded from the results, as they were considered airborne contamination.

2.4. MPs identification

The filters obtained from the digestion were observed under a stereomicroscope (Zeiss Discovery V.8). All particles were counted, measured (length and width, mm) and photographed using the AxioVs40 version 4.8.2.0 digital image processing software.

Fourier transform infrared (FT-IR) spectroscopy was used to identify plastic polymers. The plastic identification was carried out with an Agilent Cary 630 spectrometer in ATR (Attenuated Total Reflection) mode (spectrum matching over 70%). Because of the instrumental limit of detection, only particles with a size > 100 µm were examined; specific libraries (Agilent Polymer Handheld ATR Library, Agilent Elastomer Oring and Seal Handheld ATR Library, Clear Polymers, POLY_D, ATR Demo Library) were used to identify the polymer composition. Identified plastic items were classified based on their size (small-micro: 0.1–1 mm; large-micro: 1–5 mm; meso: 5–25 mm; macro: > 25 mm), shape (pellet, fibre, foam, fragment, sheet and sphere) and colour according to the protocol of the MSFD (Galgani et al., 2013). For each LC, the average number of plastic items found in the GITs was calculated based on the total number of individuals (number of plastic items/number of all examined individuals).

2.5. Statistical analysis

The relative condition factor (Kn) was chosen as general indicator of health of fish, according to Sbrana et al. (2020). Kn is more reliable than Fulton's condition factor when comparing fish with different length (Froese, 2006). Kn was calculated by comparing the observed weight of the fish (TW) to an expected weight based on the fish's observed lengths

(TL; Bottari et al., 2014).

Length and weight data were log-transformed and the linearised relationships were fitted by least squares regression to estimate “a” (intercept) and “b” (allometry) coefficients. The isometric condition (H_0: b = 3) was tested by Student’s *t*-test. The relative condition factor (Kn) was calculated for each LC according to the expression:

$$Kn = TW / (a' \times TL^{b})$$

where a’ (antilog of a) and b are the power length-weight relationship parameters (Le Cren, 1951).

Kendall’s rank correlation has been performed to assess the correlation between: i) MPs abundance *vs.* fish body size; ii) MPs abundance *vs*. fish weight; iii) MPs abundance *vs.* Kn; iv) MPs size *vs*. fish body size.

Plastic particle length data were tested for homoscedastiticy and normality by the Levene and Shapiro–Wilk test’s using the PAST software (Hammer et al., 2001). Because the data did not satisfy the supposition required to perform a parametric analysis of variance (ANOVA), even after log transformation, the Kruskal–Wallis non-parametric test was used to test whether there were any significant differences in the plastic particles size among the four LC (LC_15; LC_20; LC_25; LC_30). Differences were considered significant at $p < 0.05$ (Fig. 1).

3. Results

A total of 65 adult specimens of *B. boops* was analysed, they measured from 15.6 to 34 cm in TL and from 31.6 to 383.2 g in TW (Table 1).

3.1. Plastic ingestion

A total of 180 plastic elements (2.8 items/specimens) was detected in the bogue GITs, mainly belonging to small MPs (59%) and large MPs (40%) (Fig. 2a). Of them, fragments and fibres (63% and 30%, respectively) were the predominant shape categories, followed by sheets (7%). No plastic pellets, foams or spheres were observed (Fig. 2b). Fig. S-3 shows the length and width ranges of plastic particles, varying from 0.04 to 11.39 mm and from 0.01 to 3.84 mm, respectively.(Fig. 3) Transparent (27%), black (15%), blue (13%) and yellow (10%) were the most common colours observed, but brown, white, green, red, grey and other colours were also found (Fig. 4). The main polymer types identified by FT-IR analysis were polypropylene (PP, 25%), polyethylene (PE, 24%), both low density (LDPE) and high density (HDPE), ethylene-propylene rubber (EPR, 19%), polyvinyl chloride (PVC, 9%) and nitrile butadiene rubber (NBR, 9%), followed by polybutylene terephthalate (PBT, 5%), polychloroprene (CR, 5%), ethylene-propylene diene monomer (EPDM), polyisobutylene rubber (PIB), polytetrafluoroethylene (PTFE) and, finally, styrene-butadiene rubber (SBR), representing 1% (Fig. 5). Fig. S-6 reports images of the main isolated polymers.

Finally, only 0.8 fibres per specimen were identified, but their characterisation was not achieved by FT-IR because they were too thin. The ingested fibres were mainly green (16%), blue or red (15%) and transparent or black (11%), even though brown, grey, yellow and “other color” category were also found.

3.2. Data analysis

Table 1 provides Kn data for each LC; Kn was very similar in the LCs with small fluctuations between 0.99 in the LC_15 and 1.04 in the LC_30 and a mean value equal to 1.00.

Plastic particles were found in all four LC; their number per specimen increased from 1 (LC_15) to 5.8 (LC_30) (Table 1). A positive correlation (Tau = 1; $p < 0.05$) has been found between the MPs abundance and the fish body size. Similarly, a positive correlation was found between the MPs abundance and the fish body weight (Tau = 1; $p < 0.05$). No significant correlation between MPs abundance and Kn was evident (Tau = 0.6; $p > 0.05$).

The plastic particle length for each LC was assessed. As shown in the Fig. S-7, there was a significant difference among the sample medians (H = 17.3, $p < 0.01$). No significant correlation was found between the MPs size and the fish body length (Tau = 0.0; $p > 0.05$).

Overall, PE was the only polymer present in all groups, with a percentage ranging from 4% to 38%. The other main types of polymers found in LC_15 were EPR (43%) and PP (29%). The latter one was also the most frequent synthetic polymer (46%) in LC_20, followed by PE (27%) and PVC (21%). PE and NBR were predominant in LC_25, with a percentage of 38% and 34%, respectively. Finally, EPR (55%) and PBT (21%) were mainly observed in LC_30 (Fig. 8).

4. Discussion

The present study reported information on the MPs ingestion in *B. boops* species collected during experimental trawl fisheries in the southern Tyrrhenian Sea in 2010. The presented data represent, chronologically, the first record of MPs ingestion in this benthopelagic species and also provide additional information on marine litter pollution in

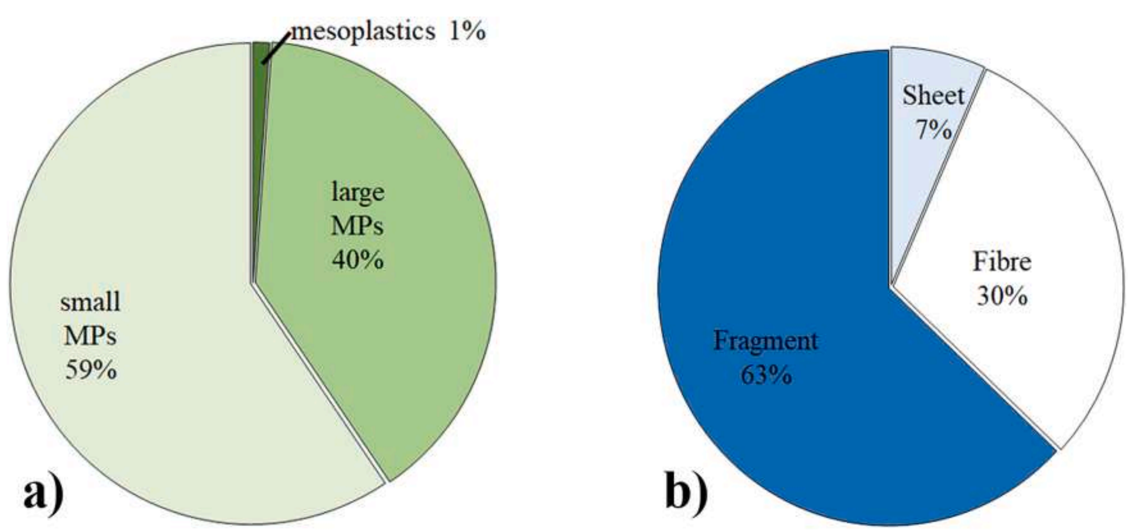

Fig. 1. Categorisation (%) by a) size (small-MPs: 0.1–1 mm; large-MPs: 1–5 mm; meso: 5–25 mm; macro: > 25 mm) and b) shape (pellet, fibre, foam, fragment, sheet and sphere) of plastics isolated from *Boops boops*.

Table 1
Number (n) of *B. boops* specimens for length class (LC), mean, standard deviation (SD) and ranges of total length (TL, cm) and total weight (TW, g), relative condition factor (Kn) and the numerical abundance of ingested plastic particles.

Length class	Sample size	TL (cm)		TW (g)		Kn	N. plastic items	N. plastic items/N. all examined individuals
	n	range	mean ± SD	range	mean ± SD			
LC_15	8	15.6–17.3	16.7 ± 0.6	31.6–56.2	44.75 ± 8.5	0.99	8	1.0
LC_20	34	17.7–22.5	20.1 ± 1.4	46–115.7	77 ± 19.8	1.01	66	1.9
LC_25	12	22.8–27.5	25.2 ± 1.8	103.8–229.5	156.7 ± 36.4	1.00	42	3.5
LC_30	11	28–34	30.3 ± 1.9	200.8–383.2	271.2 ± 53.1	1.04	64	5.8
Total	65	15.6–34	22.4 ± 4.6	31.6–383.2	120.6 ± 82.2	1.00	180	2.8

$Kn = TW / (a \bullet TL^b)$

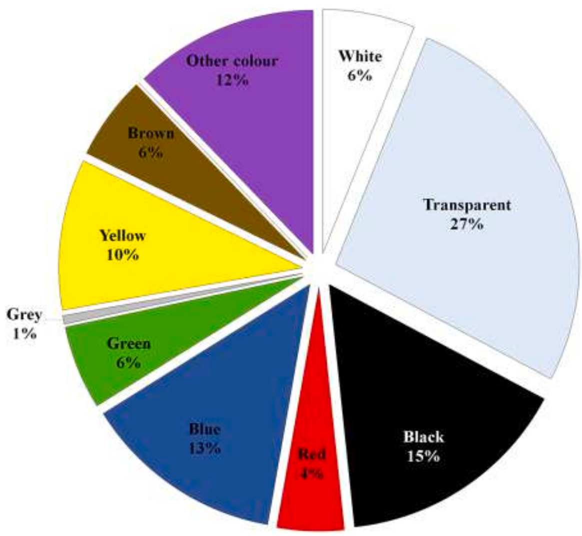

Fig. 2. Distribution percentage of plastics colour (%) ingested by *Boops boops*.

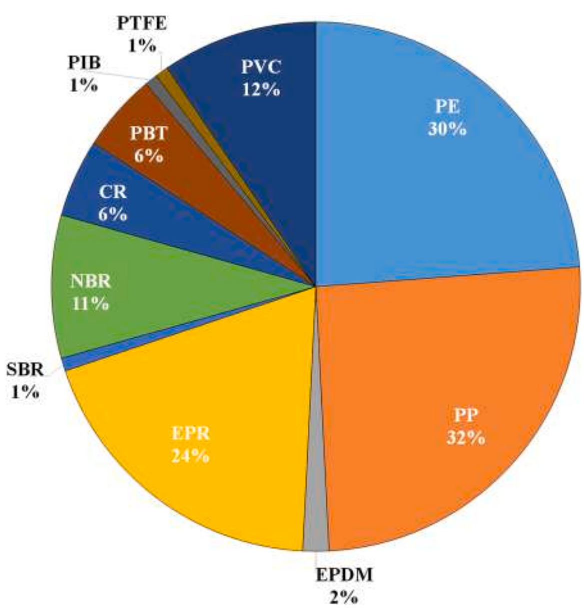

Fig. 3. Composition percentage of polymers found in GITs of *Boops boops*.

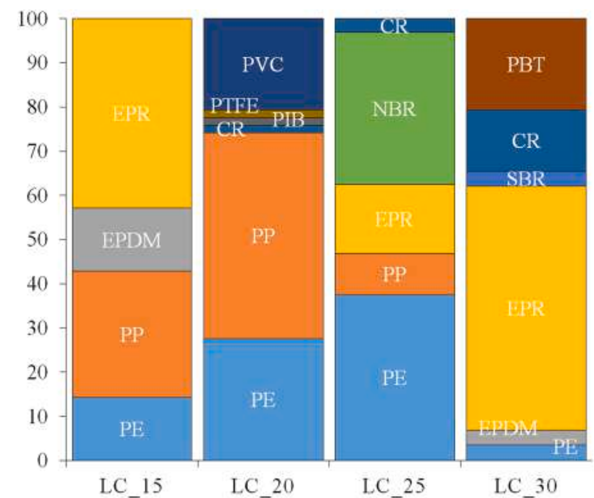

Fig. 4. Frequency of occurrence (FO %) of plastic polymers for each length class (LC, cm).

the study area, showing evidence of plastic litter as early as 2010.

About the results on MPs ingestion, our value of abundance (2.8) was higher than other Mediterranean areas, though it falls within the range (3.19 – 0.19) reported by Tsangaris et al. (2020). This result confirms an important occurrence of MPs in bogues from the Mediterranean basin as well as a high variability on spatial scale, how emphasized by Tsangaris et al. (2020). This result seems to be closely linked to the characteristics of the Gulf of Patti. In fact, as reported by Bottari et al. (2019), this is a highly urbanised coastal area strongly affected by several anthropic activities (e.g. tourism, fishing and mariculture activities, shipbuilding and boating). The introduction of marine litter could also be due to the runoff processes of neighbouring watercourses with different flow rates. In addition, the study area has been closed to trawl fishing since 1990 (Battaglia et al., 2017). These conditions, together with low water circulation levels, may make it a highly vulnerable site. Indeed, a recent study on the distribution of seabed macrolitter identified a high density of plastic in samples from 2013 to 2015 in the southern Tyrrhenian Sea, including the Gulf of Patti (Spedicato et al., 2019).

MPs (small and large) were the main size class of ingested litter. This finding in the GITs of bogues could be due to the feeding habits as also suggested by other authors (Nadal et al., 2016; Fossi et al., 2018). Indeed, the bogues are opportunistic predators, occupy an intermediate position in the marine pelagic trophic web (Cardona et al., 2012), thus they may accidentally or intentionally ingest the MPs but also eat plastics contaminated prey (secondary ingestion). For instance, bogues might confuse plastics with potential preys such as small crustaceans or feeding on the organisms attached to plastics, indirectly ingesting MPs items (Nadal et al., 2016). MPs ingestion by bogues could also occur in part during predation on gregarious prey (Fossi et al., 2018).

Furthermore, evidences of MPs ingestion were observed in other species, demersal and benthopelagic, from the same study area (Mancuso et al., 2018; Bottari et al., 2019; Capillo et al., 2020, Pedà et al., 2020).

Fragments and fibres, were the most abundant MPs shape categories observed, consistent with the finding reported in bogues from the northern coasts of Catalonia and South Sardinia (Tsangaris et al., 2020). This result may be related to different land and sea-based pollution sources in the study area (Rochman et al., 2015), although these shape categories, were also observed by different authors in other Mediterranean areas (Nadal et al., 2016; Tsangaris et al., 2020).

Another feature that differentiates these results from those obtained in other studies concerns the colour of the plastic. In fact, the most common colour for plastic items was transparent, followed by black and white. The prevalence of light-coloured MPs in the examined bogues could be due to a possible likeness to their potential preys. For instance, the exoskeleton of some crustacean's species and gelatinous planktonic organisms are transparent, translucent or white (Nadal et al., 2016). Tsangaris et al. (2020) reported different colours based on Mediterranean geographical regions. For example, black was the main colour observed in French coasts, Lazio and Liguria, whereas in Spanish coasts it was green and blue. These differences are probably due to the different sources of contamination in the areas as well as the feeding habits of bogue species.

The FT-IR analyses highlighted the presence of 11 polymer types in the GITs of the bogue coming from the southern Tyrrhenian Sea and collected in 2010. The polymer types detected in the current study were more varied than other Mediterranean areas in which the number of polymer types ranged from 2 to 5 (Tsangaris et al., 2020). The most recurring polymer in the analysed organisms were PP (25%) and PE (24%), followed by EPR (19%). PP and PE are widely used for packaging or single-use products; thus, they have a relatively short lifetime and they are also the most common polymers in the Mediterranean waters (Andrady, 2011; Rochman et al., 2013; Suaria et al., 2016). The occurrence of these polyolefins (PE and PP) have already been widely reported in demersal (*Scyliorhinus canicula*), benthopelagic (*Galeus melastomus* and *Zeus faber*), mesopelagic (*Lepidopus caudatus*) and pelagic species (*Coryphaena hippurus*, *Sardina pilchardus* and *Engraulis encrasicolus*) from the southern Tyrrhenian Sea (Bottari et al., 2019; Capillo et al., 2020; Pedà et al., 2020; Savoca et al., 2020; Schirinzi et al., 2020) and it could be also linked to the degradation of lost or abandoned fishing gears due to the intense fishing activity carried out in the study area (Pedà et al., 2020), while we may exclude they came from the net employed in our sampling, since we recorded mainly fragments and sheets. Regarding the bogue, most of the information on plastic ingestion has come from the western Mediterranean basin (Tsangaris et al., 2020), confirming PP and PE as the most common polymer type in this species from different geographical subareas (GSA 6 – Northern Spain; GSA 9 – Ligurian Sea and Northern Tyrrhenian Sea; GSA 10 – Central and southern Tyrrhenian Sea; GSA 11 – Sardinia; GSA 20 – Eastern Ionian Sea). Nevertheless, other synthetic polymers of the elastomer class (e.g., EPR, NBR, EPDM, PIB and SBR) were found in this study. In particular, EPR was the third most abundant polymer (19%) and it was observed in three of the four LC. It is used in the automotive and road construction industry such as tyres, automotive components, tubes, O-rings, gaskets, accumulator bladders, wire and cable connector manufacturing (Qi, 2015). Generally, the presence of all these elastomers is closely linked to motorway-related pollution and they enter the marine environment through runoff processes. This is consistent with the fact that the coastal area near the Gulf of Patti is subject to a lot of traffic along the coast. Considering that the bogue does not carry out extensive movements, the MPs were probably ingested in the sampling area (Sbrana et al., 2020; Tsangaris et al., 2020). Therefore, the types of ingested plastics may reflect the marine contamination in situ.

There was a positive correlation between the abundance of plastic particles and specimen's size and weight consistently with several studies on MPs ingestion in fish (Beer et al., 2018; Park et al., 2018; Pegado et al., 2018; Capone et al., 2020). Instead, to date, no relation between MPs abundance and fish size has been reported for the Mediterranean bogue by Tsangaris et al. (2020).

Assessment of a general health status of fish through the use of indirect biological parameters is suggested in the studies on MPs ingestion (Morgana et al., 2018; De Vries et al., 2020; Tsangaris et al., 2020). Some studies assessed the impact of MPs on the health status of fish using Fulton's condition factor (Garcia-Garin et al., 2019; Tsangaris et al., 2020). This parameter assumes that mass and length increase isometrically, but we are conscious that the different methodological analyses could be limited to compare data. In our study, Kn value, used as a proxy for fitness, was equal to 1.00 despite the presence of MPs in the gastrointestinal tract. This value was slightly higher than value reported for bogue in the same period (October 2010) in southern Tyrrhenian Sea ($Kn = 0.9$; Bottari et al., 2014). Moreover, MPs abundance was not related to Kn consistently with Sbrana et al. (2020) who examined bogues from GSA 9 (Ligurian Sea and Northern Tyrrhenian Sea) and GSA 11 (Sardinia). Probably MPs ingested were too few and/or small and did not retain long enough to influence the general health status of examined bogue. Indeed, this parameter provides information for a first assessment of health status but further studies are needed to better deepen this aspect.

Correlation between plastic size and fish size was not found in agreement with Rios-Fuster et al. (2019), who analysed plastic pollution in *B. boops* from western Mediterranean Sea. Conversely, Tsangaris et al. (2020) reported a weak but significant correlation between plastic size and fish size.

To understand the temporal variability of plastic pollution, it is useful to compare our data with data from the same area. Of note, the fibres could not be identified because of their thickness and the technical limitation of our instrument. The fibre abundance index was calculated to compare our record with Savoca et al. (2019), the only other study on the bogue in the Gulf of Patti. In fact, they observed only the presence of anthropogenic microfibres (viscose, cellulose and rayon). From this comparison, we noted an increase in fibre abundance over time. Moreover, we also found a greater variability in colour then Savoca et al. (2019), even if dark fibres were the most abundant.

The highlighted difference can be both linked to the different isolation method employed (visual sorting) as well to the temporal variability. In fact, 7 years had passed between the two investigations, during which time the abundance and composition of MPs in the sea could have changed, as suggested by recent studies highlighting the increase in anthropogenic textile microfibres in the Mediterranean Sea (Musso et al., 2019; Avio et al., 2020; Rodríguez-Romeu et al., 2020; Pedrotti et al., 2021).

5. Conclusion

Our data represent the oldest record of MPs in *B. boops* and they can be considered the first picture of MPs ingestion by this benthopelagic species. This finding can be useful to better understand changes in marine litter pollution over time, especially in the Gulf of Patti (southern Tyrrhenian Sea), with its peculiar environmental features, fishery management and anthropic pressure.

The increase in marine litter in the study area has been strongly influenced by the closure of the Gulf of Patti to trawl fishing since 1990. The absence of these activities and the limited seabed mixing combined with the confluence of different watercourses and the presence of several human activities, including roads, make it a 'waste disposal site'. This condition has arguably influenced the trend of plastic occurrence in the GITs of the bogue during the time. Our results confirm the suitability of *B. boops* to monitor the plastic ingestion, in a view of spatial-temporal variability. To this end, it would be useful to establish a connection network to retrieve older samples from different areas of the Mediterranean; to plan an annual monitoring using the bogue as a target species; to promote mitigation actions.

CRediT authorship contribution statement

Teresa Bottari: Formal analysis, Writing - Original Draft. **Monique Mancuso**: Resources, Writing - original draft. **Cristina Pedà**: Formal analysis, Investigation, Data curation, Writing - Review & Editing. **Francesca De Domenico**: Investigation, Writing - Review & Editing.

Federica Laface: Investigation, Writing - Review & Editing. **Gabriella F. Schirinzi**: Investigation, Writing - Review & Editing. **Pietro Battaglia**: Investigation, Supervision. **Pierpaolo Consoli**: Investigation, Supervision. **Nunziacarla Spanò**: Resources, Supervision. **Silvestro Greco**: Resources, Supervision. **Teresa Romeo**: Conceptualization, Writing - Review & Editing, Project administration, Funding acquisition, Supervision.

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.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2021.127669.

References

AA.V.V., 2016. Piano di Gestione Locale (PGL) dell'Unità Gestionale di Portorosa (dalla foce del Torrente Naso alla foce del Torrente Gallo). 66 pp.

Anastasopoulou, A., Fortibuoni, T., 2019. Impact of plastic pollution on marine life in the Mediterranean Sea. The Handbook of Environmental Chemistry. Springer, Berlin, Heidelberg. https://doi.org/10.1007/698_2019_421.

Andrady, A., 2011. Microplastics in the marine environment. Mar. Pollut. Bull. 62, 1596–1605. https://doi.org/10.1016/j.marpolbul.2011.05.030.

Avio, C.G., Pittura, L., d'Errico, G., Abel, S., Amorello, S., Marino, G., Gorbi, S., Regoli, F., 2020. Distribution and characterization of microplastic particles and textile microfibers in Adriatic food webs: general insights for biomonitoring strategies. Environ. Pollut. 258, 113766 https://doi.org/10.1016/j.envpol.2019.113766.

Battaglia, P., Andaloro, F., Consoli, P., Pedà, C., Raicevich, S., Spagnolo, M., Romeo, T., 2017. Baseline data to characterize and manage the small-scale fishery (SSF) of an oncoming Marine Protected Area (Cape Milazzo, Italy) in the western Mediterranean Sea. Ocean Coast. Manag. 148, 231–244.

Beer, S., Garm, A., Huwer, B., Dierking, J., Nielsen, T.G., 2018. No increase in marine microplastic concentration over the last three decades - A case study from the Baltic Sea. Sci. Total Environ. 621, 1272–1279. https://doi.org/10.1016/j.scitotenv.2017.10.101.

Bottari, T., Spanò, N., Mancuso, M., Capillo, G., Panarello, G., Bonsignore, M., Romeo, T., Luna, G., Savoca, S., Fazio, E., 2019. Plastics occurrence in the gastrointestinal tract of *Zeus faber* and *Lepidopus caudatus* from the Tyrrhenian Sea. Mar. Pollut. Bull. 146, 408–416. https://doi.org/10.1016/j.marpolbul.2019.07.003.

Bottari, T., Micale, V., Liguori, M., Rinelli, P., Busalacchi, B., Bonfiglio, R., Ragonese, S., 2014. The reproductive biology of *Boops boops* (Linnaeus, 1758) (Teleostei: Sparidae) in the southern Tyrrhenian Sea (central mediterranean). Cah. Biol. Mar. 55, 281e292.

Bray, L., Digka, N., Tsangaris, C., Camedda, A., Gambaiani, D., de Lucia, G.A., et al., 2019. Determining suitable fish to monitor plastic ingestion trends in the Mediterranean Sea. Environ. Pollut. 247, 1071–1077. https://doi.org/10.1016/j.envpol.2019.01. 100.

Capillo, G., Savoca, S., Panarello, G., Mancuso, M., Branca, C., Romano, V., D'Angelo, G., Bottari, T., Spanò, N., 2020. Quali-quantitative analysis of plastics and synthetic microfibers found in demersal species from southern Tyrrhenian Sea (Central Mediterranean). Mar. Pollut. Bull. 150, 110596 https://doi.org/10.1016/j.marpolbul.2019.110596.

Capone, A., Petrillo, M., Misic, C., 2020. Ingestion and elimination of anthropogenic fibres and microplastic fragments by the European anchovy (*Engraulis encrasicolus*) of the NW Mediterranean Sea. Mar. Biol. 167, 166. https://doi.org/10.1007/s00227-020- 03779-7.

Cardona, L., de Quevedo, Alvarez, Borrell, I., Aguilar, A., 2012. Massive consumption of gelatinous plankton by mediterranean apex predators. PLoS One 7, e31329. https://doi.org/10.1371/journal.pone.0031329.

de Vries, A.N., Govoni, D., Árnason, S.H., Carlsson, P., 2020. Microplastic ingestion by fifish: body size, condition factor and gut fullness are not related to the amount of plastics consumed. Mar. Pollut. Bull. 151, 110827.

Fossi, M.C., Pedà, C., Compa, M., Tsangaris, C., Alomard, C., Claro, F., Ioakeimidis, C., Galgani, F., Hema, T., Deuderod, S., Romeo, T., Battaglia, P., Andaloro, F., Caliani, I., Casini, S., Panti, C., Baini, M., 2018. Bioindicators for monitoring marine litter ingestion and its impacts on Mediterranean biodiversity. Environ. Pollut. 237, 1023–1040. https://doi.org/10.1016/j.envpol.2017.11.019.

Froese, R., 2006. Cube law, condition factor and weight-length relationships: history, meta-analysis and recommendations. J. Appl. Ichthyol. 22, 241–253.

Galgani, F., Hanke, G., Maes, T., 2015. Global distribution, composition and abundance of marine litter. In: Bergmann, M., Gutow, L., Klages, M. (Eds.), Marine Anthropogenic Litter. Springer, Cham, pp. 29–56.

Galgani, F., Hanke, G., Werner, S.D.V.L., De Vrees, L., 2013. Marine litter within the European marine strategy framework directive. ICES J. Mar. Sci. 70, 1055–1064. https://doi.org/10.1093/icesjms/fst122.

Garcia-Garin, O., Vighi, M., Aguilar, A., Tsangaris, C., Digka, N., Kaberi, H., Borrell, A., 2019. *Boops boops* as a bioindicator of microplastic pollution along the Spanish Catalan coast. Mar. Pollut. Bull. 149, 110648 https://doi.org/10.1016/j.marpolbul.2019.110648.

Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. PAST: paleontological statistics software package for education and data analysis. Palaeontol. Electron. 4 (1), 9pp. ⟨http://palaeo-electronica.org/20011/past/issue101.htm⟩.

Le Cren, E.D., 1951. The length–weight relationship and seasonal cycle in gonad weight and condition in the perch (Perca fluviatilis). J. Anim. Ecol. 20 (2), 201–219. https://doi.org/10.2307/1540.

Lleonart, J., Maynou, F., 2003. Fish stock assessments in the Mediterranean: state of the art. Sci. Mar. 67 (1), 37–49. https://doi.org/10.3989/scimar.2003.67s137.

Llorca, M., Álvarez-Muñoz, D., Ábalos, M., Rodríguez-Mozaz, S., Lúcia, H.M.L.M., Santos, L.H.M.L.M., Lèon, V.M., Campillo, A., Martìnez-Gòmez, C., Abad, E., Farrè, M., 2020. Microplastics in Mediterranean coastal area: toxicity and impact for the environment and human health. Trends Environ. Anal. Chem. 27, e00090 https://doi.org/10.1016/j.teac.2020.e00090.

Mancuso, M., Savoca, S., Bottari, T., 2018. First record of microplastics ingestion by European hake (*Merluccius merluccius*) in Tyrrhenian Sicilian coast (Central Mediterranean Sea). J. Fish Biol. 3, 517–519. https://doi.org/10.1111/jfb.13920.

Morgana, S., Ghigliotti, L., Estévez-Calvar, N., Stifanese, R., Wieckzorek, A., Doyle, T., Christiansen, J.S., Faimali, M., Garaventa, F., 2018. Microplastics in the Arctic: a case study with sub-surface water and fish samples off Northeast Greenland. Environ. Pollut. 242, 1078–1086. https://doi.org/10.1016/j.envpol.2018.08.001.

Musso, M., Achtypi, A., Bassotto, D., Suaria, G., Aliani, S., 2019. Textile microfibers in Mediterranean surface waters. Rapp. Comm. Int. Mer. Médit. 42 (2019).

Nadal, M.A., Alomar, C., Deudero, S., 2016. High levels of microplastic ingestion by the semipelagic fish bogue *Boops boops* (L.) around the Balearic Islands. Environ. Pollut. 214, 517–523. https://doi.org/10.1016/j.envpol.2016.04.054.

Park, T.J., Lee, S.H., Lee, M.S., Lee, J.K., Lee, S.H., Zoh, K.D., 2018. Occurrence of microplastics in the Han River and riverine fish in South Korea. Sci. Total Environ. 708, 134535 https://doi.org/10.1016/j.scitotenv.2019.134535.

Pedà, C., Battaglia, P., D'alessandro, M., Laface, F., Malara, D., Consoli, P., Vicchio, T.M., Longo, F., Andaloro, F., Baini, M., Galli, M., Bottari, T., Fossi, M.C., Greco, S., Romeo, T., 2020. Coupling gastro-intestinal tract analysis with an airborne contamination control method to estimate litter ingestion in demersal elasmobranchs. Front. Environ. Sci. 8, 119. https://doi.org/10.3389/fenvs.2020.00119.

Pedrotti, M.L., Petit, S., Eyheraguibel, B., Kerros, M.E., Elineau, A., Ghiglione, J.F., Loret, J.F., Rostan, A., Gorsky, G., 2021. Pollution by anthropogenic microfibers in North-West Mediterranean Sea and efficiency of microfiber removal by a wastewater treatment plant. Sci. Total. Environ. 758, 144195 https://doi.org/10.1016/j.scitotenv.2020.144195.

Pegado, T.S.S., Shmid, K., Winemiller, K.O., Chelazzi, D., Cincinelli, A., Dei, L., Giarrizzo, T., 2018. First evidence of microplastic ingestion by fishes from the Amazon River estuary. Mar. Pollut. Bull. 133, 814–821. https://doi.org/10.1016/j.marpolbul.2018.06.035.

Qi, L., 2015. The Application of Ethylene Propylene Rubber Production Technology Research. International Conference on Mechatronics, Electronic, Industrial and Control Engineering (MEIC 2015): 1610–1613. https://doi.org/10.2991/meic-15.2015.368.

Rios-Fuster, B., Alomar, C., Compa, M., Guijarro, B., Deudero, S., 2019. Anthropogenic particles ingestion in fish species from two areas of the western Mediterranean Sea. Mar. Pollut. Bull. 144, 325–333. https://doi.org/10.1016/j.marpolbul.2019.04.064.

Rodríguez-Romeu, O., Constenla, M., Carrassón, M., Campoy-Quiles, M., Soler-Membrives, A., 2020. Are anthropogenic fibres a real problem for red mullets (*Mullus barbatus*) from the NW Mediterranean? Sci. Total. Environ. 733, 139336 https://doi.org/10.1016/j.scitotenv.2020.139336.

Rochman, C.M., Tahir, A., Williams, S.L., Baxa, D.V., Lam, R., Miller, J.T., Teh, F.-C., Werorilangi, S., Teh, S.J., 2015. Anthropogenic debris in seafood: plastic debris and fibers from textiles in fish and bivalves sold for human consumption. Sci. Rep. 5, 14340.

Rochman, C.M., Hoh, E., Kurobe, T., Teh, S.J., 2013. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. Sci. Rep. 3, 3263. https://doi.org/10.1038/srep03263.

Savoca, S., Bottari, T., Fazio, E., Bonsignore, M., Mancuso, M., Luna, G.M., Romeo, T., D'Urso, L., Capillo, G., Panarello, G., Greco, S., Compagnini, G., Lanteri, G., Crupi, R., Neri, F., Spanò, N., 2020. Plastics occurrence in juveniles of *Engraulis encrasicolus* and *Sardina pilchardus* in the Southern Tyrrhenian Sea. Sci. Total. Environ. 718, 137457 https://doi.org/10.1016/j.scitotenv.2020.137457.

Savoca, S., Capillo, G., Mancuso, M., Panarello, G., Crupi, R., Bonsignore, M., D'Urso, L., Compagnini, G., Neri, F., Fazio, E., Romeo, T., Bottari, T., Spanò, N., 2019. Detection of anthropogenic cellulose microfibers in *Boops boops* from the northern coasts of Sicily (Central Mediterranean). Sci. Total. Environ. 15 (691), 455–465. https://doi.org/10.1016/j.scitotenv.2019.07.148.

Sbrana, A., Valente, T., Scacco, U., Bianchi, J., Silvestri, C., Palazzo, L., de Lucia, G.A., Valerani, C., Ardizzone, G., Matiddi, M., 2020. Spatial variability and influence of biological parameters on microplastic ingestion by *Boops boops* (L.) along the Italian coasts (Western Mediterranean Sea). Environ. Pollut. 263 (Part A), 114429 https://doi.org/10.1016/j.envpol.2020.114429.

Schirinzi, G.F., Pedà, C., Battaglia, P., Laface, F., Galli, M., Baini, M., Consoli, P., Scotti, G., Esposito, V., Faggio, C., Farré, M., Barceló, D., Fossi, M.C., Andaloro, F., Romeo, T., 2020. A new digestion approach for the extraction of microplastics from gastrointestinal tracts (GITs) of the common dolphinfish (*Coryphaena hippurus*) from the western Mediterranean Sea. J. Hazard. Mater. 397, 122794 https://doi.org/10.1016/j.jhazmat.2020.122794.

Spedicato, M.T., Zupa, W., Carbonara, P., Fiorentino, F., Follesa, M.C., Galgani, F., García- Ruiz, C., Jadaud, A., Ioakeimidis, C., Lazarakis, G., Lembo, G., Mandic, M., Maiorano, P., Sartini, M., Serena, F., Cau, A., Esteban, A., Isajlovic, I., Micallef, R., Thasitis, I., 2019. Spatial distribution of marine macro-litter on the seafloor in the northern Mediterranean Sea: the MEDITS initiative. Sci. Mar. 83 (S1), 257–270. https://doi.org/10.3989/scimar.04987.14A.

Suaria, G., Avio, C., Mineo, Lattin, G.L., Magaldi, M.G., Belmonte, G., Moore, C.J., Regoli, F., Aliani, S., 2016. The Mediterranean Plastic Soup: synthetic polymers in Mediterranean surface waters. Sci. Rep. 6, 37551. https://doi.org/10.1038/srep37551.

Tsangaris, C., Digka, N., Valente, T., Aguilar, A., Borrell, A., de Lucia, G.A., Gambaiani, D., Garcia-Garin, O., Kaberi, H., Martin, J., Mauriño, E., Miaud, C., Palazzo, L., del Olmo, A.P., Raga, J.A., Sbrana, A., Silvestri, C., Skylaki, E., Vighi, M., Wongdontree, P., Matiddi, M., 2020. Using *Boops boops* (Osteichthyes) to assess microplastic ingestion in the Mediterranean Sea. Mar. Pollut. Bull. 158, 111397 https://doi.org/10.1016/j.marpolbul.2020.111397.

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The waste collector: information from a pilot study on the interaction between the common octopus (*Octopus vulgaris*, Cuvier, 1797) and marine litter in bottom traps fishing and first evidence of plastic ingestion

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ABSTRACT

Few studies focused on behaviour adaptations of organisms to marine litter (ML) pollution in Mediterranean Sea. This research, investigates on some behavior traits of *Octopus vulgaris*, focusing on the interaction with ML during the artisanal fishing activities by the bottom traps in a small coastal area of the southern Tyrrhenian Sea. For the first time, this pilot study uses an integrated approach based on the Fishermen Ecological Knowledge as well as the analysis of ML found in the traps. First assessment of plastic ingestion in this species are also reported.

Plastic and metal were the predominant ML categories observed into the bottom traps. A total of 62 plastics, mainly small microplastics and fibres shaped, were ingested. The ML finding in the bottom traps suggests an interesting behavior of the common octopus regarding its interaction with ML, in fact, it seems to bring ML inside its dens, as a collector.

1. Introduction

The impact of marine litter (ML), and especially plastics, on marine life and habitats represents one of the main concerns investigated by scientific community. The seafloor is the ultimate sink for ML (Pham et al., 2014), where litter composition, density and accumulation rates are influenced by the interaction between the anthropic pressure and environmental factors (Galgani et al., 1995; Pham et al., 2014). Debris can be accumulated on the seafloor and reach high densities both in shores close to populated areas, and in submarine canyons (Galgani et al., 2015; Pham et al., 2014). Moreover, benthic habitats are very sensitive to ML pollution where fauna may be affected by multiple impacts with important ecological implications (Angiolillo and Fortibuoni, 2020; Canals et al., 2020; Katsanevakis et al., 2007).

In the Mediterranean basin, ML pollution has been documented in different marine compartments and high ML densities on the seabed were recorded (Consoli et al., 2020, 2019, 2018a, 2018b; Galgani et al., 1996, 1995; Strafella et al., 2015; Vlachogianni et al., 2017). Lately, many studies focused both on the seafloor ML occurrence and its effects on benthic biodiversity (Angiolillo et al., 2021, 2015; Angiolillo and Fortibuoni, 2020; Bo et al., 2014; Consoli et al., 2020, 2019, 2018b, 2018a; Melli et al., 2017). Despite the increased number of studies conducted in the Mediterranean region, to date most of them have concerned the deep rocky bottoms and only few information is available on shallow coastal areas (Consoli et al., 2020, 2019, 2018b, 2018a; Scotti et al., 2021).

Evidences of direct and indirect interactions between ML and benthic fauna have been widely demonstrated (Angiolillo et al., 2021, 2015;

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Angiolillo and Fortibuoni, 2020; Bo et al., 2014; Consoli et al., 2020, 2019, 2018b, 2018a; Katsanevakis et al., 2007; Kühn et al., 2015). These interactions include entanglement, ingestion, covering, transport vector of alien and/or invasive species, and formation of artificial substrata for fauna settling. Furthermore, high ML densities in the benthic environment may cause changes in the behaviour of some marine organisms. Indeed, these species may take advantage of litter lying on the seabed, using it as camouflage, shelter and/or refuge from potential predators or other threat source (Angiolillo, 2019; Angiolillo and Fortibuoni, 2020; Mecho et al., 2018; Pierdomenico et al., 2019). This behaviour can occur especially in soft sediment or degraded environment, where natural three-dimensional shelters are limited (Angiolillo, 2019; Angiolillo and Fortibuoni, 2020; Katsanevakis et al., 2007).

To the best of our knowledge, behaviour adaptations of marine organisms to ML pollution in Mediterranean Sea have not been much investigated. A recent review (Angiolillo and Fortibuoni, 2020) reports ML-related behaviour changes in several species, including crustaceans (*Munida, Paromola cuvieri, Plesionika spp)* and fish (*Anthias anthias, Callanthias ruber, Coelorinchus caelorhincus, Helicolenus dactylopterus, Muraena helena, Scorpaena elongate, Scorpaena scrofa*, *Trachurus mediterraneus*). However, some aspects of the behaviour response to the presence of ML in marine species are still unexplored and field observations are necessary to better understand the potential impact of ML on benthic fauna.

About cephalopods, an interesting interaction with ML was observed in the common octopus (*Octopus vulgaris*), which can use ML as dens on soft substrates, where rocks and crevices are scarce (Katsanevakis et al., 2007; Katsanevakis and Verriopoulos, 2004). In this respect, this pilot study investigates on the behavior traits of the common octopus, focusing on the interaction with ML, on soft substrates in a small area of the Sicilian coast in the southern Tyrrhenian Sea (western Mediterranean Sea). The observations were carried out during the artisanal fishing activities by bottom traps using, for the first time, an integrated approach based on fishers' Local Ecological Knowledge (LEK) as well as the analysis of ML found in the traps.

Furthermore, the present research aims to assess the plastic ingestion, for the first time, in this species that plays a crucial rule in the food web and represents an important fishing resource.

2. Materials and methods

2.1. Study area

The small study area is located in the southern Tyrrhenian Sea (western Mediterranean Sea), along the Sicilian coast from Sant'Agata di Militello to Cape Tindari (Fig. 1). The whole area is part of the geographical sub-area 10 (GSA; GFCM, 2009). The coastline is characterized by high and rocky coasts (Cape Calavà, Cape d'Orlando and Cape Tindari) alternated with low and sandy shores. The seabed mainly consists of sandy or silty bottoms, *Posidonia oceanica* and *Cymodocea nodosa* meadows (Esposito et al., 2014), hard substrates and rocky outcrops. The investigated area is subject to several torrent inputs (i.e. Zappulla, Naso, Timeto), having a considerable flow during the winter, but dried in hot periods (e.g. summer). This area is characterized by the presence of different human activities affecting the marine environment: tourism (seasonal), maritime traffic and professional and recreational fishing activities. The presence of Cape d'Orlando and Sant'Agata Militello harbors facilitates the development of marine-related activities. Moreover, the eastern sector of the study area falls in the Gulf of Patti, where a ban of trawl fishing has been enforced since 1990 (Battaglia et al., 2017).

2.2. Bottom traps fishing

Bottom traps are selective gear used by local artisanal fishermen to target *O. vulgaris*. In the study area, this fishing activity is employed in

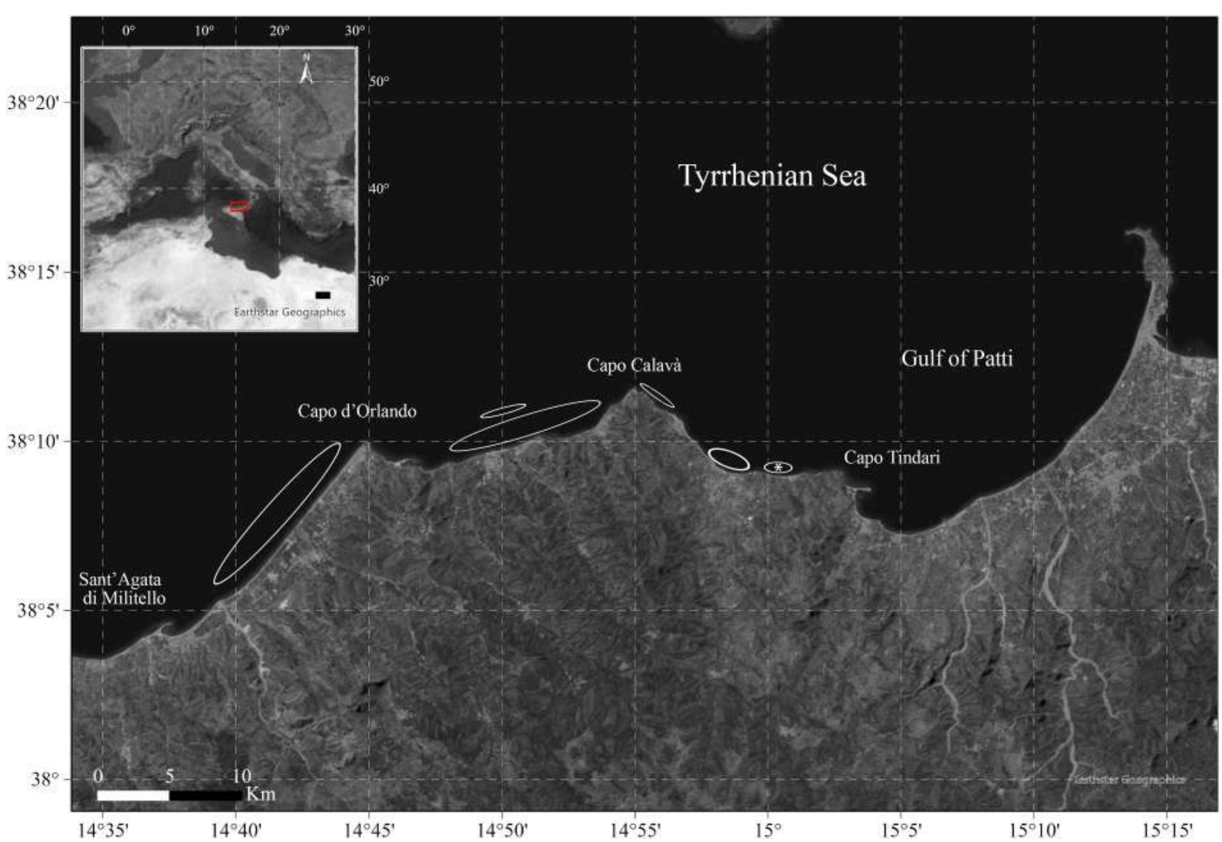

Fig. 1. A) Map of the study area in the western Mediterranean Sea showing the fishing grounds. * indicate the sampling site.

some fishing grounds (Fig. 1) and is usually carried out exclusively by 10 boats because it is not very productive. The equipment consists of cylindrical traps made of plastic material (mostly high-density polyethylene; HDPE), having an entrance of about 13 cm diameter and stones or blocks placed inside to set them on the bottom (Fig. 2). Traps are connected to a main rope (usually made of polyester and nylon) and placed on the seabed at about 20-50 m. This equipment is usually left on the bottom all year-round (with the exception of a ban period from 15 July to 15 August) and sometimes checked by hauling the traps and then immediately repositioning them in the same location. This fishing activity is regulated by the Decree n. 13129 of 30 December 2019 of the Italian Ministry of Agricultural Food and Forestry Policies.

2.3. Local Ecological Knowledge data

A LEK survey was conducted in the study area to collect information on *O. vulgaris* and to obtain information on this fishing activity and fishermen perception on the interaction between the common octopus and ML. A fishing questionnaire was submitted to professional artisanal fishermen, interviewing one fisher from each boat, between January 2021 and April 2021. In the study area there are 10 vessels which usually practice bottom trap fishing, our interviews cover the 100% of sample. Interviews were anonymous and fishermen were previously informed about the aim of the questionnaire and the use of these data (their oral informed consent was recorded). This questionnaire (Appendix A) consisted in two sections:

- section 1: focused on the fishing activity with bottom traps in the year 2020. It included questions on fishing technologies and exploited grounds, fishing effort (number of traps, fishing days) and catches (abundance, seasonality);
- section 2: focused on the ML occurrence inside the traps, with questions regarding also the ML typology and its temporal trend. Fishers were previously informed by pictures on the ML typologies, which are usually considered to categorize ML.

The fishing catch-effort information were used to better understand the interaction between ML and the common octopus caught by bottom traps.

Catch per unit effort (CPUE) were calculated as the ratio between catches (kg) and unit of effort (i.e., total number of traps per fishing trips for trap checking/50 traps), according to Battaglia et al. (2017). ML observed inside the traps by fishermen were categorized following the Material Categories for marine Macrolitter Monitoring, reported in the Marine Strategy Framework Directive (MSFD) protocol (Fleet et al., 2021).

2.4. Sampling and laboratory analysis

Overall, we got the 6 specimens of *O. vulgaris* at the landing places, already dead, from fishermen using bottom traps. All the specimens came from the same fishing ground, marked in Fig. 1, from February to November 2020.

In the laboratory, each specimen was measured (mantle length in cm), weighed (total weight in g) and then washed with ultrapure water before dissection. The entire gastrointestinal tract (GIT) (i.e., oesophagus, digestive gland, stomach, caecum and intestine) was removed from each individual and weighed. GIT was placed into a glass beaker and stored at −20 °C until the chemical digestion analysis.

2.5. Microplastics extraction and classification

Microplastics (MPs) extraction from the GITs of common octopus was performed according to the digestion protocol of Tsangaris et al. (2021) with modifications. Each GIT was subjected to chemical digestion with a 10% potassium hydroxide (KOH) solution in 1:5 (*w/v*) ratio, incubated in a stove at 50 °C for 6 h and, then, left at room temperature overnight. After the digestion of the organic matter, samples were filtered through fiberglass filters (pore size 1.6 μm, GF/A Whatman) using a vacuum pump. Filters were analysed by visual sorting under a stereomicroscope. The sorted particles were counted, measured (length and width in mm) and photographed with a stereomicroscope Zeiss Discovery V.8. coupled with Axiocam 208 color microscope camera, using ZEN 3.1 blue Edition software.

Polymer's identification was achieved by Fourier transform infrared (FT-IR) spectroscopy technique. Samples were analysed by the Agilent Cary 630 spectrometer supplied with specific polymer libraries and the level of certainty to match the sample spectrum with reference spectra was set up to > 70% (Schirinzi et al., 2020). Due to the instrumental technical limitation, too small (< 300 μm) or too thin (i.e., fibre) items could not be always analyzed. In this case, the plastic nature of samples were confirmed by applying the hot needle technique: the plastic particles in contact with hot needle tip become sticky and their surface is marked (Bellas et al., 2016; Giani et al., 2019). Then, plastic items were classified according to the protocol of the Marine Strategy Framework Directive (Directive, 2013; Galgani et al., 2013; Schirinzi et al., 2020) by size range in macroplastics (> 25 mm) mesoplastics (5 - 25 mm), large MPs (5 - 1 mm) and small MPs (1 - 0.1 mm), shape (pellet, film, filament, fibre, foam and fragment) and color. In process of classification, in accordance with Tanaka and Takada (2016) we also distinguished

Fig. 2. A) Plastic bottom trap before use. B) Plastic bottom traps after use.

between fibre and filament, because the fibres of textile origin generally have a different structure and thickness than threadlike items coming from degraded fishing gear (i.e., multifilament ropes, monofilament lines and nets).

The average number of plastic items found in the octopuses GITs were calculated as number of plastic items per number of all individuals. Finally, the identified plastics were compared with constituent polymers of bottom traps (rope and trap).

2.6. Quality control

During each step of laboratory analysis, several mitigation measures to reduce airborne contamination in samples were carried out. Cotton lab coats were worn by all researchers and the windows, doors, and air conditioners were closed in order to reduce the air currents. Glassware and metal laboratory equipment had been pre-cleaned with ultrapure water. Working surfaces were cleaned with denatured alcohol. GIT's dissection and sample rinsing and filtration were performed under the fume hood. GIT samples placed into glass beakers were always covered with aluminum foil during the digestion and filtration steps. According to Schirinzi et al. (2020) a blank sample without tissue consist of 20 mL of 10 % KOH was also run simultaneously with the 6 digested samples. Besides, a moistened filter with ultrapure water, was placed in a Petri dish and exposed during the step's digestion under the fume hood and during the filter's observation near the stereomicroscope. When airborne contamination was found in blank sample, all observed particles having similar features (i.e., structure, color) in the filters of common octopus GITs were not considered.

3. Results

The interviewed fishermen had a different year of experience, since they began their fishing activity.

The most experienced started in 2009 whereas the less skilled ones in

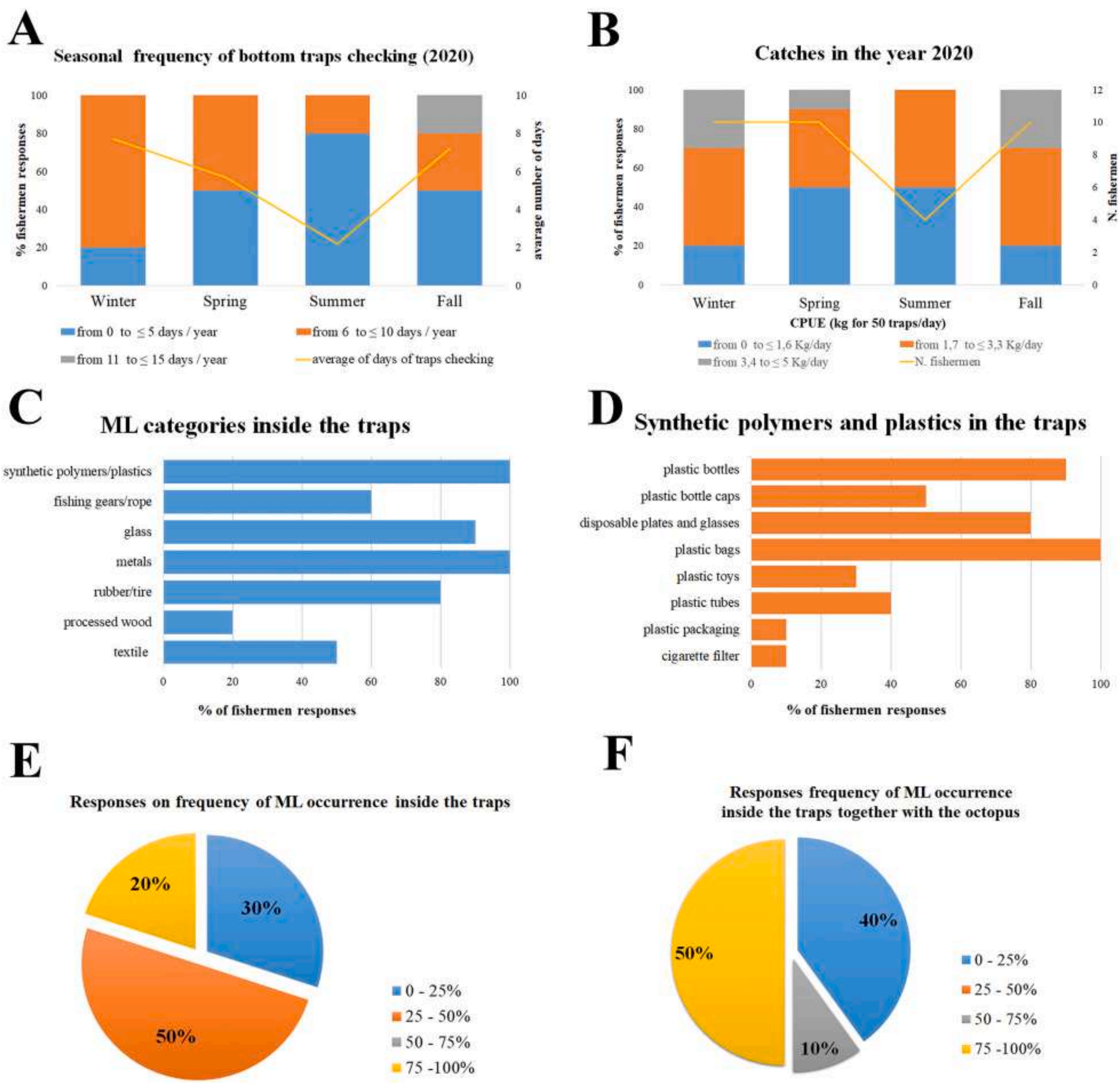

Fig. 3. Results of the questionnaire submitted to 10 professional artisanal fishermen: A) Seasonal frequency of bottom traps fishing activity in the year 2020; B) Percentage of average CPUE values (kg for 50 traps/day) of *O. vulgaris* in the year 2020; C) Composition of ML usually observed in the bottom traps; D) Composition of synthetic polymers and plastic litter usually found in the bottom traps; E) ML frequency of occurrence found in bottom traps; F) Percentage cases when the common octopus was found together with ML inside bottom traps.

2019, the average years of fishing experience was 5.7 ± 3.2 (range 2-–12 years).

3.1. Fishery, effort and catch data

The number of the bottom traps used by each local fisherman for the common octopus fishing in the year 2020 varied between 30 to 250. In the 2020, the 50% of the interviewed fishermen used a total of 250 traps; the 20% of them used 200 traps; another 20% set 100 traps; the 10% (only one fisherman) employed 30 traps.

Fishermen deployed the traps on the bottoms ranging between 15 m and 50 m, at an average depth from 27.9 ± 6.3 (minimum depth) to 40.5 ± 5.5 (maximum depth). The Fig. 1 shows the fishing grounds usually exploited in the study area by bottom trap fishery.

The number of days at sea devoted to trap fishing changed according to season. The total working days of 2020 for the whole trap fleet were higher during winter (77 days) and fall (72 days) but they decreased in spring (52 days) and in summer (22 days). Generally, fishermen checked the traps few days each season, because they carried out a multi-gear activity. Their seasonal working days ranged from 1 to 15 (Fig. 3A).

Fig. 3B reports the percentage of average CPUE values of common octopus caught by bottom traps in each season. The variability of CPUEs was observed in winter, spring and fall, with values ranging between 0 to 5.0 kg per 50 traps/day, 1.0 to 4.2 kg per 50 traps/day and 1.6 to 4.0 kg per 50 traps/day, respectively. The highest CPUEs were recorded in winter and fall whereas during summer CPUE values were around 1.0 kg per 50 traps/day.

3.2. Interaction between the common octopus and ML

The second section of the questionnaire focused on the interaction between the common octopus and ML during the professional fishing activities with bottom traps in the study area. All interviewed fishermen found ML inside their bottom traps. Fig. 3C shows the ML categories usually observed inside these bottom traps. Synthetic polymers/plastics and metal (mainly metal drink cans and bottle caps) were the most frequent ML, followed by glass (mostly bottles) and rubber/tire. Fishing gears/ropes and textile materials were also observed inside the traps by 60% and 50% of respondents, respectively. In few cases, the fishermen observed the presence of processed wood. Relatively to the category of plastic litter, plastic bags were observed by all fishermen, whereas the 90% and 80% of respondents also found plastic bottles and disposable plates and glasses, respectively. Fishermen also noted the presence of plastic bottle caps, plastics tubes, plastic toys, plastic packaging and cigarette filters (Fig. 3D).

The interviewed also provided information on the ML frequency of occurrence observed in the bottom traps and most of them (50%) detected anthropogenic materials in the 25–50% of the traps (Fig. 3E). Moreover, 50% of fishermen referred that in the 75–100% of cases they found the octopus together with ML inside the traps (Fig. 3F), although some of them (40%) reported this finding only in few cases (0–25%).

Although the interviewed fishermen had different fishing experience (in terms of years of activity), all of them observed the ML inside the traps during their fishing activity. Furthermore, data on their perception of the temporal trend of ML occurrence in the traps during the last 5 years indicated that this trend was considered stable in 50% of cases and increasing in the other 50%.

With regard to the ML disposal, only the 30% of the fishermen landed debris after fishing operations, whereas most of them (70%) threw it again into the sea. Images of ML found in the bottom traps by local fishermen are reported in (Fig. S1).

3.3. Microplastics ingestion in *O. vulgaris*

The individuals of *O. vulgaris* ranged from 9 to14 cm of mantle length (average ± SD 10.70 ± 1.75 cm) and their weight varied from 224 to 1400 g (average ± SD 483.17 ± 455.79 g). A total of 62 plastics were isolated from all 6 common octopus' specimens ranged from 1 to 38 items per individual (average ± SD 10.30 ± 16.66). The average length and width ± SD of all plastics ingested from octopuses were 1.56 ± 2.26 mm (range 0.05 - 13.37 mm) and 0.07 ± 0.11 mm (range 0.01 - 0.64 mm), respectively.

Due to the instrumental technical limitation, FT-IR analysis allowed to identify only 38 items out of 62 isolated samples (61% of the isolated particles) whereas the 24 remaining particles were tested by the hot needle method in order to confirm their plastic polymers nature and then were recorded as undetermined polymers (UPs). Among these, 9 fragments and 1 film with a range size from 0.05 to 0.28 mm and 2 filaments and 12 fibres with thickness varying from 0.01 to 0.06 mm. Images of some plastic items whose polymer could not be identified by FT-IR analysis are shown in the (Fig. S2). The most frequent polymer observed in the GITs of *O. vulgaris* was polyethylene terephthalate (PET/polyester; 68%) followed by polyethylene (PE; 13%), polyvinylchloride (PVC; 11%), silicone rubber (5%) and nylon (polyamide, PA; 3%). More than 50% of plastics belonged to small MPs category but also large MPs (37%) and mesoplastics (6%) were found. Instead, not macroplastics were recorded. PET/polyester items and the UPs included all identified size classes, in particular both large and small MPs items were the most frequent size classes. PE, silicone rubber and PVC consisted only of small MPs items, while the single nylon/PA sample was present as mesoplastic (Fig. 4A).

With regards to the shape type, the recovered plastics included 38 fibres (61%), 17 fragments (28%), 3 filaments (5%), 2 films and 2 foams (all having 3%). The UPs were present with the most type of shapes: fibres (50%), fragments (37.5%), filaments (8.3%) and films (4.2%). PE was found in the form of fragments (80%) and film (20%). On the other hand, the remaining polymers were each characterized by a different shape category. Among them have been observed PET/polyester fibres, PVC fragments, pieces of silicone rubber foams and a nylon filament (Fig. 4B).

The most frequent colors of plastic ingested by the common octopus were transparent (50%) and red (19%), but items black, white, blue, and other different colors were also observed. Samples of PE and UPs exhibited color variability, in particular transparent (60%) and red (33%) represented the main colors, respectively. On the contrary, all PET/polyester fibres and the single nylon filament were transparent whereas PVC fragments and silicone rubber foams were red and white, respectively (Fig. 4C). Fig. 5 reports some images of plastic samples found in the octopuses GITs together with the corresponding FT-IR spectra.

Moreover, some observed items, clearly came from fishing rope of the bottom traps gear. The FT-IR analysis of these components showed that the rope is composed by nylon multifilament externally and by polyester fibres internally. In the octopuses GITs were observed PET/polyester fibres but also undetermined red filaments. The latter, are attributable by structure and color to nylon multifilament. Fig. 6 shows comparison between fishing rope of the bottom traps composed by multifilament of nylon and polyester fibres with parts of fishing gear found in the GITs of the common octopus.

4. Discussion

This investigation is a pilot study, and it provides information on the presence of ML in the bottom traps and on its interaction with common octopus suggesting an interesting behaviour by this cephalopod species. Furthermore, the research reports first evidence of plastic in the GITs of *O. vulgaris*.

4.1. Fishermen perceptions

O. vulgaris is one of the most studied cephalopods (Katsanevakis and Verriopoulos, 2004). Several studies provided information on its

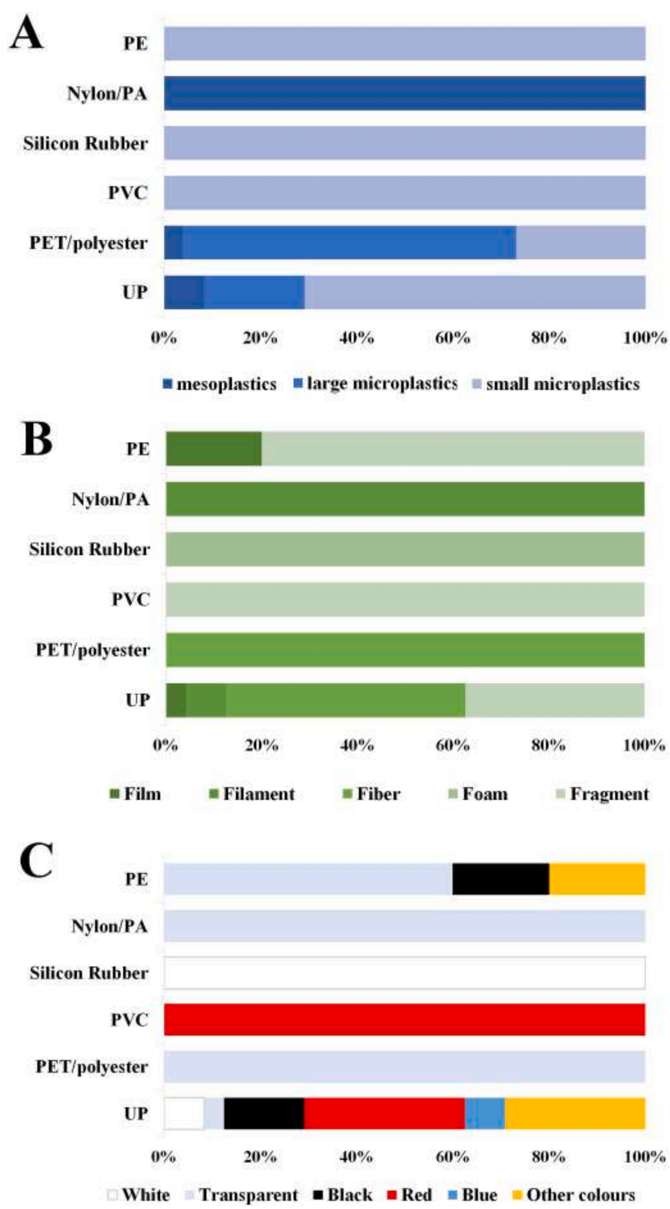

Fig. 4. Percentage of polymer types in relation to A) size B) shape and C) color of plastics detected in 6 GITs of *O. vulgaris*.

biological, ecological and behavior traits (Di Cosmo et al., 2018; Fiorito and Gherardi, 1999; Guerra et al., 2014; Katsanevakis and Verriopoulos, 2004; Maselli et al., 2020; Mereu et al., 2015). According to Battaglia et al. (2017) and Raicevich et al. (2009) the fishermen LEK is an important source of information for understanding of species behaviour and interactions. Indeed, the fishermen have a direct and often long-term experience on marine environment and organisms, and they are able to provide information on species ecology, biology, abundance and composition over time and space. The biology and ecology of this species, which mainly lives in coastal waters, has led the fishermen to evolve and develop different fishing tactics (Tsangridis et al., 2002). The use of bottom traps simulates the presence of a potential den for *O. vulgaris*, in particular on soft bottoms where natural crevices are less abundant. Bottom trap fishing in the study area shows seasonal fluctuations, being affected by a short life cycle and a particular reproductive behaviour (Boyle, 1990; Boyle and Boletzky, 1996). A remarkable seasonality for octopus catches has been already observed in the Mediterranean (Tsangridis et al., 2002) as well as in the Spanish Atlantic coasts (Sobrino et al., 2011). Moreover, in the study area, this fishing activity is less practiced during summer, because of national management measure (trap fishing closure between 15 July to 15 August), but also because most of the fishermen prefer do not retrieve traps when octopus lay down eggs. Based on the outcomes of questionnaires on fishermen perceptions, we could infer that the common octopus spawning peak occurs in summer in the study area, whereas fall and winter seasons coincide with the recruitment period according to the Mediterranean literature (Belcari et al., 2002; Cuccu et al., 2013; Guerra, 1992, 1975; Mangold-Wirz, 1963; Sánchez and Obarti, 1993).

Based on questionnaire results, the finding of ML together with stones, cobbles and shells in artificial dens (Fig. S3A, B) is certainly related both to the ability of *O. vulgaris* to construct its dens on sandy or muddy substrates using different materials (Katsanevakis and Verriopoulos, 2004), and to the wide availability of debris in the study area (Spedicato et al., 2019). Dens represent a fundamental element of the octopus habitat, inside which it spends most of its time, especially during the daylight in order to avoid predation and competition (Kayes, 1973; Mather, 1988; Katsanevakis and Verriopoulos, 2004; Guerra et al., 2014). The choice of dens is an important and frequent step in the life of the octopus, which may often modify the shelters as it need, even in some cases placing solid items to block the entrance (Guerra et al., 2014; Mather, 1994). In particular, the ability to modify the shelter help this species to increase the shelter availability (Anderson, 1997; Katsanevakis and Verriopoulos, 2004). Their survival and of its eggs also, are strictly dependent on the safety and solidity of the den. In the light of this, we can speculate that octopuses collect ML because it is highly available in the area as solid materials on the seafloor (Spedicato et al., 2019) and, also, use it in the same way as stones and shells. Therefore, this species brings ML inside the trap in order to arrange den or to create a safe and suitable shelter for itself and its eggs. This result, suggests that the common octopus considers the ML as an integral part of the habitat and uses it according to its requirements (Angiolillo, 2019; Angiolillo and Fortibuoni, 2020; Mecho et al., 2018; Pierdomenico et al., 2019).

The frequency of ML occurrence inside the traps was found to be variable, albeit most of the fishermen found ML in 25 - 50% of traps. Moreover, for half of the fishermen (50%) the octopus was found together with the ML in 75-100% of cases, while the 40% of them observed this condition in 0-25% of cases. These results indicate that the artificial traps may be used by octopus as daily and temporary shelters and also as spawning dens in agreement with the study of Mereu et al. (2018). The degree of dens occupation by the octopus may be variable and depends on biologic require of species. Indeed, the octopus's movement and excursion are influenced by several factors such as moonlight and lunar cycle, availability of food and growth (Katsanevakis and Verriopoulos, 2004; Mereu et al., 2018). About the finding of octopus together with ML inside the traps, some fishermen also claimed to find the octopus locked in the traps with partially closed entrances by a well-engineered protective barrier, made of cobbles or stones (Fig. S3C) and specially of anthropic waste such as disposable plates. This behaviour has been reported in literature both in juvenile individuals (Mather, 1994) and in female specimens inside the spawning dens (Garci et al., 2016; Hanlon and Messenger, 1996; Mereu et al., 2018, 2014). In the case of female specimens, they usually inspect several shelters before choosing the most suitable and safe for spawning and then they lock themselves inside the lair for protection (Mereu et al., 2018, 2015). The construction of barricaded spawning shelters has also been observed for other octopus species such as *Octopus tetricus* and *Octopus bimaculatus* (Anderson, 1997).

Although, the ML use as a shelter or substrate by benthic fauna may be an advantage, on the other hand, it could represent a hazard for the biodiversity safeguard. Indeed, ML can interfere with marine organisms, modifying the natural environment and altering the community structure and ecosystem functioning (Angiolillo, 2019; de Carvalho-Souza et al., 2018; Litter, 2009).

The composition of ML observed into the traps generally consisted of several types of debris (plastic and fishing gears, metal, glass, rubber and

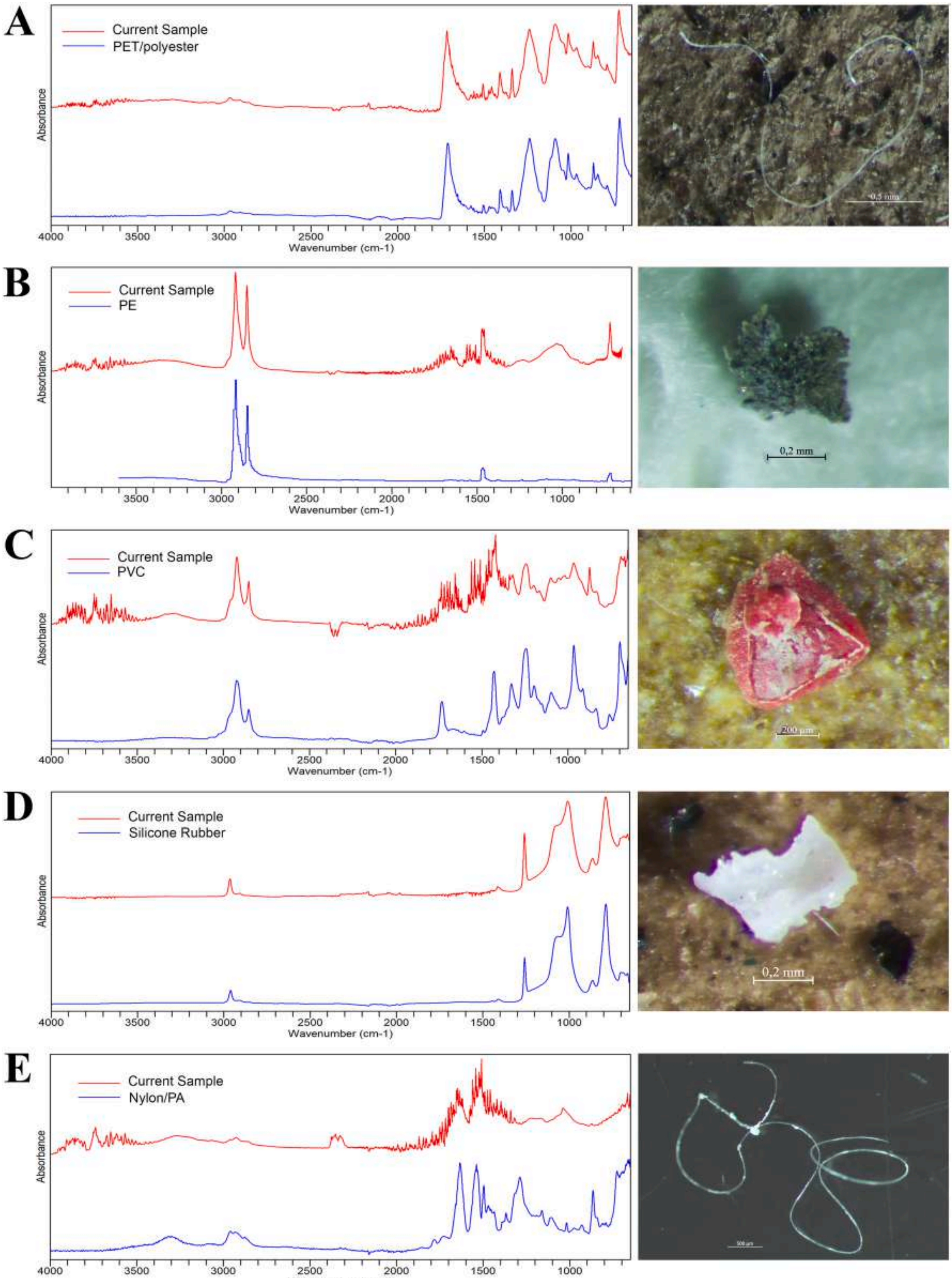

Fig. 5. Images of plastic samples detected in the GITs of *O. vulgaris.* A) fiber in PET/polyester; B) film in PE; C) fragment in PVC; D) foam in silicone rubber; E) filament in nylon/PA.

tires, textile, processed wood), with the highest frequency of plastic and metal. The finding of different ML categories in the bottom traps reflects the ML pollution levels of the study area (Consoli et al., 2020; Scotti et al., 2021; Spedicato et al., 2019). Considering the litter typology found by the fishermen in the bottom traps, we can highlight that in the study area, the density of litter on the seafloor is strongly linked by the anthropic pressure and by geomorphological and hydrodynamic features.

Although the fishermen perceptions could be affected by the fishing experience years, this research also highlights that the ML pollution in the study area during the last 5 years has persisted and has also increased, on the other hand underlines the problem of waste

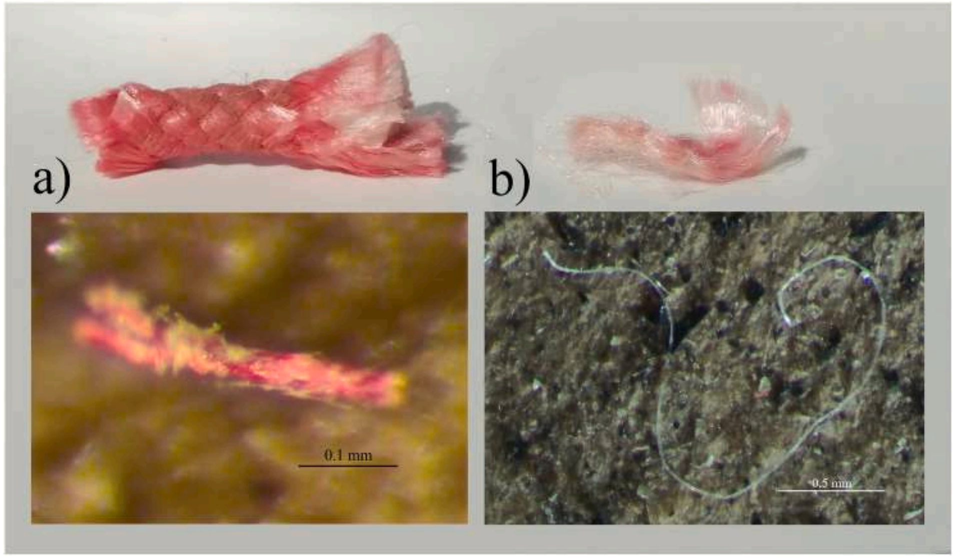

Fig. 6. Parts of multifilament of nylon A) and polyester fibers B) from fishing rope of bottom traps fishing compared with the plastic items identified in the GITs of *O. vulgaris*.

management retrieved during the fishing activities. For this purpose, specific actions are needed. Directive 2008/98/CE as amended and corrected to date, lays down measures to protect environment and human health improving waste management as a fundamental pillar of circular economy. In this context the common octopus' attitude, seems in line with prescription of EU regulation applying concretely the fundamental principles of waste hierarchy. ML is collected in marine environment and "re-used" by octopus for his purpose starting unconsciously the waste-chain management. Thus, the bottom traps should be considered as a dual-use instruments, primarily used for fishing activities and simultaneously to retrieve ML from marine ecosystem.

4.2. Microplastics in the common octopus

Currently, ingestion of plastics in cephalopod species has been poorly investigated. Indeed, plastic items were documented only in the digestive system of pelagic squid *Dodiscus gigas* and cuttlefish, both *Sepia officinalis* and *Sepia pharaonic* species (Gong et al., 2021; Oliveira et al., 2020; Prasetyo and Putri, 2021; Rosas-Luis, 2016).

The present research provides, for the first time, information on plastics ingestion in the *O. vulgaris* in the western Mediterranean Sea. Plastics ingestion has been observed in all octopus' specimens examined. The plastic litters recorded in the GITs consists mainly of PET/polyester polymer, on the other hand PE, PVC, nylon/PA and silicone rubber were also found. Most of these polymer types like PET/polyester, PE, PVC and nylon/PA are commonly used in the fishing sector and may originate by degraded or abandoned fishing gear (Consoli et al., 2018a). In particular, PET/polyester is a component of fishing rope also employed in the bottom trap fishery. Nevertheless, the polyolefins and the PVC are also the most plastics worldwide produced (Europe, 2015) and consequently very widespread in the marine environment (Andrady, 2011). Silicone rubber is an important organic rubber widely used in the aerospace, automobile, construction, electric and electronics, medical and food industry. Because this elastomer is a good alternative to petrochemical products, its production have increased in the last years (Shit and Shah, 2013). In consistency with this finding, some polymers (PE, nylon/PA and rubber) were previously observed in the stomach contents of demersal and benthopelagic species from the same study area (Bottari et al., 2021; Pedà et al., 2021). In particular, the elastomers occurrence confirmed the motorway-related pollution (rubbers from automotive manufacturers and component suppliers) through runoff processes in the southern Tyrrhenian Sea (Bottari et al., 2021).

MPs were the most abundant items found in the gastrointestinal tract, in particular, the small MPs. These results are consistent with previous studies on other cephalopods species (Gong et al., 2021; Oliveira et al., 2020) and its could be related to the feeding habits and the morphological traits of *O. vulgaris* species. The common octopus lives in direct contact with the seafloor and is characterized by an opportunistic feeding behavior mainly feeds on benthic crustaceans, mollusks (also conspecifics), polychaetes and fish (Fiorito and Gherardi, 1999; Guerra, 1978; Jereb et al., 2014; Jereb et al., 2015; Sánchez and Obarti, 1993). Its predator behavior consists of tactile exploration of different substrates including rocks, stones, soft bottoms, clumps of algae and shells by arms and suckers to identify and capture the prey (Fiorito and Gherardi, 1999; Jereb et al., 2014). This feeding behavior may induce an increase of the chance to accidentally ingest plastic litter, which can be trapped together the prey by arms and suckers. Moreover, the presence of plastic in their GITs could be also associated to the secondary ingestion because a lot of their prey has been reported affected by ML ingestion (Fossi et al., 2018). Therefore, an intentionally ingestion of plastic litter by the common octopus is very unconvincing for different reasons. Octopus is an intelligent swift-moving species characterized by a highly sophisticated sense organs and also highly developed eyes (Guerra, 2019; Maselli et al., 2020). Indeed, it cannot confuse its prey with plastic because it is able to combine both chemical (sensory inputs) and visual information (shape and general appearance) during predation behaviour (Di Cosmo et al., 2018; Fiorito and Gherardi, 1999; Maselli et al., 2020). Moreover, a recent study of Maselli et al. (2020) also highlighted that the food selection in the *O. vulgaris* is mainly dependent on chemical signals and in particular by olfactory organ. It is also known that the common octopus is able to tear the edible tissue of prey using the chitinous mandibles (beaks) and also fragment it into smaller pieces in the oral cavity by means the radula (Fiorito and Gherardi, 1999; Guerra, 2019). Then, this morphological tract could explain the large amount of small MPs in the octopuses GITs. Remarkable is the record in a specimen of particles similar in color, polymer and shape but different in size, that confirm the octopus' ability to fragment litter.

Fibres and fragments were the most frequent plastic shape categories observed. This result is in agreement with other recent studies on the *S. officinalis* and *D. gigas* species (Gong et al., 2021; Oliveira et al., 2020), confirming the predominance of fibres as mostly attributable to the degraded fishing gear (i.e. multifilament ropes). Indeed, the 68% of isolated fibres derive from the internal part of rope (main and branch rope) used in the bottom traps fishing by the local fishermen in the study area.

The colors of plastic ingested were mainly transparent and red but also black, white, blue and other colors were observed. The similar color variability agreed with the findings of Pedà et al. (2021); Schirinzi et al. (2020) and Bottari et al. (2021) in some fish species sampled in the same study area, such as *Scyliorhinus canicular*, *Galeus melastomus*, *Raja clavata, Coryphaena hippurus* and *Boops boops*. The color variability of plastic samples may depend on the composition of ML in the environment.

5. Conclusions

This pilot study could be useful to transfer this experience in other Mediterranean areas where this fishing activity plays a more important role (Chedia et al., 2010; Petetta et al., 2021; Tsangridis et al., 2002) in order also to support our observations and to encourage the adoption of this integrated approach.

The ML availability seems to make the octopus a waste collector, which brings the ML inside its dens. This approach based on the fishermen's knowledge represents an important tool to assess the ML interaction with marine organisms and also to characterize the ML. At the same time, the preliminary data on the ingestion of plastics by *O. vulgaris* provides a significant contribution for the understanding of MPs impact on cephalopod species and suggests to improve the ML ingestion studies in this taxon, especially for the common octopus, which plays an important role in the marine food webs.

This research pays also attention on the issue about the use of plastic made fishing gear and on the waste management from fishing activities. One of the major challenges in the marine research field is to ensure the sustainability of fisheries. In this respect, a roadmap could be identified for future mitigation initiatives, starting with the replacement of artificial polymer materials "plastic" with biodegradable polymers "bioplastics" in the bottom traps manufacture. Secondarily, it suggests to promote "waste management procedures" specifically bound to the fishing boat license in order: to encourage the fishermen to retrieve ML from the bottom traps; to collect the waste on board; to dispose them ashore to adequate Port Reception Facilities. The development of these management measures could represent a tool for achieving one of the most significant aims of Directive 2019/883/UE that is to align European regulations to international level as drawn by the International Convention for the Prevention of Pollution from Ships (Marpol, 2020). These actions will be helpful to reduce the ML dispersion in the ecosystem, and prevent MPs impact on the fishing resources and their potential implications on the human health, considering that waste are continuously abandoned and discarded illegally at sea representing a concrete form of "dumping" (London Convention, 2016) creating negative impact at socio-economic level and represent one of the most dangerous hazard for marine environment and ecosystem.

Finally, other proposals are needed to regulate, implement and encourage the contribution coming from "fisheries clean up actions" that could represent an example of good governance and best practice to improve the sustainable development of oceans and seas (World Ocean Assessment II, 2021).

CRediT authorship contribution statement

Cristina Pedà: Methodology, Formal analysis, Investigation, Visualization, Writing – original draft. **Francesco Longo:** Methodology, Resources, Investigation, Visualization, Writing – original draft. **Claudio Berti:** Investigation, Supervision, Visualization, Writing – review & editing. **Federica Laface:** Investigation, Writing – review & editing. **Francesca De Domenico:** Investigation, Writing – review & editing. **Pierpaolo Consoli:** Investigation, Supervision. **Pietro Battaglia:** Investigation, Supervision, Writing – review & editing. **Silvestro Greco:** Resources, Supervision. **Teresa Romeo:** Conceptualization, Supervision, Writing – review & editing, Project administration, Funding acquisition.

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.

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Appendix A. Questions (Q) from the fishing questionnaire survey submitted to the fishermen from the study area on the fishing activity by the bottom traps, in the year 2020:

Section 1 - Fishing catch and effort of *O. vulgaris*

Q1. How many years have you been catching the common octopus with the bottom traps?
Q2. How many bottom traps did you use to catch the common octopus in 2020 (number of traps/fishing day)?
Q3. How many times did you use the bottom traps to catch the common octopus in 2020 (number of days per season the traps are checked)?
Q4. What has been your average catch per season (Kg/day) in 2020?
Q5. How deep did you put your bottom traps? Please, indicate the min-max depth in meters.
Q6. In which area do you fish the common octopus with the bottom traps? Could you indicate the spatial distribution of the bottom traps on this map?

Section 2 - Interaction between marine litter and *O. vulgaris*

Q7. During the octopus' fishing, do you find ML inside the bottom traps?
Q8. What kind of ML do you usually find inside the bottom traps?
Q9. How frequent (%) did you find ML inside the traps?
Q10. Could you provide the percentage cases when the common octopus was found together with ML inside bottom traps?
Q11. Considering your fishing experience, did you observe any temporal trend about the occurrence of ML in the octopus bottom traps during the last 5 years?
Q12. How do you dispose the retrieved ML by the bottom traps?

Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2021.113185.

References

Anderson, T.J., 1997. Habitat selection and shelter use by Octopus tetricus. Mar. Ecol. Prog. Ser. 150, 137–148.
Andrady, A.L., 2011. Microplastics in the marine environment. Mar. Pollut. Bull. 62, 1596–1605.
Angiolillo, M., 2019. Debris in deep water. In: World Seas: An Environmental Evaluation. Elsevier, pp. 251–268.
Angiolillo, M., Fortibuoni, T., 2020. Impacts of marine litter on Mediterranean reef systems: from shallow to deep waters. Front. Mar. Sci. 7, 826.
Angiolillo, M., di Lorenzo, B., Farcomeni, A., Bo, M., Bavestrello, G., Santangelo, G., Cau, Angelo, Mastascusa, V., Cau, Alessandro, Sacco, F., 2015. Distribution and assessment of marine debris in the deep Tyrrhenian Sea (NW Mediterranean Sea, Italy). Mar. Pollut. Bull. 92, 149–159.
Angiolillo, M., Gérigny, O., Valente, T., Fabri, M.-C., Tambute, E., Rouanet, E., Claro, F., Tunesi, L., Vissio, A., Daniel, B., 2021. Distribution of seafloor litter and its interaction with benthic organisms in deep waters of the Ligurian Sea (Northwestern Mediterranean). Sci. Total Environ. 788, 147745.
Battaglia, P., Andaloro, F., Consoli, P., Pedà, C., Raicevich, S., Spagnolo, M., Romeo, T., 2017. Baseline data to characterize and manage the small-scale fishery (SSF) of an

oncoming marine protected area (Cape Milazzo, Italy) in the western Mediterranean Sea. Ocean Coast. Manag. 148, 231–244.
Belcari, P., Cuccu, D., González, M., Srairi, A.L.I., Vidoris, P., 2002. Distribution and abundance of Octopus vulgaris Cuvier, 1797 (Cephalopoda: Octopoda) in the Mediterranean Sea. Sci. Mar. 66, 157–166.
Bellas, J., Martínez-Armental, J., Martínez-Cámara, A., Besada, V., Martínez-Gómez, C., 2016. Ingestion of microplastics by demersal fish from the Spanish Atlantic and Mediterranean coasts. Mar. Pollut. Bull. 109, 55–60.
Bo, M., Bava, S., Canese, S., Angiolillo, M., Cattaneo-Vietti, R., Bavestrello, G., 2014. Fishing impact on deep Mediterranean rocky habitats as revealed by ROV investigation. Biol. Conserv. 171, 167–176.
Bottari, T., Mancuso, M., Pedà, C., De Domenico, F., Laface, F., Schirinzi, G.F., Battaglia, P., Consoli, P., Spanò, N., Greco, S., Romeo, T., 2021. Microplastics in bogue, Boops boops: a snapshot of the past from the southern Tyrrhenian Sea. J. Hazard. Mater., 127669 https://doi.org/10.1016/j.jhazmat.2021.127669. In press.
Boyle, P.R., 1990. Cephalopod biology in the fisheries context. Fish. Res. 8, 303–321.
Boyle, P.R., Boletzky, S.V., 1996. Cephalopod populations: definition and dynamics. Philos. Trans. R. Soc. London. Ser. B Biol. Sci. 351, 985–1002.
Canals, M., Pham, C.K., Bergmann, M., Gutow, L., Hanke, G., Van Sebille, E., Angiolillo, M., Buhl-Mortensen, L., Cau, A., Ioakeimidis, C., 2020. The quest for seafloor macrolitter: a critical review of background knowledge, current methods and future prospects. Environ. Res. Lett. 16 (2), 023001.
Chedia, J., Widien, K., Amina, B., 2010. Role of sea surface temperature and rainfall in determining the stock and fishery of the common octopus (Octopus vulgaris, Mollusca, Cephalopoda) in Tunisia. Mar. Ecol. 31, 431–438.
Consoli, P., Andaloro, F., Altobelli, C., Battaglia, P., Campagnuolo, S., Canese, S., Castriota, L., Cillari, T., Falautano, M., Peda, C., 2018a. Marine litter in an EBSA (Ecologically or biologically significant Area) of the Central Mediterranean Sea: abundance, composition, impact on benthic species and basis for monitoring entanglement. Environ. Pollut. 236, 405–415.
Consoli, P., Falautano, M., Sinopoli, M., Perzia, P., Canese, S., Esposito, V., Battaglia, P., Romeo, T., Andaloro, F., Galgani, F., 2018b. Composition and abundance of benthic marine litter in a coastal area of the Central Mediterranean Sea. Mar. Pollut. Bull. 136, 243–247.
Consoli, P., Romeo, T., Angiolillo, M., Canese, S., Esposito, V., Salvati, E., Scotti, G., Andaloro, F., Tunesi, L., 2019. Marine litter from fishery activities in the Western Mediterranean Sea: the impact of entanglement on marine animal forests. Environ. Pollut. 249, 472–481.
Consoli, P., Scotti, G., Romeo, T., Fossi, M.C., Esposito, V., D'Alessandro, M., Battaglia, P., Galgani, F., Figurella, F., Pragnell-Raasch, H., 2020. Characterization of seafloor litter on Mediterranean shallow coastal waters: evidence from dive against Debris®, a citizen science monitoring approach. Mar. Pollut. Bull. 150, 110763.
Cuccu, D., Mereu, M., Cau, Alessandro, Pesci, P., Cau, Angelo, 2013. Reproductive development versus estimated age and size in a wild Mediterranean population of Octopus vulgaris (Cephalopoda: Octopodidae). J. Mar. Biol. Assoc. U. K. 93, 843–849.
de Carvalho-Souza, G.F., Llope, M., Tinôco, M.S., Medeiros, D.V., Maia-Nogueira, R., Sampaio, C.L.S., 2018. Marine litter disrupts ecological processes in reef systems. Mar. Pollut. Bull. 133, 464–471.
Di Cosmo, A., Maselli, V., Polese, G., 2018. Octopus vulgaris: an alternative in evolution. Mar. Org. Model Syst. Biol. Med. 585–598.
Directive, S.F., 2013. Guidance on monitoring of marine litter in European seas. Luxemb, 10, 99475.
Esposito, V., Andaloro, F., Bianca, D., Natalotto, A., Romeo, T., Scotti, G., Castriota, L., 2014. Diet and prey selectivity of the red mullet, Mullus barbatus (Pisces: Mullidae), from the southern Tyrrhenian Sea: the role of the surf zone as a feeding ground. Mar. Biol. Res. 10, 167–178.
Europe, P., 2015. An analysis of European plastics production, demand and waste data. Plast. facts, 147.
Fiorito, G., Gherardi, F., 1999. Prey-handling behaviour of Octopus vulgaris (Mollusca, Cephalopoda) on bivalve preys. Behav. Process. 46, 75–88.
Fleet, D., Vlachogianni, T., Hanke, G., 2021. A joint list of litter categories for marine macrolitter monitoring. In: EUR 30348 EN Publications Office of the European Union, JRC121708. European Union, Luxembourg, p. 52.
Fossi, M.C., Pedà, C., Compa, M., Tsangaris, C., Alomar, C., Claro, F., Ioakeimidis, C., Galgani, F., Hema, T., Deudero, S., Romeo, T., Battaglia, P., Andaloro, F., Caliani, I., Casini, S., Panti, C., Baini, M., 2018. Bioindicators for monitoring marine litter ingestion and its impacts on Mediterranean biodiversity. Environ. Pollut. 237, 1023–1040. https://doi.org/10.1016/j.envpol.2017.11.019.
Galgani, F., Jaunet, S., Campillo, A., Guenegen, X., His, E., 1995. Distribution and abundance of debris on the continental shelf of the North-Western Mediterranean Sea. Mar. Pollut. Bull. 30, 713–717.
Galgani, F., Souplet, A., Cadiou, Y., 1996. Accumulation of debris on the deep sea floor off the french Mediterranean coast. Mar. Ecol. Prog. Ser. 142, 225–234.
Galgani, F., Hanke, G., Werner, S., Oosterbaan, L., Nilsson, P., Fleet, D., Kinsey, S., Thompson, R.C., Van Franeker, J., Vlachogianni, T., 2013. Monitoring guidance for marine litter in European seas. In: JRC Sci. Policy Reports, EUR 26113.
Galgani, F., Hanke, G., Maes, T., 2015. Global distribution, composition and abundance of marine litter. In: Marine Anthropogenic Litter. Springer, Cham, pp. 29–56.
Garci, M.E., Hernández-Urcera, J., Gilcoto, M., Fernández-Gago, R., González, Á.F., Guerra, Á., 2016. From brooding to hatching: new insights from a female Octopus vulgaris in the wild. J. Mar. Biol. Assoc. U. K. 96, 1341–1346.
GFCM, 2009. Establishment of Geographical Sub-Areas in the GFCM area amending the resolution GFCM/31/2007/2, GFCM/33/2009/2.
Giani, D., Baini, M., Galli, M., Casini, S., Fossi, M.C., 2019. Microplastics occurrence in edible fish species (Mullus barbatus and Merluccius merluccius) collected in three different geographical sub-areas of the Mediterranean Sea. Mar. Pollut. Bull. 140, 129–137.
Gong, Y., Wang, Y., Chen, L., Li, Y., Chen, X., Liu, B., 2021. Microplastics in different tissues of a pelagic squid (Dosidicus gigas) in the northern Humboldt current ecosystem. Mar. Pollut. Bull. 169, 112509.
Guerra, A., 1975. Determination of the different stages of the sexual development of the common octopus by a maturity index. Investig. Pesq. 39 (2), 398–416.
Guerra, Á., 1978. Sobre la alimentación y el comportamiento alimentario de Octopus vulgaris.
Guerra, A., 1992. Mollusca Cephalopoda. Fauna Ibérica. Vol. 1. Mus. Nat. Ciencias Nat. CSIC, Madrid.
Guerra, Á., 2019. Functional anatomy: macroscopic anatomy and post-mortem examination. In: Handbook of Pathogens and Diseases in Cephalopods. Springer, pp. 11–38.
Guerra, Á., Hernández-Urcera, J., Garci, M.E., Sestelo, M., Regueira, M., González, Á.F., Cabanellas-Reboredo, M., Calvo-Manazza, M., Morales-Nin, B., 2014. Dwellers in dens on sandy bottoms: ecological and behavioural traits of Octopus vulgaris. Sci. Mar. 78, 405–414.
Hanlon, R.T., Messenger, J.B., 1996. Cephalopod Behaviour. Cambridge University Press, Cambridge, UK.
Jereb, et al., 2014. Cephalopods of the world. An annotated and illustrated catalogue of cephalopod species known to date. In: Octopods and Vampire Squids, 3.
Jereb, P., Allcock, L.A., Lefkaditou, E., Piatkowski, U., Hastie, L.C., Pierce, G.J., 2015. Cephalopod biology and fisheries in Europe: II. In: Species Accounts. ICES.
Katsanevakis, S., Verriopoulos, G., 2004. Den ecology of Octopus vulgaris Cuvier, 1797, on soft sediment: availability and types of shelter. Sci. Mar. 68, 147–157.
Katsanevakis, S., Verriopoulos, G., Nicolaidou, A., Thessalou-Legaki, M., 2007. Effect of marine litter on the benthic megafauna of coastal soft bottoms: a manipulative field experiment. Mar. Pollut. Bull. 54, 771–778.
Kayes, R.J., 1973. The daily activity pattern of octopus vulgar is in a natural habitat. Mar. Freshw. Behav. Phy 2, 337–343.
Kühn, S., Rebolledo, E.L.B., Van Franeker, J.A., 2015. Deleterious effects of litter on marine life. Mar. Anthropog. Litter 75–116.
Litter, U.M., 2009. In: A Global Challenge, 232. UNEP, Nairobi, p. 354.
London Convention, 2016. London Convention and London Protocol 2016 Edition, Convention on the Prevention of Marine Pollution by Dumping of Wastes and Other Matter, International Maritime Organization. London.
Mangold-Wirz, K., 1963. Biologie des cephalopodes bentiques et nectoniques de la mer catalan. Vie milieu 13, 1–285.
Marpol, 2020. International Convention for the Prevention of Pollution from Ships 1973, as modified by the Protocol of 1978 and the protocol of 1997. London.
Maselli, V., Al-Soudy, A.-S., Buglione, M., Aria, M., Polese, G., Di Cosmo, A., 2020. Sensorial hierarchy in Octopus vulgaris's food choice: chemical vs. visual. Animals 10, 457.
Mather, J.A., 1988. Daytime activity of juvenile Octopus vulgaris in Bermuda. Malacologia 29, 69–76.
Mather, J.A., 1994. 'Home'choice and modification by juvenile Octopus vulgaris (Mollusca: Cephalopoda): specialized intelligence and tool use? J. Zool. 233, 359–368.
Mecho, A., Aguzzi, J., De Mol, B., Lastras, G., Ramirez-Llodra, E., Bahamon, N., Canals, M., 2018. Visual faunistic exploration of geomorphological human-impacted deep-sea areas of the North-Western Mediterranean Sea. Mar. Biol. Assoc. United Kingdom. J. Mar. Biol. Assoc. U. K. 98, 1241–1252.
Melli, V., Angiolillo, M., Ronchi, F., Canese, S., Giovanardi, O., Querin, S., Fortibuoni, T., 2017. The first assessment of marine debris in a site of community importance in the North-Western Adriatic Sea (Mediterranean Sea). Mar. Pollut. Bull. 114, 821–830.
Mereu, M., Agus, B., Alvito, A., Atzori, G., Fois, M., Cuccu, D., 2014. Artificial dens for octopus vulgaris cuvier, 1797 in the sardinian SEA/TANE artificiali per octopus vulgaris cuvier, 1797 nel mar di sardegna. Biol. Mar. Mediterr. 21, 287.
Mereu, M., Agus, B., Addis, P., Cabiddu, S., Cau, A., Follesa, M.C., Cuccu, D., 2015. Movement estimation of Octopus vulgaris Cuvier, 1797 from mark recapture experiment. J. Exp. Mar. Bio. Ecol. 470, 64–69.
Mereu, M., Cau, A., Agus, B., Cannas, R., Follesa, M.C., Pesci, P., Cuccu, D., 2018. Artificial dens as a management tool for Octopus vulgaris: evidence from a collaborative fisheries research project (central western Mediterranean Sea). Ocean Coast. Manag. 165, 428–433.
Oliveira, A.R., Sardinha-Silva, A., Andrews, P.L.R., Green, D., Cooke, G.M., Hall, S., Blackburn, K., Sykes, A.V., 2020. Microplastics presence in cultured and wild-caught cuttlefish, Sepia officinalis. Mar. Pollut. Bull. 160, 111553.
Pedà, C., Battaglia, P., D'Alessandro, M., Laface, F., Malara, D., Consoli, P., Vicchio, T.M., Longo, F., Andaloro, F., Baini, M., 2021. Coupling gastro-intestinal tract analysis with an airborne contamination control method to estimate litter ingestion in demersal elasmobranchs. In: Microplastics Mar. Environ. Sources, Distrib. Biol. Eff. Socio-Economic Impacts.
Petetta, A., Virgili, M., Guicciardi, S., Lucchetti, A., 2021. Pots as alternative and sustainable fishing gears in the Mediterranean Sea: an overview. Rev. Fish Biol. Fish. 1–23.
Pham, C.K., Ramirez-Llodra, E., Alt, C.H.S., Amaro, T., Bergmann, M., Canals, M., Davies, J., Duineveld, G., Galgani, F., Howell, K.L., 2014. Marine litter distribution and density in European seas, from the shelves to deep basins. PLoS One 9, e95839.
Pierdomenico, M., Cardone, F., Carluccio, A., Casalbore, D., Chiocci, F., Maiorano, P., D'Onghia, G., 2019. Megafauna distribution along active submarine canyons of the Central Mediterranean: relationships with environmental variables. Prog. Oceanogr. 171, 49–69.

Prasetyo, D., Putri, L.S.E., 2021. Cuttlefish (Sepia pharaonis ehrenberg, 1831) as a bioindicator of microplastic pollution. Aquac. AquariumConserv. Legis. 14, 918–930.

Raicevich, S., Fortibuoni, T., Franceschini, G., Celic, I., Giovanardi, O., 2009. The use of Local Ecological Knowledge to Reconstruct the History of Marine Animal Populations. Potentials and limitations. When Humanit. Meet Ecol. Proc. HMAP Int. Summer Sch. Hist. Chang. Mediterr. Black Sea Mar. Biodivers. Ecosyst. since Rom. period until nowadays. Lang. Methodol. Perspect. 81–95.

Rosas-Luis, R., 2016. Description of plastic remains found in the stomach contents of the jumbo squid Dosidicus gigas landed in Ecuador during 2014. Mar. Pollut. Bull. 113, 302–305.

Sánchez, P., Obarti, R., 1993. In: The biology and fishery of Octopus vulgaris caught with clay pots on the Spanish Mediterranean coast. Recent Adv. Fish. Biol. Tokai Univ. Press, Tokyo, pp. 477–487.

Schirinzi, G.F., Pedà, C., Battaglia, P., Laface, F., Galli, M., Baini, M., Consoli, P., Scotti, G., Esposito, V., Faggio, C., 2020. A new digestion approach for the extraction of microplastics from gastrointestinal tracts (GITs) of the common dolphinfish (Coryphaena hippurus) from the western Mediterranean Sea. J. Hazard. Mater. 397, 122794.

Scotti, G., Esposito, V., D'Alessandro, M., Panti, C., Vivona, P., Consoli, P., Figurella, F., Romeo, T., 2021. Seafloor litter along the italian coastal zone: an integrated approach to identify sources of marine litter. Waste Manag. 124, 203–212.

Shit, S.C., Shah, P., 2013. A review on silicone rubber. Natl. Acad. Sci. Lett. 36, 355–365.

Sobrino, I., Juarez, A., Rey, J., Romero, Z., Baro, J., 2011. Description of the clay pot fishery in the Gulf of Cadiz (SW Spain) for Octopus vulgaris: selectivity and exploitation pattern. Fish. Res. 108, 283–290.

Spedicato, M.T., Walter, Z., Pierluigi, C., Fabio, F., Follesa, M.C., François, G., Cristina, G.-R., Angélique, J., Christos, I., Giorgos, L., 2019. Spatial distribution of marine macro-litter on the seafloor in the northern Mediterranean Sea: the MEDITS initiative.

Strafella, P., Fabi, G., Spagnolo, A., Grati, F., Polidori, P., Punzo, E., Fortibuoni, T., Marceta, B., Raicevich, S., Cvitkovic, I., 2015. Spatial pattern and weight of seabed marine litter in the northern and Central Adriatic Sea. Mar. Pollut. Bull. 91, 120–127.

Tanaka, K., Takada, H., 2016. Microplastic fragments and microbeads in digestive tracts of planktivorous fish from urban coastal waters. Sci. Rep. 6, 1–8.

Tsangaris, C., Panti, C., Compa, M., Pedà, C., Digka, N., Baini, M., D'Alessandro, M., Alomar, C., Patsiou, D., Giani, D., 2021. Interlaboratory comparison of microplastic extraction methods from marine biota tissues: a harmonization exercise of the plastic busters MPAs project. Mar. Pollut. Bull. 164, 111992.

Tsangridis, A., Sánchez, P., Ioannidou, D., 2002. Exploitation patterns of Octopus vulgaris in two Mediterranean areas. Sci. Mar. 66, 59–68.

Vlachogianni, T., Anastasopoulou, A., Fortibuoni, T., Ronchi, F., Zeri, C., 2017. Marine litter assessment in the Adriatic and Ionian Seas. In: IPA-Adriatic DeFishGear Proj. MIO-ECSDE, HCMR ISPRA, 168.

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Chapter 25
May Mesopelagic Fishes Play an Important Role as Vector of Microplastics Across the Mediterranean Trophic Web? A Case of Study in the Strait of Messina

Federica Laface, Cristina Pedà, Matteo Nannini, Giuseppe Cangemi, Valentina Sciutteri, Pietro Battaglia, and Teresa Romeo

25.1 Introduction

Currently, the issue of Microplastics (MPs) pollution has become of global concern. MPs are defined as particles < 5 mm [1] and can be manufactured as industrial microbeads and scrubbers (primary MPs) or originate from the fragmentation of larger plastics (secondary MPs) free in the marine environment resulting from abiotic and/or biotic factors [2]. MPs, ubiquitous in marine environment worldwide, can enter the trophic web through direct or secondary ingestion by marine fauna [3]. MPs ingestion represents a serious threat to marine organisms, causing physical/mechanical damage and chemical damage [4].

Although many studies have documented the MPs ingestion [4], the research challenge focused now on assessing the transfer of MPs across marine trophic levels.

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MPs ingestion has also been reported in deep environment and specifically in mesopelagic fish from several oceanic and Mediterranean areas [5–12].

However, the role of mesopelagic fish in the MPs' transfer across the marine trophic web is still poorly explored. They play an important ecological role in the food web, performing diel vertical migrations and contributing to the connection between epipelagic waters to deep-sea ecosystems [12, 13]. Indeed, they are involved in the energy transfer from zooplankton to higher trophic levels, representing an important food source for medium and large pelagic predators [14, 15].

In this view, the present paper investigates: (i) the MPs ingestion in five mesopelagic fish belonging to the families Myctophidae (*Electrona risso, Hygophum benoiti*, *Myctophum punctatum*) and Sternoptychidae (*Argyropelecus hemigymnus* and *Maurolicus muelleri*) from the Strait of Messina; (ii) the potential transfer of MPs to intermediate and large predators, across the pelagic trophic web, mediated by mesopelagic food resources.

25.2 Experimental

An integrated approach based on the study of both MPs ingestion by mesopelagic species and their trophic relationships in the study area was applied to assess MPs transfer in the trophic web.

25.2.1 Sample Collection

Sample collection was carried out along the shore of the Sicilian coast of the Strait of Messina (central Mediterranean Sea) in 2020–2021. This area is characterized by peculiar hydrodynamic system, which periodically allows the stranding of deep-sea fauna [16]. Three Myctophidae (*E. risso, H. benoiti* and *M. punctatum*) and two Sternoptychidae (*A. hemigymnus* and *M. muelleri*) were selected for this study. For each species, the gastrointestinal tracts (GITs) were removed and grouped into size classes (*E. risso*: <20 mm, 20–40 mm, >40 mm; *H. benoiti*: <25 mm, 25–40 mm, >40 mm; *M. punctatum*: 30–50 mm, >50 mm; *A. hemigymnus*: <20 mm, 20–30 mm, >30 mm; *M. muelleri*: <20 mm; 20–40 mm; >40 mm). The GITs were weighed and placed into glass beakers for the analysis.

25.2.2 *Isolating Microplastics*

25.2.2.1 Chemical Digestion

MPs were extracted from GITs using the chemical digestion protocol of Schirinzi et al. [17], which involves two digestion steps. During the first step, each size group was treated with a potassium hydroxide solution (10% KOH, ratio 1:3 (w/v)) and incubated in a stove at 60 °C/6 h. The digested solutions were vacuum filtered through a glass fibre membrane (pore size 1.6 µm). During a second digestion step, a 20% HNO_3 solution was added on the clogged filters for 60 min at room temperature to remove most of the organic and inorganic material.

25.2.2.2 Polymer Identification

Filters were examined under a stereomicroscope Zeiss Discovery V.8. coupled with Axiocam 208 camera. All isolated particles were counted, measured (length and width in mm) and photographed. Polymer nature was identified by Fourier transform infrared (FT-IR) spectroscopy technique using the Agilent Cary 630 spectrometer supplied with specific polymer libraries and the level of certainty to match the sample spectrum with reference spectra was set up to >70% [17, 18]. The average number of plastic items found in the GITs was calculated on the total number of individuals (N. plastic items/N. all examined individuals).

25.2.2.3 Microplastics Transfer Across the Trophic Web

To assess the potential transfer of MPs from mesopelagic fish to pelagic predators, we collected the available information on the feeding habits of intermediate and top predators from the study area. We selected the studies where mesopelagic species represent an important food resource for predators of the Strait of Messina [14, 15, 19] and also used unpublished data to improve information on trophic relationship between pelagic resources. Overall, we considered the top predators *Thunnus thynnus* and *Xiphias gladius* as well as the intermediate predators *Chauliodus sloani* (Stomiidae), *Trachurus picturatus* (Carangidae), *Lepidopus caudatus* (Trichiuridae) and *Ommastrephid squids* (Ommastrephidae).

Since the values of prey abundance of mesopelagic fish in stomach of these predators, we hypothesized a MPs transfer scheme across the trophic web.

25.3 Results and Discussion

A total of 1101 GITs of mesopelagic fish were examined. Table 25.1 shows the mean size (SL mm) per size group of each species.

Overall, 41 plastic particles (0.04 items/specimens) were detected in mesopelagic GITs (Table 25.1), mainly belonging to small MPs (61%) and large MPs (34%), although mesoplastics (5%) were also found.

The most abundant polymers identified by FT-IR analysis were polyvinylchloride (PVC; 12%), nylon (polyamide, PA; 10%), polytetrafluoroethylene (PTFE; 10%) and polyethylene (PE; 7%). Other polymers were polypropylene (PP; 5%), rubber (5%), polystyrene (PS; 2%) and polyurethane (PUR; 2%). The highest ratio between the ingested MPs items and the number of examined individuals was found in *H. benoiti* (0.06 plastic items/individual). This agrees with the findings of Romeo et al. [12], although this last study was only based on visual inspection of stomach contents of

Table 25.1 Number of individuals and mean size (SL mm) per size group of each mesopelagic species investigated. Abundance of plastic ingestion by each size class pool of mesopelagic species from the Strait of Messina

Family/species	Size group (SL mm)	N. specimens	Mean SL ± Standard deviation	N. MPs	N plastic items/total examined individuals
MYCTOPHIDAE					
Electrona risso	<20 mm	180	15.6 ± 2.8	3	0.02
	20–40 mm	63	28.4 ± 6.2	6	0.10
	>40 mm	10	43.1 ± 2.4	0	0.00
	Total	253	19.9 ± 8.2	9	0.04
Hygophum benoiti	<25 mm	107	23.0 ± 1.7	2	0.02
	25–40 mm	128	33.2 ± 3.5	10	0.08
	>40 mm	104	45.3 ± 3.0	9	0.09
	Total	339	33.7 ± 9.3	21	0.06
Myctophum punctatum	30–50 mm	26	44.6 ± 4.3	2	0.08
	>50 mm	96	58.4 ± 3.2	2	0.02
	Total	122	55.5 ± 6.6	4	0.03
STERNOPTYCHIDAE					
Argyropelecus hemigymnus	<20 mm	105	15.8 ± 2.9	0	0.00
	20–30 mm	117	27.6 ± 2.2	4	0.03
	>30 mm	107	29.5 ± 3.4	3	0.03
	Total	329	25.0 ± 7.0	7	0.02
Maurolicus muelleri	20–40 mm	36	27.5 ± 4.1	0	0.00
	>40 mm	22	44.6 ± 2.3	0	0.00
	Total	58	33.9 ± 9.1	0	0.00
Total		1101	30.3 ± 13.2	41	0.04

lanternfish. In addition, the greatest polymeric variability of MPs was also observed in *H. benoiti*. The variation in ingestion rates of MPs by investigated species maybe related to their different feeding and migrating behavior. Moreover, these species hunt using vision, so they may mistake plastics for prey by intentionally ingesting MPs [10, 12].

The assessment of MPs transfers throughout the trophic web of the Strait of Messina, mediated by mesopelagic food resources, is summarized in Fig. 25.1 that defined the trophic relationships between mesopelagic fish and their predators in the study area. Bluefin tuna directly prey upon mesopelagic fishes in the Strait of Messina, exploiting upwelling phenomena to forage on myctophids and sternoptychids, but also feeds on important intermediate predators such as *C. sloani*, *T. picturatus* and ommastrephid squids. Therefore, it can partially collect MPs through direct ingestion of mesopelagic fish. Instead, swordfish may only indirectly accumulate MPs of mesopelagic fish origin by preying upon ommastrephid squids and *L. caudatus*. In Fig. 25.1, the green lines indicate the trophic relationships between mesopelagic species and intermediate predators, while the red lines the relationships between top predators and both intermediate predators and mesopelagic organisms. Although the ingestion rate of MPs by mesopelagic fish was low, the analysis of the complex trophic relationships in the study area and the amounts of mesopelagic specimens ingested by predators suggest that the concentration of MPs transferred across trophic levels can reach important orders of magnitude at the higher levels.

25.4 Conclusions

The present research provides preliminary results on the potential transfer of MPs in trophic web of the Strait of Messina mediated by mesopelagic fish resources and underlines the importance of these species in the MPs transfer across the food web. Further studies are needed to better understand how long MPs stay in the GITs and how quickly they are evacuated. An important perspective will be to assess the implications for human consumption related to the potential effects of contaminants the transfer from litter to edible fish tissue.

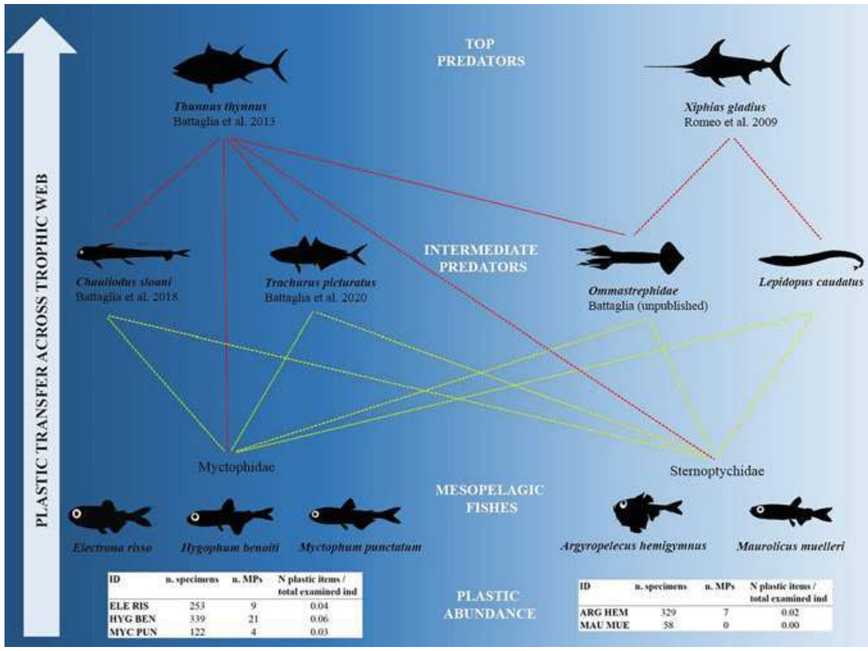

Fig. 25.1 Microplastics transfer across the trophic web in the Strait of Messina mediated by mesopelagic food resources

References

1. Arthur C, Baker JE, Bamford HA (2009) Proceedings of the international research workshop on the occurrence, effects, and fate of microplastic marine debris, September 9–11, 2008. University of Washington Tacoma, Tacoma, WA, USA
2. Andrady AL (2011) Microplastics in the marine environment. Mar Pollut Bull 62:1596–1605
3. Pedà C, Battaglia P, D'Alessandro M, Laface F, Malara D, Consoli P, Vicchio TM, Longo F, Andaloro F, Baini M, Galli M, Bottari T, Fossi MC, Greco S, Romeo T (2020) Coupling gastro-intestinal tract analysis with an airborne contamination control method to estimate litter ingestion in demersal elasmobranchs. Front Environ Sci 8:119
4. Fossi MC, Pedà C, Compa M, Tsangaris C, Alomar C, Claro F, Ioakeimidis C, Galgani F, Hema T, Deudero S, Romeo T, Battaglia P, Andaloro F, Caliani I, Casini S, Panti C, Baini M (2018) Bioindicators for monitoring marine litter ingestion and its impacts on Mediterranean biodiversity. Environ Pollut 237:1023–1040
5. Boerger CM, Lattin GL, Moore SL, Moore CJ (2010) Plastic ingestion by planktivorous fishes in the North Pacific Central Gyre. Mar Pollut Bull 60:2275–2278
6. Davison P, Asch RG (2011) Plastic ingestion by mesopelagic fishes in the North Pacific Subtropical Gyre. Mar Ecol Prog Ser 432:173–180
7. Lusher AL, O'Donnell C, Officer R, O'Connor I (2016) Microplastic interactions with North Atlantic mesopelagic fish. ICES Mar Sci Symp 73:1214–1225
8. Wieczorek AM, Morrison L, Croot PL, Allcock AL, MacLoughlin E, Savard O, Brownlow H, Doyle TK (2018) Frequency of microplastics in mesopelagic fishes from the Northwest Atlantic. Front Mar Sci 5

9. McGoran AR, Maclaine JS, Clark PF, Morritt D (2021) Synthetic and semi-synthetic microplastic ingestion by mesopelagic fishes from Tristan da Cunha and St. Helena, South Atlantic. Front Mar Sci 8
10. Justino AKS, Ferreira GVB, Schmidt N, Eduardo LN, Fauvelle V, Lenoble V, Sempéré R, Panagiotopoulos C, Mincarone MM, Frédou T, Lucena-Frédou F (2022) The role of mesopelagic fishes as microplastics vectors across the deep-sea layers from the Southwestern Tropical Atlantic. Environ Pollut 300:118988
11. Zhu L, Wang H, Chen B, Sun X, Qu K, Xia B (2019) Microplastic ingestion in deep-sea fish from the South China Sea. Sci Total Environ 677:493–501
12. Romeo T, Pedà C, Fossi MC, Andaloro F, Battaglia P (2016) First record of plastic debris in the stomach of Mediterranean lanternfishes. Acta Adriatica Int J Mar Sci 57:115–122
13. Gjøsaeter J, Kawaguchi K, Nations F, A.O. of the U. (1980) A review of the world resources of mesopelagic fish, food & agriculture org
14. Battaglia P, Andaloro F, Consoli P, Esposito V, Malara D, Musolino S, Pedà C, Romeo T (2013) Feeding habits of the Atlantic bluefin tuna, *Thunnus thynnus* (L. 1758), in the central Mediterranean Sea (Strait of Messina). Helgol Mar Res 67:97–107
15. Battaglia P, Pagano L, Consoli P, Esposito V, Granata A, Guglielmo L, Pedá C, Romeo T, Zagami G, Vicchio TM, Guglielmo R, Andaloro F (2020) Consumption of mesopelagic prey in the Strait of Messina, an upwelling area of the central Mediterranean Sea: feeding behaviour of the blue jack mackerel *Trachurus picturatus* (Bowdich, 1825). Deep-Sea Res I: Oceanogr Res Pap 155:103158
16. Battaglia P, Ammendolia G, Cavallaro M, Consoli P, Esposito V, Malara D, Rao I, Romeo T, Andaloro F (2017) Baseline data to characterize and manage the small-scale fishery (SSF) of an oncoming Marine Protected Area (Cape Milazzo, Italy) in the western Mediterranean Sea. Ocean Coast Manag 148:231–244
17. Schirinzi GF, Pedà C, Battaglia P, Laface F, Galli M, Baini M, Consoli P, Scotti G, Esposito V, Faggio C, Farré M, Barceló D, Fossi MC, Andaloro F, Romeo T (2020) A new digestion approach for the extraction of microplastics from gastrointestinal tracts (GITs) of the common dolphinfish (*Coryphaena hippurus*) from the western Mediterranean Sea. J Hazard Mater 397:122794
18. Pedà C, Longo F, Berti C, Laface F, De Domenico F, Consoli P, Battaglia P, Greco S, Romeo T (2022) The waste collector: information from a pilot study on the interaction between the common octopus (*Octopus vulgaris*, Cuvier, 1797) and marine litter in bottom traps fishing and first evidence of plastic ingestion. Mar Pollut Bull 174:113185
19. Battaglia P, Ammendolia G, Esposito V, Romeo T, Andaloro F (2018) Few but relatively large prey: trophic ecology of *Chauliodus sloani* (Pisces: Stomiidae) in deep waters of the Central Mediterranean Sea. J Ichthyol 58:8–16

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XXXI Congresso S.It.E.

Adattamenti degli Ecosistemi alle Pressioni dell'Antropocene

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S7.P17 The use of artificial baits in swordfish longline fishery: potential impacts on trophic web assessed from fishers' Local Ecological Knowledge and stomach content analysis

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In the framework of marine litter pollution, the impact of artificial baits used in Mediterranean fishery activities has been poorly investigated. In the last decades, artificial baits have been partially replaced natural ones in swordfish longline fisheries, because of their durability, reusability and cost-effectiveness. Present study aims to assess the potential impact of artificial baits used in mesopelagic longline fisheries targeting swordfish (*Xiphias gladius*) through fishers' Local Ecological Knowledge. A questionnaire was submitted to professional fishermen using as key study area in the northern Sicilian coast (southern Tyrrhenian Sea), between November 2021 and January 2022, to obtain information on swordfish fishery, fishermen perceptions and artificial baits related impacts. Interviewed fishermen used mesopelagic longline between June and August 2021, with an average CPUE of 8.6 ± 4.4 kg. Most fishermen used artificial lures combined with natural baits (sardines or frozen squids). The most common artificial baits were soft squids and mackerels of different colors, made of polyvinyl chloride. In few cases, fishermen referred that artificial baits were lacking from longline, probably ingested by swordfish which escaped the catch (1%). Our investigations were integrated by stomach content analysis of swordfish landed in the same area and fishing season, with the aim of verify the frequency of occurrence of artificial baits in the guts. Only in few cases (2%) artificial baits were found in swordfish stomachs. Interviews also referred that artificial baits were often chewed by large squids, which probably ingest lure particles. Squids are among the main prey of swordfish and then the problem of secondary ingestion of plastic in swordfish should be assessed. Considering the importance of these fishery activities on Mediterranean scale and the potential transfer of plastics across the trophic web, further investigations replicated in other fishing areas should clarify these aspects and eventually suggest management and mitigation measures.

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USE OF FOURIER TRANSFORM INFRARED (FT-IR) SPECTROSCOPY FOR THE POLYMERIC COMPOSITION ASSESSMENT OF MICROPLASTICS INGESTED BY MEDITERRANEAN FISHERY RESOURCES

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In the last decades, there has been an increasing scientific interest on the environmental threat due to microplastics (MPs, plastic particles lower than 5 mm). Because of their chemical and physical properties, these "emerging" contaminants are ubiquitous in the marine habitat worldwide, with potential biological and ecological impacts. MPs can be ingested by marine fauna and transferred along the food-web, causing physical/mechanical damages in tissues and inducing toxicological harm. Marine Strategy Framework Directive guidelines suggest to define the trends of marine plastic ingestion in bioindicator species from European waters and to identify the MPs polymer composition in the size range 0.1-5 mm, using spectroscopy techniques. In this framework, the present study aims to assess the polymeric composition of MPs ingested by some fishery resources (*Boops boops, Coryphaena hippurus, Scyliorhinus canicula, Galeus melastomus, Raja clavata, Octopus vulgaris* and *Sepia officinalis*) sampled in the Gulf of Patti (southern Tyrrhenian Sea, Sicily), through one of the most used spectroscopy techniques. MPs were isolated from the gastrointestinal tracts and analyzed by ATR-Fourier transform infrared (FT-IR) spectroscopy supplied with specific polymer libraries and the level of certainty to match the sample spectrum with reference spectra was set up to > 70%. The main polymer types identified were rubber (58%), polyethylene (55%), polypropylene (40%), polyethylene terephthalate (30%) and polyvinyl chloride (17%). This analysis allowed to characterize the MPs polymeric composition in the study area, underlining that the elastomers, grouped in the rubber category, were the most frequent MPs. This finding may be linked to motorway-related pollution through runoff processes in the coastal area near the Gulf of Patti.

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Bio-based materials for sustainable mussel production: preliminary results of a multidisciplinary study

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Keywords: Mater-Bi, Marine litter, *Mytilus galloprovincialis*

Plastic is a widely used material in aquaculture facilities that may become marine litter (ML). In Italy a thousand tonnes of polypropylene (PP) socks per year are used in mussel farm. Thus, it is important to consider alternative solutions such as the employment of bio-based materials, but the studies assessing their behaviour are still poor.

In this context, the present study aims to replace PP with Mater-Bi socks (Novamont S.p.A., Italy) and to assess: i) the polymer degradation along the production cycle; ii) the mussel's biomass (weight, size), iii) the macrofaunal community associated to *Mytilus galloprovincialis* in a farm located in the Ionian Sea (Mar Piccolo, Italy).

In October 2021, six socks in PP and six Mater-Bi were deployed in the study area (T0) and two different treatments were considered for each type of socks: socks with mussels vs socks without mussels (n=3). Socks samples from each replica were collected monthly (T1-T2-T3) while mussels and the associated biodiversity were sampled seasonally (winter and spring). Fourier transform infrared (FT-IR) spectroscopy analysis was performed using a self-generated polymer library characterised by only the reference spectra of the Mater-Bi (T0) before the start of the analysis, to assess the possible polymers alterations. The polymer identification was accepted when the match with the reference spectra had a level of certainty >70%. Taxonomic identification was carried out through microscopy observation and biometric parameters of each specimen of blue mussel were recorded. Preliminary FT-IR analysis did not show evident alterations in the different experimental levels. From preliminary results we observed that the dominant taxon in the macrofaunal community changed across seasons. Further studies, considering longer observation time, are needed to better understand the suitability of bio-based materials in aquaculture and to improve the sustainability of this crucial production sector.

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Microbial colonization in mussel farming socks made of Mater-Bi, an innovative bio-based material

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Keywords: Mater-Bi, microbial colonization, plastics, pathogens, aquaculture

The use of innovative materials to be used for applications in the field of aquaculture is an important alternative to favor the reduction of the impact due to the use of recalcitrant plastic materials. Indeed, in addition to causing direct damage to marine organisms, plastics and microplastics can favor quick colonization by microorganisms on their surface by hosting a real microbial community, called plasticsphere [1]. Plastics and microplastics could also act as transport vectors for toxin-producing and pathogenic microbial species [2,3], thus promoting the spread of potential infectious agents. During this study, the bio-based material Mater-Bi (Novamont S.p.A., Italy) was tested as an alternative material to the classic polypropylene (PP) stockings for mussel farming (*Mytilus galloprovincialis*) in the Gulf of Taranto, for an experimental period of 6 months. At pre-established interval times, three fragments of each sock (with and without mussels) have been collected and stored. The total bacterial abundance was determined with DAPI-staining. For the enumeration of cultivable bacteria, aliquots of diluted suspensions were spread plated on common medium for marine heterotrophic microrganisms. The presence of microbial fecal pollution indicator (i.e. total coliforms, *Enterococci*, *E. coli*, *Shigella*, *Salmonella*, *Proteus*, *Klebsiella*, *V. cholerae*, *V. parahaemolyticus, P. aeruginosa*, *S. aureus* and *C. perfringens*) have been also assessed by using specific media. All bacterial strains were also tested for biofilm production, to elucidate the different adhesion and affinity level to the two matrices.

The results indicated a clear diversification of the microbial colonization on the PP and Mater-Bi in terms of abundance and presence of bacterial pathogens. Further analysis are planned to complete the study with determination of total microbial communities by metabarcoding approach.

1 - Zettler ER, Mincer TJ, Zettler LA. (2013) Life in the "plastisphere": microbial communities on plastic marine debris *Environ Sci Technol* Jul 2;47(13):7137-46. doi: 10.1021/es401288x.
2- Kirstein IV, Kirmizi S, Wichels A, Fernandez A, Erler R, Löder M, Gerdts G. (2016) Dangerous hitchhikers? Evidence for potentially pathogenic Vibrio spp. on microplastic particles *Mar Environ Res* Sep;120:1-8. doi: 10.1016/j.marenvres.2016.07.004
3 -Viršek MK, Lovšin MN, Koren S, Kržan A, Peterlin M (2017) Microplastics as a vector for the transport of the bacterial fish pathogen species Aeromonas salmonicida *Mar Pollut Bull* Dec 15;125(1-2):301-309. doi: 10.1016/j.marpolbul.2017.08.024

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Occurrence of Anthropogenic Debris in Three Commercial Shrimp Species from South-Western Ionian Sea

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Simple Summary: Plastic litter is ubiquitous in the marine environment due to its rapid dispersion and great durability. Furthermore, several environmental processes can modify the characteristics of plastics, altering their density and, consequently, their likelihood of sinking. In fact, deep-sea environments are highly threatened by plastic waste, with a greater risk for benthic species. The Ionian Sea is heavily impacted by man-made floating debris, accumulated on beaches or on the seabed. The aim of this work was to evaluate the presence of anthropogenic debris in the gastrointestinal tracts of three decapods (*Parapenaeus longirostris*, *Aristeus antennatus*, *Aristaeomorpha foliacea*) from the southwestern Ionian Sea. A total of 230 anthropogenic debris were isolated from 136 specimens, with a high frequency of occurrence in all analyzed species (76% in *P. longirostris*, 70% in *A. antennatus* and 83% in *A. foliacea*) mainly represented by fibers (92.6%) with a size between 0.10 and 0.49 mm, and with a predominance of blue color. The results of this study, highlight the importance of expanding knowledge on these Decapoda species of high commercial and ecological value, in a heavily impacted basin, such as the Sea Mediterranean, helping to monitor possible risks to human health.

Abstract: Deep Sea environments represent the final collector of anthropogenic debris mainly represented by both plastic and non-plastic materials with different size. This led to potential contamination of deep marine fauna due to direct and indirect ingestion, representing a potential hazard for the species itself and for the final consumer. In this framework, the present study explored the occurrence of anthropogenic debris in the gastrointestinal tract of three Decapoda species of high commercial and ecological value (*Parapenaeus longirostris*, *Aristeus antennatus*, and *Aristaeomorpha foliacea*) from south-western Ionian Sea. After morphometrical measurements and sex determination, the gastrointestinal tract of 136 specimens were extracted and then chemically digested. A total of 230 low density microparticles were isolated, with a high frequency of occurrence in all the analyzed species (76% in *P. longirostris*, 70% in *A. antennatus*, and 83% in *A. foliacea*) mainly represented by fibers (92.6%) with a size between 0.10 and 0.49 mm, and with a dominance of the blue color. The results of the present study report for the first time the anthropogenic debris presence in the studied Decapoda from south-western Ionian Sea, highlighting the necessity to broaden the knowledge about anthropogenic debris pollution status in Mediterranean deep-sea species.

Keywords: marine pollution; Decapoda; low density microplastics; deep-sea; Mediterranean Sea

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1. Introduction

The massive production of plastics materials, and their accumulation in the environment due to insufficient recycling practices have led the scientific community and the entire society to focus the attention on the risks associated with plastic contamination, both for ecosystems and human health [1]. Concerning the marine environments, these are hardly threatened by plastic litter so far as to induce the control authorities on food (e.g., EFSA, European Food Safety Authority) to establish monitoring on plastic contamination especially for seafood products [2,3]. Several studies [4,5] have highlighted how in 2010 between 4.8 and 12.7 million tonnes (Mt) of plastics entered the oceans, drawing the attention toward the increasing trend of plastic input into the environment which could reach 12,000 Mt by 2050.

Both terrestrial and maritime human activities are responsible for the continuous release of plastic into the marine environment. Once released into the sea, microplastics can colonize all compartments of the marine environment: coasts, water surface, water column, seabed, and biota [6,7]. These contaminants are considered ubiquitous due to their rapidity in dispersion related to positive buoyancy (plastics materials have low densities) and great durability [8]. Indeed, it has been observed that plastic accumulations on the sea surface represent only about 1% of the estimated global budget, while most of the remaining 99% of marine plastic will sink to the deep sea [9] due to of vertical transport from surface accumulation. However, it has recently been shown that the spatial distribution and final fate of microplastics are strongly controlled by bottom currents [9]. Microplastic transport is a difficult topic as transport includes physical, chemical, and biological processes [6]. Among the various difficulties, it should also be considered that the physical properties (e.g., size, shape, density, buoyancy) of microplastics can vary considerably, influencing their transport [10–12]. Their final destination seems to be mainly influenced by the density of the polymers: polymers with a density higher than that of water (>1.027 g/cm^3) will tend to settle on the bottom; while low density polymers will tend to float on the water column [6,13]. However, the presence of low-density polymers was also found at a depth of 10,000 m [14] contradicting this hypothesis. Alternative hypotheses suggest that other factors, such as biofouling, also contribute to modifying the density of microplastics and consequently their expected distribution in the water column.

Furthermore, other processes such degradation and fragmentation processes can modify the density of microplastics and consequently their distribution in the marine environment. The distribution of microplastics, mainly the floating ones, is also influenced by environmental factors, such as winds, surface currents, turbulent flows, tides, waves, storm surges, through horizontal and vertical transport [10]. Different hydrodynamic processes, such as currents, tides, waves are the main agents of horizontal dispersion of microplastics from their sources. Microplastics, particularly floating ones, are passively transported by complex physical flows, resulting in a wide variability in surface concentrations. Wind also affects the distribution of floating plastic [15,16]. Neutral microplastics can float on the surface of the water but are also suspended in the water column until they reach deep water. Several studies have highlighted a discrepancy between the observed and predicted plastic concentrations in surface waters [17,18], also obtaining very different and more or less homogeneous vertical dispersion results depending on the oceanographic characteristics of the investigated study. This observed variability has promoted research on the vertical distribution of microplastics in the water column, leading to the evaluation of all environmental factors or intrinsic properties of plastic particles that can influence their vertical transport and subsequent sinking.

This phenomenon is well documented by the high presence of plastics and other anthropogenetic debris in deep environments and sediments, with an increased risk for species strictly related with sea floor, and meso-bathy pelagic environments [19–21].

Moreover, the fragmentation processes, which induce the formation of small fragments and fibers from plastics macro litter, increase their dispersion and bioavailability for marine organisms [22]. Microplastics (plastic' fragments smaller than 5 mm [23]) are widespread distributed and ingested by marine organisms inhabiting all the domains [22,24–27], but until now their effects on organisms are less known, despite the increasing amount of experimental studies focusing on this topic. In addition to plastics, other anthropogenic debris (e.g., rayon, dyed cotton fibers) are widely distributed in the entire marine ecosystem, raising major concerns about their toxicity, bio availability, and persistence in the environment [28]. Indeed, despite it is well-known that microplastics have the capability of absorb chemical contaminants, increasing the pollutants availability for organisms due to plastics ingestion, the knowledge base on contaminants transports conveyed by other anthropogenic debris, especially natural, or semi-natural fibers, is limited, if compared with those on plastics [28,29].

The Mediterranean Sea represents one of the most polluted area in terms of anthropogenic debris in the world [30,31], with a great amounts of surface plastic and microplastics [32,33] related to the high urbanization of the coastlines and the presence of heavily polluted rivers, which act as waste source for the entire basins. Each year, 0.57 million tons of plastic enter the Mediterranean waters, and this number will continue to rise as plastic waste production is expected to quadruple by 2050 [34]. Anthropogenic debris contamination, together with the other anthropogenic impacts acting in the Mediterranean Sea, makes this semi enclose basin a hotspot for habitat degradation and environmental pollution. For this reason, it is essential to monitor and study the level of pollution and contamination of the Mediterranean Sea [35], especially in the most impacted and anthropized geographical areas [36]. The Ionian Sea is a considerably exploited area by a large trawling fleet and a developed fishery operating with different gears (longline, gillnet, purse seine). This basin is characterized by the presence of heavily impacted zones by anthropogenic debris, floating, accumulated on the beaches or on the sea bed [37–39]. Moreover, it is well documented the widespread presence of marine debris and fishing litter in deep benthic environment and sediment from the entire Mediterranean basin at different depths, with accumulation zones reported in many different areas (e.g., French Mediterranean coast, Tyrrhenian Sea, Eastern Mediterranean, Cilician Coast, Spanish continental shelf, Sardinian coast) [39–45], making essential to assess the occurrence of anthropogenic debris in deep benthic species. In this regard, it is now known that vagile benthic fauna is particularly exposed to the risk of MPs ingestion. The feeding behavior of some species of crustaceans allows them to interact with sediment-water flows and resuspended sediments, making them excellent candidates for the role of bioindicators of MPs contamination of the seabed [46,47]. This has raised several concerns, considering that some decapods species, represent an essential resource for commercial fisheries, being among the most valuable and appreciated sea food resources worldwide [48–51]. In addition to their commercial value, they play a fundamental ecological role in benthic ecosystem, being an important component of megafaunal assemblages, occupying an high trophic position, and being among the most essential preys' for many apical demersal predators [27,51–58].

In this context, the aim of the present paper was to evaluate the presence of anthropogenic debris in the gastrointestinal tracts of three decapods of high commercial value (*Parapenaeus longirostris*, H. Lucas, 1846, *Aristeus antennatus*, Risso, 1816, *Aristaeomorpha foliacea*, Risso, 1827) from south-western Ionian Sea. They are usually caught using trawling nets, according with their bathymetric distribution. They inhabit the deep benthic environment, with the highest density at depths ranging from 150 to 350 m for *P. longirostris*, 300 to 2000 m for *A. antennatus*, and 300 to 800 m for *A. foliacea*. Several studies were carried out on microplastic contamination in *P. longirostris* and *A. antennatus* from different geographical Mediterranean areas [46,59–61], while only one report of plastic ingestion exists regarding *A. foliacea* [62]. Evaluating and analyzing the contamination in these species is essential to assess both the possible risk for human health related to their consumption, and the pollution degree of the deep-sea benthic environment in the studied area.

2. Materials and Methods

2.1. Sampling Area and Samples Processing

A total of 136 specimens (50 *P. longirostris*, 50 *A. antennatus*, 36 *A. foliacea*), were obtained from the local market, caught in the south-western Ionian Sea (autumn–winter 2021) by the trawling fleets operating in the Sicilian Ionian coast. This is an oligotrophic basin characterized by a high anthropogenetic impact [63,64], with a significant fishing pressure on the stocks inhabiting this area. Once landed, collected frozen specimens were transported to the laboratory to be processed. Each individual was weighted (total weight, TW) and measured (carapace length, CL), evaluating also its sex and degree of sexual maturity, according to Follesa, M. C., and Carbonara, P. [65]. Once registered the biometrics measurements, the gastrointestinal tract of each specimen was extracted for the anthropogenic debris extraction.

2.2. Anthropogenic Debris Extraction Protocol

For anthropogenic debris extraction, chemical digestion of the intestines and stomachs was performed, adopting a modified version of the protocol designed by Savoca et al. [66]. Each intestine was placed in a 250 mL conical glass flask. A calculated quantity of 10% KOH solution (minimum ratio 1:5 w/v) was added to the flask, subsequently covering with aluminum foil to avoid sample contamination. To remove the organic matter, the flasks were placed in an oscillation incubator to be continuously stirred at 50 °C for 48 h. Each sample was then put into a graduated glass cylinder and hypersaline NaCl solution (15%) was added to separate the two phases by density. This procedure allows low density microdebris to float in the aqueous phase [67]. After that, the supernatant was collected and filtered through a glass fiber membrane having 0.7 µm pore size and 47 mm diameter (Whatman GF/F, UK) using a vacuum system (Millipore). Neat filters were used as blank, following the same procedure of the samples. The filters were placed in sterile glass Petri dishes for subsequent observations under the stereomicroscope to isolate the anthropogenic debris. The isolated samples were recorded and categorized based on their shape, size classes, and color. The origin of the isolated microparticles was verified using the hot needle test to observe the melting points [22]. The hot needle test is now an accepted, inexpensive method that allows to check for the presence of plastic particles based on their response; in fact, the temperature range at which melting occurs does provide a specific range of potential plastics [68]. Briefly, the tip of a fine needle was heated and each isolated microparticle was tested under a stereomicroscope. When the microparticles dissolved after exposure to the hot needle, they were confirmed as microplastics (MPs).

2.3. Contamination Prevention

The samples were processed in a restricted access room to prevent any accidental external contamination. Workspaces and tools were thoroughly cleaned according to [66]. During the dissection procedure the specimens were exposed to the air for the minimum time possible within a glass Petri dish. All the materials used for dissection and analysis were rigorously cleaned with ethanol and filtered deionized water. Additionally, deionized water, potassium peroxide, and hypersaline solution were always pre-filtered (0.45 mm filter). Only sterilized glass items were used for all the assays. All sample processing was performed in a clean air flow cabinet to exclude the external contamination from fibers, which might represent a major contamination source. Filter paper in Petri dishes exposed to the laboratory air was used as control blank during the analysis [69]. Procedural blanks were obtained using filtered potassium peroxide and hypersaline solution, running through the entire laboratory procedure.

2.4. Data Analysis

After excluding non-plastic particles, the abundance and size of isolated anthropogenic debris (ADs) have been compared between male and female specimens within the same species and among species by applying the one-way analysis of variance (ANOVA). Relations between specimens' body weight and total length and microplastic number or size were tested using the Pearson's correlation. The Chi-square test was used to compare the colors of ADs ingested by species. Significance level was set at $p < 0.05$. Statistical analyses were performed using the software package Prism, Version 8.2.1 (Graphpad Software Ldt., La Jolla, CA 92037, USA).

3. Results

In the present study, three major commercial shrimp species *P. longirostris*, *A. antennatus,* and *A. foliacea* were investigated for their content of anthropogenic debris (AD) in the gastrointestinal tract (GIT). The number of specimens analyzed and their morphological characteristics, including the total body length (TL, cm), body weight (W, g) of the analyzed species are reported as means ± SD in Table 1. Morphological characteristics of the specimens that did not show AD contamination are shown in Table 2. The size classes of the identified MPs are shown in Table 3.

A total of 136 specimens were examined. The non-plastic particles identified were excluded from the statistical analysis and were represented by 9, 5, and 11 microparticles isolated from *P. longirostris*, *A. antennatus,* and *A. foliacea*, respectively.

Overall, 230 MPs were isolated, mostly represented by fibers (92.6%) with a size between 0.1 and 0.49 mm (20.43%), and with a dominance of the blue color (42.6%). Representative images of the isolated MPs are shown in Figure 1. A detailed description of the results obtained for each species is reported below.

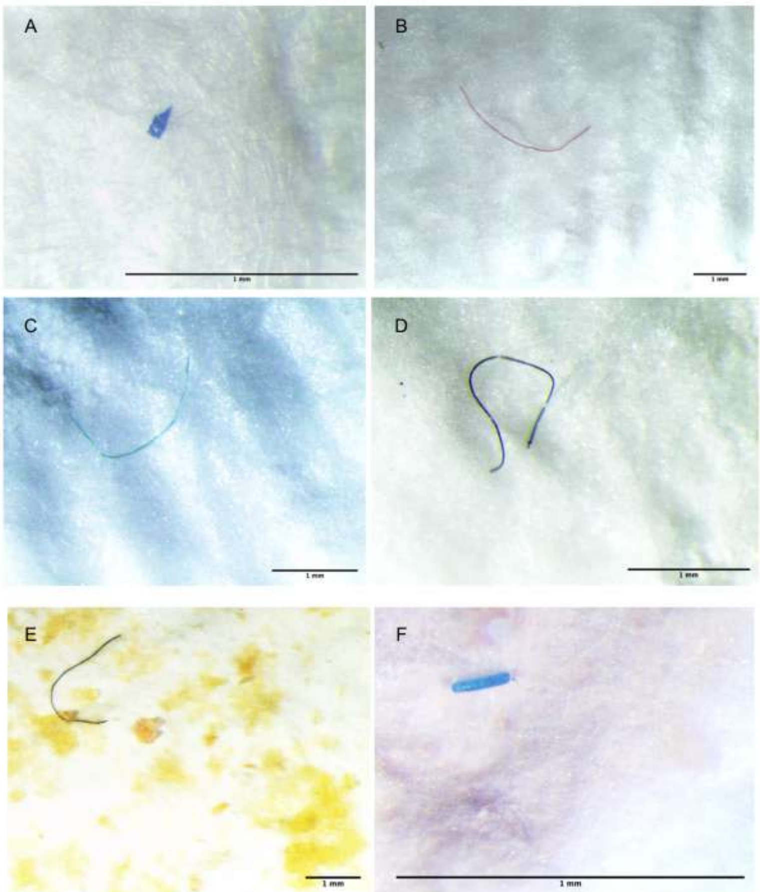

Figure 1. Representative images of AD isolated from *P. longirostris* (**A**,**B**), *A. antennatus* (**C**,**D**), and *A. foliacea* (**E**,**F**).

Table 1. Morphometric data of the analyzed crustacean species collected from the south-western Ionian Sea and the corresponding levels of particle contamination. N: number of specimens examined; Np: number of samples with detected particles.

Species	Length (mm) Means ± SD	Weight (g) Means ± SD	N° of Specimens	Np	Items/Specimen
Parapenaues longirostris	23.3 ± 1.6	8.1 ± 1.5	50	37	2.24
Aristeus antennatus	45.8 ± 5	31.5 ± 8	50	35	2.22
Aristaeomorpha foliacea	38.4 ± 6.7	19.1 ± 11	36	30	2.30

Table 2. Morphometric data of the analyzed crustacean species collected from the south-western Ionian Sea that did not show anthropogenic particles contamination. Maturity stages (see Maturity column) were detected according to the Atlas of the maturity stages of Mediterranean fishery resources [65]; 2E represents the resting adults stage in female specimens (uncolored resting ovaries with the presence of spermatophores in *A. antennatus* and *A. foliacea*) and 2B represents the recovering stage in both female (ovary developing status with a flesh, ivory and cream color in *A. foliace*, *A. antennatus* and *P. longirostris*, respectively) and male specimens (petasma completely joined, without spermatic masses in the seminar ampullae).

Species	Sample	Length (mm)	Weight (g)	Sex	Maturity	N° AD
Parapenaues longirostris	8	27.20	7.60	M	2E	0
	10	27.60	9.10	M	2B	0
	14	26.40	9.90	M	2B	0
	16	29.00	8.40	M	2E	0
	19	26.00	6.70	M	2E	0
	26	27.20	9.70	F	2B	0
	32	26.20	7.40	F	2B	0
	34	25.50	7.80	F	2B	0
	35	25.00	7.40	F	2B	0
	36	26.00	4.70	F	2B	0
	38	26.80	8.70	F	2B	0
	41	27.10	9.20	F	2B	0
	44	26.00	8.40	F	2B	0
Aristeus antennatus	51	49.60	36.50	F	2E	0
	53	51.50	41.70	F	2E	0
	56	50.30	40.80	F	2E	0
	60	37.50	19.00	F	2E	0
	61	42.50	25.10	F	2E	0
	76	50.80	41.50	F	2E	0
	77	49.30	38.80	F	2E	0
	78	41.90	23.70	F	2B	0
	80	43.00	30.70	F	2B	0
	86	50.50	34.30	F	2B	0
	87	55.50	45.90	F	2B	0
	89	48.10	33.60	F	2B	0
	93	56.00	45.90	F	2B	0
	94	52.00	41.60	F	2B	0
	98	46.80	37.40	F	2B	0
Aristaeomorpha foliacea	105	36.00	17.11	F	2E	0
	107	40.0	19.64	F	2E	0
	109	34.80	11.10	F	2E	0
	122	37.50	18.99	F	2E	0
	125	52.00	42.23	F	2E	0
	132	31.00	9.00	F	2E	0

Table 3. Size classes (mm) and number of the MPs isolated from the species analyzed this study.

Size Classes	Size Range	*P. longirostris*	*A. antennatus*	*A. foliacea*
I	0.10–0.49	12	11	24
II	0.50–0.99	15	16	8
III	1.00–1.49	11	14	10
IV	1.50–1.99	13	12	10
V	2.00–2.49	12	7	4
VI	2.50–2.99	7	7	5
VII	3.00–3.49	4	3	4
VIII	3.50–3.99	1	4	2
IX	4.00–4.99	1	3	1
X	≥5.00	7	1	1

The GITs of 50 specimens belonging to the *P. longirostris* species were examined, in which the presence of MPs was found in 76% of the specimens analyzed. From these, 83 micro debris were isolated, present both in the form of fibers (95.1%) and fragments (4.8%). The size of these microparticles was between 0.11 and 10.40 mm, the largest percentage of which fell in size class II (18%). The color composition of the microparticles was rather heterogeneous, black (27.70%) and light blue (22.89%) were the dominant ones, followed by lower representative percentages of blue (19.27%), red (9.60%) and others (see Figure 2).

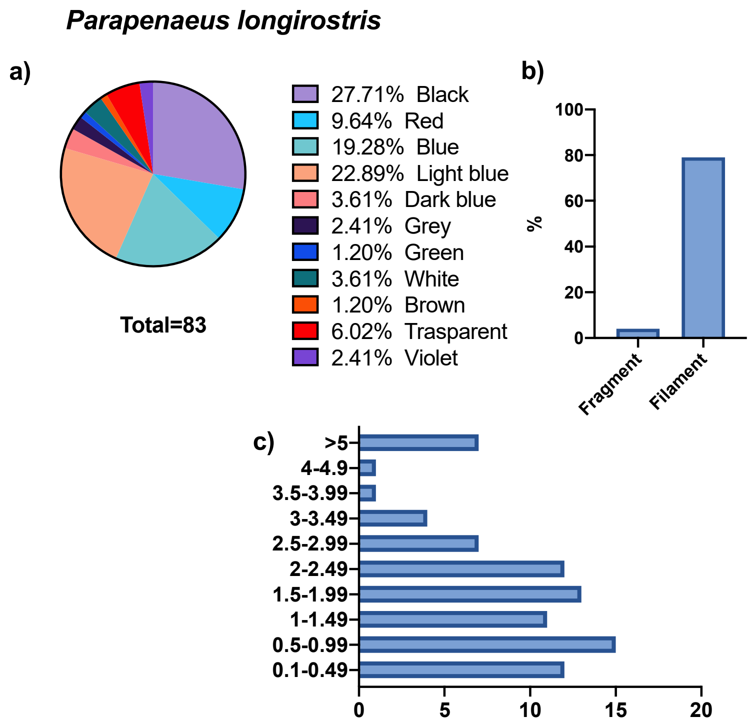

Figure 2. Abundance, colors (**a**), shape (**b**), and size (**c**) of microparticles isolated from *P. longirostris* specimens.

No difference in AD abundance was found between male and female specimens ($p > 0.05$).

The GITs of 50 specimens belonging to the *A. antennatus* species were examined, of which 70% showed the presence of MPs. From these, 78 microdebris were isolated, present only in the form of fibers. The size of these microparticles was between 0.11 and 5.50 mm, the largest percentage of which fell in size class II (20.5%). The color distribution of the microparticles was more characterized by the dominance of blue (44.8%) and black (20.5%), followed by lower representative percentages of transparent (11.5%), gray (8.9%), and others (see Figure 3). All the specimens were females, so it was not possible to differentiate between the sexes.

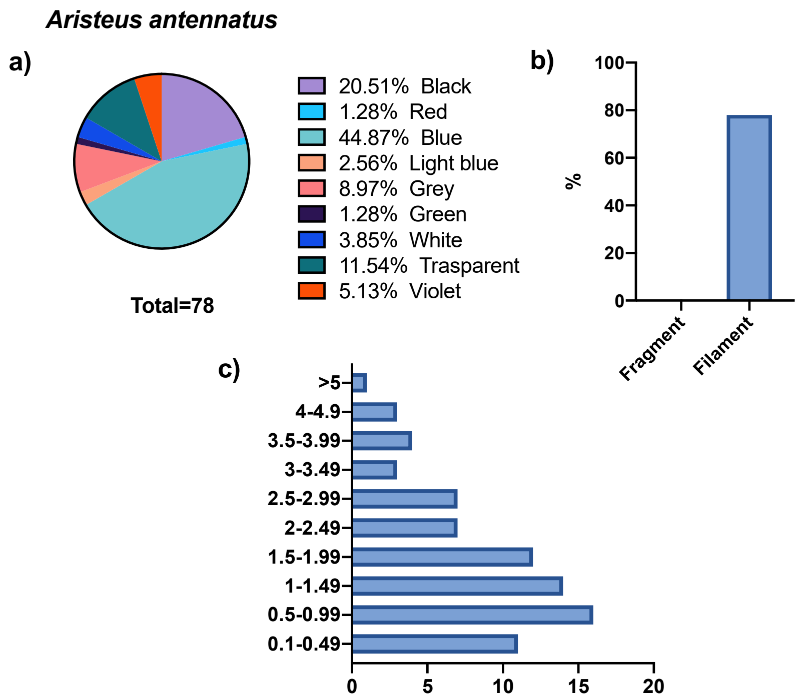

Figure 3. Abundance, colors (**a**), shape (**b**), and size (**c**) of microparticles isolated from *A. antennatus*.

Finally, 36 GITs of *A. foliacea* were examined, showing the presence of MPs in 83% of the specimens analyzed. From these, a total of 69 microdebris were isolated, of which 81% had a fibrous form and 18.8% a fragment form. The size of these microparticles was between 0.01 and 7.50 mm, the largest percentage of which fell in size class I (34.7%). Blue colored microparticles were dominant (68.0%), followed by transparent ones (11.6%) (see Figure 4). All the specimens were females except two, so it was not possible to differentiate between the sexes, as the result would have been inaccurate.

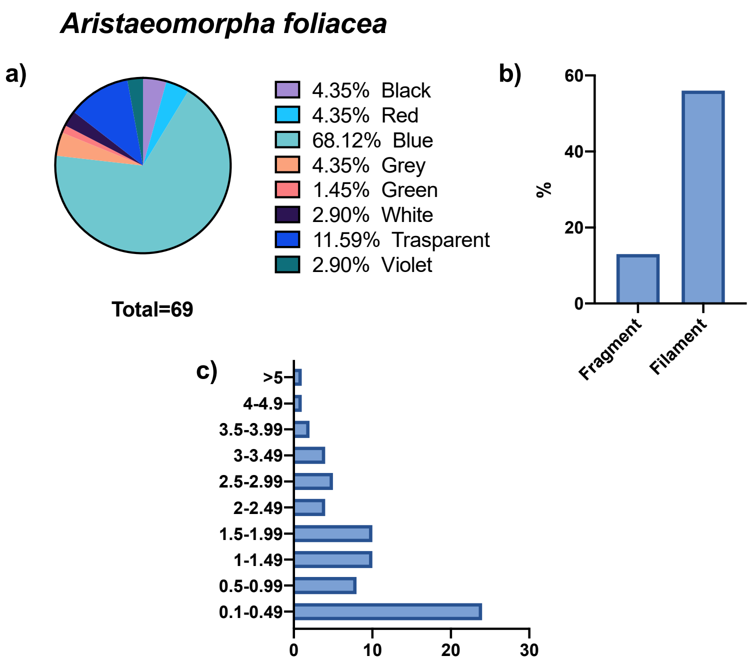

Figure 4. Abundance, colors (**a**), shape (**b**), and size (**c**) of microparticles isolated from *A. foliacea*.

No significant differences between the MPs abundances were found between the species. Furthermore, there was no correlation between the size of the specimens of each species and the dimensional characteristics and abundances of the MPs ($p > 0.05$, Figure 5).

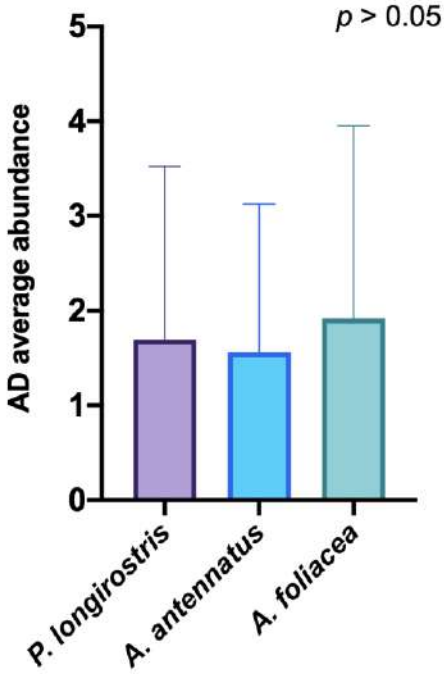

Figure 5. Microparticles abundance comparison between the three species analyzed.

However, significant AD size differences were found between *P. longirostris* and *A. foliacea* specimens ($p = 0.02$) (Figure 6). Indeed, larger MPs were found in the first species than in the second, as shown in Table 3.

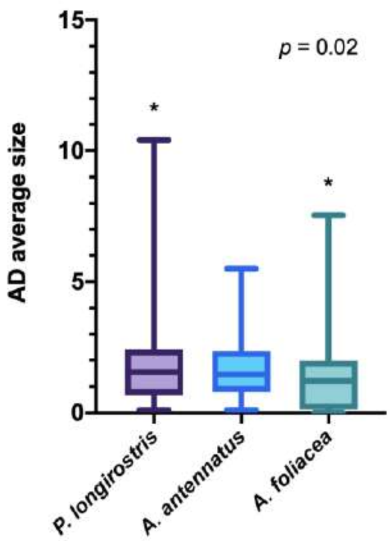

Figure 6. Microparticles size comparison between the three species analyzed. * indicates significant differences $p < 0.05$.

Significant differences were identified in the color composition of the MPs isolated from the three species ($p < 0.05$).

4. Discussion

To our best knowledge, the present paper was the first investigation on the AD presence in gastrointestinal tract of *P. longirostris*, *A. antennatus*, and *A. foliacea* from south-western Ionian Sea. Results showed a high frequency of debris' occurrence in all the analyzed species (respectively 76%, 70% and 83%), which, if compared with the literature from heavily contaminated areas [70], confirm the high and worrying degree of anthropogenic debris contamination in Mediterranean Sea deep environment. Indeed, this is considered a contamination hotspot for AD (especially micro and macro plastics) both in water column, on seafloor, and in sediments [33,41,45,71–74]. Concerning the south-western Ionian Sea, as widely reported in many Mediterranean geographical sub areas, the presence of submarine canyons [42,45,75], together with the peculiar water mass circulation [76–80], the presence of high urbanization degree near the coast, and the large amount of fisheries activities [49,76] could increase the accumulation of debris, especially fishing gear and waste of various nature which settle on sea floor [41,43]. The fragmentation and degradation processes acting on these debris induce the formation of small fragments and microfibers (such as microplastics), enhancing their availability for benthic organisms, which accidentally (through gills [81]) or intentionally may ingest them. According to the literature, it is widely reported how marine organisms can mistake small AD for food [82], ingesting them by a direct way or via indirect intake through trophic transfer [83].

Concerning investigated species, these are active benthic predators, with secondary scavenging habits [55,84,85]. *P. longirostris* alternate a hunting phase, in which it preys on swimming benthopelagic species (e.g., crustaceans, cephalopods and small fishes), with a digging phase, in which it digs in the mud searching for food, such as polychaetae, echinoderms, and bivalves [85]. It is widely distributed in depth not exploited by *A. antennatus* and *A. foliacea*, showing a different bathymetrical distribution (from 50 to 700 m, with highest densities in Mediterranean Sea reported between 150 to 350 m), fundamental for a resource partitioning with the other bathyal penaeoideans [51,86]. This difference in distribution was highlighted also by the color of micro debris isolated from analyzed specimens, with a dominance of black (27.70%) and light blue (22.89%) fibers, bigger than those found from the other species. The color and size composition of anthropogenic debris isolated from *P. longirostris* specimens could be strictly related to bathymetry and habitats exploited by the species. Indeed, *A. antennatus* and *A. foliacea*, inhabiting deeper environments than *P. longirostris*, showed a similar dimensional range (0.11–5.50 mm and 0.10–7.50 mm, respectively) with a closer color composition (blue 44.8% and black 20.5%, blue 68.0%, respectively) of micro debris isolated from GIT. According to previous literature on AD contamination in *P. longirostris*, only one study was performed on specimens from the Strait of Sicily [60]. Results obtained by Bono et al. [60] had been very different from those obtained in the present paper, with a lower frequency of occurrence (21%), the presence of spherical fragments, and a relation between plastic occurrence and shrimps' size. These differences could be related to the different sampling area, highlighting the high contamination degree of south-western Ionian Sea deep environments. Concerning the relation between debris occurrence and shrimps' length, further analysis with a larger dimensional range of samples is required to analyze the potential connection between length and debris contamination. As reported by several authors, *P. longirostris* diets show ontogenetic variation, with large specimens which show the most efficiency as active predators than smaller ones [85,87]. This variation in predation dynamics could also influence the anthropogenic debris intake, facilitated or not by the increase in active predation.

As stated before, *A. antennatus* and *A. foliacea* showed a similar composition for fibers color and size, with a difference in micro debris shape. All the AD isolated from *A. antennatus* samples were fibers, while *A. foliacea* samples showed the highest occurrence of fragments (18.8%) among the studied species. This may be related to their different feeding habits. Indeed, as widely reported in the literature, these two sympatric species have been adapting to exploit different resources to facilitate their coexistence in similar areas [88]. *A. antennatus* is an euryphagous species adapted to hunt endobenthic invertebrates in the mud [89,90]. Otherwise, *A. foliacea* diet is mainly based on planktonic and pelagic species (e.g., euphausiids, myctophids) [55,91,92]. These different feeding habits could influence the intake dynamics of plastics and other AD, allowing the differences in debris shape showed by results. The AD contamination in *A. antennatus* GIT was previously assessed in the literature from other Mediterranean geographical area. Carreras-Colom E. [59,61,93] analyzed the contamination with microplastics in this species from the Balearic Basin (north-western Mediterranean Sea), investigating also the seasonal and geographical dynamics in plastics occurrence and their impact on shrimps health condition. The frequency of occurrence in 2020 [59] was higher (85.8%) than that reported in results from the present paper, with the massive presence of single fibers and tangled ball of fibers. This high degree of plastic contamination in GIT of *A. antennatus* from high impacted Mediterranean geographical areas, such as Balearic Basin area near Barcelona city, confirms once again the importance of monitoring the contamination of anthropogenic debris in deep benthic organism, and how this can be strictly related to the degree of environmental pollution.

Concerning *A. foliacea,* to our best knowledge, the present paper represents the first assessment on the presence of AD in GIT, since, according to the literature [55], only one study on diet and trophic ecology had reported the presence of plastic debris in stomach contents of samples from Western Mediterranean Sea. The high frequency of occurrence showed by results (83%), with the dominance of blue fibers isolated from samples, underlines the necessity to improve the knowledge base on the presence of plastics and other AD in GIT of deep benthic crustaceans, especially of those with high commercial value. Indeed, despite it is widely reported in the contamination in many animal species inhabiting marine environments [82,94], relative less studies have been performed worldwide on shrimps and other decapod crustacean species despite their high commercial and ecological value [70]. For this reason, it is essential to broaden the knowledge base on this essential invertebrate class in a highly impacted basin, such as the Mediterranean Sea, focusing the attention on the most commercially viable species, and also monitoring the possible risks for human health.

5. Conclusions

The present study assessed the presence of anthropogenic debris in the gastrointestinal tracts of the studied species, *P. longirostris*, *A. antennatus*, and *A. foliacea*. A total of 230 low density microparticles were isolated, with a high frequency of occurrence in all the analyzed species (76% in *P. longirostris*, 70% in *A. antennatus*, and 83% in *A. foliacea*) mainly represented by fibers (92.6%) with a size between 0.10 and 0.49 mm, and with a dominance of the blue color. To our best knowledge the results obtained in this study report for the first time the anthropogenic debris presence in the studied Decapoda from south-western Ionian Sea, highlighting the necessity to broaden the knowledge about anthropogenic debris pollution status in Mediterranean deep-sea species. This could help also to monitor possible risks of ingestion in humans, only in case of consumption of the individual's whole body (without evisceration). Additionally, it will be of fundamental importance to perform studies on the potential presence of nano-sized debris in edible tissues to better assess the risks of these pollutants' ingestion.

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References

1. Hardesty, B.D.; Wilcox, C. A risk framework for tackling marine debris. *Anal. Methods* **2017**, *9*, 1429–1436. [CrossRef]
2. Hardesty, B.D.; Wilcox, C.A. Presence of microplastics and nanoplastics in food, with particular focus on seafood. *EFSA J.* **2016**, *14*, e04501. [CrossRef]
3. Jeftic, L.; Sheavly, S.; Adler, E. *Marine Litter: A Global Challenge Marine Litter: A Global Challenge*; UNEP: Nairobi, Kenya, 2009; ISBN 9789280730296.
4. Jambeck, J.R.; Geyer, R.; Wilcox, C.; Siegler, T.R.; Perryman, M.; Andrady, A.; Narayan, R.; Law, K.L. Plastic waste inputs from land into the ocean. *Science* **2015**, *347*, 768–771. [CrossRef] [PubMed]
5. Geyer, R.; Jambeck, J.R.; Law, K.L. Production, use, and fate of all plastics ever made. *Sci. Adv.* **2017**, *3*, e1700782. [CrossRef]
6. Andrady, A.L. Microplastics in the marine environment. *Mar. Pollut. Bull.* **2011**, *62*, 1596–1605. [CrossRef]
7. Consoli, P.; Falautano, M.; Sinopoli, M.; Perzia, P.; Canese, S.; Esposito, V.; Battaglia, P.; Romeo, T.; Andaloro, F.; Galgani, F.; et al. Composition and abundance of benthic marine litter in a coastal area of the central Mediterranean Sea. *Mar. Pollut. Bull.* **2018**, *136*, 243–247. [CrossRef]
8. Ryan, P.G.; Moore, C.J.; Van Franeker, J.A.; Moloney, C.L. Monitoring the abundance of plastic debris in the marine environment. *Philos. Trans. R. Soc. B Biol. Sci.* **2009**, *364*, 1999–2012. [CrossRef]
9. Kane, I.A.; Clare, M.A.; Miramontes, E.; Wogelius, R.; Rothwell, J.J.; Garreau, P.; Pohl, F. Seafloor microplastic hotspots controlle by deep-sea circulation. *Science* **2020**, *368*, 1140–1145. [CrossRef]
10. Ballent, A.; Purser, A.; de Jesus Mendes, P.; Pando, S.; Thomsen, L. Physical transport properties of marine microplastic pollution. *Biogeosci. Discuss.* **2012**, *9*, 18755–18798. [CrossRef]
11. Ballent, A.; Pando, S.; Purser, A.; Juliano, M.F.; Thomsen, L. Modelled transport of benthic marine microplastic pollution in the Nazaré Canyon. *Biogeosciences* **2013**, *10*, 7957–7970. [CrossRef]
12. Kowalski, N.; Reichardt, A.M.; Waniek, J.J. Sinking rates of microplastics and potential implications of their alteration by physical, biological, and chemical factors. *Mar. Pollut. Bull.* **2016**, *109*, 310–319. [CrossRef] [PubMed]
13. Cózar, A.; Sanz-Martín, M.; Martí, E.; González-Gordillo, J.I.; Ubeda, B.; Gálvez, J.Á.; Irigoien, X.; Duarte, C.M. Plastic accumulation in the mediterranean sea. *PLoS ONE* **2015**, *10*, e0121762. [CrossRef] [PubMed]
14. Peng, X.; Chen, M.; Chen, S.; Dasgupta, S.; Xu, H.; Ta, K.; Du, M.; Li, J.; Guo, Z.; Bai, S. Microplastics contaminate the deepest part of the world's ocean. *Geochem. Perspect. Lett.* **2018**, *9*, 1–5. [CrossRef]
15. Kako, S.; Isobe, A.; Kataoka, T.; Hinata, H. A decadal prediction of the quantity of plastic marine debris littered on beaches of the East Asian marginal seas. *Mar. Pollut. Bull.* **2014**, *81*, 174–184. [CrossRef]
16. Liubartseva, S.; Coppini, G.; Lecci, R.; Creti, S. Regional approach to modeling the transport of floating plastic debris in the Adriatic Sea. *Mar. Pollut. Bull.* **2016**, *103*, 115–127. [CrossRef]
17. Cózar, A.; Echevarría, F.; González-Gordillo, J.I.; Irigoien, X.; Úbeda, B.; Hernández-León, S.; Palma, Á.T.; Navarro, S.; García-de-Lomas, J.; Ruiz, A.; et al. Plastic debris in the open ocean. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 10239–10244. [CrossRef]
18. Eriksen, M.; Lebreton, L.C.M.; Carson, H.S.; Thiel, M.; Moore, C.J.; Borerro, J.C.; Galgani, F.; Ryan, P.G.; Reisser, J. Plastic Pollution in the World's Oceans: More than 5 Trillion Plastic Pieces Weighing over 250,000 Tons Afloat at Sea. *PLoS ONE* **2014**, *9*, e0111913. [CrossRef]
19. Woodall, L.C.; Robinson, L.F.; Rogers, A.D.; Narayanaswamy, B.E.; Paterson, G.L.J. Deep-sea litter: A comparison of seamounts, banks and a ridge in the Atlantic and Indian Oceans reveals both environmental and anthropogenic factors impact accumulation and composition. *Front. Mar. Sci.* **2015**, *2*, 3. [CrossRef]
20. Chiba, S.; Saito, H.; Fletcher, R.; Yogi, T.; Kayo, M.; Miyagi, S.; Ogido, M.; Fujikura, K. Human footprint in the abyss: 30 year records of deep-sea plastic debris. *Mar. Policy* **2018**, *96*, 204–212. [CrossRef]
21. Choy, C.A.; Robison, B.H.; Gagne, T.O.; Erwin, B.; Firl, E.; Halden, R.U.; Hamilton, J.A.; Katija, K.; Lisin, S.E.; Rolsky, C.; et al. The vertical distribution and biological transport of marine microplastics across the epipelagic and mesopelagic water column. *Sci. Rep.* **2019**, *9*, 7843. [CrossRef]
22. Lusher, A.L.; Welden, N.A.; Sobral, P.; Cole, M. Sampling, isolating and identifying microplastics ingested by fish and invertebrates. *Anal. Methods* **2017**, *9*, 1346–1360. [CrossRef]
23. Hartmann, N.B.; Hüffer, T.; Thompson, R.C.; Hassellöv, M.; Verschoor, A.; Daugaard, A.E.; Rist, S.; Karlsson, T.; Brennholt, N.; Cole, M.; et al. Are We Speaking the Same Language? Recommendations for a Definition and Categorization Framework for Plastic Debris. *Environ. Sci. Technol.* **2019**, *53*, 1039–1047. [CrossRef] [PubMed]
24. Imhof, H.K.; Sigl, R.; Brauer, E.; Feyl, S.; Giesemann, P.; Klink, S.; Leupolz, K.; Löder, M.G.J.; Löschel, L.A.; Missun, J.; et al. Spatial and temporal variation of macro-, meso- and microplastic abundance on a remote coral island of the Maldives, Indian Ocean. *Mar. Pollut. Bull.* **2017**, *116*, 340–347. [CrossRef] [PubMed]
25. Alomar, C.; Deudero, S. Evidence of microplastic ingestion in the shark *Galeus melastomus* Rafinesque, 1810 in the continental shelf off the western Mediterranean Sea. *Environ. Pollut.* **2017**, *223*, 223–229. [CrossRef]

26. Albano, M.; Panarello, G.; Di Paola, D.; D'Angelo, G.; Granata, A.; Savoca, S.; Capillo, G. The mauve stinger *Pelagia noctiluca* (Cnidaria, Scyphozoa) plastics contamination, the Strait of Messina case. *Int. J. Environ. Stud.* **2021**, *78*, 977–982. [CrossRef]
27. D'Iglio, C.; Savoca, S.; Rinelli, P.; Spanò, N. Diet of the Deep-Sea Shark *Galeus melastomus* Rafinesque, 1810, in the Mediterranean Sea: What We Know and What We Should Know. *Sustainability* **2021**, *13*, 3962. [CrossRef]
28. Ladewig, S.M.; Bao, S.; Chow, A.T. Natural Fibers: A Missing Link to Chemical Pollution Dispersion in Aquatic Environments. *Environ. Sci. Technol.* **2015**, *49*, 12609–12610. [CrossRef]
29. Li, L.; Frey, M.; Browning, K.J. Biodegradability study on cotton and polyester fabrics. *J. Eng. Fibers Fabr.* **2010**, *5*, 42–53. [CrossRef]
30. García-Rivera, S.; Lizaso, J.L.S.; Millán, J.M.B. Spatial and temporal trends of marine litter in the Spanish Mediterranean seafloor. *Mar. Pollut. Bull.* **2018**, *137*, 252–261. [CrossRef]
31. UNEP/MAP. *Updated Report on Marine Litter Assessment in the Mediterranean*; UNEP(DEPI)/MED WG.421/Inf.18; European Environment Agency: Athens, Greece, 2015.
32. Suaria, G.; Aliani, S. Floating debris in the Mediterranean Sea. *Mar. Pollut. Bull.* **2014**, *86*, 494–504. [CrossRef]
33. Suaria, G.; Avio, C.G.; Mineo, A.; Lattin, G.L.; Magaldi, M.G.; Belmonte, G.; Moore, C.J.; Regoli, F.; Aliani, S. The Mediterranean Plastic Soup: Synthetic polymers in Mediterranean surface waters. *Sci. Rep.* **2016**, *6*, 37551. [CrossRef] [PubMed]
34. Dalberg Advisors, W.M.M. *Initiative Stop the Flood of Plastic: How Mediterranean Countries Can Save Their Sea. A Guide for Policy Makers in Morocco*; WWF: Gland, Switzerland, 2019.
35. Costa, R.; Albergamo, A.; Piparo, M.; Zaccone, G.; Capillo, G.; Manganaro, A.; Dugo, P.; Mondello, L. Multidimensional gas chromatographic techniques applied to the analysis of lipids from wild-caught and farmed marine species. *Eur. J. Lipid Sci. Technol.* **2017**, *119*, 1600043. [CrossRef]
36. Alimba, C.G.; Faggio, C. Microplastics in the marine environment: Current trends in environmental pollution and mechanisms of toxicological profile. *Environ. Toxicol. Pharmacol.* **2019**, *68*, 61–74. [CrossRef] [PubMed]
37. Prevenios, M.; Zeri, C.; Tsangaris, C.; Liubartseva, S.; Fakiris, E.; Papatheodorou, G. Beach litter dynamics on Mediterranean coasts: Distinguishing sources and pathways. *Mar. Pollut. Bull.* **2018**, *129*, 448–457. [CrossRef] [PubMed]
38. Politikos, D.V.; Tsiaras, K.; Papatheodorou, G.; Anastasopoulou, A. Modeling of floating marine litter originated from the Eastern Ionian Sea: Transport, residence time and connectivity. *Mar. Pollut. Bull.* **2020**, *150*, 110727. [CrossRef]
39. Vlachogianni, T.; Fortibuoni, T.; Ronchi, F.; Zeri, C.; Mazziotti, C.; Tutman, P.; Varezić, D.B.; Palatinus, A.; Trdan, Š.; Peterlin, M.; et al. Marine litter on the beaches of the Adriatic and Ionian Seas: An assessment of their abundance, composition and sources. *Mar. Pollut. Bull.* **2018**, *131*, 745–756. [CrossRef]
40. Galgani, F.; Leaute, J.P.; Moguedet, P.; Souplet, A.; Verin, Y.; Carpentier, A.; Goraguer, H.; Latrouite, D.; Andral, B.; Cadiou, Y.; et al. Litter on the sea floor along European coasts. *Mar. Pollut. Bull.* **2000**, *40*, 516–527. [CrossRef]
41. Angiolillo, M.; di Lorenzo, B.; Farcomeni, A.; Bo, M.; Bavestrello, G.; Santangelo, G.; Cau, A.; Mastascusa, V.; Cau, A.; Sacco, F.; et al. Distribution and assessment of marine debris in the deep Tyrrhenian Sea (NW Mediterranean Sea, Italy). *Mar. Pollut. Bull.* **2015**, *92*, 149–159. [CrossRef]
42. Tubau, X.; Canals, M.; Lastras, G.; Rayo, X.; Rivera, J.; Amblas, D. Marine litter on the floor of deep submarine canyons of the Northwestern Mediterranean Sea: The role of hydrodynamic processes. *Prog. Oceanogr.* **2015**, *134*, 379–403. [CrossRef]
43. Cau, A.; Alvito, A.; Moccia, D.; Canese, S.; Pusceddu, A.; Rita, C.; Angiolillo, M.; Follesa, M.C. Submarine canyons along the upper Sardinian slope (Central Western Mediterranean) as repositories for derelict fishing gears. *Mar. Pollut. Bull.* **2017**, *123*, 357–364. [CrossRef]
44. García-Rivera, S.; Lizaso, J.L.S.; Millán, J.M.B. Composition, spatial distribution and sources of macro-marine litter on the Gulf of Alicante seafloor (Spanish Mediterranean). *Mar. Pollut. Bull.* **2017**, *121*, 249–259. [CrossRef]
45. Pierdomenico, M.; Casalbore, D.; Chiocci, F.L. The key role of canyons in funnelling litter to the deep sea: A study of the Gioia Canyon (Southern Tyrrhenian Sea). *Anthropocene* **2020**, *30*, 100237. [CrossRef]
46. Cau, A.; Avio, C.G.; Dessì, C.; Follesa, M.C.; Moccia, D.; Regoli, F.; Pusceddu, A. Microplastics in the crustaceans *Nephrops norvegicus* and *Aristeus antennatus*: Flagship species for deep-sea environments? *Environ. Pollut.* **2019**, *255*, 113107. [CrossRef] [PubMed]
47. Cau, A.; Avio, C.G.; Dessì, C.; Moccia, D.; Pusceddu, A.; Regoli, F.; Cannas, R.; Follesa, M.C. Benthic Crustacean Digestion Can Modulate the Environmental Fate of Microplastics in the Deep Sea. *Environ. Sci. Technol.* **2020**, *54*, 4886–4892. [CrossRef] [PubMed]
48. STECF. *Mediterranean Assessments Part 1 (STECF-15-18)*; JRC 98676; EUR 27638; Publications Office of the European Union: Luxembourg, 2015; Volume 1, ISBN 978-92-79-54141-4.
49. STECF. *Scientific, Technical and Economic Committee for Fisheries (STECF)—Stock Assessments: Demersal Stocks in the Western Mediterranean Sea (STECF-20-09)*; EUR 28359; Publications Office of the European Union: Luxembourg, 2020; ISBN 978-92-76-11288-4.
50. Carvalho, N.; Doerner, H. *Scientific, Technical and Economic Committee for Fisheries (STECF) Balance Indicators*; Publications Office of the European Union: Luxembourg, 2014. [CrossRef]
51. Perdichizzi, A.; D'Iglio, C.; Giordano, D.; Profeta, A.; Ragonese, S.; Rinelli, P. Comparing life-history traits in two contiguous stocks of the deep-water rose shrimp *Parapenaeus longirostris* (H. Lucas, 1846) (Crustacea: Decapoda) in the Southern Tyrrhenian Sea (Central Mediterranean Sea). *Fish. Res.* **2022**, *248*, 106206. [CrossRef]
52. Cartes, J.E.; Company, J.B.; Maynou, F. Deep-water decapod crustacean communities in the Northwestern Mediterranean: Influence of submarine canyons and season. *Mar. Biol.* **1994**, *120*, 221–229. [CrossRef]

53. García, T.; Sobrino, I. Biology and fishery of the deepwater Rose shrimp, *Parapenaeus longirostris* (Lucas, 1846), from the Atlantic Moroccan coast. *Sci. Mar.* **1994**, *58*, 299–305.
54. Modica, L.; Cartes, J.E.; Velasco, F.; Bozzano, A. Juvenile hake predation on Myctophidae and Sternoptychidae: Quantifying an energy transfer between mesopelagic and neritic communities. *J. Sea Res.* **2015**, *95*, 217–225. [CrossRef]
55. Cartes, J.E. Diets of, and trophic resources exploited by, bathyal penaeoidean shrimps from the western Mediterranean. *Mar. Freshw. Res.* **1995**, *46*, 889–896. [CrossRef]
56. D'Iglio, C.; Porcino, N.; Savoca, S.; Profeta, A.; Perdichizzi, A.; Armeli, E.; Davide, M.; Francesco, S.; Rinelli, P.; Giordano, D. Ontogenetic shift and feeding habits of the European hake (*Merluccius merluccius* L., 1758) in Central and Southern Tyrrhenian Sea (Western Mediterranean Sea): A comparison between past and present data. *Ecol. Evol.* **2022**, *12*, e8634. [CrossRef]
57. D'Iglio, C.; Albano, M.; Tiralongo, F.; Famulari, S.; Rinelli, P.; Savoca, S.; Spanò, N.; Capillo, G. Biological and Ecological Aspects of the Blackmouth Catshark (*Galeus melastomus* Rafinesque, 1810) in the Southern Tyrrhenian Sea. *J. Mar. Sci. Eng.* **2021**, *9*, 967. [CrossRef]
58. D'Iglio, C.; Famulari, S.; Albano, M.; Giordano, D.; Rinelli, P.; Capillo, G.; Spanò, N.; Savoca, S. Time-Scale Analysis of Prey Preferences and Ontogenetic Shift in the Diet of European Hake *Merluccius merluccius* (Linnaeus, 1758) in Southern and Central Tyrrhenian Sea. *Fishes* **2022**, *7*, 167. [CrossRef]
59. Carreras-Colom, E.; Constenla, M.; Soler-Membrives, A.; Cartes, J.E.; Baeza, M.; Carrassón, M. A closer look at anthropogenic fiber ingestion in *Aristeus antennatus* in the NW Mediterranean Sea: Differences among years and locations and impact on health condition. *Environ. Pollut.* **2020**, *263*, 114567. [CrossRef] [PubMed]
60. Bono, G.; Falsone, F.; Falco, F.; Di Maio, F.; Gabriele, M.; Gancitano, V.; Geraci, M.L.; Mancuso, M.; Okpala, C.; Luisa, P.; et al. Microplastics and Alien Black Particles as Contaminants of Deep-Water Rose Shrimp (*Parapenaeus longistroris* Lucas, 1846) in the Central Mediterranean Sea. *J. Adv. Biotechnol. Bioeng.* **2020**, *8*, 23–28. [CrossRef]
61. Carreras-Colom, E. Unravelling the (Micro)Plastic Threat: The Case Study of Plastic Ingestion in *Aristeus antennatus* and *Nephrops norvegicus* from the NW Mediterranean Sea and Its Potential Impact on Health Condition. Ph.D. Thesis, Universitat Autònoma de Barcelona, Barcelona, Spain, 2021.
62. Kapiris, K.; Thessalou-Legaki, M.; Petrakis, G.; Conides, A. Ontogenetic shifts and temporal changes in the trophic patterns of the deep-sea red shrimp, *Aristaeomorpha foliacea* (Decapods: Aristeidae), in the Eastern Ionian Sea (Eastern Mediterranean). *Mar. Ecol.* **2010**, *31*, 341–354. [CrossRef]
63. Mangano, M.C.; Kaiser, M.J.; Porporato, E.M.D.; Lambert, G.I.; Spanò, N. Trawling disturbance effects on the trophic ecology of two co-generic Astropectinid species. *Mediterr. Mar. Sci.* **2015**, *16*, 538–549. [CrossRef]
64. Porporato, E.M.D.; Lo Giudice, A.; Michaud, L.; de Domenico, E.; Spanò, N. Diversity and Antibacterial Activity of the Bacterial Communities Associated with Two Mediterranean Sea Pens, *Pennatula phosphorea* and *Pteroeides spinosum* (Anthozoa: Octocorallia). *Microb. Ecol.* **2013**, *66*, 701–714. [CrossRef]
65. Follesa, M.C.; Carbonara, P. *Atlas of the Maturity Stages of Mediterranean Fishery Resources. Studies and Reviews n. 99*; FAO: Rome, Italy, 2019; ISBN 9789251319758.
66. Savoca, S.; Matanović, K.; D'Angelo, G.; Vetri, V.; Anselmo, S.; Bottari, T.; Mancuso, M.; Kužir, S.; Spanò, N.; Capillo, G.; et al. Ingestion of plastic and non-plastic microfibers by farmed gilthead sea bream (*Sparus aurata*) and common carp (*Cyprinus carpio*) at different life stages. *Sci. Total Environ.* **2021**, *782*, 146851. [CrossRef]
67. Besley, A.; Vijver, M.G.; Behrens, P.; Bosker, T. A standardized method for sampling and extraction methods for quantifying microplastics in beach sand. *Mar. Pollut. Bull.* **2017**, *114*, 77–83. [CrossRef]
68. Silva, A.B.; Bastos, A.S.; Justino, C.I.L.; da Costa, J.P.; Duarte, A.C.; Rocha-Santos, T.A.P. Microplastics in the environment: Challenges in analytical chemistry—A review. *Anal. Chim. Acta* **2018**, *1017*, 1–19. [CrossRef]
69. Giani, D.; Baini, M.; Galli, M.; Casini, S.; Fossi, M.C. Microplastics occurrence in edible fish species (Mullus barbatus and *Merluccius merluccius*) collected in three different geographical sub-areas of the Mediterranean Sea. *Mar. Pollut. Bull.* **2019**, *140*, 129–137. [CrossRef] [PubMed]
70. Yin, J.; Li, J.Y.; Craig, N.J.; Su, L. Microplastic pollution in wild populations of decapod crustaceans: A review. *Chemosphere* **2022**, *291*, 132985. [CrossRef] [PubMed]
71. Collignon, A.; Hecq, J.H.; Galgani, F.; Collard, F.; Goffart, A. Annual variation in neustonic micro- and meso-plastic particles and zooplankton in the Bay of Calvi (Mediterranean-Corsica). *Mar. Pollut. Bull.* **2014**, *79*, 293–298. [CrossRef] [PubMed]
72. Fastelli, P.; Blašković, A.; Bernardi, G.; Romeo, T.; Čižmek, H.; Andaloro, F.; Russo, G.F.; Guerranti, C.; Renzi, M. Plastic litter in sediments from a marine area likely to become protected (Aeolian Archipelago's islands, Tyrrhenian sea). *Mar. Pollut. Bull.* **2016**, *113*, 526–529. [CrossRef]
73. Cau, A.; Bellodi, A.; Moccia, D.; Mulas, A.; Pesci, P.; Cannas, R.; Pusceddu, A.; Follesa, M.C. Dumping to the abyss: Single-use marine litter invading bathyal plains of the Sardinian margin (Tyrrhenian Sea). *Mar. Pollut. Bull.* **2018**, *135*, 845–851. [CrossRef] [PubMed]
74. Mistri, M.; Scoponi, M.; Granata, T.; Moruzzi, L.; Massara, F.; Munari, C. Types, occurrence and distribution of microplastics in sediments from the northern Tyrrhenian Sea. *Mar. Pollut. Bull.* **2020**, *153*, 111016. [CrossRef]
75. Rossi, S.; Gabbianelli, G. Geomorfologia del Golfo di Taranto. *Bull. Soc Geol.* **1978**, *97*, 423–437.
76. Cataudella, S.; Spagnolo, M. *The State of Italian Marine Fisheries and Aquaculture*; MiPAAF: Rome, Italy, 2011.

77. Theocharis, A.; Georgopoulos, D.; Lascaratos, A.; Nittis, K. Water masses and circulation in the central region of the Eastern Mediterranean: Eastern Ionian, South Aegean and Northwest Levantine, 1986–1987. *Deep Sea Res. Part II* **1993**, *40*, 1121–1142. [CrossRef]
78. Canals, M.; Danovaro, R.; Heussner, S.; Lykousis, V.; Puig, P.; Trincardi, F.; Calafat, A.M.; de Madron, X.D.; Palanques, A.; Sànchez-Vidal, A. Cascades in mediterranean submarine grand canyons. *Oceanography* **2009**, *22*, 26–43. [CrossRef]
79. Klein, B.; Roether, W.; Manca, B.B.; Bregant, D.; Beitzel, V.; Kovacevic, V.; Luchetta, A. The large deep water transient in the Eastern Mediterranean. *Deep Sea Res. Part I Oceanogr. Res. Pap.* **1999**, *46*, 371–414. [CrossRef]
80. Manca, B.B.; Ursella, L.; Scarazzato, P. New development of eastern mediterranean circulation based on hydrological observations and current measurements. *Mar. Ecol.* **2002**, *23*, 237–257. [CrossRef]
81. Watts, A.J.R.; Urbina, M.A.; Goodhead, R.; Moger, J.; Lewis, C.; Galloway, T.S. Effect of Microplastic on the Gills of the Shore Crab Carcinus maenas. *Environ. Sci. Technol.* **2016**, *50*, 5364–5369. [CrossRef] [PubMed]
82. Mishra, S.; Rath, C.C.; Das, A.P. Marine microfiber pollution: A review on present status and future challenges. *Mar. Pollut. Bull.* **2019**, *140*, 188–197. [CrossRef] [PubMed]
83. Desforges, J.P.W.; Galbraith, M.; Ross, P.S. Ingestion of Microplastics by Zooplankton in the Northeast Pacific Ocean. *Arch. Environ. Contam. Toxicol.* **2015**, *69*, 320–330. [CrossRef]
84. Cartes, J.E.; Fanelli, E.; Kapiris, K.; Bayhan, Y.K.; Ligas, A.; López-Pérez, C.; Murenu, M.; Papiol, V.; Rumolo, P.; Scarcella, G. Spatial variability in the trophic ecology and biology of the deep-sea shrimp *Aristaeomorpha foliacea* in the Mediterranean Sea. *Deep Sea Res. Part I Oceanogr. Res. Pap.* **2014**, *87*, 1–13. [CrossRef]
85. Kapiris, K. Feeding ecology of *Parapenaeus longirostris* (Lucas, 1846) (Decapoda: Penaeidae) from the Ionian Sea (Central and Eastern Mediterranean Sea). *Sci. Mar.* **2004**, *68*, 247–256. [CrossRef]
86. Sbrana, M.; Viva, C.; Belcari, P. Fishery of the deep-water rose shrimp *Parapenaeus longirostris* (Lucas, 1846) (Crustacea: Decapoda) in the northern Tyrrhenian Sea (western Mediterranean). *Hydrobiologia* **2006**, *557*, 135–144. [CrossRef]
87. Burukovskii, R.N. On the bathymetric distribution and feeding of the shrimp *Parapenaeus longirostris* (Lucas). *Ices C. M.* **1969**, 1–7.
88. Lagardère, J.P. Recherches sur la distribution et sur l'alimentation des crustacés décapodes benthiques de la pente continentale du Golfe de Gascogne. Analyse des groupements carcinologiques. *Bull. Cent. Étud. Rech. Sci. Biarritz* **1977**, *11*, 367–440.
89. Cartes, J.E. Influence of depth and season on the diet of the deep-water aristeid *Aristeus antennatus* along the continental slope (400 to 2300 m) in the Catalan Sea (western Mediterranean). *Mar. Biol.* **1994**, *120*, 639–648. [CrossRef]
90. Cartes, J.; Sarda, F. Feeding ecology of the deep-water aristeid crustacean *Aristeus antennatus*. *Mar. Ecol. Prog. Ser.* **1989**, *54*, 229–238. [CrossRef]
91. Rainer, S.F. Diet of prawns from the continental slope of north-western Australia. *Bull. Mar. Sci.* **1992**, *50*, 258–274.
92. Lagardere, J.P. Recherches sur l' alimentation des crevettes de la pente continentale marocaine. *Tethys* **1972**, *3*, 655–675.
93. Carreras-Colom, E.; Constenla, M.; Soler-Membrives, A.; Cartes, J.E.; Baeza, M.; Padrós, F.; Carrassón, M. Spatial occurrence and effects of microplastic ingestion on the deep-water shrimp *Aristeus antennatus*. *Mar. Pollut. Bull.* **2018**, *133*, 44–52. [CrossRef] [PubMed]
94. D'Costa, A.H. Microplastics in decapod crustaceans: Accumulation, toxicity and impacts, a review. *Sci. Total Environ.* **2022**, *832*, 154963. [CrossRef]

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A glimpse into the future: A suitable methodological approach for the detection and identification of micro-bioplastics in biota

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ABSTRACT

In the frame of the circular economy, bioplastics are considered a good alternative to conventional plastic materials. Until recently, only a few studies have focused on the occurrence and impact of bio-microplastics (bio-MPs) in aquatic environments, and there is a lack of a methodological approach to measure their amount in marine compartments. This research aimed to identify and validate a method for bio-MPs extraction from biota. A chemical digestion protocol suitable for conventional MPs, using potassium hydroxide (KOH), was applied for the detection, in mussels, of MPs made with Mater-Bi (MBi) from socks used in mussel farming. This method was tested on virgin MBi (VMBi) and aged (AMBi) MPs, ranging from 200 to 1000 μm in presence and absence of mussel tissues.

Samples were analysed in pre- and post-digestion steps to assess the recovery rate, potential visual and size changes and polymer alteration in different bio-MPs size ranges. Results showed that MBi seems to be affected by KOH under pre-production conditions (VMBi), whereas in the AMBi treatment, which represents the environmentally realistic condition, the presence of fouling due to deployment at sea preserves MBi from the action of the alkaline agent. This approach allowed the recovery of small MPs, generally difficult to extract from biota, in an optimal visual condition and without polymer alteration. Despite the fraction of organic material in the MBi, these results suggested the suitability of this method and provided the assessment of the KOH effects on MBi-MPs under different environmental conditions. Finally, validation tests proved that the KOH protocol represents a reliable approach for detecting bio-MPs in marine organisms.

This study is an important starting point for assessing the impact of the bio-MPs on the marine environment and suggests future studies to improve these issues in order to fill the gaps in the field of bioplastics.

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1. Introduction

In recent years, one of the main challenges of the global scientific community has been to address the issue of plastic pollution by providing new tools and inputs for the protection of both the terrestrial and marine environments and biodiversity (UNEP, 2021; Santos et al., 2022). In the frame of sustainable development, the technological innovation and scientific research have led to several solutions to decrease plastic input into the environment, including the design of new bio-based and biodegradable materials (UNEP, 2021). In this respect, the bioplastics, namely a large family of materials with different properties and applications, are considered a good alternative to conventional plastic materials, and they are being explored as materials for a wide range of uses (Fojt et al., 2020; Shruti and Kutralam-Muniasamy, 2019; Weinstein et al., 2020; European Bioplastics, 2022).

According to European Bioplastics (2022), a bioplastic is defined as a plastic material that is either bio-based, biodegradable or has both properties. Biodegradable products are materials that are able to completely decompose into natural substances under the action of microorganisms under certain environmental conditions and within a certain period of time. Biodegradable plastics may also be bio-based, totally or partly derived from biomass (*e.g.* corn, sugarcane or cellulose). High biomass percentage does not necessarily indicate biodegradability. Indeed, bio-based plastics such as polylactic acid (PLA), polyhydroxy alkenoates (PHA) and polybutylene succinate (PBS) are biodegradable in specific conditions, while bio-polyethylene (Bio-PE), bio-polyethylene terephthalate (Bio-PET) and bio-poly-acrylate (Bio-PA) are non-biodegradable.

The global production of bioplastics is set to increase from about 2.23 million tonnes in 2022 to around 6.3 million tonnes in 2027. These alternative materials are being used in several applications such as packaging, which accounts for almost half of the demand, followed by consumer goods and textiles, as well as other uses in sectors such as agriculture, transport and construction (European Bioplastics, 2022). To date, in the European context, bioplastics make up <1 % plastic produced annually (>390 million tonnes) (Plastics Europe, 2022). Several policy frameworks and legislation provisions and implementations were provided and improved in terms of the circular economy and bioeconomy (COM/2018/028; COM/2019/640; 2019/904; COM/2020/98), including the EU policy framework on biobased, biodegradable and compostable plastics (COM/2022/682). In particular, many of the regulatory aspects were strictly concerned with the marine environment and the strategies to contrast and monitor the issue of plastic pollution such as the Barcelona Convention (L/240, 19.9.77), the Marine Strategy Framework Directive (MSFD; 2008/56/CE) and the Port Reception Facilities Directive (2019/883).

Although the development and application of bioplastics is still in progress, several aspects still need to be assessed in order to understand their sustainability and effective environmental advantages compared to conventional plastic. Currently, it is important to recognise whether their production and use contribute to increased waste and pollution, climate-changing emissions and loss of biodiversity (Weinstein et al., 2020).

For this reason, both field and laboratory studies are investigating the behaviour of bioplastics in marine environment at different environmental, biological and physicochemical conditions. Most of these studies assessed the degradability of bioplastics as well as their physical and chemical properties (Briassoulis et al., 2019; Pires et al., 2022; Weinstein et al., 2020). Although bioplastics are biodegradable, their complete degradation occurs only under specific conditions, which are rarely achieved in ecosystems in comparison to optimal laboratory conditions and the industrial composting facilities (Song et al., 2009; Fojt et al., 2020). Therefore, when they are disposed of in an uncontrolled or improper manner, they may accumulate in the environment and fragment faster than conventional plastics into small fragments, lesser than 5 mm in the longest dimension, known as bio-microplastics (bio-MPs) (Shruti and Kutralam-Muniasamy, 2019; Fojt et al., 2020; Weinstein et al., 2020).

Information about the fragmentation of bioplastics into bio-microplastics (bio-MPs) in aquatic environments is very poor (Lambert and Wagner, 2016; González-Pleiter et al., 2019), although bio-MPs have shown to have similar adverse effects to those of conventional MPs on both biodiversity and ecosystem functioning (Green et al., 2016; González-Pleiter et al., 2019; Shruti and Kutralam-Muniasamy, 2019; Quade et al., 2022; Xie et al., 2022). Meanwhile, there is a lack of methods for determining the amount of bio-MPs in the marine compartments (Shruti and Kutralam-Muniasamy, 2019). Although consumer demand of bioplastics has increased, it cannot be excluded that they are already another critical source of MPs in the environment, threatening ocean biodiversity and functioning.

Therefore, given the knowledge gap about the bioplastic's behaviour, fate and persistence as litter in the marine environment, especially in the form of MPs, scientific research efforts should also focus on the methodological approach for the identification and extraction of them from both abiotic (sediment, sea water) and biotic (organisms) matrices.

In this regard, the aim of this research is to prospect and document, for the first time, the extraction of bio-MPs from biota and to validate the methodological approach according standardised protocols for reliable assessment of the MPs occurrence in marine biota (Schirinzi et al., 2020; Tsangaris et al., 2021).

To this purpose, a chemical digestion protocol suitable for conventional MPs was tested and validated for the identification and detection, in *Mytilus galloprovincialis*, of MPs made with Mater-Bi (MBi) from socks used in mussel farming. In particular, the behaviour of MPs in MBi exposed to KOH and the potential changes in different bio-MPs sizes ranges were investigated.

MBi was selected as the bioplastic in this study because it is used in a wide variety of fields including agriculture, packaging, carrier bags and food service, and recently, it was also employed in the production of socks for mussel farming (Pedà et al., 2023). MBi represents an important family of biodegradable and compostable bioplastic produced by a Novamont S.p.A certified company and obtained from starches, cellulose, vegetable oils and their combinations using innovative technologies (https://materbi.com/). The biodegradability and compostability of all MBi grades are certified according to the main International and European standards. In particular, at the European level, it is suitable for the manufacture of products that comply with the essential requirements of the Packaging and Packaging Waste Directive (94/62/EC) and also meets the relevant standards for recycling (EN 13430), for energy recovery (EN 13431) and, finally, for organic recovery (EN 13432).

2. Materials and methods

2.1. MBi-MPs and treatments

In this study, MBi socks for mussel farming were employed and their polymeric nature was confirmed by Fourier transform infrared (FTIR) spectroscopy technique (Fig. S1; see details further in the text).

To assess the suitability and the potential effects of the selected protocol on MBi-MPs under different environmental conditions, the experiment included two different treatments: virgin MBi (VMBi) socks and aged MBi socks (AMBi). The latter were deployed for five months in a mussel farm (Mar Piccolo, Taranto; Italy) to simulate the natural physical-chemical and biological processes experienced by the VMBi sock in the environment during the mussel production. Representative images of both types of MBi surfaces obtained by scanning electron microscopy are shown in the supplementary material (Fig. S2a and b).

For each treatment (VMBi; AMBi), irregularly shaped fragments were obtained using a steel scalpel. The fragments were photographed, measured with a stereomicroscope Zeiss Discovery V.8. coupled with Axiocam 208 colour microscope camera, using ZEN 3.1 blue Edition software, and grouped into five size classes (a) 200–400 μm; (b) 400–600 μm; (c) 600–800 μm; (d) 800–1000 μm; (e) >1000 μm for spiked sample preparation according to Tsangaris et al. (2021) in order to reproduce the variable sizes of MPs as seen in field biota samples. Table S1 shows the length ranges and the corresponding mean values for the size classes.

2.2. Microplastic extraction and test design

Bio-MP extraction in 1:5 (*w/v*) with 10 % potassium hydroxide (KOH) digestion was selected as a chemical protocol according to Tsangaris et al. (2021). Homogenised *M. galloprovincialis* tissue samples (about 1 g of wet weight) were placed in a glass beaker with KOH, spiked with two fragments for each size class (protocol with mussel tissues, MT), using a metal tweezer, and incubated in a stove at 50 °C for 6 h for digestion. After digestion, the MT samples were filtered through fibreglass filters (Whatman GF/C, pore size 1.2 μm) using a glass vacuum filtration system. The obtained filters were placed in Petri dishes and left to dry at room temperature overnight.

Since in the literature, the effect of KOH on MBi has only been verified on the particle weight (Kühn et al., 2017), it was assessed to simultaneously determine if this bioplastic was strongly or negligibly affected by the alkaline agent. Therefore, the same test was performed without mussel tissue as a control protocol (CTRL) for each treatment, adding one fragment for each class size according to Schirinzi et al. (2020). This test was carried out in triplicate for each protocol (MT; CTRL) of both treatments (VMBi; AMBi).

2.3. Validation tests

In the post-digestion step, the filters were observed under stereomicroscope to recover the particles resistant to the chemical digestion protocol.

The number of recovered fragments was recorded to calculate the percentage of bio-MP recovery for each protocol (MT; CTRL) of both treatments (VMBi; AMBi) as follows:

$$\text{Number of bio} - \text{MPs recovered after digestion}/\text{number of spiked bio} - \text{MPs before digestion}*100.$$

Each recovered fragment was also isolated, photographed, measured and compared to a respective pre-digestion sample to assess the potential change in size. In addition, morphological modifications such as changes in surface (degradation), changes in colour (discolouration), loss of small pieces (fragmentation), and possible shape alterations (deformation) were assessed. A degree of alteration (high, moderate and low) was also assigned based on the classification criteria reported in Table S2. The percentage of bio-MP modifications for each protocol (MT; CTRL) of both treatments (VMBi; AMBi) was estimated as follows:

$$\text{Number of bio} - \text{MPs with morphological modifications}/\text{number of spiked bio} - \text{MPs before digestion}*100.$$

Finally, polymer identification and degradation were performed on recovered particles for each protocol (MT; CTRL) of both treatments (VMBi; AMBi) by FTIR analysis. The samples were analysed by Agilent's Cary 630 spectrometer in attenuated total reflectance (ATR), using a self-generated library containing only a reference spectrum of VMBi. Polymer identification was accepted when the match with reference spectra had a level of certainty >70 % (Schirinzi et al., 2020; Tsangaris et al., 2021).

2.4. Statistical analysis

Differences in recovery rate among protocols were assessed using the Kruskal Wallis test, while the pairwise differences between protocols were evaluated using the post-hoc Dunn's test and Holm adjusted *p*-value method. In addition, the Wilcoxon test was used to assess differences in recovery rate in each size class between protocols of each treatment. The above statistical analyses were performed using R and R-studio software (Posit, 2023; R Core Team, 2022).

Statistical differences in length for each size class among protocols (CTRL; MT) of both treatments (VMBi; AMBi) at different step of digestion (pre- and post-digestion) were assessed by a three-way crossed non-parametric multivariate analysis (PERMANOVA) using "treatment", "protocol" and "step digestion" as factors. Prior to analysis, data matrices were square root transformed and analysed on the basis of Euclidean distance, using 4999 permutations. When significant differences ($p < 0.05$) among factor levels were detected, Pair-wise comparisons were computed. This analysis was performed using the statistical software PRIMER6 & PERMANOVA+ (Anderson et al., 2008; Clarke et al., 2014).

3. Results

3.1. MBi recovery rate

The recovery rate of the bio-MBi fragments extracted for each size class from each protocol (CTRL; MT) of the two treatments (VMBi; AMBi) is shown in Fig. 1. Overall, a total of 32 fragments were recovered out of the 45 used in all replicates from the VMBi treatment: 12 in the CTRL (80 %) and 20 in the MT (67 %) protocol. On the other hand, in the AMBi treatment, 100 % of the spiked fragments were recovered from both the CTRL ($n = 15$) and the MT ($n = 30$) protocols. The two protocols (CTRL; MT) were compared in terms of recovery rate in each treatment and among treatments (VMBi; AMBi). Statistical difference among recovery rates between protocols was observed (Kruskal Wallis test; $p < 0.05$; Fig. 1A). The subsequent pairwise comparisons (Dunn's test) indicated significant differences between protocol MT_{VMBi} and $CTRL_{AMBi}$ ($p = 0.014$) and MT_{AMBi} ($p = 0.0015$) protocols (Fig. 1A). No other difference was found to be statistically significant.

Recovery rates were influenced by the size class. Indeed, large size class (>1000 and 800 μm; e and d) particles tested in the VMBi treatment showed a complete recovery (100 %) in both protocols. Decreasing in recovery rates was instead observed in the MT protocol at size classes < 800 μm (a, b and c) as well as in the CTRL at size classes <600 μm (a and b). Despite this, no differences between protocols of the same size class have been observed (Wilcoxon test; $p > 0.05$; Fig. 1B).

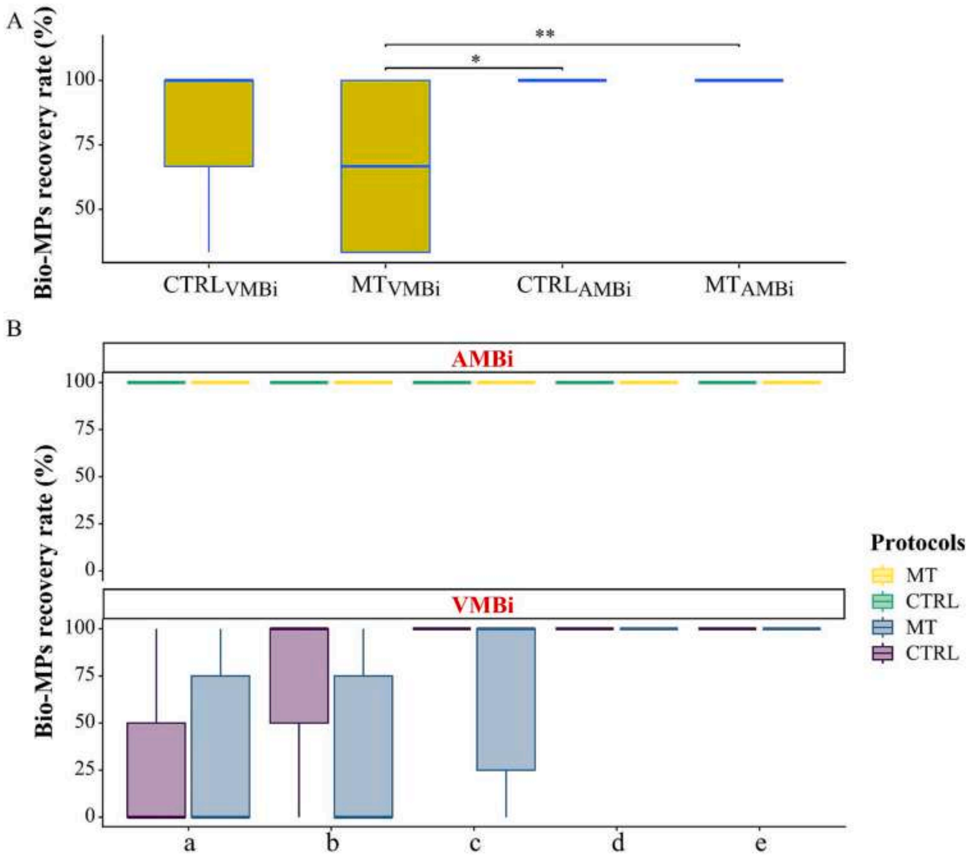

Fig. 1. Box plots showing recovery rates of bio-MPs extracted from biota after KOH 10 % digestion; A) Comparison of recovery rate of two protocols (CTRL; MT) in each treatment and among treatments (VMBi; AMBi); B) Differences in recovery rate for each bio-MP size classes tested in both protocols (CTRL; MT) of each treatment (VMBi; AMBi). $n = 15$ (CTRL); $n = 30$ (MT). Significant statistical differences: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

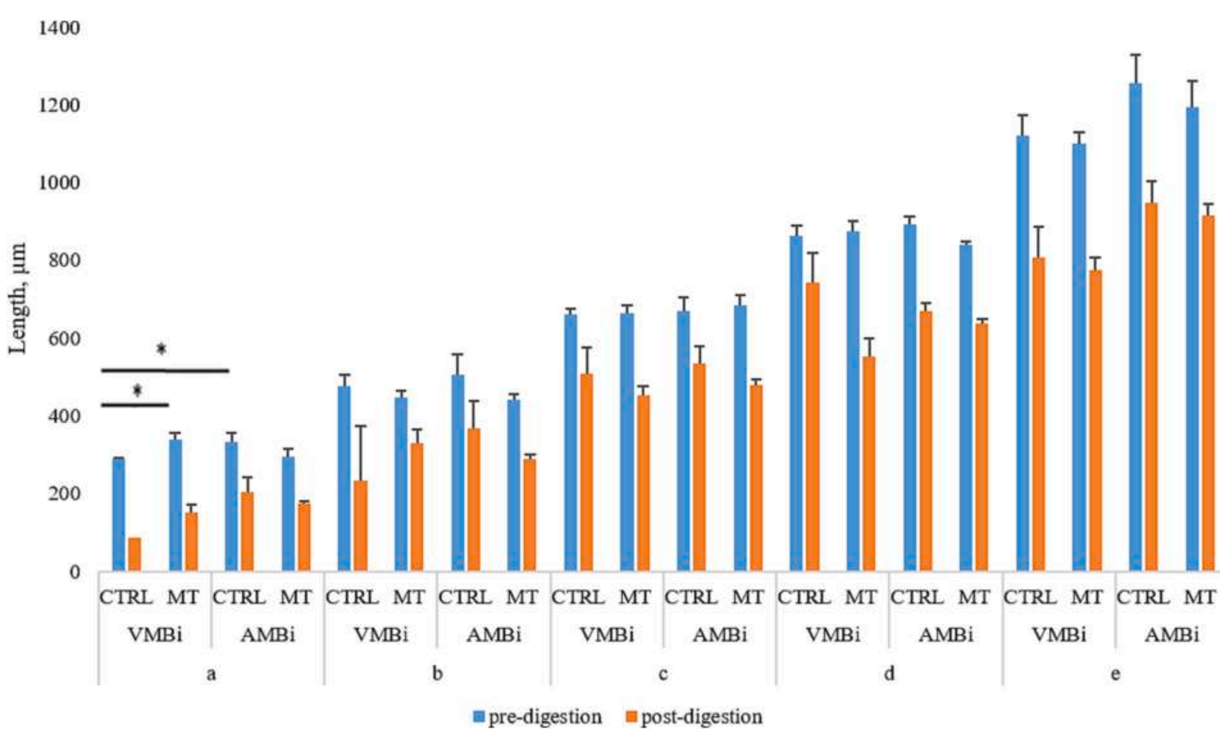

Fig. 2. Size (length, μm) of recovered Mater-Bi fragments before (pre-digestion) and after application of digestion protocol (post-digestion) in absence (CTRL) and presence (MT) of mussel tissues for each size classes in both treatment (VMBi; AMBi). $n = 15$ (CTRL); $n = 30$ (MT). Data are expressed as mean ± SEM. Significant statistical differences: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

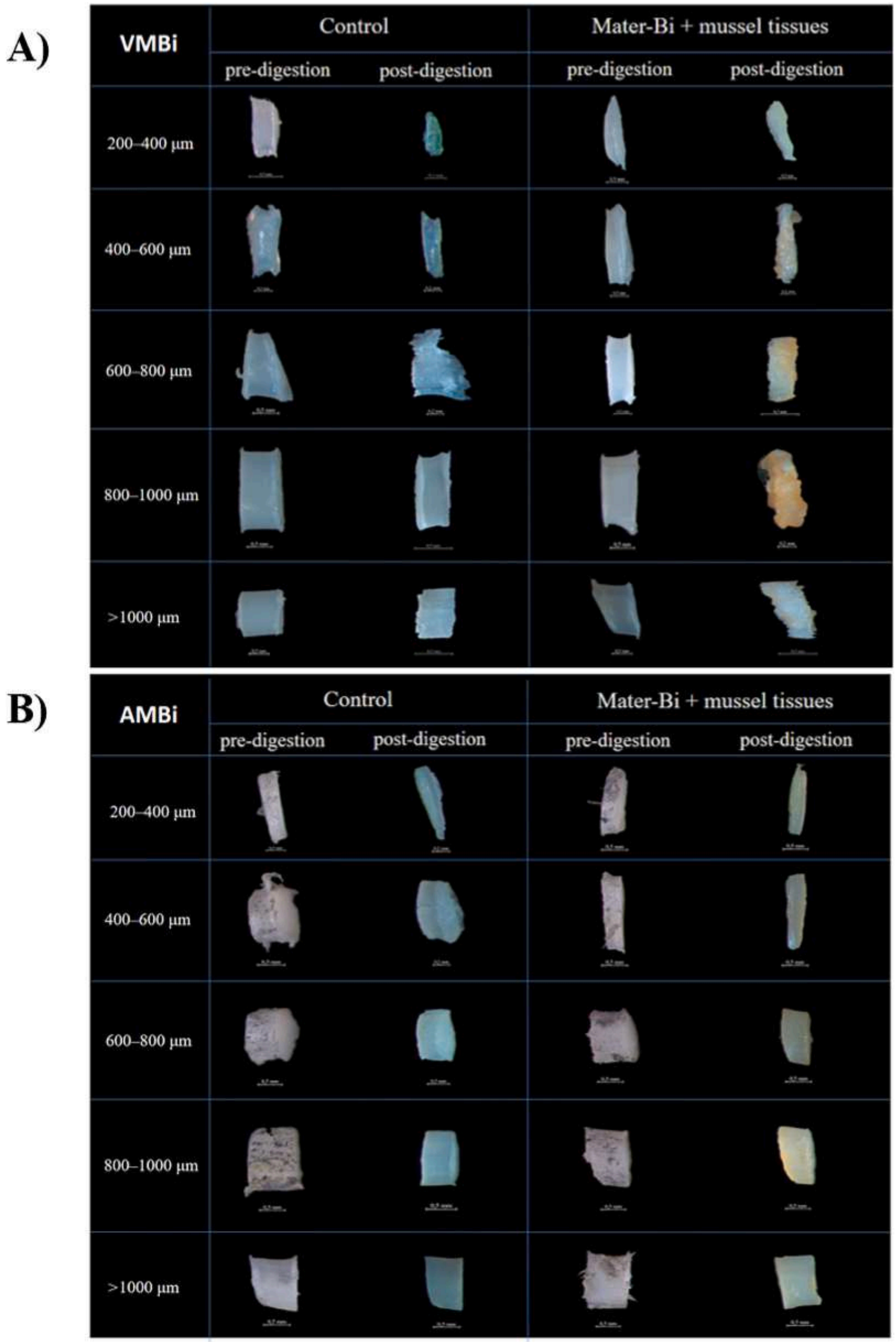

Fig. 3. Examples of spiked Mater-Bi fragments pre- and post-digestion. Morphological modifications of Mater-Bi samples post-digestion (1:5 *w/v* 10%KOH) on five size classes for each CTRL (control; $N = 3$) and MT (Mater-Bi + mussel tissues; $N = 6$) protocol in both treatments A) VMBi and B) AMBi.

3.2. Morphological modifications of MBi

Changes in size (length, μm) of recovered MBi fragments after the application of chemical digestion (post-digestion) for each size class are reported in Fig. 2. After the digestion step, the length decreased by 207.50 ± 34.4 μm (mean ± SEM) and 264.25 ± 27.7 μm (mean ± SEM) in $CTRL_{VMBi}$ and MT_{VMBi} protocols, respectively. On the other hand, the length in the $CTRL_{AMBi}$ and MT_{AMBi} protocols were decreased by 186.93 ± 22.8 μm (mean ± SEM) and 190.97 ± 15.3 μm (mean ± SEM), respectively. Highly significant differences were observed between the two digestion steps for each size class ($p < 0.01$) and within the factor “protocol” (CTRL *vs.* MT; $p < 0.01$) for size class d and within the factor "treatment" (VMBi and AMBi; $p < 0.01$) for size classes a and e. In particular, changes in size length were most evident for size class a between digestion steps of both treatments ($p < 0.01$) with significant differences in the VMBi treatment between protocols in absence and presence of mussel's tissues (CTRL *vs.* MT; $p < 0.05$) and in the CTRL protocol between the virgin and aged MBi treatments (VMBi *vs.* AMBi; $p < 0.05$).

All recovered fragments from each protocol (CTRL; MT) in both treatments (VMBi; AMBi) showed degradation, discolouration, fragmentation and deformation after applying chemical digestion. Fig. 3 summarises the morphological modifications of MBi samples post-digestion on five size classes. In the VMBi treatment, high changes in surface appearance were the most frequent condition observed in both protocol CTRL (67 %) and MT (80 %; Figs. 3A, S3a). Conversely, only low and moderate degradation were found in $CTRL_{AMBi}$ (100 %) and MT_{AMBi} (90 % and 10 %) protocols (Figs. 3B, S3a).

The colour of recovered fragments was quite variable between the two protocols of the treatments (Fig. S3b). High change in colour from white to yellowish was evident in 70 % of the MT_{VMBi} samples while 67 % of the $CTRL_{VMBi}$ fragments showed a moderate change from white to transparent (Figs. 3A, S3b). All recovered items (100 %) from the AMBi treatment (Figs. 3B, S3b) showed low changes in colour after the digestion from white to transparent (CTRL) and from white to yellowish (MT).

Most of the recovered fragments from the VMBi treatment showed high fragmentation in both the CTRL (83 %) and MT (80 %) protocols (Figs. 3A, S3d). Otherwise, low fragmentation was recorded in all recovered items from the $CTRL_{AMBi}$ (100 %) and MT_{AMBi} (100 %) protocols (Figs. 3B, S3d).

Additionally, a high degree of deformation was detected in both 58 % of the $CTRL_{VMBi}$ and 75 % of the MT_{VMBi} samples (Figs. 3A, S3e), while low and moderate deformation were instead observed in the $CTRL_{AMBi}$ (100 %) and MT_{AMBi} (90 % and 10 %) protocols (Figs. 3B, S3e).

3.3. Degradation of the MBi polymer

Polymer identification was achieved in 58 out of 78 recovered fragments, in 42 % of VMBi and 97 % of AMBi treatments, respectively. The level of certainty of polymer identification ranged from 71 % to 84 % and from 73 % and 85 % in the $CTRL_{VMBi}$ and MT_{VMBi} protocols, respectively. In the AMBi treatment, the recovered fragments were identified with a level of certainty varying between 71 % and 87 % in the CTRL and between 70 % and 90 % in the MT protocol. Fig. 4 shows the FTIR spectra of MBi from samples of both protocols (CTRL and MT) for each treatment after digestion.

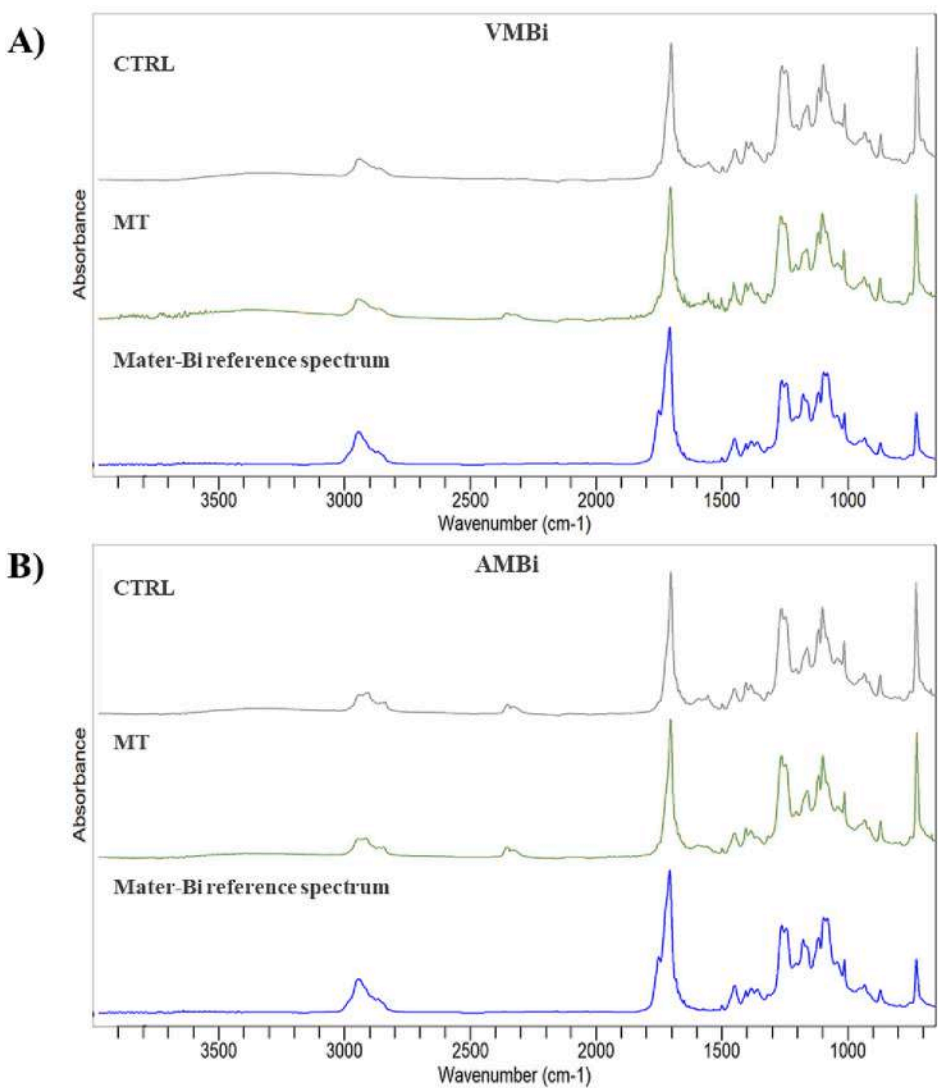

Fig. 4. FTIR spectra of Mater-Bi from samples of both protocols (CTRL; MT) for each treatment A) VMBi and B) AMBi: blue) Mater-Bi reference spectrum; green) protocol with mussel tissues (MT); grey) protocol without mussel tissues (CTRL).

4. Discussion

This research aimed to identify and validate, for the first time, a suitable method for bio-MP extraction from biota. In this study, determination of MP from MBi socks for mussels farming was performed in mussel tissues. MBi can be found as constituent of many products from packaging to carrier bags, and in recent times, its application has also been tested in the mussel farming. Indeed, MBi socks were designed for mussel farming and tested to assess their feasibility of using them in mussel production (Pedà et al., 2023). Its use in aquaculture could be a viable alternative to conventional plastic as in other productive sectors, but at the same time, new tools to exclude the possibility of pollution by MBi-MPs and their potential effects on marine organisms need to be promoted.

For these reasons, a first approach for extraction of MPs in MBi from a marine organism has been devised. In this scenario, *M. galloprovincialis* was identified as the most eligible species for this experiment due to its commercial importance and because it is affected by MP ingestion, both in the natural environment and in farming conditions (Digka et al., 2018; Fossi et al., 2018). Moreover, this bivalve is studied worldwide for MP ingestion and considered, in Mediterranean waters, to be a valuable bioindicator for monitoring marine litter ingestion and its impacts on biodiversity (Fossi et al., 2018).

So far, scientific effort has mainly focused on the detection of conventional MPs from different environmental matrices through the development and validation of suitable chemical digestion methods (Lusher et al., 2017; Schirinzi et al., 2020; Tsangaris et al., 2021; Karami et al., 2017).

To the best of our knowledge, few studies have been conducted on bioplastics including MBi to identify and quantify MPs in environmental samples such as sludge, wastewater, biosolids, marine sediments and drinking/storm water samples (Fojt et al., 2020; Ruggero et al., 2021; Okoffo et al., 2022) so by inspiring on methods already adopted for conventional MPs (Fojt et al., 2020; Ruggero et al., 2021). These studies have developed and validated methodology using the chemical digestion approach as well as an analytical technique coupling Pyrolysis-Gas and Chromatography-Mass Spectrometry (Pyr-GC/MS). There is only one study that tested the effects of KOH solution on MBi assuming a potential biota extraction (Kühn et al., 2017). Generally, the methodology based on the removal of organic materials by chemical digestion using acid, alkaline and oxidative reagents, is mostly used in this field, representing an important step in MP extraction from biota (Karami et al., 2017; Lusher et al., 2017). KOH or hydrogen peroxide (H_2O_2) reagents are considered the most suitable because they degrade organic matter facilitating MP detection without damaging them (Avio et al., 2015; Enders et al., 2017; Munno et al., 2018; Karami et al., 2017). Regarding bioplastics and MBi in detail, a previous study developed a multi-step methodology including oxidation with H_2O_2 to identify and quantify MBi-MPs from shopper bags in sludge with a preliminary assessment of the negligible effect of H_2O_2 on this bioplastic (Ruggero et al., 2021). Furthermore, Kühn et al. (2017) did not observe changes in the weight of MBi samples exposed to a lower concentration of KOH solution (20 ml of 1 Molar) at room temperature for two days.

In this respect, a chemical digestion protocol, already usually applied for the extraction of conventional MPs from biota using 10 % KOH at 50 °C for 6 h, was proposed as a starting point in the present study. Indeed, potassium hydroxide at the lowest concentration (10 %) is widely reported in the scientific literature and proved to be more advantageous than the H_2O_2 in terms of time, technical difficulties, costs, recovery and quality of isolated MPs for quantifying and monitoring conventional MP ingestion in marine organisms (Tsangaris et al., 2021). Furthermore, although this chemical alkaline agent (KOH) is a strong base, it is well known that it does not affect the integrity of most common synthetic polymers (*e.g.* polyethylene, polypropylene and expanded polystyrene) as well as the weight of MBi especially at low concentrations and temperatures (Dehaut et al., 2016; Foekema et al., 2013; Kühn et al., 2017; Lusher et al., 2017; Schirinzi et al., 2020; Tsangaris et al., 2021). Therefore, an assessment of the effects of KOH on MBi-MPs under different environmental conditions (VMBi; AMBi) was provide in this study, using a CTRL protocol. Afterwards, the identified method was tested and validated to prove its replicability and to improve the reliability of the data.

Based on the recovery rate results, it can be assumed that the MBi particles were recovered efficiently in the same way of conventional plastics (Lusher et al., 2017; Schirinzi et al., 2020; Tsangaris et al., 2021). This result is supported by the control data that showed no difference from the respective protocol in the presence of mussel tissue (MT) for both treatments (VMBi; AMBi). Furthermore, the recovery of all particles from the aged treatment and the differences in the MT protocol recovery rate between VMBi and AMBi treatments indicate that the presence of fouling due to deployment at sea preserves MBi from the action of the alkaline agent. This condition, that simulates the natural physical-chemical and biological processes experienced by the MBi sock in the environment during the mussel production, confirms the importance and strength of this study. Indeed, it is very important to take into account the interaction between plastics and the environment in ambient conditions. The plastics in marine environment are exposed to abiotic (*e.g.* action of light and temperature) and biotic (*e.g.* microorganisms) factors that change their natural state (Andrady, 2017, 2011) and often their surface properties change resulting in the creation of new functional groups (Fotopoulou and Karapanagioti, 2019). In the same way, bioplastics, including the MBi, may exhibit a wide range of degradation at rates faster than conventional plastics as reported in some investigations (Napper and Thompson, 2019; Weinstein et al., 2020). Nevertheless, most laboratory studies on MPs use pre-produced plastics that are not representative of their natural condition in the environment (Lusher et al., 2017; Pedà et al., 2022b). Although the differences in recovery rate between size classes were not significant in both protocols of VMBi treatment, the decreased recovery in the smaller size classes (a, b and c) should be noted. Similar results have been previously reported by Ruggero et al. (2021) for MBi samples treated with H_2O_2 and by Tsangaris et al. (2021), albeit for conventional virgin polymers digested with KOH.

To evaluate the effects of the selected protocol on MBi, MP samples were analysed before and after each digestion step, and changes in morphology and size were recorded. Size class < 200 μm (a) showed the highest decrease in length, especially in the VMBi treatment (CTRL *vs.* MT) and CTRL protocol (VMBi *vs.* AMBi) although a decrease in length after digestion was found in all spiked fragments. In the studies of Schirinzi et al. (2020) and Tsangaris et al. (2021), the decrease in the largest dimension of the conventional MP samples was not as evident. The contrasting results are fairly obvious due to the nature of MBi. Given the fraction of organic material constituting MBi samples, they are more vulnerable than conventional polymers to the KOH action, especially samples from the virgin treatment (VMBi). In addition, smaller size classes are more prone to changes given their greater surface area of exposure to the chemical reagent.

Exposure to KOH caused morphological changes in the MBi-MP samples. Although MBi-MPs were degraded, fragmented and deformed by the extraction method, the degree of alteration was high on the total number of MP items tested, mainly in the virgin treatment, confirming the protection of the fouling layer. These results are closely related to the recovery rate data reported in this study. Indeed, fragmentation as well as degradation may explain the observed decrease in size after particle digestion, due to the loss of fragments from the samples and also changes in surface area. The colour alteration was always more noticeable in the samples from the virgin treatment, but it affected mostly the protocol with the mussel tissue. In the latter case, bio-MPs were covered by biofilm formation due to organic matter attached to the sample surface showing a brownish colouration. This condition, observed in the virgin treatment, also affected the FTIR analysis. Most bio-MPs from the MT_{VMBi} protocol could not be identified due to the absorbed organic matter. Regarding the FTIR analysis, in the study of Tsangaris et al. (2021), items in the size range of 290–684 μm could not be identified due to technical limitations. By contrast, polymer identification of particles <800 μm was generally achieved in the present work. Indeed, regardless of size class, all MPs from the AMBi were identified by FTIR analysis, and the fragments identified from the VMBi treatment also showed variable sizes. This result suggests that MP-AMBi can be identified by this spectroscopy technique, including small size classes, despite the exposure to KOH and the instrumental limitations of FT-IR ATR (Pedà et al., 2022a). These results are in contrast to the study of Kühn et al. (2017), which showed no degradation of MBi following KOH treatment, although only the particle weight was investigated. Differences in results suggest validation and standardisation of protocols for more reliable assessments of bio-MP occurrence in marine wildlife and to promote comparability among studies.

5. Conclusions

Environmental exposure to bio-MPs may represent another important threat for marine ecosystems and biodiversity, and further investigations are needed to understand their real impact on marine environments. This research suggests that the challenge of the scientific community should also address the identification and validation of methods for the extraction of bioplastics from biological matrices. For instance, marine organisms may already be exposed to ingestion of bio-MPs. This study has provided a step in the right direction, giving new tools and input to support and promote bioplastics research in the field of MPs. In the present study, the application of an already consolidated methodology for conventional plastics allowed the extraction of MBi-MPs from mussel tissue under different environmental conditions (pre-production and aged). Despite the fraction of organic material in the MBi, our results demonstrated the suitability of this method due to a good recovery rate and an optimal condition of the recovered fragments. In particular, MBi seems to be affected by the alkaline reagent KOH under pre-production conditions as shown by high morphological changes detected in the VMBi treatment. Instead, in the case of the AMBi treatment, which represents the environmentally realistic condition, the extraction and validation in terms of recovery rate, MPs alterations and polymer identification confirm the effectiveness and validity of this method.

However, further investigations are needed to deepen and improve some aspects. In this regard, the behaviour of bioplastics and the effects of chemical agents on biopolymers may be different based on the selected bioplastic and also the tested product (*i.e.*, shopper bag *vs.* mussel sock). The latter issue is closely related to the percentage of organic material required for the feasibility and functionality of the final product. Therefore, the fraction of organic matter could affect the performance of the chemical digestion, so different methodologies should be developed and tested to assess the resistance to chemical digestion in different products made of the same polymer. Finally, the effects of ingestion by marine organisms on biopolymers cannot be neglected and studies *in vivo* or model analysis should be recommended.

CRediT authorship contribution statement

Federica Laface: Methodology, Validation, Formal analysis, Investigation, Data curation, Visualization, Writing – original draft. **Cristina Pedà:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Visualization, Writing – original draft. **Chiara Giommi:** Validation, Investigation, Writing – review & editing. **Serena Scozzafava:** Investigation, Writing – review & editing. **Carmen Rizzo:** Supervision, Visualization, Writing – review & editing. **Danilo Malara:** Validation, Formal analysis, Writing – review & editing. **Silvestro Greco:** Resources, Supervision, Funding acquisition. **Teresa Romeo:** Conceptualization, Resources, Visualization, Writing – review & editing, Supervision, Project administration, Funding acquisition.

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.scitotenv.2023.165613.

References

Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods. Plymouth, UK, PRIMER-E.

Andrady, A.L., 2011. Microplastics in the marine environment. Mar. Pollut. Bull. 62, 1596–1605. https://doi.org/10.1016/j.marpolbul.2011.05.030.

Andrady, A.L., 2017. The plastic in microplastics: a review. Mar. Pollut. Bull. 119, 12–22. https://doi.org/10.1016/j.marpolbul.2017.01.082.

Avio, C.G., Gorbi, S., Regoli, F., 2015. Experimental development of a new protocol for extraction and characterization of microplastics in fish tissues: first observations in commercial species from Adriatic Sea. Mar. Environ. Res. 111, 18–26. https://doi.org/10.1016/j.marenvres.2015.06.014. Particles in the Oceans: Implication for a safe marine environment.

Briassoulis, D., Pikasi, A., Briassoulis, Chr, Mistriotis, A., 2019. Disintegration behaviour of bio-based plastics in coastal zone marine environments: a field experiment under natural conditions. Sci. Total Environ. 688, 208–223. https://doi.org/10.1016/j.scitotenv.2019.06.129.

Clarke, K.R., Gorley, R.N., Somerfield, P.J., Warwick, R.M., 2014. Change in Marine Communities: An Approach to Statistical Analysis and Interpretation, 3nd edition. PRIMER-E, Plymouth.

Dehaut, A., Cassone, A.-L., Frère, L., Hermabessiere, L., Himber, C., Rinnert, E., Rivière, G., Lambert, C., Soudant, P., Huvet, A., Duflos, G., Paul-Pont, I., 2016. Microplastics in seafood: benchmark protocol for their extraction and characterization. Environ. Pollut. 215, 223–233. https://doi.org/10.1016/j.envpol.2016.05.018.

Digka, N., Tsangaris, C., Torre, M., Anastasopoulou, A., Zeri, C., 2018. Microplastics in mussels and fish from the Northern Ionian Sea. Mar. Pollut. Bull. 135, 30–40. https://doi.org/10.1016/j.marpolbul.2018.06.063.

Enders, K., Lenz, R., Beer, S., Stedmon, C.A., 2017. Extraction of microplastic from biota: recommended acidic digestion destroys common plastic polymers. ICES J. Mar. Sci. 74, 326–331. https://doi.org/10.1093/icesjms/fsw173.

European Bioplastics. Bioplastics market development update 2022. https://docs.european-bioplastics.org/publications/market_data/2022/Report_Bioplastics_Market_Data_2022_short_version.pdf.

Foekema, E.M., De Gruijter, C., Mergia, M.T., van Franeker, J.A., Murk, A.J., Koelmans, A.A., 2013. Plastic in north sea fish. Environ. Sci. Technol. 47, 8818–8824. https://doi.org/10.1021/es400931b.

Fojt, J., David, J., Přikryl, R., Řezáčová, V., Kučerík, J., 2020. A critical review of the overlooked challenge of determining micro-bioplastics in soil. Sci. Total Environ. 745, 140975 https://doi.org/10.1016/j.scitotenv.2020.140975.

Fossi, M.C., Pedà, C., Compa, M., Tsangaris, C., Alomar, C., Claro, F., Ioakeimidis, C., Galgani, F., Hema, T., Deudero, S., Romeo, T., Battaglia, P., Andaloro, F., Caliani, I., Casini, S., Panti, C., Baini, M., 2018. Bioindicators for monitoring marine litter ingestion and its impacts on Mediterranean biodiversity. Environ. Pollut. 237, 1023–1040. https://doi.org/10.1016/j.envpol.2017.11.019.

Fotopoulou, K.N., Karapanagioti, H.K., 2019. Degradation of various plastics in the environment. In: Takada, H., Karapanagioti, H.K. (Eds.), Hazardous Chemicals Associated with Plastics in the Marine Environment, The Handbook of Environmental Chemistry. Springer International Publishing, Cham, pp. 71–92. https://doi.org/10.1007/698_2017_11.

González-Pleiter, M., Tamayo-Belda, M., Pulido-Reyes, G., Amariei, G., Leganés, F., Rosal, R., Fernández-Piñas, F., 2019. Secondary nanoplastics released from a biodegradable microplastic severely impact freshwater environments. Environ. Sci. Nano 6, 1382–1392. https://doi.org/10.1039/C8EN01427B.

Green, D.S., Boots, B., Sigwart, J., Jiang, S., Rocha, C., 2016. Effects of conventional and biodegradable microplastics on a marine ecosystem engineer (Arenicola marina) and sediment nutrient cycling. Environ. Pollut. 208, 426–434. https://doi.org/10.1016/j.envpol.2015.10.010.

Karami, A., Golieskardi, A., Choo, C.K., Romano, N., Ho, Y.B., Salamatinia, B., 2017. A high-performance protocol for extraction of microplastics in fish. Sci. Total Environ. 578, 485–494. https://doi.org/10.1016/j.scitotenv.2016.10.213.

Kühn, S., van Werven, B., van Oyen, A., Meijboom, A., Bravo Rebolledo, E.L., van Franeker, J.A., 2017. The use of potassium hydroxide (KOH) solution as a suitable approach to isolate plastics ingested by marine organisms. Mar. Pollut. Bull. 115, 86–90. https://doi.org/10.1016/j.marpolbul.2016.11.034.

Lambert, S., Wagner, M., 2016. Formation of microscopic particles during the degradation of different polymers. Chemosphere 161, 510–517. https://doi.org/10.1016/j.chemosphere.2016.07.042.

Lusher, A., Hollman, P.C.H., Mendoza-Hill, J., 2017. Microplastics in Fisheries and Aquaculture: Status of Knowledge on Their Occurrence and Implications for Aquatic Organisms and Food Safety. FAO fisheries and aquaculture technical paper. Food and Agriculture Organization of the United Nations, Rome.

Munno, K., Helm, P.A., Jackson, D.A., Rochman, C., Sims, A., 2018. Impacts of temperature and selected chemical digestion methods on microplastic particles. Environ. Toxicol. Chem. 37, 91–98. https://doi.org/10.1002/etc.3935.

Napper, I.E., Thompson, R.C., 2019. Environmental deterioration of biodegradable, oxo-biodegradable, compostable, and conventional plastic carrier bags in the sea, soil, and open-air over a 3-year period. Environ. Sci. Technol. 53, 4775–4783. https://doi.org/10.1021/acs.est.8b06984.

Okoffo, E.D., Chan, C.M., Rauert, C., Kaserzon, S., Thomas, K.V., 2022. Identification and quantification of micro-bioplastics in environmental samples by pyrolysis–gas chromatography–mass spectrometry. Environ. Sci. Technol. 56, 13774–13785. https://doi.org/10.1021/acs.est.2c04091.

Pedà, C., Giommi, C., Rizzo, C., Arcadi, E., Scozzafava, S., Laface, F., Greco, S., Romeo, T., 2023. Convenzione di Ricerca - Relazione finale Monitoraggio e valutazione di materiali biodegradabili innovativi per l'impiego sostenibile in miticoltura. Pagg 53.

Pedà, C., Longo, F., Berti, C., Laface, F., De Domenico, F., Consoli, P., Battaglia, P., Greco, S., Romeo, T., 2022a. The waste collector: information from a pilot study on the interaction between the common octopus (Octopus vulgaris, Cuvier, 1797) and marine litter in bottom traps fishing and first evidence of plastic ingestion. Mar. Pollut. Bull. 174, 113185 https://doi.org/10.1016/j.marpolbul.2021.113185.

Pedà, C., Romeo, T., Panti, C., Caliani, I., Casini, S., Marsili, L., Campani, T., Baini, M., Limonta, G., de Rysky, E., Caccamo, L., Perdichizzi, A., Gai, F., Maricchiolo, G., Consoli, P., Fossi, M.C., 2022b. Integrated biomarker responses in European seabass Dicentrarchus labrax (Linnaeus, 1758) chronically exposed to PVC microplastics. J. Hazard. Mater. 438, 129488 https://doi.org/10.1016/j.jhazmat.2022.129488.

Pires, J.R.A., Souza, V.G.L., Fuciños, P., Pastrana, L., Fernando, A.L., 2022. Methodologies to assess the biodegradability of bio-based polymers—current knowledge and existing gaps. Polymers 14, 1359. https://doi.org/10.3390/polym14071359.

Plastics Europe, 2022, Plastics the Facts 2021, data specified in personal communication with Charlotte Delvaux de Fenffe, Plastics Europe, 10/03/2022, (https://plasticseurope.org/knowledge-hub/plastics-thefacts-2021/).

Posit, 2023. RStudio: Integrated Development for R. Posit Software, PBC, Boston, MA.

Quade, J., López-Ibáñez, S., Beiras, R., 2022. Mesocosm trials reveal the potential toxic risk of degrading bioplastics to marine life. Mar. Pollut. Bull. 179, 113673 https://doi.org/10.1016/j.marpolbul.2022.113673.

R Core Team, 2022. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Ruggero, F., Porter, A.E., Voulvoulis, N., Carretti, E., Lotti, T., Lubello, C., Gori, R., 2021. A highly efficient multi-step methodology for the quantification of micro-(bio) plastics in sludge. Waste Manag. Res. 39, 956–965. https://doi.org/10.1177/0734242X20974094.

Santos, M.R., Dias, L.C., Cunha, M.C., Marques, J.R., 2022. Multicriteria decision analysis addressing marine and terrestrial plastic waste management: a review. Front. Mar. Sci. 8.

Schirinzi, G.F., Pedà, C., Battaglia, P., Laface, F., Galli, M., Baini, M., Consoli, P., Scotti, G., Esposito, V., Faggio, C., Farré, M., Barceló, D., Fossi, M.C., Andaloro, F., Romeo, T., 2020. A new digestion approach for the extraction of microplastics from gastrointestinal tracts (GITs) of the common dolphinfish (Coryphaena hippurus) from the western Mediterranean Sea. J. Hazard. Mater. 397, 122794 https://doi.org/10.1016/j.jhazmat.2020.122794.

Shruti, V.C., Kutralam-Muniasamy, G., 2019. Bioplastics: missing link in the era of microplastics. Sci. Total Environ. 697, 134139 https://doi.org/10.1016/j.scitotenv.2019.134139.

Song, J.H., Murphy, R.J., Narayan, R., Davies, G.B.H., 2009. Biodegradable and compostable alternatives to conventional plastics. Philos. Trans. R. Soc. B Biol. Sci. 364, 2127–2139. https://doi.org/10.1098/rstb.2008.0289.

Tsangaris, C., Panti, C., Compa, M., Pedà, C., Digka, N., Baini, M., D'Alessandro, M., Alomar, C., Patsiou, D., Giani, D., Romeo, T., Deudero, S., Fossi, M.C., 2021. Interlaboratory comparison of microplastic extraction methods from marine biota tissues: a harmonization exercise of the plastic busters MPAs project. Mar. Pollut. Bull. 164, 111992 https://doi.org/10.1016/j.marpolbul.2021.111992.

UNEP, 2021. United Nations Environment Programme (2021). From Pollution to Solution: A Global Assessment of Marine Litter and Plastic Pollution (Nairobi).

Weinstein, J.E., Dekle, J.L., Leads, R.R., Hunter, R.A., 2020. Degradation of bio-based and biodegradable plastics in a salt marsh habitat: another potential source of microplastics in coastal waters. Mar. Pollut. Bull. 160, 111518 https://doi.org/10.1016/j.marpolbul.2020.111518.

Xie, M., Xu, P., Zhou, W., Xu, X., Li, H., He, W., Yue, W., Zhang, L., Ding, D., Suo, A., 2022. Impacts of conventional and biodegradable microplastics on juvenile Lates calcarifer: bioaccumulation, antioxidant response, microbiome, and proteome alteration. Mar. Pollut. Bull. 179, 113744 https://doi.org/10.1016/j.marpolbul.2022.113744.

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Oceanographic and anthropogenic variables driving marine litter distribution in Mediterranean protected areas: Extensive field data supported by forecasting modelling

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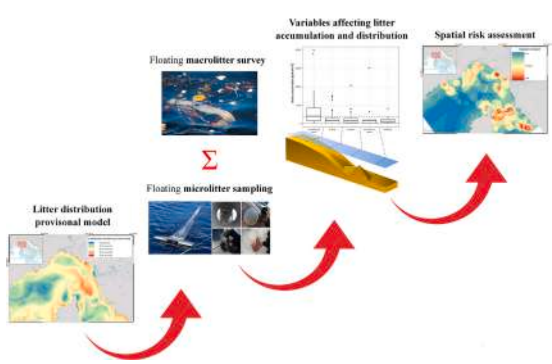
HIGHLIGHTS

- New model-guided methodological approach to simultaneously monitor floating macrolitter and microplastics
- Largest data collection on marine litter distribution in the NW Mediterranean Sea
- Macrolitter and microplastic items accumulated in the same hotspot areas.
- Secondary-origin plastic items were the most abundant.
- Anthropogenic factors deeply influence litter inputs into the Pelagos Sanctuary.

GRAPHICAL ABSTRACT



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ABSTRACT

Marine litter concentration in the Mediterranean Sea is strongly influenced both by anthropogenic pressures and hydrodynamic factors that locally characterise the basin. Within the Plastic Busters MPAs (Marine Protected Areas) Interreg Mediterranean Project, a comprehensive assessment of floating macro- and microlitter in the Pelagos Sanctuary and the Tuscan Archipelago National Park was performed. An innovative multilevel

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Marine litter sources
Pelagos Sanctuary
Oceanographic factors
Spatial risk assessment

experimental design has been planned ad-hoc according to a litter provisional distribution model, harmonising and implementing the current sampling methodologies. The simultaneous presence of floating macro- and microlitter items and the potential influences of environmental and anthropogenic factors affecting litter distribution have been evaluated to identify hotspot accumulation areas representing a major hazard for marine species. A total of 273 monitoring transects of floating macrolitter and 141 manta trawl samples were collected in the study areas to evaluate the abundance and composition of marine litter. High mean concentrations of floating macrolitter (399 items/km^2) and microplastics (259,490 items/km^2) have been found in the facing waters of the Gulf of La Spezia and Tuscan Archipelago National Park as well in the Genova canyon and Janua seamount area. Accordingly, strong litter inputs were identified to originate from the mainland and accumulate in coastal waters within 10–15 nautical miles. Harbours and riverine outfalls contribute significantly to plastic pollution representing the main sources of contamination as well as areas with warmer waters and weak oceanographic features that could facilitate its accumulation. The results achieved may indicate a potentially threatening trend of litter accumulation that may pose a serious risk to the Pelagos Sanctuary biodiversity and provide further indications for dealing with plastic pollution in protected areas, facilitating future management recommendations and mitigation actions in these fragile marines and coastal environments.

1. Introduction

Nowadays, the irreversibility and global ubiquity of marine litter pollution, and plastic in particular, make this material a potential threat to planetary boundaries (Nash et al., 2017; Rockström et al., 2009; Villarrubia-Gómez et al., 2018). Information on litter quantities and trends are widely reported across the ocean basins (Cózar et al., 2014; Suaria et al., 2016), although comprehensive approaches evaluating and linking their presence and distribution with the ecological impacts (including human health and socio-economic impacts) are still poorly adopted. The lack of standardized and harmonized measurements and reporting, as well as the uncertainty concerning definition and baseline values, sources, transport and accumulation, and effects of litter and plastic, still represent existing knowledge gaps to address. These challenges need consideration in the establishment of future harmonized monitoring programmes at the global level and related strategies, both in terms of scientific approaches and feasibility, providing a valuable basis for the development of effective protection and mitigation measures to be taken forward.

In the Mediterranean Sea, the management and monitoring of marine litter fall within the framework of two main regional drivers: the Regional Plan on Marine Litter Management in the Mediterranean (UN Environmental Programme/Mediterranean Action Plan), and the Marine Strategy Framework Directive (MSFD; 2008/56/EC, Descriptor 10) for the European marine waters. In this context, the Interreg Mediterranean Project Plastic Busters MPAs (PB MPAs) was conceived to consolidate the fight against marine litter in specific Mediterranean protected areas (MPAs) through a harmonized multidisciplinary approach. A new simultaneous sampling model-guided strategy was developed and tested to create a standardized protocol providing comparable and reliable data on the abundance of litter in pelagic and coastal surface waters as well as on beaches, highlighting potential hotspot and coldspot areas of litter accumulation in one of the heavily polluted basins at the global scale, the Mediterranean Sea. Concurrently, the spatial distribution of marine mammals and others species (seabirds, sea turtles, rays and fishes) were assessed as well as several endangered and bioindicator species were collected to figure out the threat posed to the organisms and the potential related physical impacts and biological effects.

This study focuses on the Pelagos Sanctuary, the most extended protected area of the Mediterranean Sea and the Tuscan Archipelago National Park, located in its eastern part. The Pelagos Sanctuary for Mediterranean Marine Mammals, hereafter Pelagos Sanctuary, is a Special Protection Area of Mediterranean Importance (SPAMI) established in 1999, to protect marine mammals and their habitat and to assess the actual and potential threats to cetacean populations (e.g. intense shipping traffic, fishing, whale-watching activities, chemical pollution, coastal development, military exercises, seismic prospecting, and global climate change) (Coomber et al., 2016; Grossi et al., 2021; Mackelworth, 2016; Notarbartolo di Sciara et al., 2008; Notarbartolo di Sciara and Birkun Jr., 2010; Panigada et al., 2017). Recently, it has been recognized as an area particularly affected by high concentrations of microplastics (MPs) and plastic additives, which may constitute an additional threat to the endangered species inhabiting this basin (baleen whales, sea turtles, filter-feeding sharks) (Baini et al., 2017; Fossi et al., 2012, 2014, 2016, 2017, 2018a; Germanov et al., 2018; Panti et al., 2015) and to the overall biodiversity of the Mediterranean Sea (Compa et al., 2019; Deudero and Alomar, 2015; Galgani et al., 2015; Romeo et al., 2015). The Tuscan Archipelago National Park is the largest marine park in the Mediterranean as well as being classified as a biosphere reserve (Angeletti et al., 2010). It consists of 7 main islands, Gorgona, Capraia, Elba, Pianosa, Montecristo, Giglio and Giannutri managed according to different levels of protection: protection zone 1 or integral reserve referring to adjacent strips of water up to 1 km offshore and protection zone 2 (general protection) (Fratini et al., 2013; Renzi et al., 2010). The Tuscan Archipelago represents a crucial ecosystem for the central Tyrrhenian Sea due to its geographical position, geomorphological structure and high biological value given by the presence of several fish nursery areas (Renzi et al., 2010; Sbrana et al., 2016; Serena et al., 1998). Nevertheless, the intense maritime traffic, the pressure of tourist activities and the presence of several local pollution sources (e.g., maritime and commercial ports, Arno and Serchio riverine inputs, agricultural land and industrial activities) (Renzi et al., 2010) make this area highly anthropized and prone to the accumulation of floating plastic. This is confirmed by the temporary formation of a well-known retention area near the island of Capraia, where floating litter may accumulate (Fossi et al., 2017; Suaria et al., 2016). According to the different oceanographical features, habitats and extent of the selected study areas, the aims of this study were: i) to review the litter pollution status of MPAs, identifying the current knowledge gaps; ii) to define and test a new simultaneous multilevel experimental design within ad hoc sampling campaigns guided by litter distribution provisional model; iii) to provide a comprehensive assessment of the quantities and composition of floating marine litter in pelagic and coastal MPAs, highlighting hot and coldspot areas of litter accumulation; iv) to evaluate the potential influences of environmental and anthropogenic factors affecting the litter inputs and accumulation; v) to develop a map highlighting areas at higher risk of exposure for the marine organisms.

2. Materials and methods

2.1. Marine litter distribution provisional model

Sampling campaigns for floating litter were planned a priori based on a lagrangian model, developed by the LaMMA consortium, simulating the dispersion of floating passive litter particles (Fossi et al., 2017). The purpose of this model was to validate the predicted distribution and concentration of marine litter with on-field data to verify the strength and usefulness of litter prediction and as a result to identify potential hotspots (accumulation areas), coldspots (dispersion areas), and convergence litter areas in the Pelagos Sanctuary and Tuscan Archipelago National Park. Dedicated bulletins were edited to directly guide the monitoring efforts in the study area according to litter pollution estimates by the Lagrangian model adopted (Fossi et al., 2017) and habitats and feeding grounds of investigated species. To compare marine litter abundances with simulation results, the linear correlation coefficient (Pearson correlation test) between the model data and the observed data was calculated, taking into consideration the estimations done at the same positions and times.

2.2. Sampling strategy

The ad hoc sampling activities for simultaneous monitoring of floating macrolitter and microplastic are shown in Fig. 1A and B. In the Pelagos Sanctuary SPAMI, monitoring transects were performed starting from one nautical mile (nm) offshore and repeated every 10 nm in pelagic areas (Fig. 1A). Additional macrolitter transects were conducted before the simultaneous sampling described above. In the Tuscan Archipelago National Park, a star-shaped experimental design was adopted on the coastal waters off the 7 main islands to assess potential differences in marine litter distribution as a function of different levels of protection (monitoring zones inside and outside the protected areas) and distance from the coast (Fig. 1B). Simultaneous transects were started one nautical mile offshore and repeated at 3 nm, while the macrolitter object monitoring was carried out throughout the circumnavigation of each island (Fig. 1B). Simultaneously at the floating litter evaluation, in both areas several bioindicator species (e.g. invertebrates, fishes and cetaceans) of plastic ingestion were collected or sampled to assess the potential physical and chemical impacts of plastics and their additive compounds (i.e., phthalic acid esters) (data not shown).

2.3. Marine litter monitoring activities

The sea surface floating litter (macrolitter and microplastic) sampling campaign in the Pelagos Sanctuary was carried out both in pelagic and neritic areas focusing on the northern sector (including the coasts of Liguria and Tuscany, Italy) and the central-western sector (including the coasts of Italy and France) of the Ligurian Sea from May to June 2019 and the northeast and northwest coasts of the island of Corsica (Fig. 1) in September 2019. Sampling effort was carried out taking into account the litter distribution provisional model outputs, different depths and slopes of the study area, focusing on the submarine canyons of Genova, Imperia and Saint-Florent, considered special feeding areas for cetaceans and fin whales in particular (Moulins et al., 2007, 2008; Würtz, 2012), as well as near potential sources of marine litter pollution, such as the port of Livorno and Marina di Pisa (along the Tuscan coast) and La Spezia, Genova and Loano (along the Ligurian coast), as well as the river discharges of Arno, Serchio and Magra. The sampling campaign was conducted onboard the 54-ft sailing catamaran "Headwind" property of CIMA Research Foundation. A total of 1568 nautical miles were navigated, with 168 floating macrolitter monitoring transects and 84 manta trawls conducted to assess the floating microplastic.

The Tuscan Archipelago National Park sampling campaign was conducted in July 2019 aboard the oceanographic ISPRA research vessel ASTREA, focussing on the coastal waters off the 7 main islands of the archipelago. A total of 585 nautical miles were covered, with 105 transects monitoring the floating macrolitter and 57 manta trawls assessing the floating microplastic.

2.4. Sea surface floating macrolitter: monitoring and characterization

The distribution, abundance and composition of floating macrolitter (>2.5 cm) were assessed using the fixed-width strip transect method as recommended in the monitoring guidelines developed by the EU MSFD Technical Subgroup on Marine Litter (Hanke et al., 2013) and the MEDSEALITTER project (Arcangeli et al., 2020). This method allows counting the number of objects detected within a fixed-width strip, which should be representative of the visibility conditions during the survey and depends mainly on the speed of the vessel and the height of the observer above sea level. All observations were made with the naked eye from the bow of the ship (3 m above sea level) at a mean speed of 4 knots for 30 min. Due to the characteristics of our observation set-up, a relatively narrow strip of 7 m was monitored, following the recommendation of Galgani et al. (2013). Each item was characterized according to the main list of litter categories (Galgani et al., 2013), which revised the original OSPAR and UNEP categories (Cheshire et al., 2009) and indicated the type (Artificial Polymer Materials, Rubber, Cloth/Textile, Paper/Cardboard; Processed/Worked Wood and Metal), size classes (B. 2.5–5 cm, C. 5–10 cm, D.10–20 cm, E. 20–30 cm, F. 30–50 cm, G. > 50 cm) and colours (W. White; T. Transparent; B. Black; C. Cyan/Blue; R. Red; G. Green; Y. Yellow; O. Other) of the floating objects. Finally, counts of scattered objects were converted to density values (*Di*) by dividing the total number of objects sighted by the effective area sampled in each transect:

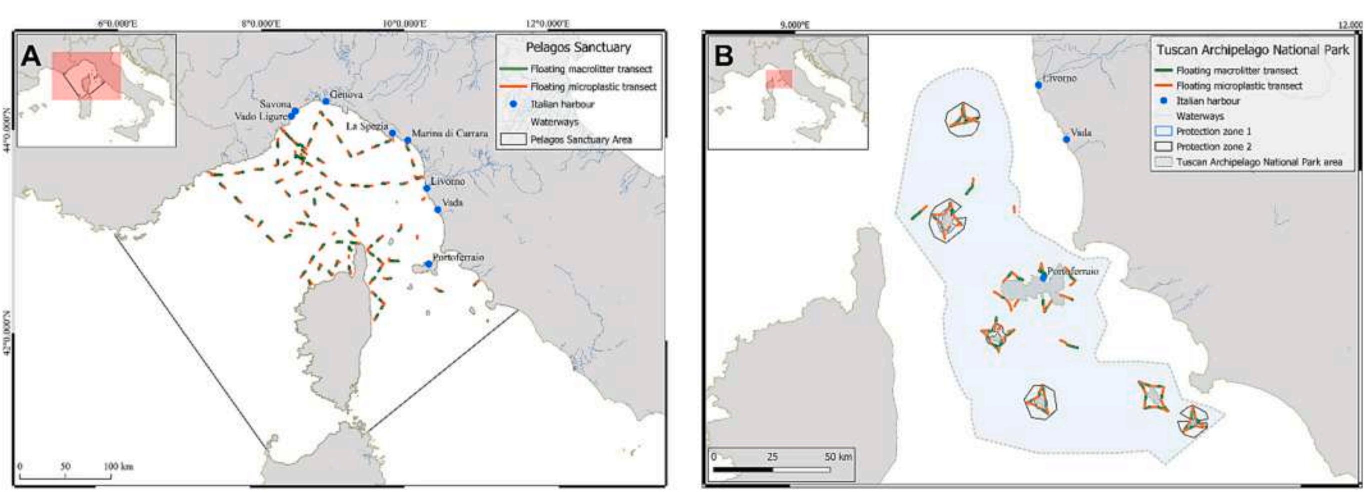

Fig. 1. Monitoring activities carried out during the Pelagos Sanctuary (A) and Tuscan Archipelago National Park (B). Macrolitter (green) and microplastic transects (orange) were performed simultaneously starting one nautical mile from the coast and repeated every 3 and 10 nautical miles in the Pelagos Sanctuary and Tuscan Archipelago National Park, respectively.

$$\mathrm{Di} = \frac{n}{L^{*}W}$$

Where *n* is the number of items seen on the transect*, L* is the length of the transect*, and W* (7 m) is the fixed width of the strip observed and expressed as items/km^2.

2.5. Sea surface floating microplastic: sampling and characterization

Floating microplastic samples were collected using a manta trawl (330 μm mesh size, 16 × 60 cm mouth opening) (simultaneously to the floating macrolitter survey) towed at 2–3 knots on the water surface for 30 min, held to the side of the boat to avoid the turbulence caused by the wake of the vessel. At the end of each transect, the net was thoroughly rinsed from the outside to ensure that both neuston and microplastics were washed into the end of the net. Samples were filtered through a 300 μm metal sieve and stored in a 70 % ethanol solution for synthetic particle analysis. To avoid contamination throughout the sampling activities, all the materials used for sample collection, including the nets, were carefully cleaned and rinsed before each tow.

In the laboratory, the floating microplastic samples were filtered through a sieve (mesh size: 300 μm) and observed under an NBS stereo zoom microscope (Mod. NBS-STMDLX -T) equipped with an LED light and a micro metered eyepiece. The microplastics were manually isolated in a glass Petri dish and allowed to dry overnight at room temperature. Each Petri dish was then photographed and analysed for particle size measurement (expressed in mm) using ImageJ software (Fiji Distribution). Natural buoyant materials such as plant leaves, wood and bird feather were manually removed from the samples and rinsed with microfiltered waters (0.45 μm) to collect all the plastic particles sticking to them. Samples visually characterized both by heavy plastic particles and organic matter content were re-suspended with a NaCl solution (1.2 g/cm^3) and let settled down overnight. Moreover, the dubious chemical origin of smaller natural particles such as chitin residues, was promptly tested through the hot needle technique (Bellas et al., 2016). The isolated particles were characterized according to different size classes into small microplastic (SMPs) (0.3–1 mm), large microplastic (LMPs) (1–5 mm), mesoparticles (5–25 mm), and macroparticles (>25 mm), shape (pellet, fragment, film, filament, microbead, and foam), and colour (black, blue, white/transparent, white/opaque, red, green, and others) and weighed using an OHAUS Explorer precision balance (±0.1 mg) (Fossi et al., 2017). Glassware was used in the laboratory procedures, and special care was taken to prevent airborne contamination by performing sample analysis in a clean airflow cabinet and using two glass Petri dishes placed on either side of the stereomicroscope as blank controls. Despite the use of contamination control procedures, fibres and paint were not included in the total count of the particles due to the risk of external contamination during sampling.

The data obtained, expressed as concentration items/km^2 and mg/m^2 were normalised, if necessary, by applying the correction factor proposed by Kukulka et al. (2012). This factor, widely accepted in the scientific literature (Baini et al., 2018; Faure et al., 2015; Fossi et al., 2017; Kooi et al., 2016) takes into account the unfavourable meteorological and maritime conditions (wave >0.50 m and wind speed >4 m/s) that may affect the accumulation of floating microplastic in surface waters due to the wind mixing effect, leading to an underestimation of their concentrations, and proposes an appropriate value to correct the final concentrations of the samples. Finally, the polymeric composition of 10 % of the isolated microplastics was evaluated and selected proportionally according to the relative abundance in the different sizes, shapes and colour classes for each sample. Using Fourier infrared spectroscopy (FTIR), each particle was scanned 16 times using an Agilent Cary 630 spectrophotometer. To identify the polymers, the spectrum obtained was processed using Agilent Micro Lab FTIR software and compared to a database of reference spectra. Only results that showed >80 % overlap were accepted (Baini et al., 2018).

2.6. Environmental and anthropogenic influences on marine litter distribution

The whole dataset related to floating macrolitter and microplastic was examined considering the habitat types (bathyal, canyon, seamount, slope and continental shelf) and the main environmental (SST: sea surface temperature; SSH: sea surface height; MLD: depth of mixed layer and current velocity) and anthropogenic factors (vessel traffic, distance from ports, distance from the coast and distance from estuaries) that may influence its distribution. Habitat types were identified using GEBCO bathymetric data (GEBCO, 2022); canyons were identified following Tepsich et al., 2014. Oceanographic data were taken daily from the Copernicus Marine Environmental Service, associating each floating macrolitter and microplastic sample with the corresponding daily value of the environmental variables considered. Vessel traffic and port data were downloaded from the European Marine Observation and Data Network (EMODnet, n.d., www.emodnet.eu). The vessel traffic data have a monthly resolution and include data from different vessel types (tankers, cargo vessels, fishing vessels, passenger vessels, sailing vessels and recreational vessels). Vessel densities are reported in hours/km^2/month; each floating waste concentration sample was linked to the corresponding monthly traffic data. Discharge location data were obtained in QGIS using river data downloaded from the ISPRA website (http://www.sinanet.isprambiente.it/it/sia-ispra/download-mais/reticolo-idrografico/view, n.d) for Italy and the French government (https://www.data.gouv.fr/fr/datasets/cours-deau-metropole-2017-bd-carthage, n.d) for France. In Italy, rivers were classified into two different groups (torrents and streams) according to their flow rate and classified as minor and major discharges, respectively. In France, rivers were divided into two classes according to their length: rivers longer than 25 km belong to class 1 (major discharges), and those longer than 10 km belong to class 2 (minor discharges).

2.7. GIS (geographical information systems) and statistical analysis

Floating marine litter concentration data were imported and processed using the Quantum GIS platform (version 3.10.1 A Coruña), and Rstudio (version 1.1.4.1106) to perform spatial and statistical analysis respectively. Descriptive statistics and normality tests (Shapiro-Wilk normality test and Anderson-Darling test) were performed to examine the entire floating litter datasets to determine whether parametric or non-parametric statistical analyses were appropriate.

Mann-Whitney-Wilcoxon for pairwise comparisons and Kolmogorov-Smirnov tests were used to compare differences in floating litter mean concentrations (items/km^2) and characteristics of the items (size, shape and macrolitter categories) between the Pelagos Sanctuary and the Tuscan Archipelago National Park areas and the zoning protection among islands. The Kruskal-Wallis test for multiple comparisons and post hoc test analysis was conducted to compare differences in the distribution of floating litter among different habitats.

The analysis of environmental and anthropogenic factors that can influence the distribution of floating litter was performed in two steps, following the method of Kanhai et al. (2017). A Spearman's rank correlation test was performed between the factors considered and the scattering litter concentration. Then, generalised additive models (GAMs) were used to evaluate the influence of each variable on the distribution of floating litter. The response variable was always litter abundance (macrolitter or MPs), while the initial explanatory variables were potential pollution sources. The variables were considered separately so that a GAM was created for each variable for each type of floating litter (macrolitter or MPs). For the variables characterized by the presence of outliers, two models were created: one in which all values were included, and the other in which the outliers were excluded. Outliers have been identified by examining box-plots built separately for each variables, thus identifying points located outside the 1.5 times the interquartile range above the upper quartile and bellow the lower quartile. Among the two GAMs built, the final model was then selected applying REML (Rpakage mgcv). This procedure was chosen to evaluate the influence of extreme situations that might not be representative of the general situation in the study area. To better understand the relationships represented by the GAM plots, a null line was used to define the positive effect of the predictors on litter accumulation, in a process called GAMvelope (Torres et al., 2008; Correia et al., 2015). The GAMvelope allowed the highlighting of areas favourable to litter accumulation in the Pelagos Conservation Area. A significance level ($p < 0.05$) was considered for all analyses.

2.8. Litter distribution risk map

Average oceanographic conditions in terms of SST, SSH and current velocity were determined using monthly maps corresponding to the period of the sampling campaigns (June–September 2019). A 5 km grid was overlaid to the Pelagos Sanctuary area, assigning a value of 1 to each cell characterized by environmental and anthropogenic variables that positively affect the litter distribution, as resulted from the GAMvelope model. A comprehensive hazard map was then generated, based on the distribution of floating macrolitter, with hazard indices ranging from 1 to 8 (considering the maximum number of variables influencing the litter distribution and accumulation) and indicating the areas at higher risk of exposure for the marine organisms.

3. Results and discussions

3.1. Marine litter distribution in marine protected areas: State of the art

Litter abundance and distribution in the Mediterranean Sea were reviewed with a particular focus on the sampling efforts carried out in the MPAs, their pollution status and the potential gaps to be covered to fully addressed the impact and effect of marine litter.

Although there are several studies on floating litter in the Mediterranean Sea covering different areas (Fig. 2), slightly >30 % have focused on marine protected areas (Supplementary material Tables S1 and S2). The Langrangian modelling analysis of plastic fluxes on six selected coasts of marine protected areas in the Mediterranean proposed by Liubartseva et al. (2019) represents one of the first attempts to predict marine litter distribution and potential impacts in MPAs. It showed that the input of litter was relatively low (0.4–3.6 kg (km/day) compared to the average flux of 6.2 ± 0.8 kg (km/day) calculated for the Mediterranean Sea in 2013–2017 (Liubartseva et al., 2019), assessing the synergistic role of anthropogenic factors generating plastic and hydrodynamic transport influencing its distribution within MPAs. A different approach was proposed by Fossi et al. (2017) in the Pelagos Sanctuary SPAMI, where simulated and in situ MP concentrations were evaluated to verify the accuracy and strength of the predictive plastic distribution model and to highlight the potential risk associated with ingestion by fin whales in this important ecological MPA. This area is one of the most investigated in the Mediterranean Sea (Supplementary material Tables S1 and S2), 70 % of the studies conducted in MPAs are carried out here, with an average abundance of floating objects and MPs of 0.73 ± 82.3 items/km^2 and 85,122 ± 35,726 items/km^2 (0.30 ± 0.23 items/m^3), respectively. This MPA also seems to be affected by the temporary formation of the marine litter convergence area between the islands of Corsica and Capraia, where a high concentration of plastic has been reported (Baini et al., 2018; Fossi et al., 2017; Suaria et al., 2016). In the western part of the Mediterranean, the presence and distribution of marine litter in other MPAs have been studied in the Menorca Channel (Ruiz-Orejón et al., 2019), the Cabrera National Park (Fagiano et al., 2022), Calanque National Park (Schmidt et al., 2021), Ischia and Ventotene Marine Protected Area (de Lucia et al., 2018) and Torre Flavia wetland (Battisti et al., 2019; Cesarini et al., 2021). Exceptionally high concentrations of floating macrolitter were detected in surface waters of the MPAs of Gozo and Malta (Ionian Sea and Central Mediterranean sub-

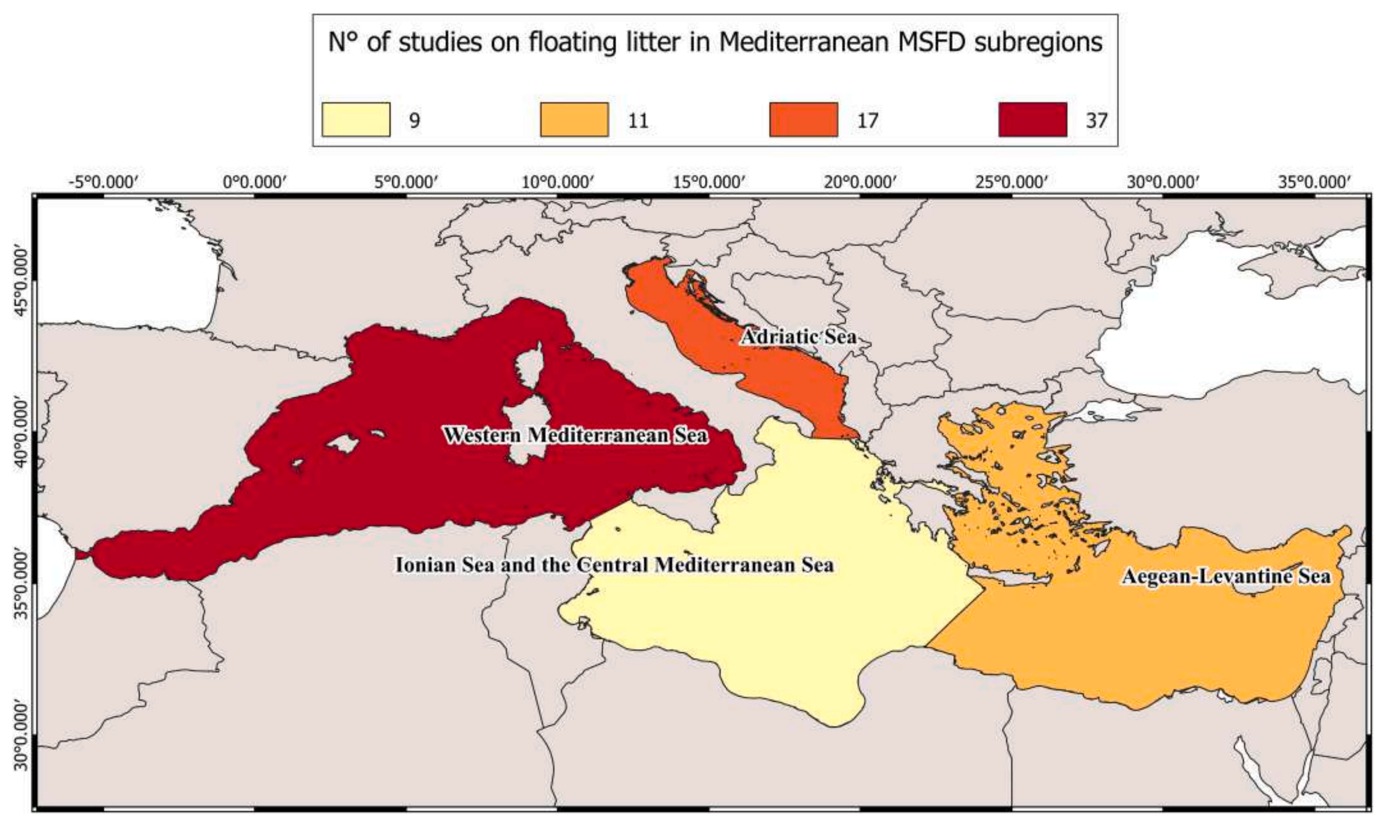

Fig. 2. Number of studies evaluating floating marine litter (macrolitter and microplastic) carried out in the Mediterranean Sea from 1980 to 2022, according to the Marine Strategy Framework Directive (MSFD) subregions.

regions). This study highlights the potential influence of seasonal variation and distance from the coast on the distribution and accumulation of litter, showing the highest levels during the winter season (2392 ± 7477 items/km^2) and in coastal areas (6371 ± 11,968 items/km^2) (Curmi and Axiak, 2021). Despite the data reported by previous studies carried out in the MPAs, no information is still available regarding the potential influence of oceanographic and anthropogenic variables affecting the litter accumulation and stranding as well as the potential sources of pollution insisting in this basin. In this context, the present study reports relevant data that shed light on the main inputs of litter contamination and factors driving litter dispersion, proposing for the first time a model-guided sampling approach.

3.2. Floating litter: abundances and composition

The experimental designs performed ad hoc in the selected areas showed valuable efficacy in the collection of data providing reliable information on the abundance, distribution and composition of macrolitter and microplastic. An overall correlation index (Pearson correlation test; $R = 0.83$, $p < 0.05$), between on-field marine litter abundances and simulation results computed by the Lagrangian model, was highlighted confirming the effectiveness of the adopted sampling strategy and validating the modelling indirect approach to correctly predict the concentration of floating litter.

3.2.1. Floating macrolitter

A total of 273 transects were conducted to monitor the presence of floating macrolitter throughout the study area. A total of 2169 items ranging from 0 to 3974 items/km^2 were sighted, with an average concentration of 399.01 ± 485.84 items/km^2 (Fig. 3A). This value is one-two orders of magnitude higher than the threshold level proposed by UNEP/MAP (2020) (5 objects/km^2) and the average concentration calculated considering the published data on the assessment of litter in the western part of the Mediterranean (Supplementary material Table S1) (29.7 ± 46.8 items/km^2). As far as we know, this value represents the highest concentration of floating macrolitter recorded so far in the study area and could indicate a potential worsening of the macrolitter status in an important ecological area as the Pelagos Sanctuary (Arcangeli et al., 2018; Campanale et al., 2019; Di-Méglio and Campana, 2017; Fossi et al., 2017; Suaria and Aliani, 2014). As for the other Mediterranean subregions, few studies reported similar litter concentrations in the surface waters of MPAs located respectively in the Adriatic Sea (Palatinus et al., 2019) and near the islands of Malta and Gozo (Curmi and Axiak, 2021). Nevertheless, most published papers have been conducted with oceanographic vessels sailing at >6 knots and from an observing height of 6 to 25 m. Variability in observation conditions can affect the detection of small macrolitter objects (Class B. 2.5–5 cm), as previously acknowledged (Galgani et al., 2013; Zeri et al., 2018). Only recently studies have started to report the minimum size class detected (Compa et al., 2019; Di-Méglio and Campana, 2017; Fossi et al., 2017; Vlachogianni et al., 2018, 2020; Zeri et al., 2018), and relative information on the size characterization of sighted items (Zeri et al., 2018). Against this background, the application of harmonized monitoring protocols at the Mediterranean level, as proposed and implemented in the PB MPAs project, will improve the accuracy, and comparability of reported marine litter densities and effective identification of hot spot areas.

Litter and in particular artificial polymer materials items (99 % of the total) were observed in 90 % (245/273 transects) of the transects conducted. These results are consistent with previous studies published throughout the Mediterranean Sea (Campanale et al., 2019; Compa et al., 2019; Fossi et al., 2017; Tata et al., 2020). The majority of the sighted objects (80 %) had a size of <20 cm and a light-coloured characterization (>80 %), with size class B (2.5–5 cm; 58 %) being the most common. The account of this dimensional range as the most frequently sighted is consistent with other studies conducted aboard small vessels at low speed, which allowed a homogeneous detection of all floating objects encountered in the sampled striped waters (Palatinus et al., 2019; Vlachogianni et al., 2018; Zeri et al., 2018).

Analysis of the most common objects revealed that >70 % of all objects floating on the sea surface were represented by 10 categories of litter (Supplementary material Table S3). Objects of secondary origin belonging to the categories G67 (Sheets and industrial packaging) and G79 (Plastic pieces 2.5 cm><50 cm) were most frequently sighted. Their presence could be an indication of the degradation processes and fragmentation that affect the litter objects once dispersed in the marine environment, allowing the formation of smaller items.

A statistical difference in the distribution of samples between the two monitored areas (W = 5413.5, *p*-value = 9.70e-10) was found, confirming a lower concentration of floating macrolitter in the Pelagos Sanctuary (280.36 ± 423.88 items/km^2) than in the surface waters of the Tuscan Archipelago National Park (617.76 ± 599.15 items/km^2). A statistical difference in mean concentration was also observed between the two areas considering different size classes (B. 2.5–5 cm, C. 5–10 cm, D. 10–20 cm, E. 20–30 cm, F. 30–50 cm, G. > 50 cm). Only for class E (20–30 cm), no difference in average concentration was observed, while the concentration for all other classes was higher in the Tuscan Archipelago National Park than in the Pelagos Sanctuary (Supplementary material Fig. 1). The highest abundances in surface waters of the islands of the Tuscany region, both in terms of the number of items and size classes, may be due to more recent inputs of pollution from land, as this area was particularly affected by tourist and recreational activities during the summer period of the sampling campaigns. Moreover, the stability of hydrodynamic features that characterise the Tuscan Archipelago during the summer season could favour the floating of larger objects in coastal waters once they are dispersed in the marine environment, delaying their potential accumulation in pelagic areas.

Considering the different types of litter, the categories with the highest average concentration in the Pelagos Sanctuary were G67 (Sheets and industrial packaging) and G79 (Plastic pieces 2.5 cm><50 cm), for which an average of >100 items/km^2 was recorded (Supplementary material Table S3). These categories were resulted the most abundant also in the Tuscan Archipelago National Park, reaching >300 items/km^2 and 150 items/km^2, respectively. The categories “G58: Fish boxes”, “G94: Tablecloth”, “G145: Other textiles” and “G149: Paper packaging” were only sighted in the Pelagos Sanctuary; while “G3: Buoys”, “G74: foam packaging”, “G135: Clothing”, “G142: Rope, string, and nets” and “G160: Pallets” were only present in the Tuscan Archipelago.

Among the categories sampled in both study areas, “G6: Bottles”, “G18: Crates and containers/baskets”, “G45: Mussel nets/Oyster nets”, “G48: Synthetic rope”, “G67: Sheets, industrial packaging, plastic sheeting”, “G79: Plastic pieces 2.5 cm> <50 cm” and “G124: Other plastic/polystyrene items (identifiable)” were found to have a higher statistically significant concentration in the Tuscan Archipelago National Park than in the Pelagos Sanctuary area (Supplementary material Table S3).

Considering the differences between the islands of the Tuscan Archipelago National Park, the highest concentration of macrolitter was found in the southern sector of the archipelago near the islands of Giglio and Giannutri (792.90 ± 610.13 items/km^2) and the northern sector between the islands of Gorgona and Capraia (726.42 ± 735.20 items/km^2) (Fig. 4). These patterns of accumulation may be influenced by the inputs of litter originating directly from the coast due to the short distance of these islands and the proximity of the Tevere river identified as a plastic pollution source by de Lucia et al. (2018) in the southern sector. The influence of rivers on plastic distribution in this area was pointed out also by Galgani et al. (2019), evidencing how during the summer period, the northern part of the Tyrrhenian Sea was particularly affected by plastics riverine inputs originating from the Ombrone and Tevere rivers and spatially distributed by the superficial currents insisting on this area. Oceanographical features, and in particular the currents

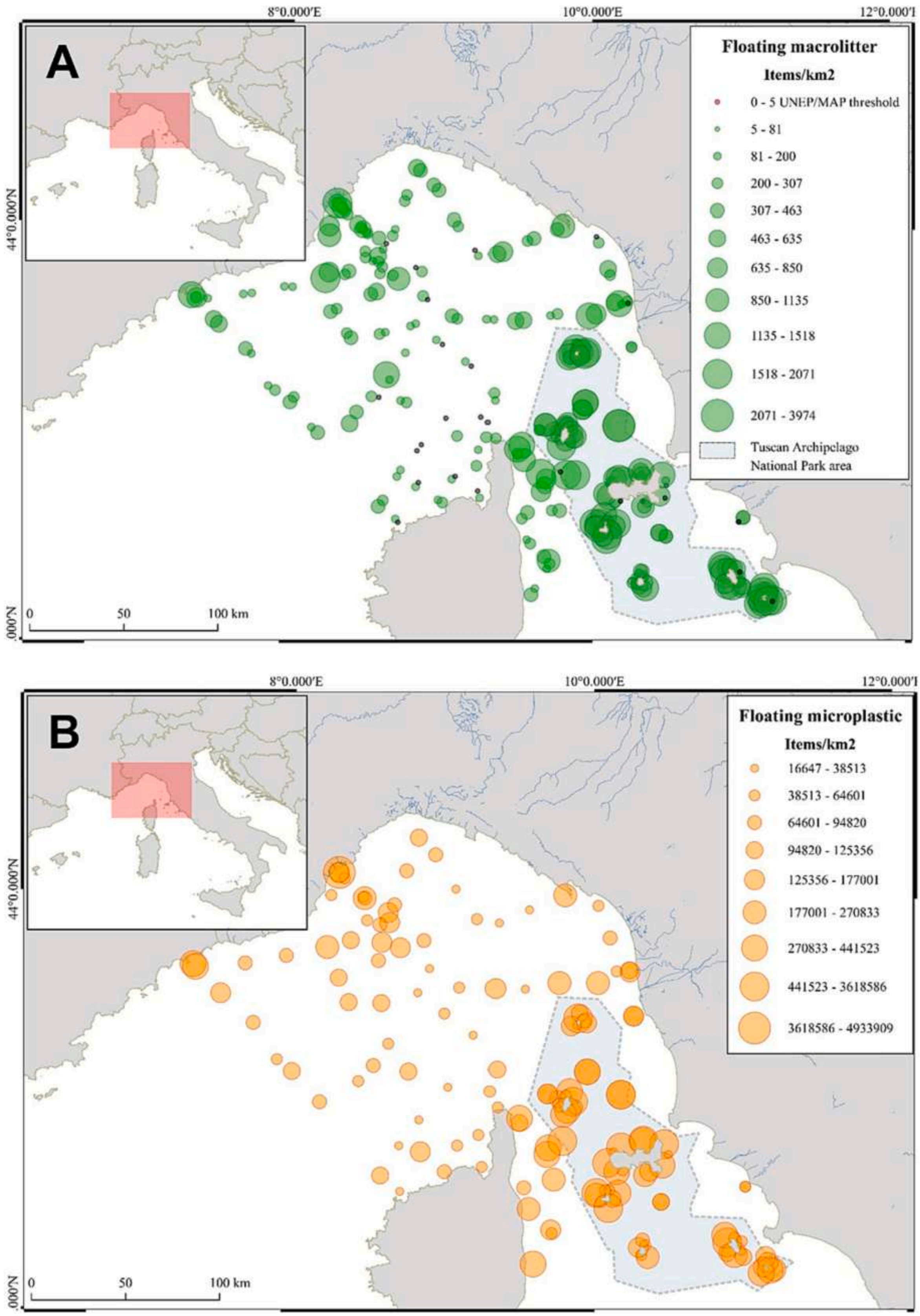

Fig. 3. Floating macrolitter (A) and microplastics (B) spatial distribution in the whole study area considered. The concentrations of litter objects sighted were expressed in items/km^2, and the floating macrolitter threshold proposed by UNEP/MAP (2020) reported.

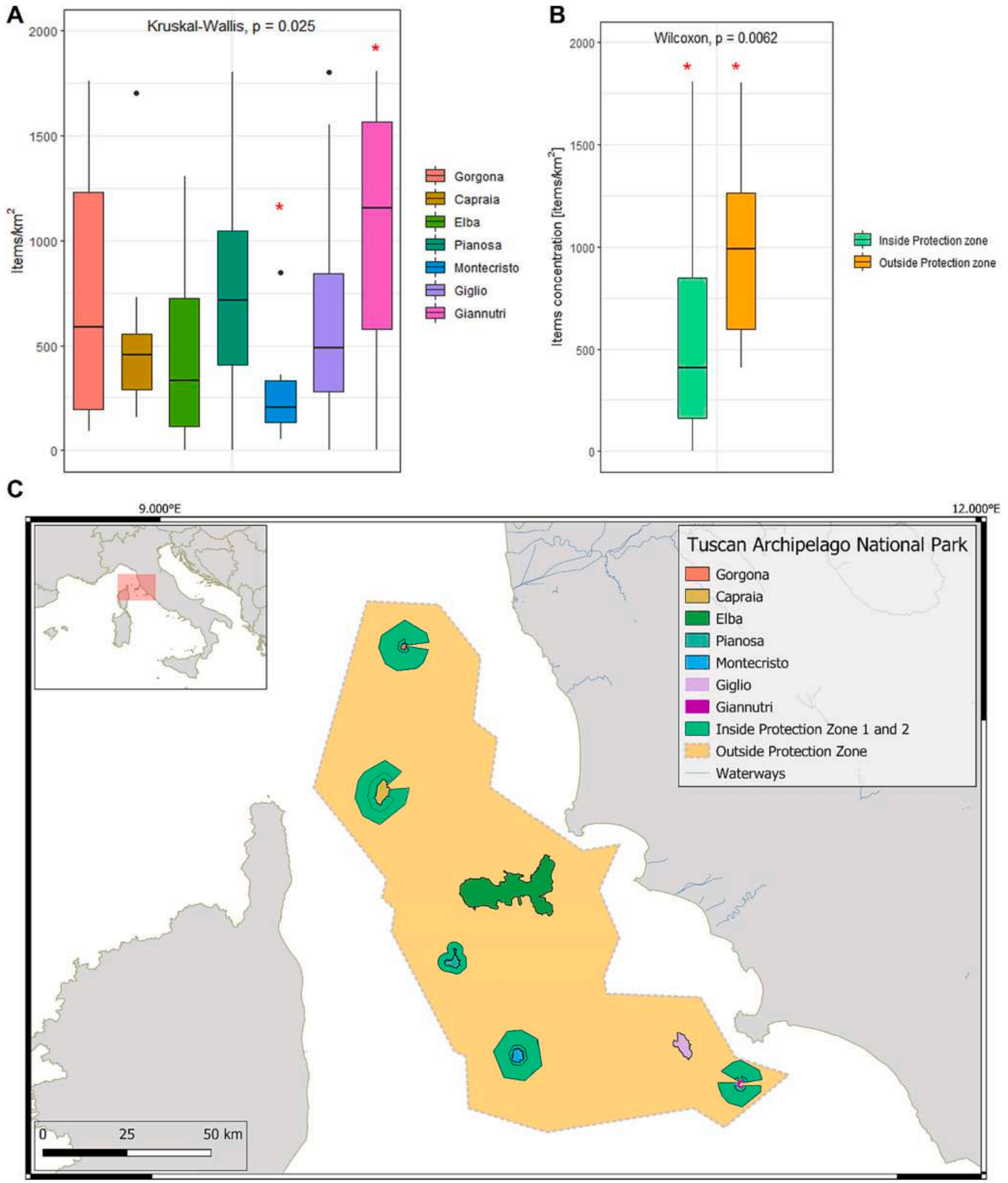

Fig. 4. Total mean concentrations of floating macrolitter found in the facing waters of Tuscan Archipelago National Park from the northernmost to southernmost island (A) and inside (green) and outside (orange) the protection zones (B). The areas considered as "Inside and Outside Protection Zone" groups are also displayed (C). * indicates statistically significant difference ($p < 0.05$).

prevailing in the northern part of the Tuscan Archipelago National Park might be considered the main factors determining plastic distribution in this area. Other studies published have highlighted the transient formation of a convergence area between the islands of Capraia, Corsica and Gorgona, which influences the distribution and concentration of litter (Fossi et al., 2017; Suaria et al., 2016).

Statistical differences among islands in terms of floating litter concentration (chi-squared = 14.401, df = 6, p-value = 2.5e-2) were found. In particular, Giannutri and Montecristo were statistically different from the other areas and were the islands with the highest (1040.35 ± 648.34

items/km^2) and lowest (264.93 ± 210.92 items/km^2) concentration values, respectively (p = 1e-3, p adjusted = 2e-2) (Fig. 4A). This difference could be explained both by the distance from the Tuscan coast and by the potential pollution sources that may affect these islands. Montecristo is the farthest island of the Tuscan Archipelago and it is located about 22 nm far from the Tuscan coast. Moreover, the waters of the island are fully protected up to a distance of 1 nm and tourist access is regulated and limited to 1000 visitors per year. For these reasons, the presence and accumulation of litter could be limited, as confirmed by our data. The second-highest concentration was found in the waters facing the island of Pianosa (748.32 ± 522.32 items/km^2) (Fig. 4A). The island is in the central sector of the Tuscan Archipelago and seems to be affected by intensive transport, accumulation and beaching ashore of litter items. So far, these data represent the first assessment of litter occurrence in this area, where surface currents seem to play a crucial role in marine litter accumulation.

As already highlighted for the whole study area, size class B was the most abundant in the different islands studied, especially in Giannutri Island with a concentration of 579.25 ± 402.43 items/km^2 (p = 1e-3 - p adjusted = 2e-2) (Supplementary material Table S4). Litter with larger dimensions (>10 cm) was found statistically significant in the surface waters off the islands of Pianosa (classes D and E p = 2e-3 p adjusted = 4e-2) and again Giannutri (class D) (p adjusted = 2.3e-2) (Supplementary material Table S4). Considering the samples carried out inside or outside the protected areas in the Tuscan Archipelago National Park, the concentration of floating litter showed statistically significant lower values inside the marine protected area than outside (p = 6.2e-3) (Fig. 4B). Categories G67 (Sheets, industrial packaging, plastic sheeting), and G79 (Plastic pieces 2.5 cm > < 50 cm) were the most frequently found objects on the different islands. Their presence was assessed in higher concentrations in the samples collected outside the protected areas in the Tuscan Archipelago National Park. However, statistical differences were only found for category G67 (679.08 ± 332.06 objects/km^2) (p-value = 7e-4).

3.2.2. Floating microplastic

A total of 141 manta trawl samples were simultaneously collected during the macrolitter monitoring to assess the concentration of smaller particles (<25 mm) at the sea surface. A total of 56,084 particles were isolated belonging 90 % (n. 50,985) and 10 % (n. 5099) to microplastics and mesoplastics, respectively. No rubber particles were found, so the following results refer to MPs only. An average concentration of 259,490 ± 586,477 items/km^2 was found throughout the whole study area, ranging from 16,647 to 4,933,909 items/km^2 (Fig. 3B). This value was in agreement with the mean value of floating MPs abundances in the western Mediterranean subregion, which was calculated considering the studies available in the literature and set at 216,399 ± 284,360 items/km^2. Although no threshold values for MPs in the Mediterranean Sea have yet been proposed, the concentration found in this study and by Caldwell et al. (2019) (233,927 ± 810,357 items/km^2) in the Pelagos Sanctuary appear to be increasing compared to those reported during the sampling campaign carried out in 2018 (Caldwell et al., 2019) and by previous studies (Baini et al., 2018; Collignon et al., 2012, 2014; Fossi et al., 2017; Pedrotti et al., 2016; Tesán Onrubia et al., 2021). This threatening trend of particle accumulation may pose a threat to organisms living in this protected area throughout the marine trophic chain, as also highlighted by Fossi et al. (2017). The average concentration observed here (1.62 ± 3.67 items/m^3), expressed as particles per m^3 to allow a proper comparison with other studies (Supplementary material Table S2), resulted higher than all those reported in the literature except for the values found by Fagiano et al. (2022) in the Cabrera National Park (3.52 ± 8.81 items/m^3) considered an area of high plastic waste density (Ruiz-Orejón et al., 2018).

MPs characterization analysis revealed that large MPs were the most abundant size class (76 %) ranging between 1 mm to 2.5 mm and accounting for 42 % in total. Fragments (86 %) and films (10 %) are the most represented shapes with 96 % of the isolated particles. These results are consistent with other studies conducted in the Mediterranean Sea (Baini et al., 2018; Compa et al., 2020; Suaria et al., 2016) and in other oceans (Cózar et al., 2014; Eriksen et al., 2013) and confirm that secondary microplastics are the most widespread in the marine environment. Colours can also influence the uptake of plastic particles. Particularly brightly coloured items, which were represented in this study at a concentration of >70 %, could increase the likelihood of ingestion as they resemble prey (Martí et al., 2020; Wright et al., 2013).

Polymer composition analysis showed that polyolefin thermoplastics, represented by polyethylene (PE) and polypropylene (PP) (95 % in total), were the most abundant. Their presence in the marine environment is widely recognized in all ocean basins (Baini et al., 2018; Enders et al., 2015; Pedrotti et al., 2016; Suaria et al., 2016), reflecting the increasing production and use of these plastic polymers. They are mainly used in packaging and disposable products and their production in Europe represents about 50 % of the total plastic demand (PlasticsEurope, 2020). Moreover, as PE and PP positively buoyant polymers (0.90–0.99 g/cm^3; 0.85–0.92 g/cm^3) are sensitive to degradation in the marine environment and have a longer residence time at the sea surface, they tend to accumulate at the sea surface as confirmed by the plastic-type here found, mainly fragments and films, made of these materials. The average weight density and concentration values of MPs were statistically lower in the Pelagos Sanctuary (0.068 ± 0.162 mg/m^2 and 226,075 ± 650,984 items/km^2) than in the surface waters of the Tuscan Archipelago National Park (0.152 ± 0.261 mg/m^2 and 355,281 ± 616,782 items/km^2) (weight density W = 1524, p-value = 1.8e-5; concentration W = 1524, p-value =3.2e-3).

This result confirms what was observed for the distribution and concentration of floating macrolitter objects and strengthens the hypothesis that the presence of larger objects (categories G67 and G79) may influence the formation of MPs as a result of degradation and fragmentation processes. According to that, a correlation between the spatial concentrations of floating macrolitter (273 monitoring transects) and microplastics (141 manta trawl samples) collected in the whole study area was investigated to reveal a statistically common distribution pattern. A significant strong direct correlation (p-value <2.2e-16, r = 0.63) (Fig. 5) was found confirming the effectiveness of the experimental plan performed and highlighting the importance of the simultaneous floating litter sampling to better address the presence and distribution of plastic pollution in the marine protected areas highlighting the presence of hotspot areas and finally providing also preliminary information on the potential impacts on marine organisms.

The shape analysis of the isolated particles revealed a significant concentration of fragments (305,065 ± 522,863 items/km^2) (p-value = 9.2e-03) and films (37,479 ± 101,232 items/km^2) (p-value = 4.1e-05) higher in the surface waters of the Tuscan Archipelago National Park than in the Pelagos Sanctuary (Fig. 6A).

According to the classification of size classes, the difference in mean concentration between the two areas was statistically significant when considering only larger particles, which resulted in a higher concentration in the Tuscan Archipelago National Park (287,744 ± 497,983 items/km^2) than in the Pelagos Sanctuary (163,084 ± 466,917 items/km^2) (Fig. 6B). This accumulation pattern was confirmed by the study of Pedrotti et al. (2016), analysing the size distributions of plastic particles at different distances from land and showed an increase in plastic abundance from large to small items moving from coastal to pelagic areas. Moreover, this result is consistent also with the general size distribution found by Cózar et al. (2014) for ocean surface waters. According to Pedrotti et al. (2016), the highest presence of large MPs in the nearshore areas could be due to the combination of efficient removals of small fragments from the surface due to their potential stratification along the water column, sinking due to the biofouling processes and the interactions with marine organisms such as invertebrates species. In addition, the gradual fragmentation processes due to physical and chemical degradation of plastic particles moving towards the pelagic

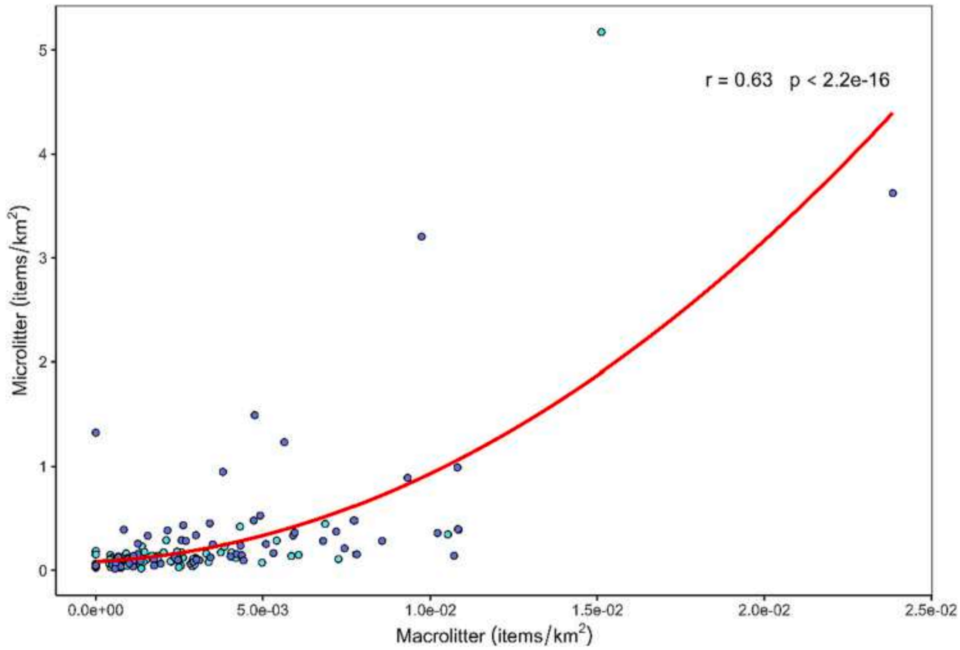

Fig. 5. Correlation scatterplots among floating macrolitter and MPs concentration evaluated in theSPAMI Pelagos Sanctuary (cyan dots) and Tuscan Archipelago National Park (blue dots). Statistical significance for p-value <0.001.

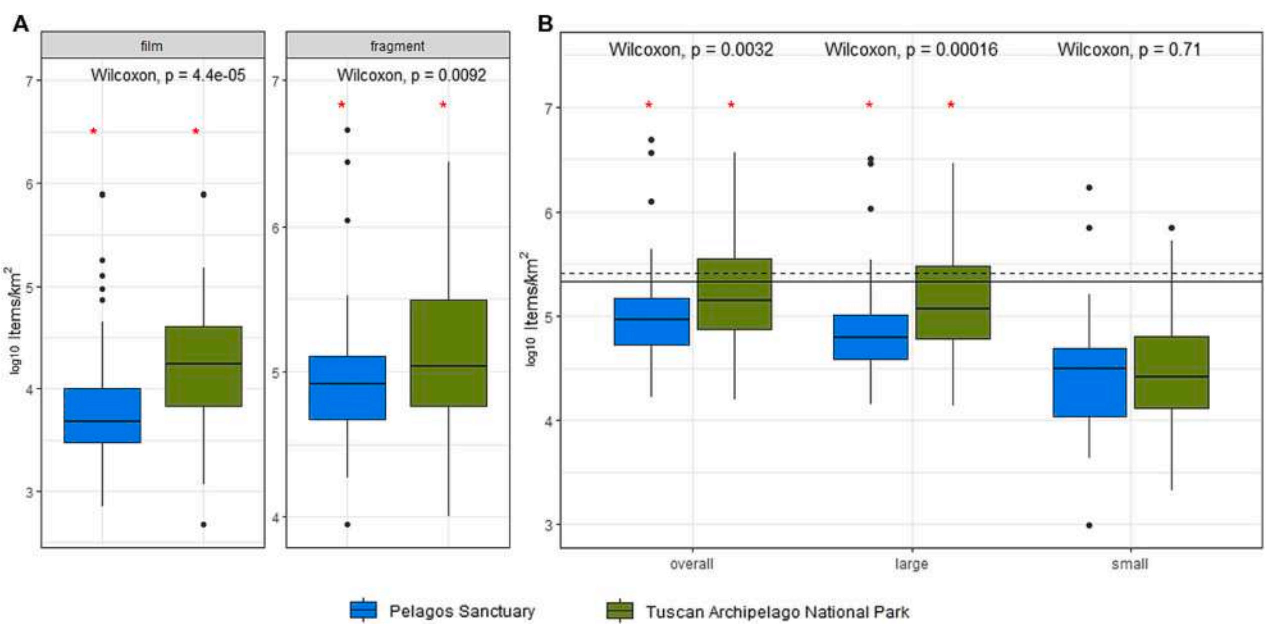

Fig. 6. Floating MPs different distribution among the two study areas considered (Pelagos Sanctuary: blue boxplots; Tuscan Archipelago National Park: green boxplots) according to shape, size classes and total average concentration. The black line shows the reference value for mean MPs concentration in the Northwestern Mediterranean Sea, while the dashed line represents the reference value for the standard deviation of MPs concentration in the Northwestern Mediterranean Sea.* indicates difference statistically significative (p < 0.05).

areas may favourite the formation of smaller particles and their accumulation in offshore waters.

However, no differences among the islands of the Tuscan Archipelago National Park were found (Supplementary material Table S5).

The highest concentrations for both number and weight density of particles were found in the northern part of the Tuscan Archipelago facing the island of Gorgona (Supplementary material Table S5). This area was previously described as the most affected by the presence of floating macroparticles, indicating the formation of a temporary accumulation zone previously described in the literature (Fossi et al., 2017; Suaria et al., 2016). High particle abundances were also detected around the islands of Elba and Pianosa in the central part of the archipelago, respectively, where MPs seem to accumulate. While the first area is under strong anthropogenic pressure, especially during the summer months, the distribution and accumulation of particles in the facing waters of Pianosa island again seem to be closely related to the surface currents that characterise the waters there. Differently from what was highlighted for macrolitter objects, the islands in the southern sector appear to be more vulnerable to recent inputs of plastic pollution from the coast. This is also confirmed by the greater extent of the sighted objects, which may be displaced to more pelagic areas of the Tyrrhenian Sea where fragmentation processes occur, as indicated by the litter dispersion model (Northern Tyrrhenian Gyre, described in Fossi et al., 2017). No differences in MPs distribution were found between the different levels of protection regulating the monitored islands.

3.3. Marine litter distribution: influence of marine habitats

The potential distribution of floating macrolitter and MPs was assessed considering the different marine habitats according to the topographic features within the Pelagos Sanctuary and the Tuscan Archipelago National Park. The monitored areas were characterized by different habitat types in the bathyal, canyon, seamount, slope and continental shelf (Supplementary material Table S6).

A statistical difference between habitats (Kruskal-Wallis chi-squared = 39.79, df = 4, *p*-value = 4.8e-08) was detected only for floating macrolitter, underlining how the continental shelf (573 ± 572 items/km^2) and seamount (205 ± 245 items/km^2) areas were separated from all other habitats (Fig. 8B). No difference was found for the distribution of MPs (chi-square = 8.91, df = 4, p-value = 6.3e-2) in the study areas (Fig. 8A).

The highest concentration of floating macrolitter was found in the correspondence of the continental shelf (573 ± 572 items/km^2). This area, which is the natural extent of the mainland, from the coastline to a depth of 200 m, is the most sensitive habitat for the accumulation of floating litter that enters the marine environment via land-based sources. Previously described as an area characterized by low litter seafloor density (Galgani et al., 1996; Pham et al., 2014) could be considered a transition zone of buoyant litter towards pelagic habitats such as submarine canyons, where marine litter has been shown to sink and accumulate (Galgani et al., 1996; Gerigny et al., 2019).

3.4. Marine litter distribution: influence of oceanographic and anthropogenic factors

The distribution of the floating macrolitter and MPs throughout the monitored study area was examined considering the main oceanographic (SST: sea surface temperature; SSH: sea surface height; MLD: mixed layer depth and current velocity) and anthropogenic factors (vessel traffic, distance from ports, distance from the coast and distance from estuaries) that may have influenced their spatial distribution during the sampling campaigns. Correlation analyses show a statistically significant relationship between many of the variables considered (76 %) and concentrations of floating macrolitter. In particular, SST, SSH, fishing vessel density and sailing vessel density showed a weak positive correlation (0 < rho < 0.3) with the amount of litter. Bathymetry showed a stronger significant positive correlation (0.3 < rho < 0.5), while a weak negative correlation (−0.3 > rho > 0) was found between floating macrolitter concentration and mixed layer depth (MLD), current velocity, tanker density, cargo vessel density, distance from nearest major outfalls, and distance from the nearest minor outfall. The correlation of floating macrolitter abundance respectively with distance from the coast and distance from the nearest port was also negative and stronger (−0.5 < rho < −0.3). The descriptive statistical values of each environmental and anthropogenic variable considered and the corresponding correlation values and scatter plots with floating macro pollution concentration were summarised in the Supplementary material (Table S7 and Fig. 2). MPs concentration was significantly related to 47 % of the variables studied. The statistically significant results show a weak positive correlation (0 < rho < 0.3) of microplastic density with sea surface temperature, sea surface height, bathymetry and sailing vessel density. Weak negative correlations (−0.3 < rho < 0) were found for currents, distance from the coast, distance from the nearest port and cargo ship density (Supplementary material Table S8 and Fig. 3A). Generalised additive models (GAM) were applied to further determine the influence of each variable on litter abundance (Supplementary

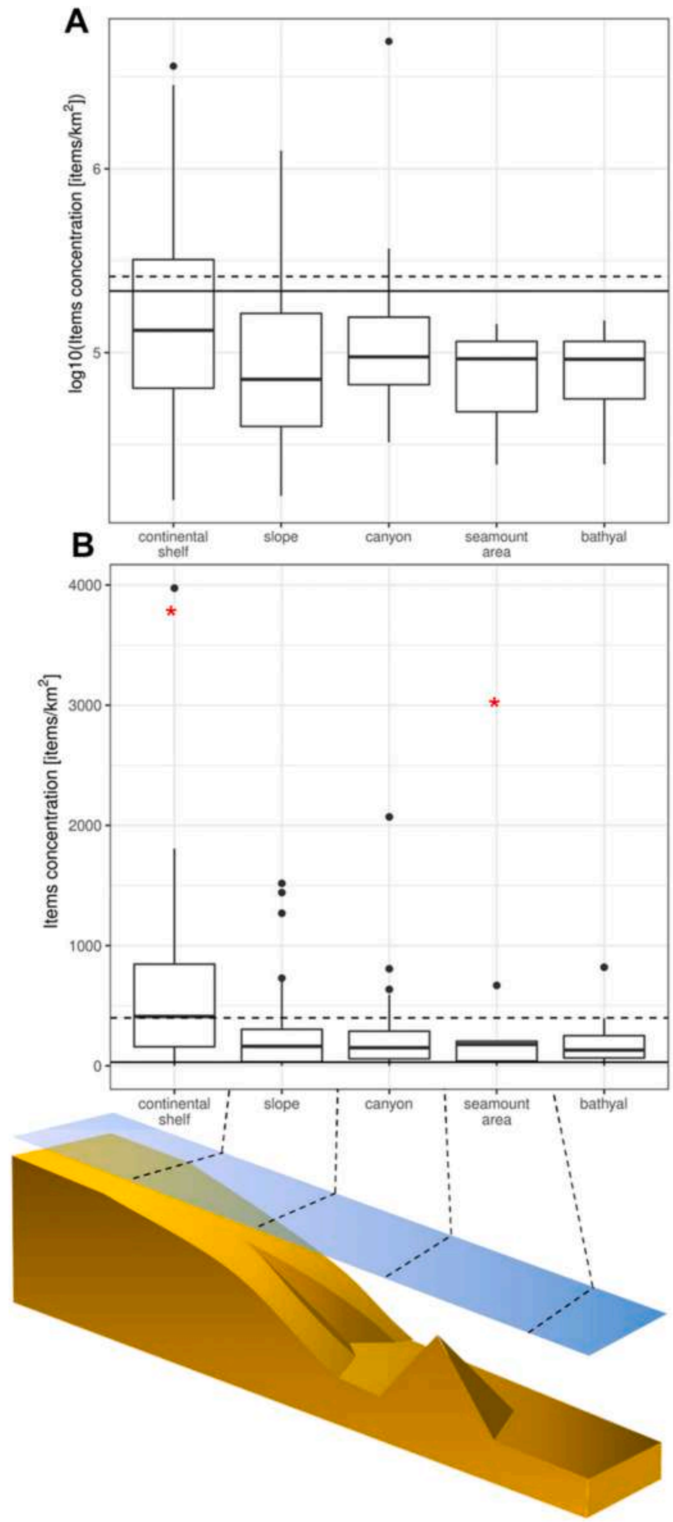

Fig. 8. Concentrations of floating macrolitter (A) and MPs (B) for different habitats within the study areas. Black lines represent the reference value of mean floating macrolitter/MPs concentration in the Western Mediterranean sub-region and the dashed line represents the mean concentration overall of Pelagos Sanctuary from the present study. * indicates statistically significant difference (p < 0.05).

material Tables S9 and S10). In addition, to better highlight the relationships represented by GAM, a zero line was used to define a positive effect of the predictors on litter accumulation. This was done in a procedure called GAMvelope, described by Torres et al. (2008) (Fig. 9A–C and D–E), and allowed the identification of areas affected by the presence of litter in the Pelagos Sanctuary.

Among the oceanographic variables, SST higher than 297.7 K (24.55 °C), SSH higher than −0.38 m, and currents slower than 0.101 m/s have a positive effect on the accumulation of floating litter (Fig. 9A–C). These results suggest that areas with warmer waters and weak oceanographic features such as lower wave height, slower currents, and no upwelling areas may favour macrolitter accumulation. The influence of certain physical and chemical parameters of oceanic waters on the distribution of litter and sampling activities was clearly outlined by Van Sebille et al. (2020). The so-called "vertical mixing effect" of plastic particles, first described by Kukulka et al. (2012) and also emphasised by Enders et al. (2015) and Reisser et al. (2015), is closely related to wave height and direct wind force, which could facilitate the stratification of plastic particles along the water column according to their physical properties (Kooi et al., 2016). A significant increase in litter has also been observed during daily ocean warming, leading to an accumulation of particles at the warmer sea surface (Kukulka et al., 2016).

Considering the anthropogenic factors, a statistical correlation was found between the amount of floating macrolitter and the distance from the coastline closer than 11 km, the distance from the nearest port closer than 25 km, and the distance from the river mouth between 8 and 37 km (Fig. 9D and E). These results confirm the findings of the spatial analysis of litter (Fig. 3A and B) and the distribution of floating plastics in the Mediterranean Sea modelled by Liubartseva et al. (2019), according to which >75 % of the litter scattered in the sea is located in the 50 km of nearshore waters. These areas can potentially be affected by large amounts of litter originating from nearby land-based sources and coastal maritime activities associated with densely populated areas, as well as inputs from rivers (Jambeck et al., 2015; Lebreton et al., 2017). In the Pelagos Sanctuary, the harbour of Livorno (one of the largest Italian ports with 30 million tonnes of cargo and 2 million tourists), the Arno (240 km long and crossing several cities, agricultural areas and industrial zones) and Serchio rivers, and the intensive aquaculture and fishing activities near La Spezia could be the main sources of waste and plastic pollution (Cincinelli et al., 2001; Cortecci et al., 2002; Giovacchini et al., 2018; Merlino et al., 2020). Other minor litter inputs could be derived from the port of Genova, which is described to play an important role in litter distribution in the coastal areas of the northern part of the Pelagos Sanctuary, as well as the influence of the Magra river in the transport and accumulation of anthropogenic particles, especially during the summer season (Galgani et al., 2019). Its contribution appears particularly evident in the Tuscan Archipelago National Park due to the mediated transport of plastic by currents towards the southern sector of the SPAMI monitored (Galgani et al., 2019). This area may also be characterized by litter originating from the Tevere and Ombrone rivers, despite their influences that seem heavily affect the Pelagos Sanctuary, especially during the winter season (Galgani et al., 2019).

Sea surface temperature (SST), bathymetry and distance to the nearest port were shown to significantly influence MP distribution (Supplementary material Table S8). However, given the lower explained variance and the paucity of significant variables, no further analysis of MPs were conducted. Moreover, considering the existing predictive models for their distribution (Fossi et al., 2017, 2018a, 2018b; Liubartseva et al., 2019; Mansui et al., 2015; Politikos et al., 2020), the GAMvelope approach was not considered more effective and was applied only to floating macrolitter at the sea surface. Nevertheless, due to the strong correlation found between the spatial concentrations of floating macrolitter and MPs, the overall risk maps (Fig. 10) produced for floating macrolitter can also provide a reliable indication for the accumulation of smaller particles.

Overall, the study area was characterized by a high input of litter coming from the mainland (e.g., harbours and river inputs) and accumulating in coastal waters within about 10–15 nautical miles. The slope area off western Liguria, the continental shelf in the eastern part and the surrounding areas in the Tuscan Archipelago National Park and north-eastern Corsica was shown to be particularly characterized by plastic

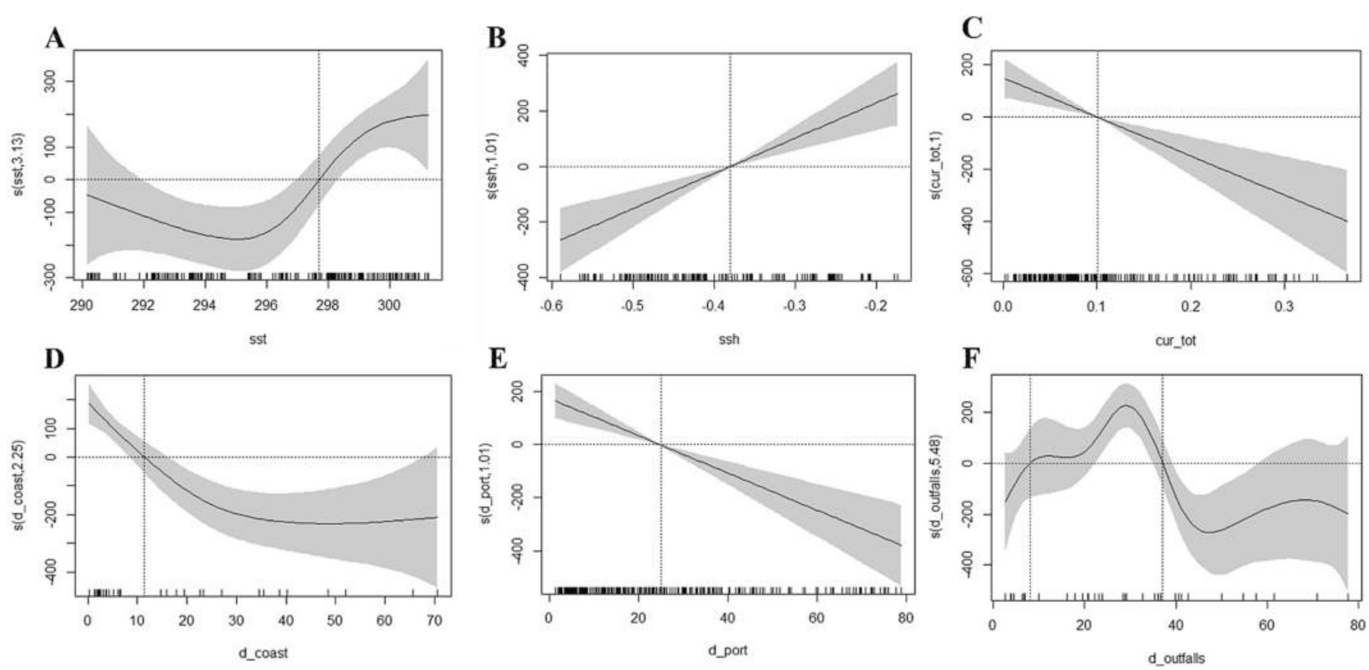

Fig. 9. GAMs plot of significative oceanographic (A: SST; B: SSH and C: current velocity) and anthropogenic variables (D: distance to the coast; E: distance to the port; and F: distance to river outfall) influencing the floating macrolitter accumulation. The degrees of freedom for non-linear fits are in parenthesis on the y-axis. Tick marks above the x-axis indicate the distribution of observations (with and without sightings). The shaded areas represent the 95 % confidence intervals of the spline functions.

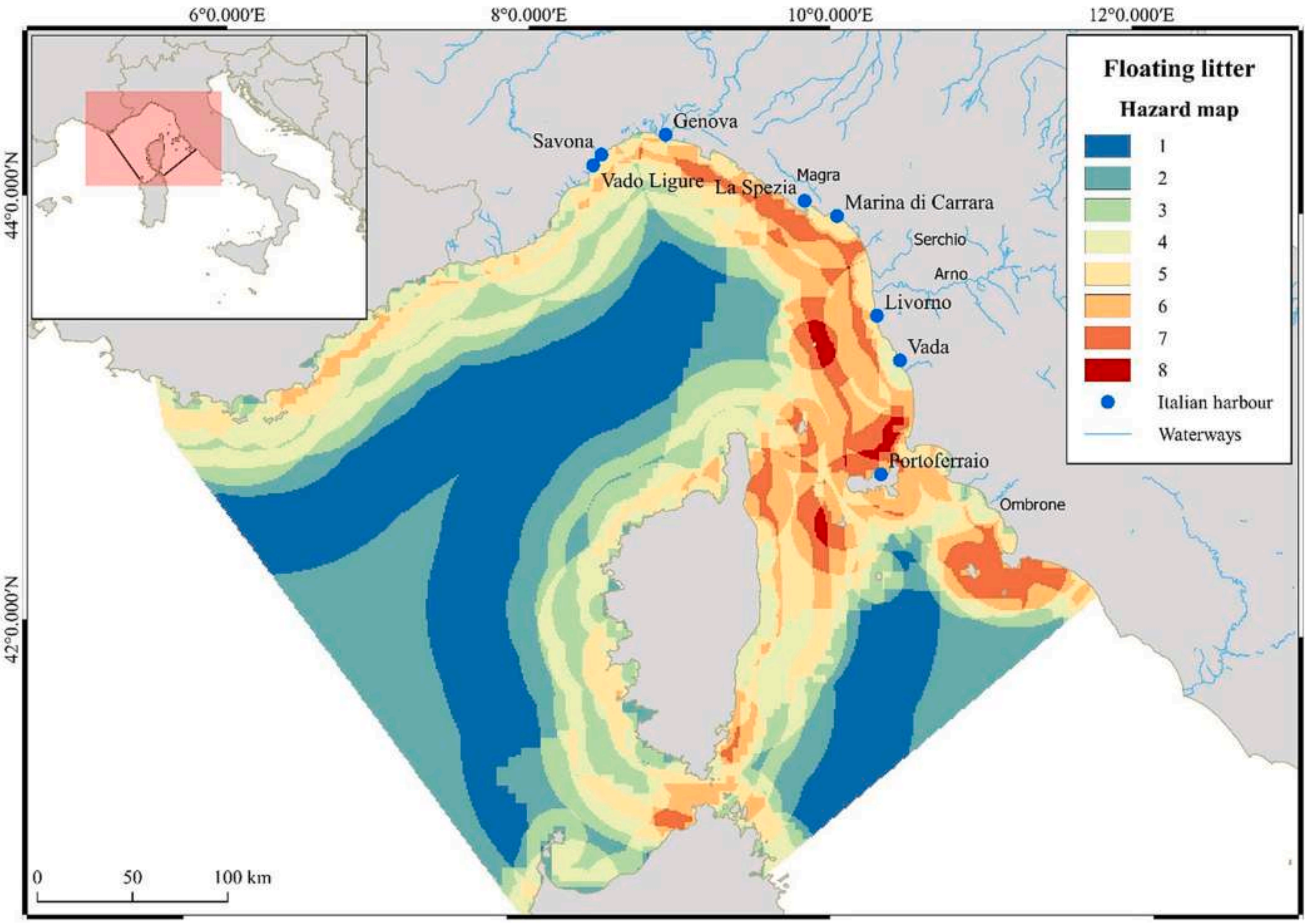

Fig. 10. Floating macrolitter spatial hazard map created considering the oceanographic and anthropogenic factors statistically influencing litter distribution. A hazard score, ranging from 1 to 8, was assigned highlighting areas with different impacts.

litter accumulation (Fig. 10). A moderate risk was present in the canyons of western Liguria and western Corsica, while the least accumulation of plastic was found in the offshore waters over the bathyal plane (Fig. 10). The critical areas highlighted by this spatial risk analysis based on the collected field data show the ecological impact of plastics on the biodiversity inhabiting the Pelagos Sanctuary, especially in the continental shelf ecosystems. The oceanographic variables and anthropogenic activities and the related plastic accumulation in these areas pose a risk to marine species which are exposed to a plethora of anthropogenic stress.

4. Conclusions

The high heterogeneity of marine litter evidence in the available literature stresses the need to create and adopt shared monitoring protocols among the scientific community to collect comparable and consistent data. The harmonized multilevel protocol adopted by this study represents a clear and innovative effort towards a comprehensive assessment of litter impact including transport and accumulation pathways, pollution sources, and potential ecotoxicological effects on marine organisms. Data collected strengthen the effectiveness of the provisional model, as a reliable indirect tool to estimate the litter pollution status of ecologically valuable environments (i.e. SPAMI and National Park and pelagic and coastal protected areas) highlighting areas more at risk for marine organisms. The role of different anthropogenic variables as litter-originating driving factors has been pointed out, confirming the evaluation of the pollution sources as one of the urgent existing gaps to be addressed and defined. The strong correlation found between the distribution of floating macrolitter objects and microplastics highlighted the significance and effectiveness of the simultaneous floating litter sampling design to better address the presence and distribution of plastic pollution in the marine environment. The multi-tier approach allowed to identify main litter sources: strong litter inputs were identified to originate from the mainland, with significant contribution of ports and estuaries as well as areas with warmer waters and weak oceanographic features (e.g., continental shelf) could facilitate plastic accumulation. Coastal waters, within 10–15 nautical miles, seem to represent litter retention zones, which in turn causes concerns about the underlying risk for marine biodiversity, especially considering the key ecological role of the protected areas of the Pelagos Sanctuary and the Tuscan Archipelago. Overall, the relevant information achieved in this study could serve as an affordable basis for implementing effective marine litter prevention, reduction and disposal policies in MPAs and facilitate future management recommendations and use of the marine and coastal environments of these protected areas.

CRediT authorship contribution statement

Matteo Galli: Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Matteo Baini:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Cristina Panti:** Conceptualization, Methodology, Investigation, Resources, Writing – original draft, Writing – review & editing. **Dario Giani:** Investigation. **Ilaria Caliani:** Investigation, Writing – review & editing. **Tommaso Campani:** Investigation. **Massimiliano Rosso:** Investigation, Visualization. **Paola Tepsich:** Formal analysis, Investigation, Writing – review & editing, Visualization. **Vanessa Levati:** Formal analysis. **Federica Laface:** Investigation. **Teresa Romeo:** Project administration, Funding acquisition. **Gianfranco Scotti:** Investigation. **Francois Galgani:** Investigation, Supervision. **Maria Cristina Fossi:** Conceptualization, Methodology, Validation, Investigation, Resources, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

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.scitotenv.2023.166266.

References

Angeletti, L., Ceregato, A., Ghirelli, M., Gualandi, B., Lipparini, E., Malatesta, D., Sperotti, A., Taviani, M., 2010. ROV-SCUBA integrated survey of the Montecristo island nature reserve (Tuscan archipelago national park, Mediterranean Sea). Underw. Technol. 29, 151–154. https://doi.org/10.3723/ut.29.151.

Arcangeli, A., Campana, I., Angeletti, D., Atzori, F., Azzolin, M., Carosso, L., Di Miccoli, V., Giacoletti, A., Gregorietti, M., Luperini, C., Paraboschi, M., Pellegrino, G., Ramazio, M., Sarà, G., Crosti, R., 2018. Amount, composition, and spatial distribution of floating macro litter along fixed trans-border transects in the Mediterranean basin. Mar. Pollut. Bull. 129, 545–554. https://doi.org/10.1016/j.marpolbul.2017.10.028.

Baini, M., Martellini, T., Cincinelli, A., Campani, T., Minutoli, R., Panti, C., Finoia, M.G., Fossi, M.C., 2017. First detection of seven phthalate esters (PAEs) as plastic tracers in superficial neustonic/planktonic samples and cetacean blubber. Anal. Methods 9, 1512–1520. https://doi.org/10.1039/c6ay02674e.

Arcangeli, A., David, L., Aguilar, A., Atzori, F., Borrell, A., Campana, I., Carosso, L., Crosti, R., Darmon, G., Gambaiani, D., Di-Méglio, N., Di Vito, S., Frau, F., Garcia-Garin, O., Orasi, A., Revuelta, O., Roul, M., Miaud, C., Vighi, M., 2020. Floating marine macro litter: density reference values and monitoring protocol settings from coast to offshore. Results from the MEDSEALITTER project. Mar. Pollut. Bull. 160, 111647. https://doi.org/10.1016/j.marpolbul.2020.111647.

Baini, M., Fossi, M.C., Galli, M., Caliani, I., Campani, T., Finoia, M.G., Panti, C., 2018. Abundance and characterization of microplastics in the coastal waters of Tuscany (Italy): the application of the MSFD monitoring protocol in the Mediterranean Sea. Mar. Pollut. Bull. 133, 543–552. https://doi.org/10.1016/j.marpolbul.2018.06.016.

Battisti, C., Kroha, S., Kozhuharova, E., De Michelis, S., Fanelli, G., Poeta, G., Pietrelli, L., Cerfolli, F., 2019. Fishing Lines and Fish Hooks as Neglected Marine Litter: First Data on Chemical Composition, Densities, and Biological Entrapment from a Mediterranean Beach. https://doi.org/10.1007/s11356-018-3753-9.

Bellas, J., Martínez-Armental, J., Martínez-Cámara, A., Besada, V., Martínez-Gómez, C., 2016. Ingestion of microplastics by demersal fish from the Spanish Atlantic and Mediterranean coasts. Mar. Pollut. Bull. 109, 55–60. https://doi.org/10.1016/j.marpolbul.2016.06.026.

Caldwell, J., Petri-Fink, A., Rothen-Rutishauser, B., Lehner, R., 2019. Assessing meso- and microplastic pollution in the Ligurian and Tyrrhenian seas. Mar. Pollut. Bull. 149, 110572 https://doi.org/10.1016/j.marpolbul.2019.110572.

Campanale, C., Suaria, G., Bagnuolo, G., Baini, M., Galli, M., De Rysky, E., Ballini, M., Aliani, S., Fossi, M.C., Uricchio, V.F., 2019. Visual observations of floating macro litter around Italy (Mediterranean Sea). Mediterr. Mar. Sci. 20, 271. https://doi.org/10.12681/mms.19054.

Cesarini, G., Cera, A., Battisti, C., Taurozzi, D., Scalici, M., 2021. Is the weight of plastic litter correlated with vegetal wrack? A case study from a Central Italian beach. Mar. Pollut. Bull. 171, 112794.

Cheshire, A., Adler, E., Barbière, J., Cohen, Y., Evans, S., Jarayabhand, S., Westphalen, G., 2009. UNEP/IOC Guidelines on Survey and Monitoring of Marine Litter.

Cincinelli, A., Stortini, A.M., Perugini, M., Checchini, L., Lepri, L., 2001. Organic pollutants in sea-surface microlayer and aerosol in the coastal environment of Leghorn - (Tyrrhenian Sea). Mar. Chem. 76, 77–98. https://doi.org/10.1016/S0304-4203(01)00049-4.

Collignon, A., Hecq, J.H., Glagani, F., Voisin, P., Collard, F., Goffart, A., 2012. Neustonic microplastic and zooplankton in the North Western Mediterranean Sea. Mar. Pollut. Bull. 64, 861–864. https://doi.org/10.1016/j.marpolbul.2012.01.011.

Collignon, A., Hecq, J.H., Galgani, F., Collard, F., Goffart, A., 2014. Annual variation in neustonic micro- and meso-plastic particles and zooplankton in the Bay of Calvi (Mediterranean-Corsica). Mar. Pollut. Bull. 79, 293–298. https://doi.org/10.1016/j.marpolbul.2013.11.023.

Compa, M., Alomar, C., Wilcox, C., van Sebille, E., Lebreton, L., Hardesty, B.D., Deudero, S., 2019. Risk assessment of plastic pollution on marine diversity in the Mediterranean Sea. Sci. Total Environ. 678, 188–196. https://doi.org/10.1016/j.scitotenv.2019.04.355.

Compa, M., Alomar, C., Mourre, B., March, D., Tintoré, J., Deudero, S., 2020. Nearshore spatio-temporal sea surface trawls of plastic debris in the Balearic Islands. Mar. Environ. Res. 158, 104945 https://doi.org/10.1016/j.marenvres.2020.104945.

Coomber, F.G., D'Incà, M., Rosso, M., Tepsich, P., di Sciara, G.N., Moulins, A., 2016. Description of the vessel traffic within the north Pelagos Sanctuary: inputs for marine spatial planning and management implications within an existing international marine protected area. Mar. Policy 69, 102–113.

Correia, A.M., Tepsich, P., Rosso, M., Caldeira, R., Sousa-Pinto, I., 2015. Cetacean occurrence and spatial distribution: habitat modelling for offshore waters in the Portuguese EEZ (NE Atlantic). J. Mar. Syst. 143, 73–85. https://doi.org/10.1016/j.jmarsys.2014.10.016.

Cortecci, G., Dinelli, E., Bencini, A., Adorni-Braccesi, A., La Ruffa, G., 2002. Natural and anthropogenic SO4 sources in the Arno river catchment, northern Tuscany, Italy: a chemical and isotopic reconnaissance. Appl. Geochem. 17, 79–92. https://doi.org/10.1016/S0883-2927(01)00100-7.

Cózar, A., Echevarría, F., González-Gordillo, J.I., Irigoien, X., Úbeda, B., Hernández-León, S., Palma, T.A., Navarro, S., García-de-Lomas, J., Ruiz, A., Fernández-de-Puelles, M.L., Duarte, C.M., 2014. Plastic debris in the open ocean. Proc. Natl. Acad. Sci. 111 (28), 10239–10244.

Curmi, M., Axiak, V., 2021. Extended study on floating litter in Malta's coastal waters (Central Mediterranean). Mar. Pollut. Bull. 166, 112200 https://doi.org/10.1016/j.marpolbul.2021.112200.

de Lucia, G.A., Vianello, A., Camedda, A., Vani, D., Tomassetti, P., Coppa, S., Palazzo, L., Amici, M., Romanelli, G., Zampetti, G., Cicero, A.M., Carpentieri, S., Di Vito, S., Matiddi, M., 2018. Sea water contamination in the vicinity of the Italian minor islands caused by microplastic pollution. Water (Switzerland) 10, 1108. https://doi.org/10.3390/w10081108.

Deudero, S., Alomar, C., 2015. Mediterranean marine biodiversity under threat: reviewing influence of marine litter on species. Mar. Pollut. Bull. 98, 58–68. https://doi.org/10.1016/j.marpolbul.2015.07.012.

Di-Méglio, N., Campana, I., 2017. Floating macro-litter along the Mediterranean French coast: composition, density, distribution and overlap with cetacean range. Mar. Pollut. Bull. 118, 155–166. https://doi.org/10.1016/j.marpolbul.2017.02.026.

EMODnet. Human activities portal. https://www.emodnet.eu/.

Enders, K., Lenz, R., Stedmon, C.A., Nielsen, T.G., 2015. Abundance, size and polymer composition of marine microplastics ≥10 μm in the Atlantic Ocean and their modelled vertical distribution. Mar. Pollut. Bull. 100, 70–81. https://doi.org/10.1016/j.marpolbul.2015.09.027.

Eriksen, M., Maximenko, N., Thiel, M., Cummins, A., Lattin, G., Wilson, S., Hafner, J., Rifman, S., Zellers, A., Rifman, S., 2013. Plastic pollution in the South Pacific subtropical gyre. Mar. Pollut. Bull. 68 (1–2), 71–76. https://doi.org/10.1016/j.marpolbul.2012.12.021.

Fagiano, V., Alomar, C., Compa, M., Soto-Navarro, J., Jordá, G., Deudero, S., 2022. Neustonic microplastics and zooplankton in coastal waters of Cabrera Marine Protected Area (Western Mediterranean Sea). Sci. Total Environ. 804, 150120 https://doi.org/10.1016/j.scitotenv.2021.150120.

Faure, F., Saini, C., Potter, G., Galgani, F., de Alencastro, L.F., Hagmann, P., 2015. An evaluation of surface micro- and mesoplastic pollution in pelagic ecosystems of the Western Mediterranean Sea. Environ. Sci. Pollut. Res. 22, 12190–12197. https://doi.org/10.1007/s11356-015-4453-3.

Fossi, M.C., Panti, C., Guerranti, C., Coppola, D., Giannetti, M., Marsili, L., Minutoli, R., 2012. Are baleen whales exposed to the threat of microplastics? A case study of the Mediterranean fin whale (B*alaenoptera physalus*). Mar. Pollut. Bull. 64, 2374–2379. https://doi.org/10.1016/j.marpolbul.2012.08.013.

Fossi, M.C., Coppola, D., Baini, M., Giannetti, M., Guerranti, C., Marsili, L., Panti, C., de Sabata, E., Clò, S., 2014. Large filter feeding marine organisms as indicators of microplastic in the pelagic environment: the case studies of the Mediterranean

basking shark (*Cetorhinus maximus*) and fin whale (*Balaenoptera physalus*). Mar. Environ. Res. 100, 17–24. https://doi.org/10.1016/j.marenvres.2014.02.002.
Fossi, M.C., Marsili, L., Baini, M., Giannetti, M., Coppola, D., Guerranti, C., Caliani, I., Minutoli, R., Lauriano, G., Finoia, M.G., Rubegni, F., Panigada, S., Bérubé, M., Urbán Ramírez, J., Panti, C., 2016. Fin whales and microplastics: the Mediterranean Sea and the Sea of Cortez scenarios. Environ. Pollut. 209, 68–78. https://doi.org/10.1016/j.envpol.2015.11.022.
Fossi, M.C., Romeo, T., Baini, M., Panti, C., Marsili, L., Campan, T., Canese, S., Galgani, F., Druon, J.N., Airoldi, S., Taddei, S., Fattorini, M., Brandini, C., Lapucci, C., 2017. Plastic debris occurrence, convergence areas and fin whales feeding ground in the Mediterranean marine protected area Pelagos Sanctuary: a modeling approach. Front. Mar. Sci. 4, 1–15. https://doi.org/10.3389/fmars.2017.00167.
Fossi, M.C., Panti, C., Baini, M., Lavers, J.L., 2018a. A review of plastic-associated pressures: cetaceans of the Mediterranean Sea and Eastern Australian Shearwaters as case studies. Front. Mar. Sci. 5, 1–10. https://doi.org/10.3389/fmars.2018.00173.
Fossi, M.C., Pedà, C., Compa, M., Tsangaris, C., Alomar, C., Claro, F., Ioakeimidis, C., Galgani, F., Hema, T., Deudero, S., Romeo, T., Battaglia, P., Andaloro, F., Caliani, I., Casini, S., Panti, C., Baini, M., 2018b. Bioindicators for monitoring marine litter ingestion and its impacts on Mediterranean biodiversity. Environ. Pollut. 237, 1023–1040. https://doi.org/10.1016/j.envpol.2017.11.019.
Fratini, S., Ragionieri, L., Cutuli, G., Vannini, M., Cannicci, S., 2013. Pattern of genetic isolation in the crab P*achygrapsus marmoratus* within the tuscan archipelago (Mediterranean Sea). Mar. Ecol. Prog. Ser. 478, 173–183. https://doi.org/10.3354/meps10247.
Galgani, F., Souplet, A., Cadiou, Y., 1996. Accumulation of debris on the deep-sea floor off the French Mediterranean coast. Mar. Ecol. Prog. Ser. 142, 225–234.
Galgani, F., Hanke, G., Werner, S., De Vrees, L., 2013. Marine litter within the European marine strategy framework directive. Dir. J. Mar. Sci. 70, 1055–1064. https://doi.org/10.1093/icesjms/fst122.
Galgani, F., Hanke, G., Maes, T., 2015. Global distribution, composition and abundance of marine litter. In: Bergmann, M., Gutow, L., Klages, M. (Eds.), Marine Anthropogenic Litter. Springer International Publishing, Cham, pp. 29–56. https://doi.org/10.1007/978-3-319-16510-3_2.
Galgani, F., Deidun, A., Liubartseva, S., Gauci, A., Doronzo, B., Brandini, C., Gerigny, O., 2019. Monitoring and Assessment Guidelines for Marine Litter in Mediterranean MPAs. Technical Report of the Interreg/MED/AMARE Project. IFREMER/AMARE editor (57 pages). https://archimer.ifremer.fr/doc/00487/59840/.
GEBCO Compilation Group, 2022. GEBCO_2022 Grid. https://doi.org/10.5285/e0f0bb80-ab44-2739-e053-6c86abc0289c.
Gerigny, O., Brun, M., Fabri, M.C., Tomasino, C., Le Moigne, M., Jadaud, A., Galgani, F., 2019. Seafloor litter from the continental shelf and canyons in French Mediterranean water: distribution, typologies and trends. Mar. Pollut. Bull. 146, 653–666.
Germanov, E.S., Marshall, A.D., Bejder, L., Fossi, M.C., Loneragan, N.R., 2018. Microplastics: no small problem for filter-feeding megafauna. Trends Ecol. Evol. 33, 227–232. https://doi.org/10.1016/j.tree.2018.01.005.
Giovacchini, A., Merlino, S., Locritani, M., Stroobant, M., 2018. Spatial distribution of marine litter along italian coastal areas in the Pelagos sanctuary (Ligurian Sea - NW Mediterranean Sea): a focus on natural and urban beaches. Mar. Pollut. Bull. 130, 140–152. https://doi.org/10.1016/j.marpolbul.2018.02.042.
Grossi, F., Lahaye, E., Moulins, A., Borroni, A., Rosso, M., Tepsich, P., 2021. Locating ship strike risk hotspots for fin whale (Balaenoptera physalus) and sperm whale (Physeter macrocephalus) along main shipping lanes in the North-Western Mediterranean Sea. Ocean Coast. Manag. 212, 105820.
Hanke, G., Galgani, F., Werner, S., Oosterbaan, L., Nilsson, P., Fleet, D., Kinsey, S., Thompson, R., Palatinus, A., Van Franeker, J., Vlachogianni, T., Scoullos, M., Veiga, J., Matiddi, M., Alcaro, L., Maes, T., Korpinen, S., Budziak, A., Leslie, H., Gago, J., Liebezeit, G., 2013. Guidance on monitoring of marine litter in European seas. Publications Office of the European Union.
http://www.sinanet.isprambiente.it/it/sia-ispra/download-mais/reticolo-idrografico/view.
https://www.data.gouv.fr/fr/datasets/cours-deau-metropole-2017-bd-carthage.
Jambeck, J., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. Mar. Pollut. 347, 768.
Kanhai, L.D.K., Officer, R., Lyashevska, O., Thompson, R.C., O'Connor, I., 2017. Microplastic abundance, distribution and composition along a latitudinal gradient in the Atlantic Ocean. Mar. Pollut. Bull. 115, 307–314. https://doi.org/10.1016/j.marpolbul.2016.12.025.
Kooi, M., Reisser, J., Slat, B., Ferrari, F.F., Schmid, M.S., Cunsolo, S., Brambini, R., Noble, K., Sirks, L.A., Linders, T.E.W., Schoeneich-Argent, R.I., Koelmans, A.A., 2016. The effect of particle properties on the depth profile of buoyant plastics in the ocean. Sci. Rep. 6, 1–10. https://doi.org/10.1038/srep33882.
Kukulka, T., Proskurowski, G., Morét-Ferguson, S., Meyer, D.W., Law, K.L., 2012. The effect of wind mixing on the vertical distribution of buoyant plastic debris. Geophys. Res. Lett. 39 https://doi.org/10.1029/2012GL051116.
Kukulka, T., Law, K.L., Proskurowski, G., 2016. Evidence for the influence of surface heat fluxes on turbulent mixing of microplastic marine debris. J. Phys. Oceanogr. 46, 809–815. https://doi.org/10.1175/JPO-D-15-0242.1.
Lebreton, L.C.M., Van Der Zwet, J., Damsteeg, J.W., Slat, B., Andrady, A., Reisser, J., 2017. River plastic emissions to the world's oceans. Nat. Commun. 8, 1–10. https://doi.org/10.1038/ncomms15611.
Liubartseva, S., Coppini, G., Lecci, R., 2019. Are Mediterranean marine protected areas sheltered from plastic pollution? Mar. Pollut. Bull. 140, 579–587. https://doi.org/10.1016/j.marpolbul.2019.01.022.
Mackelworth, P. (Ed.), 2016. Marine Transboundary Conservation and Protected Areas. Routledge.
Mansui, J., Molcard, A., Ourmières, Y., 2015. Modelling the transport and accumulation of floating marine debris in the Mediterranean basin. Mar. Pollut. Bull. 91, 249–257. https://doi.org/10.1016/j.marpolbul.2014.11.037.
Martí, E., Martin, C., Galli, M., Echevarría, F., Duarte, C.M., Cózar, A., 2020. The colors of the ocean plastics. Environ. Sci. Technol. 54, 6594–6601. https://doi.org/10.1021/acs.est.9b06400.
Merlino, S., Locritani, M., Bernardi, G., Como, C., Legnaioli, S., Palleschi, V., Abbate, M., 2020. Spatial and temporal distribution of chemically characterized microplastics within the protected area of pelagos sanctuary (Nw mediterranean sea): focus on natural and urban beaches. Water (Switzerland) 12. https://doi.org/10.3390/w12123389.
Moulins, A., Rosso, M., Ballardini, M., Würtz, M., 2008. Partitioning of the Pelagos Sanctuary (north-western Mediterranean Sea) into hotspots and coldspots of cetacean distributions. J. Mar. Biol. Assoc. UK 88 (6), 1273–1281. https://doi.org/10.1017/S0025315408000763.
Moulins, A., Rosso, M., Nani, B., Würtz, M., 2007. Aspects of the distribution of Cuvier's beaked whale (*Ziphius cavirostris*) in relation to topographic features in the Pelagos Sanctuary (north-western Mediterranean Sea). J. Mar. Biol. Assoc. U. K. 87, 177–186. https://doi.org/10.1017/S0025315407055002.
Nash, K.L., Cvitanovic, C., Fulton, E.A., Halpern, B.S., Milner-Gulland, E.J., Watson, R.A., Blanchard, J.L., 2017. Planetary boundaries for a blue planet. Nat. Ecol. Evol. 1, 1625–1634. https://doi.org/10.1038/s41559-017-0319-z.
Notarbartolo di Sciara, G., Birkun Jr., A., 2010. Conserving Whales, Dolphins and Porpoises in the Mediterranean and Black Sea: An ACCOBAMS Status Report ACCOBAMS, Monaco.
Notarbartolo di Sciara, G., Tundi, A., Hyrenbach, D., Scovazzi, T., Van Klaveren, P., 2008. The Pelagos sanctuary for Mediterranean marine mammals. Aquat. Conserv. Mar. Freshw. Ecosyst. 18, 367–391. https://doi.org/10.1002/aqc.855.
Palatinus, A., Kovač Viršek, M., Robič, U., Grego, M., Bajt, O., Šiljić, J., Suaria, G., Liubartseva, S., Coppini, G., Peterlin, M., 2019. Marine litter in the Croatian part of the middle Adriatic Sea: simultaneous assessment of floating and seabed macro and micro litter abundance and composition. Mar. Pollut. Bull. 139, 427–439. https://doi.org/10.1016/j.marpolbul.2018.12.038.
Panigada, S., Donovan, G.P., Druon, J.-N., Lauriano, G., Pierantonio, N., Pirotta, E., Zanardelli, M., Zerbini, A.N., di Sciara, G.N., 2017. Satellite tagging of Mediterranean fin whales: working towards the identification of critical habitats and the focussing of mitigation measures. Sci. Rep. 7 https://doi.org/10.1038/s41598-017-03560-9.
Panti, C., Giannetti, M., Baini, M., Rubegni, F., Minutoli, R., Fossi, M.C., Panti, C., Giannetti, M., Baini, M., Rubegni, F., Minutoli, R., Fossi, M.C., 2015. Occurrence, relative abundance and spatial distribution of microplastics and zooplankton NW of Sardinia in the Pelagos Sanctuary Protected Area, Mediterranean Sea. Environ. Chem. 12, 618–626. https://doi.org/10.1071/EN14234.
Pedrotti, M.L., Petit, S., Elineau, A., Bruzaud, S., Crebassa, J.C., Dumontet, B., Martí, E., Gorsky, G., Cózar, A., 2016. Changes in the floating plastic pollution of the mediterranean sea in relation to the distance to land. PLoS One 11, 1–14. https://doi.org/10.1371/journal.pone.0161581.
Pham, C.K., Ramirez-Llodra, E., Alt, C.H., Amaro, T., Bergmann, M., Canals, M., Company, J.B., Davies, J., Duineveld, G., Galgani, F., Howell, K.L., Huvenne, V.A.I., Isidro, E., Jones, D.O.B., Lastras, G., Morato, T., Gomes-Pereira, J.N., Purser, A., Stewart, H., Toieira, I., Tubau, X., Van Rooij, D., Tyler, P.A., 2014. Marine litter distribution and density in European seas, from the shelves to deep basins. PLoS One 9 (4), e95839.
PlasticsEurope. Plastics - The facts 2020.
Politikos, D.V., Tsiaras, K., Papatheodorou, G., Anastasopoulou, A., 2020. Modelling of floating marine litter originated from the eastern Ionian Sea: transport, residence time and connectivity. Mar. Pollut. Bull. 150, 110727 https://doi.org/10.1016/j.marpolbul.2019.110727.
Reisser, J., Slat, B., Noble, K., Du Plessis, K., Epp, M., Proietti, M., De Sonneville, J., Becker, T., Pattiaratchi, C., 2015. The vertical distribution of buoyant plastics at sea: an observational study in the North Atlantic Gyre. Biogeosciences 12, 1249–1256. https://doi.org/10.5194/bg-12-1249-2015.
Renzi, M., Perra, G., Lobianco, A., Mari, E., Guerranti, C., Specchiulli, A., Pepi, M., Focardi, S., 2010. Environmental quality assessment of the marine reserves of the Tuscan Archipelago, central Tyrrhenian Sea (Italy). Chem. Ecol. 26, 299–317. https://doi.org/10.1080/02757541003627647.
Rockström, J., Steffen, W., Noone, K., Persson, Å., Chapin, F.S., Lambin, E.F., Lenton, T. M., Scheffer, M., Folke, C., Schellnhuber, H.J., Nykvist, B., de Wit, C.A., Hughes, T., van der Leeuw, S., Rodhe, H., Sörlin, S., Snyder, P.K., Costanza, R., Svedin, U., Falkenmark, M., Karlberg, L., Corell, R.W., Fabry, V.J., Hansen, J., Walker, B., Liverman, D., Richardson, K., Crutzen, P., Foley, J.A., 2009. A safe operation space for humanity. Nature 461, 472–475.
Romeo, T., Pietro, B., Pedà, C., Consoli, P., Andaloro, F., Fossi, M.C., 2015. First evidence of presence of plastic debris in stomach of large pelagic fish in the Mediterranean Sea. Mar. Pollut. Bull. 95, 358–361. https://doi.org/10.1016/j.marpolbul.2015.04.048.
Ruiz-Orejón, L.F., Sardá, R., Ramis-Pujol, J., 2018. Now, you see me: high concentrations of floating plastic debris in the coastal waters of the Balearic Islands (Spain). Mar. Pollut. Bull. 133, 636–646. https://doi.org/10.1016/j.marpolbul.2018.06.010.
Ruiz-Orejón, L.F., Mourre, B., Sardá, R., Tintoré, J., Ramis-Pujol, J., 2019. Quarterly variability of floating plastic debris in the marine protected area of the Menorca Channel (Spain). Environ. Pollut. 252, 1742–1754. https://doi.org/10.1016/j.envpol.2019.06.063.
Sbrana, M., Musumeri, C., Sartini, M., Sartor, P., Viva, C., 2016. Artisanal fishery exploitation of European Hake, Merluccius merluccius, in the Tuscan Archipelago. Biol. Mar. Mediterr. 23, 43.

Schmidt, N., Castro-Jiménez, J., Oursel, B., Sempéré, R., 2021. Phthalates and organophosphate esters in surface water, sediments and zooplankton of the NW Mediterranean Sea: exploring links with microplastic abundance and accumulation in the marine food web. Environ. Pollut. 272 https://doi.org/10.1016/j.envpol.2020.115970.

Serena, F., Voliani, A., Auteri, R., 1998. Nursery areas and some biological information of tub gurnard (*Trigla lucerna* L., 1758) off Tuscany coasts (Italy). Rapp. Comm. Int. Pour L'exploration Sci. Mer Méditerranée 35, 482–483.

Suaria, G., Aliani, S., 2014. Floating debris in the Mediterranean Sea. Mar. Pollut. Bull. 86, 494–504. https://doi.org/10.1016/j.marpolbul.2014.06.025.

Suaria, G., Avio, C.G., Mineo, A., Lattin, G.L., Magaldi, M.G., Belmonte, G., Moore, C.J., Regoli, F., Aliani, S., 2016. The Mediterranean plastic soup: synthetic polymers in Mediterranean surface waters. Sci. Rep. 6, 1–10. https://doi.org/10.1038/srep37551.

Tata, T., Belabed, B.E., Bououdina, M., Bellucci, S., 2020. Occurrence and characterization of surface sediment microplastics and litter from North African coasts of Mediterranean Sea: preliminary research and first evidence. Sci. Total Environ. 713, 136664 https://doi.org/10.1016/j.scitotenv.2020.136664.

Tepsich, P., Rosso, M., Halpin, P.N., Moulins, A., 2014. Habitat preferences of two deep-diving cetacean species in the northern Ligurian Sea. Mar. Ecol. Prog. Ser. 508, 247–260. https://doi.org/10.3354/meps10851.

Tesán Onrubia, J.A., Djaoudi, K., Borgogno, F., Canuto, S., Angeletti, B., Besio, G., Capello, M., Cutroneo, L., Stocchino, A., Mounier, S., Lenoble, V., 2021. Quantification of microplastics in North-Western Mediterranean harbors: seasonality and biofilm-related metallic contaminants. J. Mar. Sci. Eng. 9, 337. https://doi.org/10.3390/jmse9030337.

Torres, L.G., Read, A.J., Halpin, P., 2008. Fine-scale habitat modelling of a top marine predator: do prey data improve predictive capacity? Ecol. Appl. 18, 1702–1717. https://doi.org/10.1890/07-1455.1.

UNEP/MAP 2020. Agenda Item 5: Parallel CORMON Sessions (Pollution and Marine Litter, and Biodiversity and Fisheries) Updated Baseline Values and Proposal for Threshold Values for IMAP Common Indicator 22. 1-3 December 2020.

Van Sebille, E., Aliani, S., Law, K.L., Maximenko, N., Alsina, J.M., Bagaev, A., Bergmann, M., Chapron, B., Chubarenko, I., Cózar, A., Delandmeter, P., Egger, M., Fox-Kemper, B., Garaba, S.P., Goddijn-Murphy, L., Hardesty, B.D., Hoffman, M.J., Isobe, A., Jongedijk, C.E., Kaandorp, M.L.A., Khatmullina, L., Koelmans, A.A., Kukulka, T., Laufkötter, C., Lebreton, L., Lobelle, D., Maes, C., Martinez-Vicente, V., Morales Maqueda, M.A., Poulain-Zarcos, M., Rodríguez, E., Ryan, P.G., Shanks, A.L., Shim, W.J., Suaria, G., Thiel, M., Van Den Bremer, T.S., Wichmann, D., 2020. The physical oceanography of the transport of floating marine debris. Environ. Res. Lett. 15 https://doi.org/10.1088/1748-9326/ab6d7d.

Villarrubia-Gómez, P., Cornell, S.E., Fabres, J., 2018. Marine plastic pollution as a planetary boundary threat – the drifting piece in the sustainability puzzle. Mar. Policy 96, 213–220. https://doi.org/10.1016/j.marpol.2017.11.035.

Vlachogianni, T., Fortibuoni, T., Ronchi, F., Zeri, C., Mazziotti, C., Tutman, P., Varezić, D.B., Palatinus, A., Trdan, Š., Peterlin, M., Mandić, M., Markovic, O., Prvan, M., Kaberi, H., Prevenios, M., Kolitari, J., Kroqi, G., Fusco, M., Kalampokis, E., Scoullos, M., 2018. Marine litter on the beaches of the Adriatic and Ionian seas: an assessment of their abundance, composition and sources. Mar. Pollut. Bull. 131, 745–756. https://doi.org/10.1016/j.marpolbul.2018.05.006.

Vlachogianni, T., Skocir, M., Constantin, P., Labbe, C., Orthodoxou, D., Pesmatzoglou, I., Scannella, D., Spika, M., Zissimopoulos, V., Scoullos, M., 2020. Plastic pollution on the Mediterranean coastline: generating fit-for-purpose data to support decision-making via a participatory-science initiative. Sci. Total Environ. 711, 135058 https://doi.org/10.1016/j.scitotenv.2019.135058.

Wright, S.L., Thompson, R.C., Galloway, T.S., 2013. The physical impacts of microplastics on marine organisms: a review. Environ. Pollut. 178, 483–492. https://doi.org/10.1016/j.envpol.2013.02.031.

Würtz, M., 2012. Mediterranean Submarine Canyons: Ecology and Governannce. IUCN, Gland, Switzerland and Málaga, Spain.

Zeri, C., Adamopoulou, A., Bojanić Varezić, D., Fortibuoni, T., Kovač Viršek, M., Kržan, A., Mandic, M., Mazziotti, C., Palatinus, A., Peterlin, M., Prvan, M., Ronchi, F., Siljic, J., Tutman, P., Vlachogianni, T., 2018. Floating plastics in Adriatic waters (Mediterranean Sea): from the macro- to the micro-scale. Mar. Pollut. Bull. 136, 341–350. https://doi.org/10.1016/j.marpolbul.2018.09.016.

7 Bibliography

Abiñon, B.S., Camporedondo B. S., Mercadal E. M. B., Olegario K. M.R., 2021. Abundance and Characteristics of Microplastics in Commercially sold fishes from Cebu Island, Philippines | International Journal of Aquatic Biology.

Alessi, R., Ambati, M., Gerasimov, T., Vidoli, S., De Lorenzis, L., 2018. Comparison of Phase-Field Models of Fracture Coupled with Plasticity, in: Oñate, E., Peric, D., de Souza Neto, E., Chiumenti, M. (Eds.), Advances in Computational Plasticity: A Book in Honour of D. Roger J. Owen, Computational Methods in Applied Sciences. Springer International Publishing, Cham, pp. 1–21. https://doi.org/10.1007/978-3-319-60885-3_1

Amaral-Zettler, L.A., Zettler, E.R., Mincer, T.J., 2020a. Ecology of the plastisphere. Nat. Rev. Microbiol. 18, 139–151. https://doi.org/10.1038/s41579-019-0308-0

Amaral-Zettler, L.A., Zettler, E.R., Mincer, T.J., 2020b. Ecology of the plastisphere. Nat. Rev. Microbiol. 18, 139–151. https://doi.org/10.1038/s41579-019-0308-0

Amaral-Zettler, L.A., Zettler, E.R., Slikas, B., Boyd, G.D., Melvin, D.W., Morrall, C.E., Proskurowski, G., Mincer, T.J., 2015. The biogeography of the Plastisphere: implications for policy. Front. Ecol. Environ. 13, 541–546. https://doi.org/10.1890/150017

Amelia, T.S.M., Khalik, W.M.A.W.M., Ong, M.C., Shao, Y.T., Pan, H.-J., Bhubalan, K., 2021. Marine microplastics as vectors of major ocean pollutants and its hazards to the marine ecosystem and humans. Prog. Earth Planet. Sci. 8, 12. https://doi.org/10.1186/s40645-020-00405-4

Anastasopoulou, A., Fortibuoni, T., 2019. Impact of Plastic Pollution on Marine Life in the Mediterranean Sea 1–62. https://doi.org/10.1007/698_2019_421

Anastasopoulou, A., Mytilineou, C., Smith, C.J., Papadopoulou, K.N., 2013. Plastic debris ingested by deep-water fish of the Ionian Sea (Eastern Mediterranean). Deep Sea Res. Part Oceanogr. Res. Pap. 74, 11–13. https://doi.org/10.1016/j.dsr.2012.12.008

Andaloro, F., 2006. Gruppo di studio ed elementi di base per la gestione dei grandi pelagici. Sottoprogetto – Ruolo trofico del grande pelagico Thunnus alalunga, Bonnaterre, 1788. Rapporto Finale, Ministero per le Politiche Agricole e Forestali, pp. 71.

Andrady, A.L., 2017. The plastic in microplastics: A review. Mar. Pollut. Bull. 119, 12–22. https://doi.org/10.1016/j.marpolbul.2017.01.082

Andrady, A.L., 2015. Persistence of Plastic Litter in the Oceans, in: Bergmann, M., Gutow, L., Klages, M. (Eds.), Marine Anthropogenic Litter. Springer International Publishing, Cham, pp. 57–72. https://doi.org/10.1007/978-3-319-16510-3_3

Andrady, A.L., 2011a. Microplastics in the marine environment. Mar. Pollut. Bull. 62, 1596–1605. https://doi.org/10.1016/j.marpolbul.2011.05.030

Andrady, A.L., 2011b. Microplastics in the marine environment. Mar. Pollut. Bull. 62, 1596–1605. https://doi.org/10.1016/j.marpolbul.2011.05.030

Andrady, A.L., 2003. Plastics and the Environment. John Wiley & Sons.

Avio, C.G., Gorbi, S., Milan, M., Benedetti, M., Fattorini, D., d'Errico, G., Pauletto, M., Bargelloni, L., Regoli, F., 2015a. Pollutants bioavailability and toxicological risk from microplastics to marine mussels. Environ. Pollut. 198, 211–222. https://doi.org/10.1016/j.envpol.2014.12.021

Avio, C.G., Gorbi, S., Regoli, F., 2015b. Experimental development of a new protocol for extraction and characterization of microplastics in fish tissues: First observations in commercial species from Adriatic Sea. Mar. Environ. Res., Particles in the Oceans: Implication for a safe marine environment 111, 18–26. https://doi.org/10.1016/j.marenvres.2015.06.014

Baini, M., Fossi, M.C., Galli, M., Caliani, I., Campani, T., Finoia, M.G., Panti, C., 2018. Abundance and characterization of microplastics in the coastal waters of Tuscany (Italy): The application of the MSFD monitoring protocol in the Mediterranean Sea. Mar. Pollut. Bull. 133, 543–552. https://doi.org/10.1016/j.marpolbul.2018.06.016
Baini, M., Martellini, T., Cincinelli, A., Campani, T., Minutoli, R., Panti, C., Finoia, M.G., Fossi, M.C., 2017. First detection of seven phthalate esters (PAEs) as plastic tracers in superficial neustonic/planktonic samples and cetacean blubber. Anal. Methods 9, 1512–1520. https://doi.org/10.1039/c6ay02674e
Barboza, L.G.A., Cózar, A., Gimenez, B.C.G., Barros, T.L., Kershaw, P.J., Guilhermino, L., 2019. Chapter 17 - Macroplastics Pollution in the Marine Environment, in: Sheppard, C. (Ed.), World Seas: An Environmental Evaluation (Second Edition). Academic Press, pp. 305–328. https://doi.org/10.1016/B978-0-12-805052-1.00019-X
Barnes David K. A., Galgani Francois, Thompson Richard C., Barlaz Morton, 2009. Accumulation and fragmentation of plastic debris in global environments. Philos. Trans. R. Soc. B Biol. Sci. 364, 1985–1998. https://doi.org/10.1098/rstb.2008.0205
Barnes, D.K.A., Galgani, F., Thompson, R.C., Barlaz, M., 2009. Accumulation and fragmentation of plastic debris in global environments. Philos. Trans. R. Soc. B Biol. Sci. 364, 1985–1998. https://doi.org/10.1098/rstb.2008.0205
Battaglia, P., Andaloro, F., Consoli, P., Pedà, C., Raicevich, S., Spagnolo, M., Romeo, T., 2017. Baseline data to characterize and manage the small-scale fishery (SSF) of an oncoming Marine Protected Area (Cape Milazzo, Italy) in the western Mediterranean Sea. Ocean Coast. Manag. 148, 231–244. https://doi.org/10.1016/j.ocecoaman.2017.08.014
Battaglia, P., Malara, D., Romeo, T., Andaloro, F., 2010. Relationships between otolith size and fish size in some mesopelagic and bathypelagic species from the Mediterranean Sea (Strait of Messina, Italy). Sci. Mar. 74, 605–612. https://doi.org/10.3989/scimar.2010.74n3605
Battaglia, P., Pedà, C., Musolino, S., Esposito, V., Andaloro, F., Romeo, T., 2016. Diet and first documented data on plastic ingestion of Trachinotus ovatus L. 1758 (Pisces: Carangidae) from the Strait of Messina (central Mediterranean Sea). Ital. J. Zool. 83, 121–129. https://doi.org/10.1080/11250003.2015.1114157
Bauer, D.F., 1972. Constructing Confidence Sets Using Rank Statistics. J. Am. Stat. Assoc. 67, 687–690. https://doi.org/10.1080/01621459.1972.10481279
Bellas, J., Martínez-Armental, J., Martínez-Cámara, A., Besada, V., Martínez-Gómez, C., 2016. Ingestion of microplastics by demersal fish from the Spanish Atlantic and Mediterranean coasts. Mar. Pollut. Bull. 109, 55–60. https://doi.org/10.1016/j.marpolbul.2016.06.026
Bergmann, M., Mützel, S., Primpke, S., Tekman, M.B., Trachsel, J., Gerdts, G., 2019. White and wonderful? Microplastics prevail in snow from the Alps to the Arctic. Sci. Adv. 5, eaax1157.
Bernal, A., Olivar, M.P., Maynou, F., Fernández de Puelles, M.L., 2015. Diet and feeding strategies of mesopelagic fishes in the western Mediterranean. Prog. Oceanogr. 135, 1–17. https://doi.org/10.1016/j.pocean.2015.03.005
Blasi, M.F., Boitani, L., 2014. Complex Social Structure of an Endangered Population of Bottlenose Dolphins (Tursiops truncatus) in the Aeolian Archipelago (Italy). PLOS ONE 9, e114849. https://doi.org/10.1371/journal.pone.0114849
Blasi, M.F., Boitani, L., 2012. Modelling fine-scale distribution of the bottlenose dolphin Tursiops truncatus using physiographic features on Filicudi (southern Thyrrenian Sea, Italy). Endanger. Species Res. 17, 269–288. https://doi.org/10.3354/esr00422
Blasi, M.F., Bruno, C., Boitani, L., 2020. Female reproductive output in a Mediterranean bottlenose dolphin Tursiops truncatus population. Aquat. Biol. 29, 123–136. https://doi.org/10.3354/ab00732

Blasi, M.F., Hochscheid, S., Bardelli, R., Bruno, C., Melodia, C., Salzeri, P., Rosa, P.D., Madonia, P., 2022. First report on two loggerhead turtle (Caretta caretta) nests in the Aeolian Archipelago (Southern Italy). Acta Herpetol. 17, 37–43. https://doi.org/10.36253/a_h-10188

Blasi, M.F., Roscioni, F., Mattei, D., 2016. Interaction of Loggerhead Turtles (Caretta caretta) with Traditional Fish Aggregating Devices (FADs) in the Mediterranean Sea. Herpetol. Conserv. Biol. 11, 386–401.

Boerger, C.M., Lattin, G.L., Moore, S.L., Moore, C.J., 2010. Plastic ingestion by planktivorous fishes in the North Pacific Central Gyre. Mar. Pollut. Bull. 60, 2275–2278. https://doi.org/10.1016/j.marpolbul.2010.08.007

Bottari, T., Mancuso, M., Pedà, C., De Domenico, F., Laface, F., Schirinzi, G.F., Battaglia, P., Consoli, P., Spanò, N., Greco, S., Romeo, T., 2022. Microplastics in the bogue, Boops boops: A snapshot of the past from the southern Tyrrhenian Sea. J. Hazard. Mater. 424, 127669. https://doi.org/10.1016/j.jhazmat.2021.127669

Bowley, J., Baker-Austin, C., Porter, A., Hartnell, R., Lewis, C., 2021. Oceanic Hitchhikers - Assessing Pathogen Risks from Marine Microplastic. Trends Microbiol. 29, 107–116. https://doi.org/10.1016/j.tim.2020.06.011

Bray, L., Digka, N., Tsangaris, C., Camedda, A., Gambaiani, D., de Lucia, G.A., Matiddi, M., Miaud, C., Palazzo, L., Pérez-del-Olmo, A., Raga, J.A., Silvestri, C., Kaberi, H., 2019. Determining suitable fish to monitor plastic ingestion trends in the Mediterranean Sea. Environ. Pollut. 247, 1071–1077. https://doi.org/10.1016/j.envpol.2019.01.100

Briand, J.-F., Pollet, T., Misson, B., Garnier, C., Lejars, M., Maintenay, M., Barry-Martinet, R., Portas, A., Ghiglione, J.-F., Bressy, C., 2022. Surface Characteristics Together With Environmental Conditions Shape Marine Biofilm Dynamics in Coastal NW Mediterranean Locations. Front. Mar. Sci. 8.

Brown, J., Harper, J., Humphrey, N., 2010. Cirque glacier sensitivity to 21st century warming: Sperry Glacier, Rocky Mountains, USA. Glob. Planet. Change 74, 91–98. https://doi.org/10.1016/j.gloplacha.2010.09.001

Browne, M.A., 2015. Sources and Pathways of Microplastics to Habitats, in: Bergmann, M., Gutow, L., Klages, M. (Eds.), Marine Anthropogenic Litter. Springer International Publishing, Cham, pp. 229–244. https://doi.org/10.1007/978-3-319-16510-3_9

Cadiou, J.-F., Gerigny, O., Koren, Š., Zeri, C., Kaberi, H., Alomar, C., Panti, C., Fossi, M.C., Adamopoulou, A., Digka, N., Deudero, S., Concato, M., Carbonell, A., Baini, M., Galli, M., Galgani, F., 2020. Lessons learned from an intercalibration exercise on the quantification and characterisation of microplastic particles in sediment and water samples. Mar. Pollut. Bull. 154, 111097. https://doi.org/10.1016/j.marpolbul.2020.111097

Cai, M., He, H., Liu, M., Li, S., Tang, G., Wang, W., Huang, P., Wei, G., Lin, Y., Chen, B., Hu, J., Cen, Z., 2018. Lost but can't be neglected: Huge quantities of small microplastics hide in the South China Sea. Sci. Total Environ. 633, 1206–1216. https://doi.org/10.1016/j.scitotenv.2018.03.197

Caldwell, J., Petri-Fink, A., Rothen-Rutishauser, B., Lehner, R., 2019. Assessing meso- and microplastic pollution in the Ligurian and Tyrrhenian Seas. Mar. Pollut. Bull. 149, 110572. https://doi.org/10.1016/j.marpolbul.2019.110572

Campani, T., Baini, M., Giannetti, M., Cancelli, F., Mancusi, C., Serena, F., Marsili, L., Casini, S., Fossi, M.C., 2013. Presence of plastic debris in loggerhead turtle stranded along the Tuscany coasts of the Pelagos Sanctuary for Mediterranean Marine Mammals (Italy). Mar. Pollut. Bull. 74, 225–230. https://doi.org/10.1016/j.marpolbul.2013.06.053

Capillo, G., Savoca, S., Panarello, G., Mancuso, M., Branca, C., Romano, V., D'Angelo, G., Bottari, T., Spanò, N., 2020. Quali-quantitative analysis of plastics and synthetic microfibers found in demersal species from Southern Tyrrhenian Sea (Central Mediterranean). Mar. Pollut. Bull. 150, 110596. https://doi.org/10.1016/j.marpolbul.2019.110596

Capó, X., Morató, M., Alomar, C., Rios-Fuster, B., Valls, M., Compa, M., Deudero, S., 2022. A Biomarker Approach as Responses of Bioindicator Commercial Fish Species to Microplastic Ingestion: Assessing Tissue and Biochemical Relationships. Biology 11, 1634. https://doi.org/10.3390/biology11111634

Cesarano, C., Aulicino, G., Cerrano, C., Ponti, M., Puce, S., 2023. Marine beach litter monitoring strategies along Mediterranean coasts. A methodological review. Mar. Pollut. Bull. 186, 114401. https://doi.org/10.1016/j.marpolbul.2022.114401

Chagnon, C., Thiel, M., Antunes, J., Ferreira, J.L., Sobral, P., Ory, N.C., 2018. Plastic ingestion and trophic transfer between Easter Island flying fish (Cheilopogon rapanouiensis) and yellowfin tuna (Thunnus albacares) from Rapa Nui (Easter Island). Environ. Pollut. 243, 127–133. https://doi.org/10.1016/j.envpol.2018.08.042

Chen, C.-L., 2015. Regulation and management of marine litter. Mar. Anthropog. Litter 395–428.

Chen, J.-C., Fang, C., Zheng, R.-H., Hong, F.-K., Jiang, Y.-L., Zhang, M., Li, Y., Hamid, F.S., Bo, J., Lin, L.-S., 2021. Microplastic pollution in wild commercial nekton from the South China Sea and Indian Ocean, and its implication to human health. Mar. Environ. Res. 167, 105295. https://doi.org/10.1016/j.marenvres.2021.105295

Cheng, Y.-L., Zhang, R., Tisinger, L., Cali, S., Yu, Z., Chen, H.Y., Li, A., 2022. Characterization of microplastics in sediment using stereomicroscopy and laser direct infrared (LDIR) spectroscopy. Gondwana Res., Plastisphere and its impact on Earth's environment and life 108, 22–30. https://doi.org/10.1016/j.gr.2021.10.002

Claessens, M., De Meester, S., Van Landuyt, L., De Clerck, K., Janssen, C.R., 2011. Occurrence and distribution of microplastics in marine sediments along the Belgian coast. Mar. Pollut. Bull. 62, 2199–2204. https://doi.org/10.1016/j.marpolbul.2011.06.030

Cocci, P., Gabrielli, S., Pastore, G., Minicucci, M., Mosconi, G., Palermo, F.A., 2022. Microplastics accumulation in gastrointestinal tracts of Mullus barbatus and Merluccius merluccius is associated with increased cytokine production and signaling. Chemosphere 307, 135813. https://doi.org/10.1016/j.chemosphere.2022.135813

Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants in the marine environment: a review. Mar. Pollut. Bull. 62, 2588–2597. https://doi.org/10.1016/j.marpolbul.2011.09.025

Coll, M., Piroddi, C., Steenbeek, J., Kaschner, K., Lasram, F.B.R., Aguzzi, J., Ballesteros, E., Bianchi, C.N., Corbera, J., Dailianis, T., Danovaro, R., Estrada, M., Froglia, C., Galil, B.S., Gasol, J.M., Gertwagen, R., Gil, J., Guilhaumon, F., Kesner-Reyes, K., Kitsos, M.-S., Koukouras, A., Lampadariou, N., Laxamana, E., Cuadra, C.M.L.-F. de la, Lotze, H.K., Martin, D., Mouillot, D., Oro, D., Raicevich, S., Rius-Barile, J., Saiz-Salinas, J.I., Vicente, C.S., Somot, S., Templado, J., Turon, X., Vafidis, D., Villanueva, R., Voultsiadou, E., 2010. The Biodiversity of the Mediterranean Sea: Estimates, Patterns, and Threats. PLOS ONE 5, e11842. https://doi.org/10.1371/journal.pone.0011842

Compa, M., Alomar, C., Mourre, B., March, D., Tintoré, J., Deudero, S., 2020. Nearshore spatio-temporal sea surface trawls of plastic debris in the Balearic Islands. Mar. Environ. Res. 158, 104945. https://doi.org/10.1016/j.marenvres.2020.104945

Compa, M., Alomar, C., Wilcox, C., van Sebille, E., Lebreton, L., Hardesty, B.D., Deudero, S., 2019. Risk assessment of plastic pollution on marine diversity in the Mediterranean Sea. Sci. Total Environ. 678, 188–196. https://doi.org/10.1016/j.scitotenv.2019.04.355

Compa, M., Ventero, A., Iglesias, M., Deudero, S., 2018. Ingestion of microplastics and natural fibres in Sardina pilchardus (Walbaum, 1792) and Engraulis encrasicolus (Linnaeus, 1758) along the Spanish Mediterranean coast. Mar. Pollut. Bull. 128, 89–96. https://doi.org/10.1016/j.marpolbul.2018.01.009

Consoli, P., Esposito, V., Battaglia, P., Perzia, P., Scotti, G., D'Alessandro, M., Canese, S., Andaloro, F., Romeo, T., 2021. Marine litter pollution associated with hydrothermal sites in

the Aeolian archipelago (western Mediterranean Sea). Sci. Total Environ. 773, 144968. https://doi.org/10.1016/j.scitotenv.2021.144968
Consoli, P., Romeo, T., Giongrandi, U., Andaloro, F., 2008. Differences among fish assemblages associated with a nearshore vermetid reef and two other rocky habitats along the shores of Cape Milazzo (northern Sicily, central Mediterranean Sea). J. Mar. Biol. Assoc. U. K. 88, 401–410. https://doi.org/10.1017/S0025315408000489
Consoli, P., Scotti, G., Romeo, T., Fossi, M.C., Esposito, V., D'Alessandro, M., Battaglia, P., Galgani, F., Figurella, F., Pragnell-Raasch, H., Andaloro, F., 2020. Characterization of seafloor litter on Mediterranean shallow coastal waters: Evidence from Dive Against Debris®, a citizen science monitoring approach. Mar. Pollut. Bull. 150, 110763. https://doi.org/10.1016/j.marpolbul.2019.110763
Courtene-Jones, W., Quinn, B., Ewins, C., Gary, S.F., Narayanaswamy, B.E., 2019. Consistent microplastic ingestion by deep-sea invertebrates over the last four decades (1976–2015), a study from the North East Atlantic. Environ. Pollut. 244, 503–512. https://doi.org/10.1016/j.envpol.2018.10.090
Cowger, W., Booth, A.M., Hamilton, B.M., Thaysen, C., Primpke, S., Munno, K., Lusher, A.L., Dehaut, A., Vaz, V.P., Liboiron, M., Devriese, L.I., Hermabessiere, L., Rochman, C., Athey, S.N., Lynch, J.M., Frond, H.D., Gray, A., Jones, O.A.H., Brander, S., Steele, C., Moore, S., Sanchez, A., Nel, H., 2020. Reporting Guidelines to Increase the Reproducibility and Comparability of Research on Microplastics. Appl. Spectrosc. 74, 1066–1077. https://doi.org/10.1364/AS.74.001066
Cózar, A., Echevarría, F., González-Gordillo, J.I., Irigoien, X., Úbeda, B., Hernández-León, S., Palma, Á.T., Navarro, S., García-de-Lomas, J., Ruiz, A., Fernández-de-Puelles, M.L., Duarte, C.M., 2014. Plastic debris in the open ocean. Proc. Natl. Acad. Sci. 111, 10239–10244. https://doi.org/10.1073/pnas.1314705111
Cózar, A., Sanz-Martín, M., Martí, E., González-Gordillo, J.I., Ubeda, B., Gálvez, J.Á., Irigoien, X., Duarte, C.M., 2015. Plastic Accumulation in the Mediterranean Sea. PLOS ONE 10, e0121762. https://doi.org/10.1371/journal.pone.0121762
D'Agostino, F., Bellante, A., Quinci, E., Gherardi, S., Placenti, F., Sabatino, N., Buffa, G., Avellone, G., Di Stefano, V., Del Core, M., 2020. Persistent and Emerging Organic Pollutants in the Marine Coastal Environment of the Gulf of Milazzo (Southern Italy): Human Health Risk Assessment. Front. Environ. Sci. 8.
D'Alessandro, M., Esposito, V., Giacobbe, S., Renzi, M., Mangano, M.C., Vivona, P., Consoli, P., Scotti, G., Andaloro, F., Romeo, T., 2016. Ecological assessment of a heavily human-stressed area in the Gulf of Milazzo, Central Mediterranean Sea: an integrated study of biological, physical and chemical indicators. Mar. Pollut. Bull. 106, 260–273. https://doi.org/10.1016/j.marpolbul.2016.01.021
Dang, H., Li, T., Chen, M., Huang, G., 2008. Cross-Ocean Distribution of Rhodobacterales Bacteria as Primary Surface Colonizers in Temperate Coastal Marine Waters. Appl. Environ. Microbiol. 74, 52–60. https://doi.org/10.1128/AEM.01400-07
Dang, T.C.H., Nguyen, D.T., Thai, H., Nguyen, T.C., Tran, T.T.H., Le, V.H., Nguyen, V.H., Tran, X.B., Pham, T.P.T., Nguyen, T.G., Nguyen, Q.T., 2018. Plastic degradation by thermophilic Bacillus sp. BCBT21 isolated from composting agricultural residual in Vietnam. Adv. Nat. Sci. Nanosci. Nanotechnol. 9, 015014. https://doi.org/10.1088/2043-6254/aaabaf
Darbre, P.D., 2021. Endocrine Disruption and Human Health. Academic Press.
Darbre, P.D., 2020. Chemical components of plastics as endocrine disruptors: Overview and commentary. Birth Defects Res. 112, 1300–1307. https://doi.org/10.1002/bdr2.1778
De Frond, H., Cowger, W., Renick, V., Brander, S., Primpke, S., Sukumaran, S., Elkhatib, D., Barnett, S., Navas-Moreno, M., Rickabaugh, K., Vollnhals, F., O'Donnell, B., Lusher, A., Lee, E., Lao, W., Amarpuri, G., Sarau, G., Christiansen, S., 2023. What determines accuracy

of chemical identification when using microspectroscopy for the analysis of microplastics? Chemosphere 313, 137300. https://doi.org/10.1016/j.chemosphere.2022.137300

De Lucia, G.A., Vianello, A., Camedda, A., Vani, D., Tomassetti, P., Coppa, S., Palazzo, L., Amici, M., Romanelli, G., Zampetti, G., Cicero, A.M., Carpentieri, S., Di Vito, S., Matiddi, M., 2018. Sea Water Contamination in the Vicinity of the Italian Minor Islands Caused by Microplastic Pollution. Water 10, 1108. https://doi.org/10.3390/w10081108

De Metrio, Giacchetta F., Santamaria N., 1995. Sex-ratio ed indice gonado-somatico del pesce spada (Xiphias gladius L.) dello Ionio settentrionale e dell'Adriatico meridionale.

de Oliveira, T.T.S., Andreu, I., Machado, M.C., Vimbela, G., Tripathi, A., Bose, A., 2020. Interaction of Cyanobacteria with Nanometer and Micron Sized Polystyrene Particles in Marine and Fresh Water. Langmuir 36, 3963–3969. https://doi.org/10.1021/acs.langmuir.9b03644

De Tender, C.A., Devriese, L.I., Haegeman, A., Maes, S., Ruttink, T., Dawyndt, P., 2015. Bacterial Community Profiling of Plastic Litter in the Belgian Part of the North Sea. Environ. Sci. Technol. 49, 9629–9638. https://doi.org/10.1021/acs.est.5b01093

Debroas, D., Mone, A., Ter Halle, A., 2017. Plastics in the North Atlantic garbage patch: A boat-microbe for hitchhikers and plastic degraders. Sci. Total Environ. 599–600, 1222–1232. https://doi.org/10.1016/j.scitotenv.2017.05.059

Deepika, S., Jaya, M.R., 2015. Biodegradation of low density polyethylene by microorganisms from garbage soil." J Exp Biol Agric Sci 3.

Derraik, J.G.B., 2002. The pollution of the marine environment by plastic debris: a review. Mar. Pollut. Bull. 44, 842–852. https://doi.org/10.1016/s0025-326x(02)00220-5

Desai, V.R., 1970. Studies on the fishery and biology of Tor tor (Hamilton) from river Narmada.

Deudero, S., Alomar, C., 2015. Mediterranean marine biodiversity under threat: Reviewing influence of marine litter on species. Mar. Pollut. Bull. 98, 58–68. https://doi.org/10.1016/j.marpolbul.2015.07.012

Digka, N., Tsangaris, C., Torre, M., Anastasopoulou, A., Zeri, C., 2018a. Microplastics in mussels and fish from the Northern Ionian Sea. Mar. Pollut. Bull. 135, 30–40. https://doi.org/10.1016/j.marpolbul.2018.06.063

Digka, N., Tsangaris, C., Torre, M., Anastasopoulou, A., Zeri, C., 2018b. Microplastics in mussels and fish from the Northern Ionian Sea. Mar. Pollut. Bull. 135, 30–40. https://doi.org/10.1016/j.marpolbul.2018.06.063

D'Iglio, C., Di Fresco, D., Spanò, N., Albano, M., Panarello, G., Laface, F., Faggio, C., Capillo, G., Savoca, S., 2022. Occurrence of Anthropogenic Debris in Three Commercial Shrimp Species from South-Western Ionian Sea. Biology 11, 1616. https://doi.org/10.3390/biology11111616

Dool, T., Bosker, T., 2022. Predicted microplastic uptake through trophic transfer by the short-beaked common dolphin (Delphinus delphis) and common bottlenose dolphin (Tursiops truncatus) in the Northeast Atlantic Ocean and Mediterranean Sea. Mar. Pollut. Bull. 180, 113745. https://doi.org/10.1016/j.marpolbul.2022.113745

Dussud, C., Meistertzheim, A.L., Conan, P., Pujo-Pay, M., George, M., Fabre, P., Coudane, J., Higgs, P., Elineau, A., Pedrotti, M.L., Gorsky, G., Ghiglione, J.F., 2018. Evidence of niche partitioning among bacteria living on plastics, organic particles and surrounding seawaters. Environ. Pollut. 236, 807–816. https://doi.org/10.1016/j.envpol.2017.12.027

Enders, K., Lenz, R., Stedmon, C.A., Nielsen, T.G., 2015. Abundance, size and polymer composition of marine microplastics ≥10μm in the Atlantic Ocean and their modelled vertical distribution. Mar. Pollut. Bull. 100, 70–81. https://doi.org/10.1016/j.marpolbul.2015.09.027

Eriksen, M., Lebreton, L.C.M., Carson, H.S., Thiel, M., Moore, C.J., Borerro, J.C., Galgani, F., Ryan, P.G., Reisser, J., 2014. Plastic Pollution in the World's Oceans: More than 5 Trillion

Plastic Pieces Weighing over 250,000 Tons Afloat at Sea. PloS One 9, e111913. https://doi.org/10.1371/journal.pone.0111913
Eriksson, C., Burton, H., 2003. Origins and Biological Accumulation of Small Plastic Particles in Fur Seals from Macquarie Island. AMBIO J. Hum. Environ. 32, 380–384. https://doi.org/10.1579/0044-7447-32.6.380
Esposito, V., Giacobbe, S., Cosentino, A., Minerva, C.S., Romeo, T., Canese, S., Andaloro, F., 2015. Distribution and ecology of the tube-dweller Ampelisca ledoyeri (Amphipoda: Ampeliscidae) associated with the hydrothermal field off Panarea Island (Tyrrhenian Sea, Mediterranean). Mar. Biodivers. 45, 763–768. https://doi.org/10.1007/s12526-014-0285-5
Fagiano, V., Alomar, C., Compa, M., Soto-Navarro, J., Jordá, G., Deudero, S., 2022a. Neustonic microplastics and zooplankton in coastal waters of Cabrera Marine Protected Area (Western Mediterranean Sea). Sci. Total Environ. 804, 150120. https://doi.org/10.1016/j.scitotenv.2021.150120
Fagiano, V., Compa, M., Alomar, C., García-Marcos, K., Deudero, S., 2022b. Marine plastics in Mediterranean islands: Evaluating the distribution and composition of plastic pollution in the surface waters along four islands of the Western Sea Basin. Environ. Pollut. Barking Essex 1987 305, 119268. https://doi.org/10.1016/j.envpol.2022.119268
Fanelli, E., Cartes, J.E., Rumolo, P., Sprovieri, M., 2009. Food-web structure and trophodynamics of mesopelagic–suprabenthic bathyal macrofauna of the Algerian Basin based on stable isotopes of carbon and nitrogen. Deep Sea Res. Part Oceanogr. Res. Pap. 56, 1504–1520. https://doi.org/10.1016/j.dsr.2009.04.004
Farrell, P., Nelson, K., 2013. Trophic level transfer of microplastic: Mytilus edulis (L.) to Carcinus maenas (L.). Environ. Pollut. 177, 1–3. https://doi.org/10.1016/j.envpol.2013.01.046
Fastelli, P., Blašković, A., Bernardi, G., Romeo, T., Čižmek, H., Andaloro, F., Russo, G.F., Guerranti, C., Renzi, M., 2016. Plastic litter in sediments from a marine area likely to become protected (Aeolian Archipelago's islands, Tyrrhenian sea). Mar. Pollut. Bull. 113, 526–529. https://doi.org/10.1016/j.marpolbul.2016.08.054
Felline, S., Piccardo, M., De Benedetto, G.E., Malitesta, C., Terlizzi, A., 2022. Microplastics' Occurrence in Edible Fish Species (Mullus barbatus and M. surmuletus) from an Italian Marine Protected Area. Microplastics 1, 291–302. https://doi.org/10.3390/microplastics1020021
Fenner, D., Riolo, F., Vittorio, M., 2013. New records of scleractinian corals from shallow waters of the Ionian coast of Italy. Mar. Biodivers. Rec. 6, e136. https://doi.org/10.1017/S1755267213001073
Fortuna, C.M., Canese, S., Giusti, M., Revelli, E., Consoli, P., Florio, G., Greco, S., Romeo, T., Andaloro, F., Fossi, M.C., Lauriano, G., 2007. An insight into the status of the striped dolphins, Stenella coeruleoalba, of the southern Tyrrhenian Sea. J. Mar. Biol. Assoc. U. K. 87, 1321–1326. https://doi.org/10.1017/S002531540705669X
Fossi, M.C., Marsili, L., Baini, M., Giannetti, M., Coppola, D., Guerranti, C., Caliani, I., Minutoli, R., Lauriano, G., Finoia, M.G., Rubegni, F., Panigada, S., Bérubé, M., Urbán Ramírez, J., Panti, C., 2016. Fin whales and microplastics: The Mediterranean Sea and the Sea of Cortez scenarios. Environ. Pollut. 209, 68–78. https://doi.org/10.1016/j.envpol.2015.11.022
Fossi, M.C., Panti, C., Guerranti, C., Coppola, D., Giannetti, M., Marsili, L., Minutoli, R., 2012. Are baleen whales exposed to the threat of microplastics? A case study of the Mediterranean fin whale (Balaenoptera physalus). Mar. Pollut. Bull. 64, 2374–2379. https://doi.org/10.1016/j.marpolbul.2012.08.013
Fossi, M.C., Pedà, C., Compa, M., Tsangaris, C., Alomar, C., Claro, F., Ioakeimidis, C., Galgani, F., Hema, T., Deudero, S., Romeo, T., Battaglia, P., Andaloro, F., Caliani, I., Casini, S., Panti, C., Baini, M., 2018. Bioindicators for monitoring marine litter ingestion and its

impacts on Mediterranean biodiversity. Environ. Pollut. 237, 1023–1040. https://doi.org/10.1016/j.envpol.2017.11.019
Fossi, M.C., Romeo, T., Baini, M., Panti, C., Marsili, L., Campani, T., Canese, S., Galgani, F., Druon, J.-N., Airoldi, S., Taddei, S., Fattorini, M., Brandini, C., Lapucci, C., 2017. Plastic Debris Occurrence, Convergence Areas and Fin Whales Feeding Ground in the Mediterranean Marine Protected Area Pelagos Sanctuary: A Modeling Approach. Front. Mar. Sci. 4, UNSP 167. https://doi.org/10.3389/fmars.2017.00167
Fossi, M.C., Vlachogianni T., Anastasopoulou A., Alomar C., Baini M., Caliani I., Campani T., Casini S., Consoli P., Cillari T., D'Alessandro M., Deudero S., Galgani Francois, Galli M. F., Kaberi H., Panti C, Pedà C. E., Romeo T., Scotti G., Tsangaris, C., Zeri, C., 2019. Toolkit for monitoring marine litter and its impacts on biodiversity in Mediterranean MPAs. InterregMed Plastic Busters MPAs project (D.3.3.2).
Free, C.M., Jensen, O.P., Mason, S.A., Eriksen, M., Williamson, N.J., Boldgiv, B., 2014. High-levels of microplastic pollution in a large, remote, mountain lake. Mar. Pollut. Bull. 85, 156–163. https://doi.org/10.1016/j.marpolbul.2014.06.001
Frère, L., Maignien, L., Chalopin, M., Huvet, A., Rinnert, E., Morrison, H., Kerninon, S., Cassone, A.-L., Lambert, C., Reveillaud, J., Paul-Pont, I., 2018. Microplastic bacterial communities in the Bay of Brest: Influence of polymer type and size. Environ. Pollut. 242, 614–625. https://doi.org/10.1016/j.envpol.2018.07.023
Frias, J.P.G.L., Otero, V., Sobral, P., 2014. Evidence of microplastics in samples of zooplankton from Portuguese coastal waters. Mar. Environ. Res. 95, 89–95. https://doi.org/10.1016/j.marenvres.2014.01.001
Galgani, F., Hanke, G., Maes, T., 2015. Global distribution, composition and abundance of marine litter. Mar. Anthropog. Litter 29–56.
Galgani, F., Hanke, G., Werner, S., De Vrees, L., 2013. Marine litter within the European Marine Strategy Framework Directive. ICES J. Mar. Sci. 70, 1055–1064. https://doi.org/10.1093/icesjms/fst122
Galgani, F., Jaunet, S., Campillo, A., Guenegen, X., His, E., 1995. Distribution and abundance of debris on the continental shelf of the north-western Mediterranean Sea. Mar. Pollut. Bull. 30, 713–717. https://doi.org/10.1016/0025-326X(95)00055-R
Galgani, F., Leaute, J.P., Moguedet, P., Souplet, A., Verin, Y., Carpentier, A., Goraguer, H., Latrouite, D., Andral, B., Cadiou, Y., Mahe, J.C., Poulard, J.C., Nerisson, P., 2000. Litter on the Sea Floor Along European Coasts. Mar. Pollut. Bull. 40, 516–527. https://doi.org/10.1016/S0025-326X(99)00234-9
Galgani, F., Michela, A., Gérigny, O., Maes, T., Tambutté, E., Harris, P.T., 2022. Marine Litter, Plastic, and Microplastics on the Seafloor, in: Plastics and the Ocean. John Wiley & Sons, Ltd, pp. 151–197. https://doi.org/10.1002/9781119768432.ch6
Galgani, Fleet D, Van Franeker JA, Katsanevakis S, Maes T, Mouat J, Oosterbaan L, Poitou I, Hanke G, Thompson R, Amato E., 2010. Marine strategy framework directive-task group 10 report marine litter. Office for Official Publications of the European Communities.
Galgani, L., Beiras, R., Galgani, F., Panti, C., Borja, A., 2019. Editorial: Impacts of Marine Litter. Front. Mar. Sci. 6.
Galli, M., Baini, M., Panti, C., Giani, D., Caliani, I., Campani, T., Rosso, M., Tepsich, P., Levati, V., Laface, F., Romeo, T., Scotti, G., Galgani, F., Fossi, M.C., 2023. Oceanographic and anthropogenic variables driving marine litter distribution in Mediterranean protected areas: Extensive field data supported by forecasting modelling. Sci. Total Environ. 166266. https://doi.org/10.1016/j.scitotenv.2023.166266
Galli, M., Tepsich, P., Baini, M., Panti, C., Rosso, M., Vafeiadou, A., Pantelidou, M., Moulins, A., Fossi, M.C., 2022. Microplastic abundance and biodiversity richness overlap: Identification

of sensitive areas in the Western Ionian Sea. Mar. Pollut. Bull. 177, 113550. https://doi.org/10.1016/j.marpolbul.2022.113550
Gao, S., Yan, K., Liang, B., Shu, R., Wang, N., Zhang, S., 2023. The different ways microplastics from the water column and sediment accumulate in fish in Haizhou Bay. Sci. Total Environ. 854, 158575. https://doi.org/10.1016/j.scitotenv.2022.158575
Garcia-Garin, O., Vighi, M., Aguilar, A., Tsangaris, C., Digka, N., Kaberi, H., Borrell, A., 2019. Boops boops as a bioindicator of microplastic pollution along the Spanish Catalan coast. Mar. Pollut. Bull. 149, 110648. https://doi.org/10.1016/j.marpolbul.2019.110648
GESAMP, 2019. Guidelines for the Monitoring and Assessment of Plastic Litter in the Ocean [WWW Document]. GESAMP. URL http://www.gesamp.org/publications/guidelines-for-the-monitoring-and-assessment-of-plastic-litter-in-the-ocean (accessed 9.11.23).
Geyer, R., Jambeck, J.R., Law, K.L., 2017. Production, use, and fate of all plastics ever made. Sci. Adv. 3, e1700782. https://doi.org/10.1126/sciadv.1700782
Giani, D., Andolina, C., Baini, M., Panti, C., Sciandra, M., Vizzini, S., Fossi, M.C., 2023. Trophic niche influences ingestion of micro- and mesoplastics in pelagic and demersal fish from the Western Mediterranean Sea. Environ. Pollut. 328, 121632. https://doi.org/10.1016/j.envpol.2023.121632
Giani, D., Baini, M., Galli, M., Casini, S., Fossi, M.C., 2019. Microplastics occurrence in edible fish species (Mullus barbatus and Merluccius merluccius) collected in three different geographical sub-areas of the Mediterranean Sea. Mar. Pollut. Bull. 140, 129–137. https://doi.org/10.1016/j.marpolbul.2019.01.005
Gregory, M.R., 1996. Plastic ‘scrubbers’ in hand cleansers: a further (and minor) source for marine pollution identified. Mar. Pollut. Bull. 32, 867–871. https://doi.org/10.1016/S0025-326X(96)00047-1
Guillette, L.J., Gross, T.S., Masson, G.R., Matter, J.M., Percival, H.F., Woodward, A.R., 1994. Developmental abnormalities of the gonad and abnormal sex hormone concentrations in juvenile alligators from contaminated and control lakes in Florida. Environ. Health Perspect. 102, 680–688. https://doi.org/10.1289/ehp.94102680
Güven, O., Gökdağ, K., Jovanović, B., Kıdeyş, A.E., 2017. Microplastic litter composition of the Turkish territorial waters of the Mediterranean Sea, and its occurrence in the gastrointestinal tract of fish. Environ. Pollut. 223, 286–294. https://doi.org/10.1016/j.envpol.2017.01.025
Hanke, G., Galgani, F., Werner, S., Oosterbaan, L., Nilsson, P., Fleet, D., Kinsey, S., Thompson, R., Palatinus, A., Van Franeker, J., Vlachogianni, T., Scoullos, M., Veiga, J., Matiddi, M., Alcaro, L., Maes, T., Korpinen, S., Budziak, A., Leslie, H., Gago, J., Liebezeit, G., 2013. Guidance on Monitoring of Marine Litter in European Seas: a guidance document within the Common Implementation Strategy for the Marine Strategy Framework Directive. (Report). Publications Office of the European Union. https://doi.org/10.2788/99475
Harris, P.T., Maes, T., Raubenheimer, K., Walsh, J.P., 2023. A marine plastic cloud - Global mass balance assessment of oceanic plastic pollution. Cont. Shelf Res. 255, 104947. https://doi.org/10.1016/j.csr.2023.104947
Harshvardhan, K., Jha, B., 2013. Biodegradation of low-density polyethylene by marine bacteria from pelagic waters, Arabian Sea, India. Mar. Pollut. Bull. 77, 100–106. https://doi.org/10.1016/j.marpolbul.2013.10.025
Hildebrandt, L., El Gareb, F., Zimmermann, T., Klein, O., Kerstan, A., Emeis, K.-C., Pröfrock, D., 2022. Spatial distribution of microplastics in the tropical Indian Ocean based on laser direct infrared imaging and microwave-assisted matrix digestion. Environ. Pollut. 307, 119547. https://doi.org/10.1016/j.envpol.2022.119547
Hirai, H., Takada, H., Ogata, Y., Yamashita, R., Mizukawa, K., Saha, M., Kwan, C., Moore, C., Gray, H., Laursen, D., Zettler, E.R., Farrington, J.W., Reddy, C.M., Peacock, E.E., Ward, M.W., 2011. Organic micropollutants in marine plastics debris from the open ocean and

remote and urban beaches. Mar. Pollut. Bull. 62, 1683–1692. https://doi.org/10.1016/j.marpolbul.2011.06.004
Hollander, M., Wolfe, D.A., Chicken, E., 2013. Nonparametric Statistical Methods. John Wiley & Sons.
Ibrahim, E., Ahmed, S., Abir, S.S.H., Taylor, K., Padilla-Gainza, V.M., Lozano, K., 2022. Centrifugally spun alginate-poly(lactic acid) microbeads: A promising carrier for drug delivery and tissue engineering. Int. J. Biol. Macromol. 220, 671–682. https://doi.org/10.1016/j.ijbiomac.2022.08.097
Ivar do Sul, J.A., Costa, M.F., 2014. The present and future of microplastic pollution in the marine environment. Environ. Pollut. 185, 352–364. https://doi.org/10.1016/j.envpol.2013.10.036
Jovanović, B., 2017. Ingestion of microplastics by fish and its potential consequences from a physical perspective. Integr. Environ. Assess. Manag. 13, 510–515. https://doi.org/10.1002/ieam.1913
Justino, A.K.S., Ferreira, G.V.B., Fauvelle, V., Schmidt, N., Lenoble, V., Pelage, L., Martins, K., Travassos, P., Lucena-Frédou, F., 2023. From prey to predators: Evidence of microplastic trophic transfer in tuna and large pelagic species in the southwestern Tropical Atlantic. Environ. Pollut. 327, 121532. https://doi.org/10.1016/j.envpol.2023.121532
Kalimeris, A., Kassis, D., 2020. Sea surface circulation variability in the Ionian-Adriatic Seas. Prog. Oceanogr. 189, 102454. https://doi.org/10.1016/j.pocean.2020.102454
Kannan, K., Vimalkumar, K., 2021. A Review of Human Exposure to Microplastics and Insights Into Microplastics as Obesogens. Front. Endocrinol. 12.
Karami, A., Golieskardi, A., Choo, C.K., Romano, N., Ho, Y.B., Salamatinia, B., 2017. A high-performance protocol for extraction of microplastics in fish. Sci. Total Environ. 578, 485–494. https://doi.org/10.1016/j.scitotenv.2016.10.213
Kershaw, P.J., Rochman, C.M., 2015. Sources, fate and effects of microplastics in the marine environment: part 2 of a global assessment. Rep. Stud.-IMOFAOUnesco-IOCWMOIAEAUNUNEP Jt. Group Experts Sci. Asp. Mar. Environ. Prot. GESAMP Eng No 93.
Kesy, K., Labrenz, M., Scales, B.S., Kreikemeyer, B., Oberbeckmann, S., 2021. Vibrio Colonization Is Highly Dynamic in Early Microplastic-Associated Biofilms as Well as on Field-Collected Microplastics. Microorganisms 9, 76. https://doi.org/10.3390/microorganisms9010076
Kirstein, I.V., Kirmizi, S., Wichels, A., Garin-Fernandez, A., Erler, R., Löder, M., Gerdts, G., 2016. Dangerous hitchhikers? Evidence for potentially pathogenic Vibrio spp. on microplastic particles. Mar. Environ. Res. 120, 1–8. https://doi.org/10.1016/j.marenvres.2016.07.004
Kirstein, I.V., Wichels, A., Krohne, G., Gerdts, G., 2018. Mature biofilm communities on synthetic polymers in seawater - Specific or general? Mar. Environ. Res. 142, 147–154. https://doi.org/10.1016/j.marenvres.2018.09.028
Koelmans, A.A., Bakir, A., Burton, G.A., Janssen, C.R., 2016. Microplastic as a Vector for Chemicals in the Aquatic Environment: Critical Review and Model-Supported Reinterpretation of Empirical Studies. Environ. Sci. Technol. 50, 3315–3326. https://doi.org/10.1021/acs.est.5b06069
Kooi, M., Reisser, J., Slat, B., Ferrari, F.F., Schmid, M.S., Cunsolo, S., Brambini, R., Noble, K., Sirks, L.-A., Linders, T.E.W., Schoeneich-Argent, R.I., Koelmans, A.A., 2016. The effect of particle properties on the depth profile of buoyant plastics in the ocean. Sci. Rep. 6, 33882. https://doi.org/10.1038/srep33882
Kühn, 2015. Deleterious effects of litter on marine life. Deleterious Eff. Litter Mar. Life 75–116.
Labropoulou, M., Eleftheriou, A., 2005. The foraging ecology of two pairs of congeneric demersal fish species: importance of morphological characteristics in prey selection. J. Fish Biol. 50, 324–340. https://doi.org/10.1111/j.1095-8649.1997.tb01361.x

Laface, F., Pedà, C., Giommi, C., Scozzafava, S., Rizzo, C., Malara, D., Greco, S., Romeo, T., 2023. A glimpse into the future: A suitable methodological approach for the detection and identification of micro-bioplastics in biota. Sci. Total Environ. 899, 165613. https://doi.org/10.1016/j.scitotenv.2023.165613
Laverty, A.L., Primpke, S., Lorenz, C., Gerdts, G., Dobbs, F.C., 2020. Bacterial biofilms colonizing plastics in estuarine waters, with an emphasis on Vibrio spp. and their antibacterial resistance. PLOS ONE 15, e0237704. https://doi.org/10.1371/journal.pone.0237704
Lazar, B., Gračan, R., 2011. Ingestion of marine debris by loggerhead sea turtles, Caretta caretta, in the Adriatic Sea. Mar. Pollut. Bull. 62, 43–47. https://doi.org/10.1016/j.marpolbul.2010.09.013
Lea, W.R., 1996. Plastic incineration versus recycling: a comparison of energy and landfill cost savings. J. Hazard. Mater., Municipal Waste Incineration 47, 295–302. https://doi.org/10.1016/0304-3894(95)00117-4
Lebreton, L.C.-M., Greer, S.D., Borrero, J.C., 2012. Numerical modelling of floating debris in the world's oceans. Mar. Pollut. Bull. 64, 653–661. https://doi.org/10.1016/j.marpolbul.2011.10.027
Lee, E.-H., Cho, K.-S., 2008. Characterization of cyclohexane and hexane degradation by Rhodococcus sp. EC1. Chemosphere 71, 1738–1744. https://doi.org/10.1016/j.chemosphere.2007.12.009
Lee, K.-W., Shim, W.J., Kwon, O.Y., Kang, J.-H., 2013. Size-Dependent Effects of Micro Polystyrene Particles in the Marine Copepod Tigriopus japonicus. Environ. Sci. Technol. 47, 11278–11283. https://doi.org/10.1021/es401932b
Lefebvre, C., Saraux, C., Heitz, O., Nowaczyk, A., Bonnet, D., 2019. Microplastics FTIR characterisation and distribution in the water column and digestive tracts of small pelagic fish in the Gulf of Lions. Mar. Pollut. Bull. 142, 510–519. https://doi.org/10.1016/j.marpolbul.2019.03.025
Lena, G., 1989. Vibo Valentia. Geografia E Morfologia Della Fascia Costiera E L'impianto Del Porto Antico. Ann. Della Scuola Norm. Super. Pisa Cl. Lett. E Filos. 19, 583–607.
Li, W.C., Tse, H.F., Fok, L., 2016. Plastic waste in the marine environment: A review of sources, occurrence and effects. Sci. Total Environ. 566–567, 333–349. https://doi.org/10.1016/j.scitotenv.2016.05.084
Liebezeit, G., 2008. Marine litter on the Kachelotplate, Lower Saxonian Wadden Sea. Senckenberg. Maritima 38, 147–151. https://doi.org/10.1007/BF03055291
Linde, M., Palmer, M., Gómez-Zurita, J., 2004. Differential correlates of diet and phylogeny on the shape of the premaxilla and anterior tooth in sparid fishes (Perciformes: Sparidae). J. Evol. Biol. 17, 941–952. https://doi.org/10.1111/j.1420-9101.2004.00763.x
Lithner, D., Larsson, Å., Dave, G., 2011. Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. Sci. Total Environ. 409, 3309–3324. https://doi.org/10.1016/j.scitotenv.2011.04.038
Litter, U.M., 2009. In: A Global Challenge, 232. UNEP, Nairobi, p.354.
Liu, X., Shi, H., Xie, B., Dionysiou, D.D., Zhao, Y., 2019. Microplastics as Both a Sink and a Source of Bisphenol A in the Marine Environment. Environ. Sci. Technol. 53, 10188–10196. https://doi.org/10.1021/acs.est.9b02834
Lloret, J., Shulman, G., Love, R.M., 2013. Condition and Health Indicators of Exploited Marine Fishes. John Wiley & Sons.
López-Rosales, A., Andrade, J., Fernández-González, V., López-Mahía, P., Muniategui-Lorenzo, S., 2022. A reliable method for the isolation and characterization of microplastics in fish gastrointestinal tracts using an infrared tunable quantum cascade laser system. Mar. Pollut. Bull. 178, 113591. https://doi.org/10.1016/j.marpolbul.2022.113591

Lots, F.A.E., Behrens, P., Vijver, M.G., Horton, A.A., Bosker, T., 2017. A large-scale investigation of microplastic contamination: Abundance and characteristics of microplastics in European beach sediment. Mar. Pollut. Bull. 123, 219–226. https://doi.org/10.1016/j.marpolbul.2017.08.057
Lusher, A., 2015. Microplastics in the Marine Environment: Distribution, Interactions and Effects, in: Bergmann, M., Gutow, L., Klages, M. (Eds.), Marine Anthropogenic Litter. Springer International Publishing, Cham, pp. 245–307. https://doi.org/10.1007/978-3-319-16510-3_10
Lusher, A.L., Hernandez-Milian, G., Berrow, S., Rogan, E., O'Connor, I., 2018. Incidence of marine debris in cetaceans stranded and bycaught in Ireland: Recent findings and a review of historical knowledge. Environ. Pollut. 232, 467–476. https://doi.org/10.1016/j.envpol.2017.09.070
Lusher, A.L., McHugh, M., Thompson, R.C., 2013. Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. Mar. Pollut. Bull. 67, 94–99. https://doi.org/10.1016/j.marpolbul.2012.11.028
Lusher, A.L., Welden, N.A., Sobral, P., Cole, M., 2017. Sampling, isolating and identifying microplastics ingested by fish and invertebrates. Anal. Methods 9, 1346–1360. https://doi.org/10.1039/C6AY02415G
Lusher, L.A., AWelden, N., Sobral, P., Cole, M., 2020. Sampling, isolating and identifying microplastics ingested by fish and invertebrates. Anal. Methods 9, 1346–1360. https://doi.org/10.1039/C6AY02415G
Mancuso, M., Savoca, S., Bottari, T., 2019. First record of microplastics ingestion by European hake MERLUCCIUS MERLUCCIUS from the Tyrrhenian Sicilian coast (Central Mediterranean Sea). J. Fish Biol. 94, 517–519. https://doi.org/10.1111/jfb.13920
Marrone, A., La Russa, M.F., Randazzo, L., La Russa, D., Cellini, E., Pellegrino, D., 2021. Microplastics in the Center of Mediterranean: Comparison of the Two Calabrian Coasts and Distribution from Coastal Areas to the Open Sea. Int. J. Environ. Res. Public. Health 18, 10712. https://doi.org/10.3390/ijerph182010712
Marsay, K.S., Ambrosino, A.C., Koucherov, Y., Davidov, K., Figueiredo, N., Yakovenko, I., Itzahri, S., Martins, M., Sobral, P., Oren, M., 2023. The geographical and seasonal effects on the composition of marine microplastic and its microbial communities: The case study of Israel and Portugal. Front. Microbiol. 14.
Marsili, L., Focardi, S., 1997. CHLORINATED HYDROCARBON (HCB, DDTs AND PCBs LEVELS IN CETACEANS STRANDED ALONG THE ITALIAN COASTS: AN OVERVIEW. Environ. Monit. Assess. 45, 129–180. https://doi.org/10.1023/A:1005786627533
Martellini, T., Guerranti, C., Scopetani, C., Ugolini, A., Chelazzi, D., Cincinelli, A., 2018. A snapshot of microplastics in the coastal areas of the Mediterranean Sea. TrAC Trends Anal. Chem. 109, 173–179. https://doi.org/10.1016/j.trac.2018.09.028
MEDSEALITTER consortium, 2019. Common monitoring protocol for marine litter. Deliverable 4.6.1.
Merrill, G.B., Hermabessiere, L., Rochman, C.M., Nowacek, D.P., 2023. Microplastics in marine mammal blubber, melon, & other tissues: Evidence of translocation. Environ. Pollut. 335, 122252. https://doi.org/10.1016/j.envpol.2023.122252
Mezzelani, M., Avio, C.G., Nardi, A., Regoli, F., 2017. MICROPLASTIC INVESTIGATION IN WATER AND TROPHIC CHAIN ALONG THE ITALIAN COAST 06/24/2017 – 07/15/2017.
Mizraji, R., Ahrendt, C., Perez-Venegas, D., Vargas, J., Pulgar, J., Aldana, M., Patricio Ojeda, F., Duarte, C., Galbán-Malagón, C., 2017. Is the feeding type related with the content of

microplastics in intertidal fish gut? Mar. Pollut. Bull. 116, 498–500. https://doi.org/10.1016/j.marpolbul.2017.01.008
Molino, C., Filippi, S., Giovani, G., Caccia, A., Meschini, R., Angeletti, D., 2023. Effects of phthalates on marine organisms: cytotoxicity and genotoxicity of mono-(2-ethylhexyl)-phthalate (MEHP) on European sea bass (Dicentrarchus labrax) embryonic cell line. Ann. Ist. Super. Sanita 59, 68–75. https://doi.org/10.4415/ANN_23_01_10
Mordecai, G., A. Tyler, P., G. Masson, D., Huvenne, V., 2011. Litter in submarine canyons off the west coast of Portugal. Deep Sea Res. Part II Top. Stud. Oceanogr. 58, 2489–2496. https://doi.org/10.1016/j.dsr2.2011.08.009
Morris, R.J., 1980. Floating plastic debris in the Mediterranean. Mar. Pollut. Bull. 11, 125. https://doi.org/10.1016/0025-326X(80)90073-9
Morseletto, P., 2020. A new framework for policy evaluation: Targets, marine litter, Italy and the Marine Strategy Framework Directive. Mar. Policy 117, 103956. https://doi.org/10.1016/j.marpol.2020.103956
Moss, K., Allen, D., González-Fernández, D., Allen, S., 2021. Filling in the knowledge gap: Observing MacroPlastic litter in South Africa's rivers. Mar. Pollut. Bull. 162, 111876. https://doi.org/10.1016/j.marpolbul.2020.111876
Müller, C., Erzini, K., Teodósio, M.A., Pousão-Ferreira, P., Baptista, V., Ekau, W., 2020. Assessing microplastic uptake and impact on omnivorous juvenile white seabream Diplodus sargus (Linnaeus, 1758) under laboratory conditions. Mar. Pollut. Bull. 157, 111162. https://doi.org/10.1016/j.marpolbul.2020.111162
Multisanti, C.R., Merola, C., Perugini, M., Aliko, V., Faggio, C., 2022. Sentinel species selection for monitoring microplastic pollution: A review on one health approach. Ecol. Indic. 145, 109587. https://doi.org/10.1016/j.ecolind.2022.109587
Nadal, M.A., Alomar, C., Deudero, S., 2016. High levels of microplastic ingestion by the semipelagic fish bogue Boops boops (L.) around the Balearic Islands. Environ. Pollut. 214, 517–523. https://doi.org/10.1016/j.envpol.2016.04.054
Nelms, S.E., Galloway, T.S., Godley, B.J., Jarvis, D.S., Lindeque, P.K., 2018. Investigating microplastic trophic transfer in marine top predators. Environ. Pollut. 238, 999–1007. https://doi.org/10.1016/j.envpol.2018.02.016
Neves, D., Sobral, P., Ferreira, J.L., Pereira, T., 2015. Ingestion of microplastics by commercial fish off the Portuguese coast. Mar. Pollut. Bull. 101, 119–126. https://doi.org/10.1016/j.marpolbul.2015.11.008
Oberbeckmann, S., Loeder, M.G.J., Gerdts, G., Osborn, A.M., 2014. Spatial and seasonal variation in diversity and structure of microbial biofilms on marine plastics in Northern European waters. FEMS Microbiol. Ecol. 90, 478–492. https://doi.org/10.1111/1574-6941.12409
Ogonowski, M., Gerdes, Z., Gorokhova, E., 2018. What we know and what we think we know about microplastic effects – A critical perspective. Curr. Opin. Environ. Sci. Health 1, 41–46. https://doi.org/10.1016/j.coesh.2017.09.001
Ourgaud, M., Phuong, N.N., Papillon, L., Panagiotopoulos, C., Galgani, F., Schmidt, N., Fauvelle, V., Brach-Papa, C., Sempéré, R., 2022. Identification and Quantification of Microplastics in the Marine Environment Using the Laser Direct Infrared (LDIR) Technique. Environ. Sci. Technol. 56, 9999–10009. https://doi.org/10.1021/acs.est.1c08870
Palatinus, A., Kovač Viršek, M., Robič, U., Grego, M., Bajt, O., Šiljić, J., Suaria, G., Liubartseva, S., Coppini, G., Peterlin, M., 2019. Marine litter in the Croatian part of the middle Adriatic Sea: Simultaneous assessment of floating and seabed macro and micro litter abundance and composition. Mar. Pollut. Bull. 139, 427–439. https://doi.org/10.1016/j.marpolbul.2018.12.038

Palmer, J., Herat, S., 2021. Ecotoxicity of Microplastic Pollutants to Marine Organisms: a Systematic Review. Water. Air. Soil Pollut. 232, 195. https://doi.org/10.1007/s11270-021-05155-7
Panti, C., Giannetti, M., Baini, M., Rubegni, F., Minutoli, R., Fossi, M.C., 2015. Occurrence, relative abundance and spatial distribution of microplastics and zooplankton NW of Sardinia in the Pelagos Sanctuary Protected Area, Mediterranean Sea. Environ. Chem. 12, 618–626. https://doi.org/10.1071/EN14234
Pedà, C., Battaglia, P., D'Alessandro, M., Laface, F., Malara, D., Consoli, P., Vicchio, T.M., Longo, F., Andaloro, F., Baini, M., Galli, M., Bottari, T., Fossi, M.C., Greco, S., Romeo, T., 2020. Coupling Gastro-Intestinal Tract Analysis With an Airborne Contamination Control Method to Estimate Litter Ingestion in Demersal Elasmobranchs. Front. Environ. Sci. 8. https://doi.org/10.3389/fenvs.2020.00119
Pedà, C., Giommi, C., Rizzo, C., Arcadi, E., Scozzafava, Laface, F., Greco, S., Romeo, T., 2023. Convenzione di Ricerca - Relazione finale Monitoraggio e valutazione di materiali biodegradabili innovativi per l'impiego sostenibile in miticoltura.
Pedà, C., Longo, F., Berti, C., Laface, F., De Domenico, F., Consoli, P., Battaglia, P., Greco, S., Romeo, T., 2022a. The waste collector: information from a pilot study on the interaction between the common octopus (Octopus vulgaris, Cuvier, 1797) and marine litter in bottom traps fishing and first evidence of plastic ingestion. Mar. Pollut. Bull. 174, 113185. https://doi.org/10.1016/j.marpolbul.2021.113185
Pedà, C., Romeo, T., Panti, C., Caliani, I., Casini, S., Marsili, L., Campani, T., Baini, M., Limonta, G., de Rysky, E., Caccamo, L., Perdichizzi, A., Gai, F., Maricchiolo, G., Consoli, P., Fossi, M.C., 2022b. Integrated biomarker responses in European seabass Dicentrarchus labrax (Linnaeus, 1758) chronically exposed to PVC microplastics. J. Hazard. Mater. 438, 129488. https://doi.org/10.1016/j.jhazmat.2022.129488
Pedrotti, M.L., Petit, S., Elineau, A., Bruzaud, S., Crebassa, J.-C., Dumontet, B., Martí, E., Gorsky, G., Cózar, A., 2016. Changes in the Floating Plastic Pollution of the Mediterranean Sea in Relation to the Distance to Land. PLOS ONE 11, e0161581. https://doi.org/10.1371/journal.pone.0161581
Pestana, C.J., Moura, D.S., Capelo-Neto, J., Edwards, C., Dreisbach, D., Spengler, B., Lawton, L.A., 2021. Potentially Poisonous Plastic Particles: Microplastics as a Vector for Cyanobacterial Toxins Microcystin-LR and Microcystin-LF. Environ. Sci. Technol. 55, 15940–15949. https://doi.org/10.1021/acs.est.1c05796
Pini, A., Tomassetti, P., Matiddi, M., de Lucia, G.A., Camedda, A., Zampetti, G., Lattanzi, C., Leuzzi, G., Monti, P., 2018. Microplastic samplings and inverse trajectory recognition in the Mediterranean Sea, in: 2018 IEEE International Workshop on Metrology for the Sea; Learning to Measure Sea Health Parameters (MetroSea). Presented at the 2018 IEEE International Workshop on Metrology for the Sea; Learning to Measure Sea Health Parameters (MetroSea), pp. 115–119. https://doi.org/10.1109/MetroSea.2018.8657848
Pinto, M., Polania Zenner, P., Langer, T.M., Harrison, J., Simon, M., Varela, M.M., Herndl, G.J., 2020. Putative degraders of low-density polyethylene-derived compounds are ubiquitous members of plastic-associated bacterial communities in the marine environment. Environ. Microbiol. 22, 4779–4793. https://doi.org/10.1111/1462-2920.15232
Plastic Europe, 2022. Plastics - the Facts 2022 • Plastics Europe. Plast. Eur. URL https://plasticseurope.org/knowledge-hub/plastics-the-facts-2022/ (accessed 8.30.23).
Pollet, T., Berdjeb, L., Garnier, C., Durrieu, G., Le Poupon, C., Misson, B., Briand, J.-F., 2018. Prokaryotic community successions and interactions in marine biofilms: the key role of Flavobacteriia. FEMS Microbiol. Ecol. 94, fiy083. https://doi.org/10.1093/femsec/fiy083

Potoschi, A., Cannizzaro, L., Milazzo, A., Scalisi, M., Bono, G., 1999. Sicilian dolphinfish (*Coryphaena hippurus*) fishery. Sci. Mar. 63, 439–445. https://doi.org/10.3989/scimar.1999.63n3-4439

Prata, J.C., Reis, V., da Costa, J.P., Mouneyrac, C., Duarte, A.C., Rocha-Santos, T., 2021. Contamination issues as a challenge in quality control and quality assurance in microplastics analytics. J. Hazard. Mater. 403, 123660. https://doi.org/10.1016/j.jhazmat.2020.123660

Provencher, J.F., Gaston, A.J., Mallory, M.L., O'hara, P.D., Gilchrist, H.G., 2010. Ingested plastic in a diving seabird, the thick-billed murre (Uria lomvia), in the eastern Canadian Arctic. Mar. Pollut. Bull. 60, 1406–1411. https://doi.org/10.1016/j.marpolbul.2010.05.017

R Core Team, 2022. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Railkin, A.I., 2003. Marine Biofouling: Colonization Processes and Defenses. CRC Press.

Reboa, A., Cutroneo, L., Consani, S., Geneselli, I., Petrillo, M., Besio, G., Capello, M., 2022. Mugilidae fish as bioindicator for monitoring plastic pollution: Comparison between a commercial port and a fishpond (north-western Mediterranean Sea). Mar. Pollut. Bull. 177, 113531. https://doi.org/10.1016/j.marpolbul.2022.113531

Rech, S., Macaya-Caquilpán, V., Pantoja, J.F., Rivadeneira, M.M., Jofre Madariaga, D., Thiel, M., 2014. Rivers as a source of marine litter--a study from the SE Pacific. Mar. Pollut. Bull. 82, 66–75. https://doi.org/10.1016/j.marpolbul.2014.03.019

Reisser, J., Slat, B., Noble, K., du Plessis, K., Epp, M., Proietti, M., de Sonneville, J., Becker, T., Pattiaratchi, C., 2015. The vertical distribution of buoyant plastics at sea: an observational study in the North Atlantic Gyre. Biogeosciences 12, 1249–1256. https://doi.org/10.5194/bg-12-1249-2015

Renzi, M., Blašković, A., Broccoli, A., Bernardi, G., Grazioli, E., Russo, G., 2020. Chemical composition of microplastic in sediments and protected detritivores from different marine habitats (Salina Island). Mar. Pollut. Bull. 152, 110918. https://doi.org/10.1016/j.marpolbul.2020.110918

Rios, L.M., Moore, C., Jones, P.R., 2007. Persistent organic pollutants carried by synthetic polymers in the ocean environment. Mar. Pollut. Bull. 54, 1230–1237. https://doi.org/10.1016/j.marpolbul.2007.03.022

Rochman, C.M., 2015. The complex mixture, fate and toxicity of chemicals associated with plastic debris in the marine environment. Mar. Anthropog. Litter 117–140.

Rochman, C.M., Hoh, E., Kurobe, T., Teh, S.J., 2013. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. Sci. Rep. 3, 3263. https://doi.org/10.1038/srep03263

Rochman, C.M., Kurobe, T., Flores, I., Teh, S.J., 2014. Early warning signs of endocrine disruption in adult fish from the ingestion of polyethylene with and without sorbed chemical pollutants from the marine environment. Sci. Total Environ. 493, 656–661. https://doi.org/10.1016/j.scitotenv.2014.06.051

Rodrigues, D., Pequeno, J., Pais, J., Antunes, J., Sobral, P., Costa, M.H., 2023. Comparing microplastics ingested by the bogue Boops boops (Teleostei: Sparidae) to those available in its feeding areas: A case study on the Portuguese west coast. Reg. Stud. Mar. Sci. 65, 103093. https://doi.org/10.1016/j.rsma.2023.103093

Rodríguez-Romeu, O., Constenla, M., Carrassón, M., Campoy-Quiles, M., Soler-Membrives, A., 2020. Are anthropogenic fibres a real problem for red mullets (Mullus barbatus) from the NW Mediterranean? Sci. Total Environ. 733, 139336. https://doi.org/10.1016/j.scitotenv.2020.139336

Romeo, T., Consoli, P., Florio, G., Andaloro, F., Fortuna, C.M., Fossi, M., Lauriano, G., Notarbartolo di Sciara, G., 2003. The feluca boat of the strait of messina: a platform of opportunity for cetacean sightings.

Romeo, T., Pedà, C., Fossi, M.C., Andaloro, F., Battaglia, P., 2016. First record of plastic debris in the stomach of Mediterranean lanternfishes 10.
Romeo, T., Pietro, B., Pedà, C., Consoli, P., Andaloro, F., Fossi, M.C., 2015. First evidence of presence of plastic debris in stomach of large pelagic fish in the Mediterranean Sea. Mar. Pollut. Bull. 95, 358–361. https://doi.org/10.1016/j.marpolbul.2015.04.048
Ruiz-Orejón, L.F., Sardá, R., Ramis-Pujol, J., 2016. Floating plastic debris in the Central and Western Mediterranean Sea. Mar. Environ. Res. 120, 136–144. https://doi.org/10.1016/j.marenvres.2016.08.001
Rummel, C.D., Löder, M.G.J., Fricke, N.F., Lang, T., Griebeler, E.-M., Janke, M., Gerdts, G., 2016. Plastic ingestion by pelagic and demersal fish from the North Sea and Baltic Sea. Mar. Pollut. Bull. 102, 134–141. https://doi.org/10.1016/j.marpolbul.2015.11.043
Ryan, P.G., 2015. A Brief History of Marine Litter Research, in: Bergmann, M., Gutow, L., Klages, M. (Eds.), . Springer International Publishing, Cham, pp. 1–25. https://doi.org/10.1007/978-3-319-16510-3_1
Savoca, S., Capillo, G., Mancuso, M., Faggio, C., Panarello, G., Crupi, R., Bonsignore, M., D'Urso, L., Compagnini, G., Neri, F., Fazio, E., Romeo, T., Bottari, T., Spanò, N., 2019. Detection of artificial cellulose microfibers in Boops boops from the northern coasts of Sicily (Central Mediterranean). Sci. Total Environ. 691, 455–465. https://doi.org/10.1016/j.scitotenv.2019.07.148
Sbrana, A., Valente, T., Bianchi, J., Franceschini, S., Piermarini, R., Saccomandi, F., de Lucia, A.G., Camedda, A., Matiddi, M., Silvestri, C., 2023. From inshore to offshore: distribution of microplastics in three Italian seawaters. Environ. Sci. Pollut. Res. 30, 21277–21287. https://doi.org/10.1007/s11356-022-23582-9
Schirinzi, G.F., Pedà, C., Battaglia, P., Laface, F., Galli, M., Baini, M., Consoli, P., Scotti, G., Esposito, V., Faggio, C., Farré, M., Barceló, D., Fossi, M.C., Andaloro, F., Romeo, T., 2020. A new digestion approach for the extraction of microplastics from gastrointestinal tracts (GITs) of the common dolphinfish (Coryphaena hippurus) from the western Mediterranean Sea. J. Hazard. Mater. 397, 122794. https://doi.org/10.1016/j.jhazmat.2020.122794
Scircle, A., Cizdziel, J.V., Tisinger, L., Anumol, T., Robey, D., 2020. Occurrence of Microplastic Pollution at Oyster Reefs and Other Coastal Sites in the Mississippi Sound, USA: Impacts of Freshwater Inflows from Flooding. Toxics 8, 35. https://doi.org/10.3390/toxics8020035
Scotti, G., D'Alessandro, M., Esposito, V., Vivona, P., Panti, C., 2023. Anthropogenic fibers and microplastics in the pelagic gooseneck barnacle Lepas (Lepas) anatifera in Capo Milazzo Marine Protected Area (Tyrrhenian Sea): A first characterization. Ecol. Indic. 152, 110368. https://doi.org/10.1016/j.ecolind.2023.110368
Setälä, O., Norkko, J., Lehtiniemi, M., 2016. Feeding type affects microplastic ingestion in a coastal invertebrate community. Mar. Pollut. Bull. 102, 95–101. https://doi.org/10.1016/j.marpolbul.2015.11.053
Shah, A.A., Hasan, F., Hameed, A., Ahmed, S., 2008. Biological degradation of plastics: a comprehensive review. Biotechnol. Adv. 26, 246–265. https://doi.org/10.1016/j.biotechadv.2007.12.005
Sharma, S., Chatterjee, S., 2017. Microplastic pollution, a threat to marine ecosystem and human health: a short review. Environ. Sci. Pollut. Res. 24, 21530–21547. https://doi.org/10.1007/s11356-017-9910-8
Sharma, S., Sharma, V., Chatterjee, S., 2021. Microplastics in the Mediterranean Sea: Sources, Pollution Intensity, Sea Health, and Regulatory Policies. Front. Mar. Sci. 8.
Shim, W.J., Hong, S.H., Eo, S.E., 2017. Identification methods in microplastic analysis: a review. Anal. Methods 9, 1384–1391. https://doi.org/10.1039/C6AY02558G

Silva, M.M., Maldonado, G.C., Castro, R.O., de Sá Felizardo, J., Cardoso, R.P., Anjos, R.M. dos, Araújo, F.V. de, 2019. Dispersal of potentially pathogenic bacteria by plastic debris in Guanabara Bay, RJ, Brazil. Mar. Pollut. Bull. 141, 561–568. https://doi.org/10.1016/j.marpolbul.2019.02.064

Sivan, A., 2011. New perspectives in plastic biodegradation. Curr. Opin. Biotechnol., Energy biotechnology – Environmental biotechnology 22, 422–426. https://doi.org/10.1016/j.copbio.2011.01.013

Soto-Navarro, J., Criado-Aldeanueva, F., García-Lafuente, J., Sánchez-Román, A., 2010. Estimation of the Atlantic inflow through the Strait of Gibraltar from climatological and in situ data. J. Geophys. Res. Oceans 115. https://doi.org/10.1029/2010JC006302

Stamper, M.A., Spicer, C.W., Neiffer, D.L., Mathews, K.S., Fleming, G.J., 2009. Morbidity in a Juvenile Green Sea Turtle (Chelonia mydas) Due to Ocean-Borne Plastic. J. Zoo Wildl. Med. 40, 196–198. https://doi.org/10.1638/2007-0101.1

Stanley, J.-D., Bernasconi, M.P., 2012. Buried and Submerged Greek Archaeological Coastal Structures and Artifacts as Gauges to Measure Late Holocene Seafloor Subsidence off Calabria, Italy. Geoarchaeology 27, 189–205. https://doi.org/10.1002/gea.21405

Strafella, P., López Correa, M., Pyko, I., Teichert, S., Gomiero, A., 2020. Distribution of Microplastics in the Marine Environment, in: Rocha-Santos, T., Costa, M., Mouneyrac, C. (Eds.), Handbook of Microplastics in the Environment. Springer International Publishing, Cham, pp. 1–35. https://doi.org/10.1007/978-3-030-10618-8_43-1

Suaria, G., Avio, C.G., Mineo, A., Lattin, G.L., Magaldi, M.G., Belmonte, G., Moore, C.J., Regoli, F., Aliani, S., 2016. The Mediterranean Plastic Soup: synthetic polymers in Mediterranean surface waters. Sci. Rep. 6, 1–10. https://doi.org/10.1038/srep37551

Subramanian, P.M., 2000. Plastics recycling and waste management in the US. Resour. Conserv. Recycl. 28, 253–263. https://doi.org/10.1016/S0921-3449(99)00049-X

Syranidou, E., Karkanorachaki, K., Amorotti, F., Franchini, M., Repouskou, E., Kaliva, M., Vamvakaki, M., Kolvenbach, B., Fava, F., Corvini, P.F.-X., Kalogerakis, N., 2017. Biodegradation of weathered polystyrene films in seawater microcosms. Sci. Rep. 7, 17991. https://doi.org/10.1038/s41598-017-18366-y

Tanaka, K., Takada, H., Yamashita, R., Mizukawa, K., Fukuwaka, M., Watanuki, Y., 2013. Accumulation of plastic-derived chemicals in tissues of seabirds ingesting marine plastics. Mar. Pollut. Bull. 69, 219–222. https://doi.org/10.1016/j.marpolbul.2012.12.010

Teuten, E.L., Saquing Jovita M., Knappe Detlef R. U., Barlaz Morton A., Jonsson Susanne, Björn Annika, Rowland Steven J., Thompson Richard C., Galloway Tamara S., Yamashita Rei, Ochi Daisuke, Watanuki Yutaka, Moore Charles, Viet Pham Hung, Tana Touch Seang, Prudente Maricar, Boonyatumanond Ruchaya, Zakaria Mohamad P., Akkhavong Kongsap, Ogata Yuko, Hirai Hisashi, Iwasa Satoru, Mizukawa Kaoruko, Hagino Yuki, Imamura Ayako, Saha Mahua, Takada Hideshige, 2009. Transport and release of chemicals from plastics to the environment and to wildlife. Philos. Trans. R. Soc. B Biol. Sci. 364, 2027–2045. https://doi.org/10.1098/rstb.2008.0284

Thompson, R.C., 2015. Microplastics in the Marine Environment: Sources, Consequences and Solutions, in: Bergmann, M., Gutow, L., Klages, M. (Eds.), Marine Anthropogenic Litter. Springer International Publishing, Cham, pp. 185–200. https://doi.org/10.1007/978-3-319-16510-3_7

Thompson, R.C., Swan, S.H., Moore, C.J., vom Saal, F.S., 2009. Our plastic age. Philos. Trans. R. Soc. B Biol. Sci. 364, 1973–1976. https://doi.org/10.1098/rstb.2009.0054

Tian, X., Beén, F., Bäuerlein, P.S., 2022. Quantum cascade laser imaging (LDIR) and machine learning for the identification of environmentally exposed microplastics and polymers. Environ. Res. 212, 113569. https://doi.org/10.1016/j.envres.2022.113569

Trani, A., Mezzapesa, G., Piscitelli, L., Mondelli, D., Nardelli, L., Belmonte, G., Toso, A., Piraino, S., Panti, C., Baini, M., Fossi, M.C., Zuccaro, M., 2023. Microplastics in water surface and in the gastrointestinal tract of target marine organisms in Salento coastal seas (Italy, Southern Puglia). Environ. Pollut. 316, 120702. https://doi.org/10.1016/j.envpol.2022.120702
Trecozzi, M.R., Iiritano, G., Petrungaro, G., 2022. Liveability and freight transport in urban areas: the example of the Calabria Region for City Logistics. Transp. Res. Procedia, New scenarios for safe mobility in urban areasProceedings of the XXV International Conference Living and Walking in Cities (LWC 2021), September 9-10, 2021, Brescia, Italy 60, 116–123. https://doi.org/10.1016/j.trpro.2021.12.016
Trestrail, C., Nugegoda, D., Shimeta, J., 2020. Invertebrate responses to microplastic ingestion: Reviewing the role of the antioxidant system. Sci. Total Environ. 734, 138559. https://doi.org/10.1016/j.scitotenv.2020.138559
Tsangaris, C., Digka, N., Valente, T., Aguilar, A., Borrell, A., de Lucia, G.A., Gambaiani, D., Garcia-Garin, O., Kaberi, H., Martin, J., Mauriño, E., Miaud, C., Palazzo, L., del Olmo, A.P., Raga, J.A., Sbrana, A., Silvestri, C., Skylaki, E., Vighi, M., Wongdontree, P., Matiddi, M., 2020. Using Boops boops (osteichthyes) to assess microplastic ingestion in the Mediterranean Sea. Mar. Pollut. Bull. 158, 111397. https://doi.org/10.1016/j.marpolbul.2020.111397
Tsangaris, C., Panti, C., Compa, M., Pedà, C., Digka, N., Baini, M., D'Alessandro, M., Alomar, C., Patsiou, D., Giani, D., Romeo, T., Deudero, S., Fossi, M.C., 2021. Interlaboratory comparison of microplastic extraction methods from marine biota tissues: A harmonization exercise of the Plastic Busters MPAs project. Mar. Pollut. Bull. 164, 111992. https://doi.org/10.1016/j.marpolbul.2021.111992
Tsiaras, K., Costa, E., Morgana, S., Gambardella, C., Piazza, V., Faimali, M., Minetti, R., Zeri, C., Thyssen, M., Ben Ismail, S., Hatzonikolakis, Y., Kalaroni, S., Garaventa, F., 2022. Microplastics in the Mediterranean: Variability From Observations and Model Analysis. Front. Mar. Sci. 9.
UNEP/MAP, 2015. Marine litter assessment in the Mediterranean 2105.
Vaksmaa, A., Egger, M., Lüke, C., Martins, P.D., Rosselli, R., Asbun, A.A., Niemann, H., 2022. Microbial communities on plastic particles in surface waters differ from subsurface waters of the North Pacific Subtropical Gyre. Mar. Pollut. Bull. 182, 113949. https://doi.org/10.1016/j.marpolbul.2022.113949
Valls, M., Quetglas, A., Ordines, F., Moranta, J., 2011. Feeding ecology of demersal elasmobranchs from the shelf and slope off the Balearic Sea (western Mediterranean). Sci. Mar. 75, 633–639. https://doi.org/10.3989/scimar.2011.75n4633
van der Hal, N., Ariel, A., Angel, D.L., 2017. Exceptionally high abundances of microplastics in the oligotrophic Israeli Mediterranean coastal waters. Mar. Pollut. Bull. 116, 151–155. https://doi.org/10.1016/j.marpolbul.2016.12.052
Wang, L.-C., Lin, J.C.-T., Dong, C.-D., Chen, C.-W., Liu, T.-K., 2021. The sorption of persistent organic pollutants in microplastics from the coastal environment. J. Hazard. Mater. 420, 126658. https://doi.org/10.1016/j.jhazmat.2021.126658
Watts, A.J.R., Lewis, C., Goodhead, R.M., Beckett, S.J., Moger, J., Tyler, C.R., Galloway, T.S., 2014. Uptake and Retention of Microplastics by the Shore Crab Carcinus maenas. Environ. Sci. Technol. 48, 8823–8830. https://doi.org/10.1021/es501090e
Widyastuti, S., Abidin, A.S., Hikmaturrohmi, H., Ilhami, B.T.K., Kurniawan, N.S.H., Jupri, A., Candri, D.A., Frediansyah, A., Prasedya, E.S., 2023. Microplastic Contamination in Different Marine Species of Bintaro Fish Market, Indonesia. Sustainability 15, 9836. https://doi.org/10.3390/su15129836

Worm, B., Lotze, H.K., Jubinville, I., Wilcox, C., Jambeck, J., 2017. Plastic as a Persistent Marine Pollutant. Annu. Rev. Environ. Resour. 42, 1–26. https://doi.org/10.1146/annurev-environ-102016-060700
Wright, S.L., Thompson, R.C., Galloway, T.S., 2013. The physical impacts of microplastics on marine organisms: a review. Environ. Pollut. Barking Essex 1987 178, 483–492. https://doi.org/10.1016/j.envpol.2013.02.031
Xu, S., Ma, J., Ji, R., Pan, K., Miao, A.-J., 2020. Microplastics in aquatic environments: Occurrence, accumulation, and biological effects. Sci. Total Environ. 703, 134699. https://doi.org/10.1016/j.scitotenv.2019.134699
Yang, G., Gong, M., Mai, L., Zhuang, L., Zeng, E.Y., 2021. Diversity and structure of microbial biofilms on microplastics in riverine waters of the Pearl River Delta, China. Chemosphere 272, 129870. https://doi.org/10.1016/j.chemosphere.2021.129870
Yarsley, V.E., Couzens, E.G.., 1945. Plastics. Plastics.
Ye, S., Andrady, A.L., 1991. Fouling of floating plastic debris under Biscayne Bay exposure conditions. Mar. Pollut. Bull. 22, 608–613. https://doi.org/10.1016/0025-326X(91)90249-R
Zeri, C., Adamopoulou, A., Bojanić Varezić, D., Fortibuoni, T., Kovač Viršek, M., Kržan, A., Mandic, M., Mazziotti, C., Palatinus, A., Peterlin, M., Prvan, M., Ronchi, F., Siljic, J., Tutman, P., Vlachogianni, T., 2018. Floating plastics in Adriatic waters (Mediterranean Sea): From the macro- to the micro-scale. Mar. Pollut. Bull. 136, 341–350. https://doi.org/10.1016/j.marpolbul.2018.09.016
Zettler, E.R., Mincer, T.J., Amaral-Zettler, L.A., 2013. Life in the “Plastisphere”: Microbial Communities on Plastic Marine Debris. Environ. Sci. Technol. 47, 7137–7146. https://doi.org/10.1021/es401288x
Zhang, S.-J., Zeng, Y.-H., Zhu, J.-M., Cai, Z.-H., Zhou, J., 2022. The structure and assembly mechanisms of plastisphere microbial community in natural marine environment. J. Hazard. Mater. 421, 126780. https://doi.org/10.1016/j.jhazmat.2021.126780
Zhao, S., Zhu, L., Li, D., 2015. Microplastic in three urban estuaries, China. Environ. Pollut. 206, 597–604. https://doi.org/10.1016/j.envpol.2015.08.027