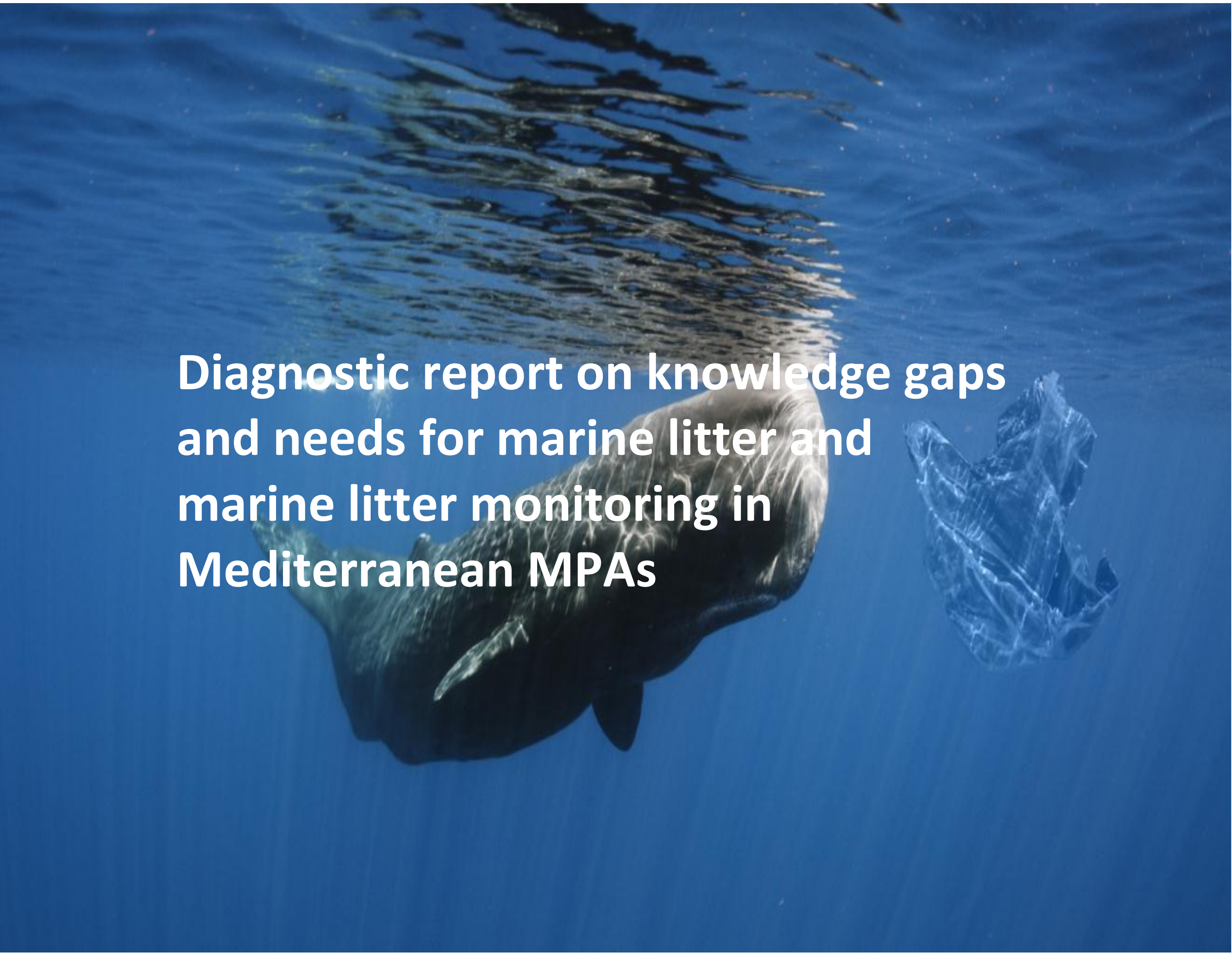

Diagnostic report on knowledge gaps and needs for marine litter and marine litter monitoring in Mediterranean MPAs

PREPARED BY

THE INTERREG MED PLASTIC BUSTERS MPAS PROJECT

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This report (Deliverable 3.2.2) consolidates the available knowledge on marine litter and its impacts and identifies the knowledge gaps and needs for marine litter and marine litter monitoring in Mediterranean MPAs.

Approvals

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1. Introduction

1.1. PlasticBusters MPAs in a nutshell

PlasticBusters MPAs, is a 4-year-long project Interreg Mediterranean funded project aiming to contribute to maintaining biodiversity and preserving natural ecosystems in pelagic and coastal marine protected areas (MPAs), by defining and implementing a harmonized approach against marine litter. The project entails actions that address the whole management cycle of marine litter, from monitoring and assessment to prevention and mitigation, as well as actions to strengthen networking between and among pelagic and coastal MPAs.

The PlasticBusters MPAs consolidates Mediterranean efforts against marine litter by:

- Diagnosing the impacts of marine litter on biodiversity in MPAs and identifying marine litter 'hotspots';
- Defining and testing tailor-made marine litter surveillance, prevention and mitigation measures in MPAs;
- Developing a common framework of marine litter actions for Interreg Mediterranean regions towards the conservation of biodiversity in Med MPAs.

The PlasticBusters MPAs project deploys the multidisciplinary strategy and common framework of action developed within the Plastic Busters initiative led by the University of Siena and the Sustainable Development Solutions Network Mediterranean. This initiative frames the priority actions needed to tackle marine litter in the Mediterranean and was labelled under the Union for the Mediterranean (UfM) in 2016, capturing the political support of 43 EuroMediterranean countries.

1.2. Aim and scope of this report

The overarching aim of this report is to consolidate the available knowledge on marine litter and its impacts in Mediterranean MPAs and identify the knowledge gaps that need to be addressed by the PlasticBusters MPAs with regards to marine litter and marine litter monitoring.

This report takes stock of all marine litter studies carried out in Mediterranean coastal and marine protected areas and builds upon the results and findings of the Plastic Busters MPAs Deliverable 3.2.1 entitled 'State-of-the-art methods to monitor marine litter and its impacts on biodiversity'.

In this respect, a thorough screening of the related scientific literature (peer-reviewed articles, technical reports, etc.) published in the last 5 years was carried out. It should be highlighted that the geographical scope of the literature review covered all Mediterranean countries; thus going beyond the scope of the Interreg Med regions. Special emphasis was given to the results of the ongoing Interreg Med projects dealing with marine litter monitoring aspects (i.e. ACT4LITTER, AMARE, MEDSEALITTER, etc.).

The present document aims to establish a common understanding within the project partnership on the available knowledge on marine litter in Mediterranean MPAs (amounts, composition, sources and impacts) in order to design in a more targeted and effective way the Plastic Busters MPAs marine litter monitoring strategy (Deliverable 3.3.1). Furthermore, the present document aims to shortlist the gaps and shortcomings that need to be addressed by the Plastic Busters MPAs with regards to the marine litter monitoring approaches.

1.3. Definitions and policy context

Within this document marine litter is defined as any persistent, manufactured or processed solid material discarded, disposed of or abandoned in the marine and coastal environment. Marine litter can be classified in size classes as follows: macrolitter referring to items above 25mm in the longest dimension; mesolitter from 5mm to 25 mm; and microlitter from 1µm to 5mm. Sometimes the later size class is further broken down to large microplastics from 1mm to 5 mm and small microplastics from 1µm to 1mm.

The main legislative frameworks related to marine litter monitoring are the EU Marine Strategy Framework Directive (2008/56/EC, 2010/477/EC, 2017/848/EC) and the Barcelona Convention Ecosystem Approach (COP19 IMAP Decision IG.22/7) (see Box 1.1 and Box 1.2).

Box. 1.1. *The Marine Litter Descriptor, criteria, and respective Indicators within the framework of the EU MSFD.*

Marine Litter within the EU MSFD

Properties and quantities of marine litter do not cause harm to the coastal and marine environment (Descriptor 10)

Criteria D10C1 - Primary: The composition, amount and spatial distribution of litter on the coastline, in the surface layer of the water column, and on the seabed, are at levels that do not cause harm to the coastal and marine environment.

- ✓ amount of litter washed ashore and/or deposited on coastlines, including analysis of its composition, spatial distribution and, where possible, source (10.1.1)
- ✓ amount of litter in the water column (including floating at the surface) and deposited on the seafloor, including analysis of its composition, spatial distribution and, where possible, source (10.1.2)

Criteria D10C2 - Primary: The composition, amount and spatial distribution of micro-litter on the coastline, in the surface layer of the water column, and in seabed sediment, are at levels that do not cause harm to the coastal and marine environment.

- ✓ amount, distribution and, where possible, composition of microparticles (in particular microplastics) (10.1.3)

Criteria D10C3 - Secondary: 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.

- ✓ amount and composition of litter ingested by marine animals (10.2.1)

Criteria D10C4 - Secondary: The number of individuals of each species which are adversely affected due to litter, such as by entanglement, other types of injury or mortality, or health effects.

Box. 1.2. *The Marine Litter Operational Objectives and respective Indicators within the framework of the Barcelona Convention Ecosystem Approach and the Integrated Monitoring and Assessment Programme (IMAP)*

Marine Litter and the Barcelona Convention Ecosystem Approach

Ecological Objective 10 (EO10): Marine and coastal litter do not adversely affect the coastal and marine environment.

IMAP Common Indicator 22: Trends in the amount of litter washed ashore and/or deposited on coastlines (including analysis of its composition, spatial distribution and, where possible, source).

IMAP Common Indicator 23: Trends in the amount of litter in the water column including micro plastics and on the seafloor.

IMAP Candidate Indicator 24: Trends in the amount of litter ingested by or entangling marine organisms focusing on selected mammals, marine birds, and marine turtles.

2. Marine litter in Mediterranean MPAs: data gaps

2.1. Marine litter on beaches

Macro-litter

Systematic efforts to collect data on the amounts, distribution, composition and sources of marine litter along the coastline of Mediterranean coastal and marine protected areas are rather limited. In terms of the geographical distribution of the data collected, these refer mainly to the coastal and marine protected areas located in the Adriatic and Ionian Seas (Munari et al., 2016; Vlachogianni et al., 2018; Vlachogianni et al., 2019) and the Pelagos Sanctuary in Italy (Giovacchini et al., 2018) (see Table 2.1).

To-date, one of the most comprehensive efforts to assess marine litter deposited on the beaches of coastal and marine protected areas has been made within the framework of the Interreg Med ACT4LITTER project. The project run four editions of the ACT4LITTER Marine Litter Watch Month (winter 2017, spring 2018, summer 2018, autumn 2018) where MPA managers carried out some 50 beach litter surveys in Albania, Croatia, France, Greece, Italy, Slovenia, Spain and Turkey. According to the results of the winter edition marine litter seems to be 'building up' in what should be pristine coastal and marine protected areas of the Mediterranean. More than one fourth of the twenty two beaches surveyed were characterized by high litter densities ranging from 430 to 12,896 items per 100-metre stretch (Vlachogianni et al., 2019). This amounts to a relatively high average litter density of 1,706 items/100m stretch or 1.2 items/m^2. The majority of litter items were made of artificial polymer materials which accounted for 82% of all litter collected. Litter from shoreline sources, such as tourism and recreational activities and poor waste management practices, accounted for 26.5% of all litter collected; while the amount of litter from fisheries and aquaculture was in the range of some 10%. The most frequently found items included small plastic and polystyrene pieces (23%), plastic caps and lids (11%), mussel nets (5.6%) and single-use plastic items (5.3%) such as drink bottles and shopping bags.

Similar results were obtained by Giovacchini et al (2018) who performed beach litter surveys in several sites located at the Pelagos Sanctuary in Italy. A density of 1.06 items/m^2 was reported. Plastic was found to be the prevailing material.

The IPA-Adriatic DeFishGear project (Vlachogianni et al., 2018) also surveyed beach litter at several coastal and marine protected areas of the Adriatic and Ionian Seas, namely the Mljet National Park (Croatia), the Protected Area of Kalamas-Acherontas-Corfu (Greece); the MPA Torre del Cerrano (Italy) and the Strunjan Landscape Park (Slovenia). The recorded litter densities varied from 92 to 10,554 items/100m stretch and 0.09 - 0.41 items/m^2. These densities were substantially higher than the ones recorded in the aforementioned studies.

Munari et al (2016) carried out beach litter surveys in the Po River Delta Park and several sites located in Italian Natura 2000 sites, namely: Rosolina in the Veneto Regional Park, Volano, Bellocchio, Casalborsetti, and Bevano in the Emilia Romagna Regional Park. The average density was 0.2 litter items/m^2 which was much lower than the densities found in the aforementioned studies. Some 80% of the items recorded were made of plastic. The most abundant items were cigarette butts which accounted for 22.9% of items collected, followed by plastic pieces (13.5%), bottle caps (9.2%), mesh bags (7.2%), plastic bottles (6.5%) and cutlery (6.4%).

The Interreg Med AMAre project carried out beach litter monitoring along the coast of the north-eastern part of the MPA of the Maltese Islands via the use of drones. A total of 473 images were captured at two survey sites (115 images at Qawra Point and 358 images at Baħar iċ-Ċagħaq). The results show, that the total number of litter items recorded were 30 items and 578 items, respectively.

Table 2.1. *Literature review on beach macro-litter studies performed from 2013-2018.*

Study areas	Sampling unit	Frequency & timing	Classification list	Litter densities	Reference
Protected areas located in Italy: Po River Delta Park and several locations in Natura 2000 sites	50-m transect	May-June	UNEP/IOC Litter classification list	0.2 items/m^2	*Munari et al., 2016*
Several coastal sites located at the Pelagos Sanctuary, Italy	100-m transect	Seasonal	OSPAR List	1.06 items/m^2	*Giovacchini et al., 2018*
Mljet National Park (Croatia), Protected Area of Kalamas-Acherontas-Corfu (Greece); MPA Torre del Cerrano (Italy); the Strunjan Landscape Park (Slovenia)	100-m transect	Every 3 months	MSFD TG10 Masterlist	92 - 10,554items/100m; 0.09 - 0.41 items/m^2	*Vlachogianni et al., 2018*
Coastal and marine protected areas located in Albania, Croatia, France, Greece, Italy, Slovenia, Spain and Turkey	100-m transect	Every 3 months	MSFD TG10 Masterlist	1,706 items/100m; 1.2 items/m^2	*Vlachogianni et al., 2019 (Results from the Interreg Med ACT4LITTER)*
Northeastern part of the MPA of the Maltese Islands	N/A	N/A	N/A	30 items and 578 items	*Interreg Med AMAre, 2018*

In conclusion, despite the fact that beach litter surveys are the most common mode of marine litter monitoring in the Mediterranean, it is evident that there are substantial data gaps with regards to the amounts, distribution, composition and sources of macro-litter deposited on the beaches of coastal and marine protected areas and thus beach macro-litter monitoring should be included within the Plastic Busters MPAs monitoring strategy. Furthermore, the use of drones or other related means for the rapid mapping of marine litter hotspots can be instrumental in terms of implementing targeted mitigation measures.

Micro-litter

A review of the literature on microplastics in beach sediments for the period 2013 - 2018 revealed the lack of data on micro-litter in beach sediments for sites located in coastal marine protected areas in the Mediterranean. The majority of the studies reported the spatial distribution (abundance, mass, type, and/or size) of microplastics in beaches which are subjected to various human pressures.

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2.2. Marine litter on the sea surface

Macro-litter

Very few studies in the Mediterranean have focused on floating macro- litter and even fewer are those that have been carried out in marine protected areas (see Table 2.2).

Palatinus et al. (2019) carried out floating macro-litter surveys in the archipelago of Zadar that includes the Kornati Islands National Park and the Telascica Nature Park. Seventeen transects with total length of 36.6 km were carried out and the calculated densities for macro-litter ranged from 0 to 689 items/km^2,with an average concentration of 175.3 ± 180.6 items/km^2 throughout the entire study area (Palatinus et al., 2019).

Another study investigated the abundance, composition and distribution of floating macro-litter in the Liguro Provençal basin and correlated these data with the presence of cetaceans. A number of 246 survey transects were performed during summer within the period from 2006 to 2015 by sailing vessels with simultaneous cetaceans observations for a total survey effort of 4443.73 Km (Di-Méglio and Campana, 2017). The visual observation surveys of floating macro-litter (>1 cm) were conducted during 98 days at sea. Using the strip transect method, the density of macrolitter was calculated (15 ± 23 items/km^2) using a double range of distance from the boat (50 m and 10 m) depending on the different items categories (Di-Méglio and Campana, 2017). This study showed that foraging habitats of cetaceans overlap by 50% with litter floating at the sea surface, representing a high possibility of encounters.

Floating macro-litter monitoring was undertaken along fixed transects connecting maritime commercial routes from Italy to France, Spain, Greece, and Tunisia (South Tyrrhenian Sea, Central Tyrrhenian Sea, Adriatic Sea and Ionian Sea) (Arcangeli et al., 2018). The study covered areas that are subject to protection measures (Ligurian Sea, Bonifacio Strait and Central Tyrrhenian Sea). The sampled transects were chosen in order to cover large high sea areas in the Mediterranean basin and ferries were used as research platforms. Only items bigger than 20 cm (longest dimension) were recorded given the high elevation level of the observer (17 – 25 m). The recorded litter abundances were in the range of 2–5 items/km^2).

Another study carried out in the Adriatic and Ionian Seas performed floating macro-litter surveys in several areas including transects in the gulf of Trieste, in the vicinity of the Miramare Marine Protected area with a density in the order of 142 ± 142 items/km^2 (Zeri et al., 2018). Fossi et al. (2017) surveyed floating macro-litter in the Pelagos Sanctuary while sampling micro-liter and meso-litter, however the related findings have not been reported (Fossi et al., 2017).

Table 2.2. *Literature review on floating macro-litter studies performed from 2013-2018.*

Study area	Observation method	Litter size classes surveyed	Litter densities	Reference
Archipelago of Zadar	Strip transect	≥2.5 cm	175.3 ± 180.6 items/km^2	*Palatinus et al., 2019*
Liguro Provençal basin	Strip transect	>1 cm	15 ± 23 items/km^2	*Di-Méglio and Campana, 2017*
Ligurian Sea, Bonifacio Strait and Central Tyrrhenian Sea	Strip transect	20 cm	2–5 items/km^2	*Arcangeli et al., 2018*
Gulf of Trieste	Strip transect	≥2.5 cm	142 ± 142 items/km^2	*Zeri et al., 2018*
Pelagos Sanctuary	Strip transect	≥2.5 cm	Not reported	*Fossi et. al., 2017*

The heterogeneity of the spatial abundance is high in many studied areas worldwide, in terms of total counts of floating macro-litter and observed size classes. These differences can be attributed to the variability in observational conditions (i.e. sea state, observation height, vessel type) and deployed survey methods, as well as the location of the survey sites (i.e. coastal waters, open sea) and the vicinity to marine litter sources (i.e. urban and touristic centres, shipping lanes, fishing areas) and pathways (i.e. rivers, wastewater treatment plants - WWTPs). In the Mediterranean Sea, the variable floating macro-litter distribution can be also attributed to the variability of the sea surface circulation patterns. According to Lagrangian circulation models, currents in the western part of the Mediterranean Sea promote particle circulation, while circulation patterns in the eastern part act more like attractors, thus contributing to high levels of beaching (Mansui et al., 2015). Differences also exist between nearshore waters and open seas. Some coastal accumulations can appear in the western part of the basin, along the Ligurian coast and around Corsica, despite the presence of the Northern Current (Mansui et al., 2015).

In conclusion, it is evident that there are substantial data gaps with regards to the amounts, distribution, composition and sources of macro-litter on the sea surface in coastal and pelagic marine protected areas.

Micro-litter

Several studies in the Mediterranean Sea have focused on assessing floating microplastics, however fewer studies have been performed in marine protected areas (Table 2.3). Microplastics have been sampled throughout the Mediterranean Sea (Cózar et al., 2015; Ruiz-Orejón et al., 2016), including the Pelagos Sanctuary (Panti et al., 2015, Fossi et al., 2017), the Aegean-Levantine Sea (Güven et al., 2017; Van der hal et al., 2017; Gündoğdu and Çevik, 2017), off the western Sardinian coast (de Lucia et al., 2014), the Adriatic Sea (Gajšt et al., 2016; Suaria et al., 2016; Zeri et al., 2018), Corsica (Collignon et al., 2014) and the Western Mediterranean Sea (Schmidt et al., 2018; Pedrotti et al., 2016; Fossi et al., 2012; 2016; Faure et al., 2015).

Cozar et al., (2015) reported that in total, 83% of the sampled microlitter in the Mediterranean Sea surface was microplastics. A recent study revealed a high occurrence of microplastics (mean: 82,000 ± 79,000 items/km^2) in the Pelagos Santuary, with the highest values recorded close to the Genoa harbor and in the proximity of Capraia Island (Fossi et al., 2017). The average concentration of plastic particles is similar to those previously found in other surveys carried out in the Pelagos Sanctuary (Collignon et al., 2012; Fossi et al., 2016). The Tuscan Archipelago area (Capraia Island) was also found to be one of the areas with high concentration values and weight of microplastics (Baini et al., 2018), as also recently highlighted by other authors (Suaria et al., 2016). Baini et al., (2018) obtained mean values of 69,162 ± 83,243 items / km^2 in sea surface samples and 0.16 ± 0.47 Items/m^3 in water column samples. In this study an increase of floating microplastics with distance to the coast was observed and seasonality as well as sampling area did not affect the abundance of microplastics. The most abundant size class was 1–2.5 mm including fragments and sheets suggesting that fragmentation of larger polyethylene and polypropylene items could be the main source of microplastics in the area (Baini et al., 2018). Palatinus et al. (2019) carried out floating micro-litter surveys in the archipelago of Zadar that includes the Kornati Islands National Park and the Telascica Nature Park. A total of 2985 micro-litter particles were isolated from the samples. The calculated average concentration was 127,135 ± 294,847 particles/km^2 (0.9 ± 1.9 particles/m^3), ranging from 17,875 particles/km^2 (0.1 particles/m^3) to 1,549,549 particles/km^2 (9.7 particles/m^3). Zeri et al., (2018) reported also a high concentration range of micro-litter particles, ranging from 6,502 particles/km^2 to 1,636,111 particles/km^2, for the gulf of Trieste located in the vicinity of the Miramare Marine Protected area (Zeri et al., 2018).

Even though there are several studies on floating micro-plastics that have been carried out in the Mediterranean Sea, covering different areas, very few have focused on Marine Protected Areas, with the Pelagos Sanctuary being one of the most researched areas (Panti et al., 2015; Fossi et al., 2017).

Table 2.3. *Sampling location, survey date, and reported density of micro- litter from published articles in the period 2012-2019.*

Location	Habitat	Date	Density	References
North Western Mediterranean Sea Tuscan coast	Sea surface/Water column	2013-2014	Surface: 69,161.3 ± 83,243.9 items/km^2 Vertical: 0.16 ± 0.47 Items/m^3	*Baini et al., 2018*
Western Mediterranean Sea Pelagos Sanctuary	Sea surface	2014	82,000 ± 79,000 items/km^2	*Fossi et al., 2017*
Western Mediterranean Sea (Ligurian Sea)	Sea surface	2013	125,930 ± 132,485 Items/km^2 ± SD	*Pedrotti et al., 2016*
Western Mediterranean Sea and Adriatic Sea	Sea surface	2013	400,000 ± 740,000 items/km^2 1.00 ± 1.84 Items/m^3	*Suaria et al., 2016*
Adriatic Sea- Slovenian coastal waters	Sea surface	2014	472,000 ± 201,000 Items/km^2 ± SD	*Gajšt et al., 2016*
Gulf of Trieste	Sea surface	2014- 2015	444,182 ± 563,190 items/km^2	*Zeri et al., 2018*
Western Mediterranean Sea	Sea surface	2012	0.31 ± 1.17 Items/m3 ± SD	*Fossi et al., 2016*
Western Mediterranean Sea, Asinara National Park, Pelagos Sanctuary	Sea surface	2012–2013	0.17 ± 0.32 Items/m^3 ± SD	*Panti et al., 2015*
Whole Mediterranean	Sea surface	2013	243,853 Items/km^2	*Cózar et al., 2015*
Western Mediterranean Sea (Sardinian coast)	Sea surface	2013	0.15 ± 0.11 Items/m^3	*de Lucia et al., 2014*
Ligurian and Sardinian Sea	Sea surface	2011	0.62 ± 2.00 Items/m^3 ± SD	*Fossi et al. 2012*
Western Mediterranean Sea	Sea surface	2010	116,000 Items/km^2	*Collignon et al., 2012*
Archipelago of Zadar	Sea surface	2015	127,135 ± 294,847 particles/km^2	*Palatinus et al., 2019*

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2.3. Marine litter on the seafloor

Macro-litter

Only few studies have focused on marine litter lying on the seafloor in Mediterranean Marine Protected Areas. Fortibuoni et al. (2019) reported seafloor litter densities for coastal Slovenian waters located within and/or in the vicinity of the Strunjan Landscape Park. Scuba visual surveys recorded a very low litter density of 0.12 items/100 m^2 (Fortibuoni et al., 2019). This low litter density was attributed to the clean-up actions organized by divers several times per year. Another study covered a large part of the Spanish Mediterranean coast, tracking both the continental shelf and upper slope from the Strait of Gibraltar to the French border, however despite the large amount of areas being under conservation status in the overall study area, protected areas were avoided since the seafloor litter surveys were implemented with trawling (García-Rivera et al., 2018). However, the same authors investigated marine litter on the seafloor of the Gulf of Alicante, where three protected areas are located, namely the Cabo de Palos-Islas Hormigas, the Isla de Tabarca and the Isla Grosa. According to this study items made out of artificial polymers were the most abundant with a density range of 0–11.6 kg/km^2 and overall, the density increased close to the coast but some isolated/remote areas were also characterized by a high density of plastics (García-Rivera et al., 2017). Consoli et al. (2018) studies seafloor litter in an EBSA (Ecologically or Biologically Significant Area) located at the central Mediterranean Sea. Remotely Operated Vehicles (ROVs) were used for this study that recorded litter densities ranging from 0 items/100 m^2 to 14.02 items/100 $m^2$with a mean of 2.13 items/100 m2. The observed average density was higher (5.2 items/100 m^2) at depths>100 m than at shallower depths (<100 m, 0.71 items/100 m^2). Lost or abandoned fishing lines contributed to 98.07% of the overall litter density.

Micro-litter

There is lack of data concerning micro-litter on the seafloor in the Mediterranean region. Especially for the seafloor of MPAs there are only two studies to-date. One was carried out in the Cabrera Archipelago National Maritime-Terrestrial Park (Alomar et al., 2016), where sediment samples were collected from shallow areas (8-10 m water depth). The average microlitter density reported was 0.90 ± 0.10 particles/g of dry weight. Palatinus et al. (2019) carried out floating micro-litter surveys in the archipelago of Zadar that includes the Kornati Islands National Park and the Telascica Nature Park. In total, 720 micro-litter particles were extracted from the samples and the average concentration of micro-litter was calculated to be 36 ± 16.91 particles/100 g dry weight.

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2.4. Marine litter in rivers/river outflows

Macro-litter

A high percentage of the litter entering the marine environment is assumed to come from land-based sources, but freshwater litter inputs have not been quantified. A riverine litter monitoring network that has been set-up by the European Commission Joint Research Centre within the RIMMEL project has investigated floating macro-litter in rivers outflowing in the Mediterranean Sea (González et al., 2016). Some of these rivers outflow in coastal and marine protected areas however the results of this initiative have not yet been published. There are no other studies that have addressed this issue.

Micro-litter

The only study found for monitoring riverine inputs of marine litter was published in 2015 (Van der Wal et al., 2015) and it was carried out in the river Po, within approximately 50 km from the river mouth. The sampling was done in one two-week sampling period and three methods were tested for monitoring floating microlitter. The average maximum number of microparticles per km^2 was found in the Po River to be some 2 million/km^2.

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2.5. Marine litter in biota

Despite the increasing number of studies in recent years, information on the impacts of marine litter on Mediterranean biota remains very poor and inconsistent. Marine litter can impact biodiversity in a number of ways, namely through litter ingestion, entanglement (e.g., in ghost nets), facilitation of the transportation of marine organisms via rafting on litter items, damages to benthic habitats and communities, reduced oxygenation or 'smothering' of species communities as well as through release of toxic compounds that can potentially lead to bio-accumulation and bio-magnification of toxic chemicals.

Systematic efforts to collect data on the impacts of marine litter on biota dwelling in Mediterranean coastal and marine protected areas are rather limited. In terms of the geographical distribution of the data collected, the studies refer mainly to the coastal and marine protected areas located in the Pelagos Sanctuary (north-western Mediterranean) (Fossi et al., 2012, 2014, 2016; Baini et al., 2017; Giani et al., 2019; Bonello et al., 2018, Remy et al., 2015; Camedda, 2014; Cau et al., 2017; Dussud et al., 2018; Jorissen, 2014; Fabri et al., 2014). Additionally, data is available for other Mediterranean MPAs: the Balearic Islands (Alomar et al., 2016, 2017; Bellas et al., 2016; Compa et al., 2018); the Strait of Sicily, the Strait of Messina and the Eolian archipelago (central Mediterranean) (Battaglia et al., 2015; Romeo et al., 2015, 2016); MPAs in Malta (Deidun et al., 2015); coastal and marine protected areas located in the Adriatic and Ionian Sea in Croatia, Italy, Greece and Slovenia (Anastasopoulou et al., 2018; Melli et al., 2017; Anastatopolou et al., 2013, Digka et al., 2016).

Among the different interactions of marine litter with biota, ingestion is the most studied one; while several methodological approaches have been deployed according to the type of studies species and the dimension of the ingested litter. On the contrary, entanglement and marine litter as a transport vector and habitat are far less studied.

From 2013 to 2018, more than 25 papers have been published focusing on the investigation of marine litter ingestion by marine organisms in Mediterranean MPAs, while only 6 studies have addressed the issue of entanglement and marine litter as a transport vector and habitat. Different marine species have been researched; ranging from Mollusca to marine mammals (see Tab 2.2 and Fig 2.1), with fish being the most investigated species for ingestion and cnidaria for entanglement and colonization. The percentage of marine litter occurrence in the organisms investigated varied from 0 to 100% as depicted below (Table 2.4).

In the Balearic Islands (western Mediterranean Sea), microplastics were observed in the gastrointestinal tracts of 195 Boops boops (Nadal et al., 2016). Out of 337 analyzed fish, 58% had ingested plastic filaments (Nadal et al., 2016). Results showed that Boops boops from Cap Blanc, which is between the Marine Reserve of Palma Bay and the Marine Reserve of Migjorn, had the lowest percentage of microplastics occurrence than fish from the other sampling sites (Cala Ratjada in Mallorca; Espardell and Cala Tarida in Eivissa). Remarkably, mean ingested items per fish in Cap Blanc was greater than in any other sampling location (Nadal et al., 2016). Along the Spanish Mediterranean coast, the small pelagic fish species were found to have ingested microplastic particles, Engraulis encrasicolus (0.18-0.20 items/individual) and Sardina pilchardus (0.21-0.23 items/individual) (Compa et al., 2018). Just south of the Gulf of Leon in the MPA of Empordà, both species were found to contain microplastic items in their stomach contents. This was also true for the individuals analyzed within the MPA of Delta de l'Ebre and Illes Columbretes; where 6.67 - 26.67 % of the individuals of E. encrasicolus contained microplastic items and 33.3 % of S. pilchardus contained microplastic items. Finally, in the southern regions entering the Alboran Sea, in the MPA of Tabarca-Cabo de Palos some 20% of individuals contained microplastic items.

Other studies investigated marine litter ingestion in the central Mediterranean Sea. Plastic litter was found in the stomach contents of top predators, mesopelagic fish and in the Carangide Trachinotus ovatus. Romeo et al. (2015) reported a high percentage of macro and microplastics in Thunnus thynnus (%O = 32 .4%) followed by Thunnus alalunga (%O = 12.9%) and Xiphias gladius (%O = 12.5%). According to Battaglia et al. (2016) a high level of macro and microplastic litter was found in the Carangide T. ovatus (%O = 24.3%), a species of commercial interest for some small-scale fishery activities. Finally, occurrence of microplastics was found in some Mediterranean lanternfishes (Myctophidae) i.e. Electrona risso (%O = 6.1%), Diaphus metopoclampus (%O = 0.3%), Hygophum benoiti (%O = 6.8%), and Myctophum punctatum (%O = 4.2%) (Romeo et al., 2016).

Studies carried out in the central Mediterranean Sea and in the north western Mediterranean Sea have investigated the presence of fauna-colonized litter on sea bed (Tab.2.5). Consoli et al. (2018) reported that only the 9.28% of litter items presented high fouling levels (epibionts covering > 50% of their surface) in the Ecologically or Biologically Significant Area (EBSA). According to Angiolillo et al. (2015), in the Sicilian areas (around the Egadi Archipelago) the frequency (%) of colonization of litter items by macro-benthic invertebrates was 69.3%.

The Intereg Med MEDSEALITTER project has collected data on microplastics ingestion in *Boops boops* from 4 Mediterranean MPAs located in Spain, France, Italy and Greece, however the results have not yet been published. Existing and new data on litter ingestion in the candidate bioindicator species of marine litter impacts *Caretta caretta* from several areas across the Mediterranean Sea has been collected by the INDICIT project, but these results are also not published yet.

In the Central Mediterranean sea, marine litter ingestion was reported for species representing different levels of the food web. Plastic litter was found in the stomach contents of top predators, mid-water predators and in *Trachinotus ovatus,* an opportunistic predator, which occupies an intermediate position in the marine pelagic trophic web. Romeo et al. (2015) reported a high percentage of plastics in *Thunnus thynnus* (%O = 32 .4%) followed by *Thunnus alalunga* (%O = 12.9%) and *Xiphias gladius* (%O = 12.5%). According to Battaglia et al. (2016) a high level of plastic litter was found in the Carangide *T . ovatus* (%O = 24.3%), a species of commercial interest for some small-scale fishery activities. Finally, occurrence of plastic litter was found in some Mediterranean lantern fishes (Myctophidae) i.e. *Electrona risso* (%O = 6.1%), *Diaphus metopoclampus* (%O = 0.3%), *Hygophum benoiti* (%O = 6.8%), and *Myctophum punctatum* (%O = 4.2%) (Romeo et al., 2016). Despite the fact that the frequency of ingested plastics was not high, lantern fishes are among the most important prey species of large pelagic predators. For this reason, they can contribute to the presence of plastic litter in top predators through secondary ingestion.

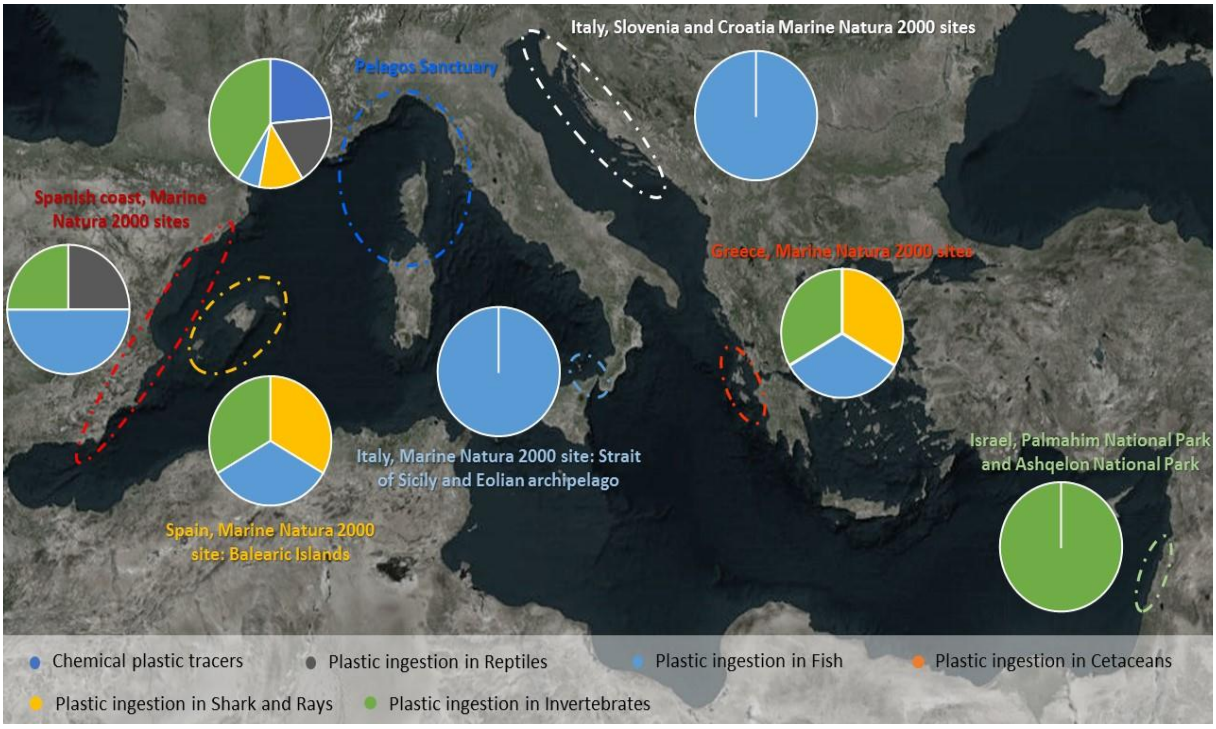

Figure 2.1. *Distribution of studies on plastic ingestion by biota in Mediterranean MPAs, subdivided by taxa, area and typology of investigation.*

In the Central Mediterranean sea, Consoli et al. (2018) reported that only the 9.28% of litter items presented high fouling levels (epibionts covering > 50% of their surface). The most common epibiontic species identified included hydroids, algae and ophiuroids.

Angiolillo et al. (2015) studied litter deposited on the deep seafloor (30–300 m depth) in 26 areas, off the coast of three Italian regions in the Tyrrhenian Sea, using a ROV. In the Sicilian areas (around the Egadi Archipelago) the frequency (%) of colonization of litter items by macro-benthic invertebrates was 69.3%. The most frequently recorded taxa on litter in all three regions were hydroids sponges and polychaetes, followed by bryozoans, anthozoans and ascidians. Moreover, in Banco Scuso a Sicilian area, ghost net colonized by epibionts such as sertulariid hydroids, *Paramuricea macrospina, Alcyonium palmatum* and Cidaridae sea urchins, were detected.

In conclusion, it is evident that there are substantial data gaps with regards to:

- the number of Mediterranean MPAs investigated;
- the number of different species investigated in order to reflect the real impact on biodiversity
- the type of biondicator species, which should be selected taking into consideration different habitats and different home ranges in order to provide greater ecological and spatial coverage of the investigated impact;
- the studied effect as most studies are limited to assessing the presence and occurrence of ingested plastics (including microplastics), and only few studies have researched the adverse effects of marine litter.

Table 2.4. *Literature review on macro- and micro-litter ingestion studies published from 2013 to 2018.*

Study Area	Species	Laboratory method	Litter size	Occurence	Reference
North-western Mediterranean sea (Pelagos Sanctuary)	Mammalia *Balaenoptera physalus*	Detection of phthalate esters as plastic tracers	n.a.	n.a.	Fossi *et al.*, 2012
		Detection of phthalate esters as plastic tracers			Fossi *et al.*, 2014
		Detection of phthalate esters as plastic tracers and biomarker responses			Fossi *et al.*, 2016
	Mammalia *Tursiops truncatus*	Detection of phthalate esters as plastic tracers	n.a.	n.a.	Baini *et al.*, 2017
	Mammalia *Grampus griseus*				
	Mammalia *Stenella coeruleoalba*				
	Anapsida *Caretta caretta*	Dissection and visual identification with stereomicroscope	Macro- and microplastic	71%	Campani *et al.*, 2013
	Chondrichthyes *Prionace glauca*	Dissection, filtration through sieves and visual identification with stereomicroscope	Macro- and microplastic	25%	Bernardini *et al.*, 2018
	Chondrichthyes *Cetorhinus maximus*	Detection of phthalate esters as plastic tracers	n.a.	n.a.	Fossi *et al.*, 2014
	Osteichthyes *Merluccius merluccius*	Dissection, digestion with KOH 10% and visual identification with stereomicroscope	Microplastic	8%	Giani *et al.*, 2019
	Osteichthyes *Mullus barbatus*			17%	
	Mollusca Mitylus galloprovincialis	Dissection, digestion with 30% H2O2 and visual identification with stereomicroscope	Microplastic	0.05 item/g dry weight	Bonello *et al.*, 2018
	Mollusca *Anomia ephippium*			0.12 item/g dry weight	
	Mollusca *Crassotrea gigas*			0.11 item/g dry weight	
	Ascidiacea *Ascidia spp.*			0.62 item/g dry weight	
	Arthropoda *Planktonic organisms*	Detection of phthalate esters as plastic tracers	n.a.	n.a.	Baini *et al.*, 2017
	Arthropoda *Gammarella fucicola*	Dissection and visual identification with	Microplastic	Average: 1.38 ± 0.79 item/individua	Remy *et al.*, 2015

	Arthropoda *Gammarus aequicauda*	stereomicroscope		l	
	Arthropoda *Melita hergensis*				
	Arthropoda *Nototropis guttatus*				
	Arthropoda *Nebalia strausi*				
	Arthropoda *Palaemon xiphias*				
	Arthropoda *Liocarcinus navigator*				
	Arthropoda *Athanas nitescens*				
	Arthropoda *Galathea intermedia*				
	Arthropoda *Euphausia krohnii*	Detection of phthalate esters as plastic tracers	n.a.	n.a.	Fossi *et al*., 2014
North western Mediterranean sea (Pelagos Sanctuary and marine protected areas of national status and Marine Natura 2000 sites)	Anapsida *Caretta caretta*	Dissection and visual identification with stereomicroscope	Macroplastic	14%	Camedda *et al*., 2014
				85%	Matiddi *et al*., 2017
Western mediterranean sea (Spain, Marine Natura 2000 site: Valencian coast)	Anapsida *Caretta caretta*	Dissection and visual identification with stereomicroscope	Macroplastic	78%	Domenech *et al*., 2019
Western Mediterranean sea (Balearic Islands, Spain)	Chondrichthyes *Etmopterus spinax*	Dissection and visual identification with stereomicroscope	Microplastic	50%	Alomar and Deudero, 2017
	Chondrichthyes Chimaera monstrosa			0%	
	Chondrichthyes *Galeus melastomus*			17%	
	Chondrichthyes *Scyliorhinus canicula*			0%	
	Osteichthyes *Boops boops*	Dissection and visual identification with stereomicroscope	Microplastic	68%	Nadal *et al*., 2016
	Echinodermata *Holothuria forskali*	Faecal pellets	Microplastic	6%	Alomar *et al*., 2016

Western Mediterranean sea (Malaga, Cartagena and Menorca island, Spain)	Osteichthyes *Mullus barbatus*	Dissection, digestion with NaOH 1 M and visual identification with stereomicroscope	Microplastic	19%	Bellas *et al.*, 2016
Western Mediterranean sea (Spain)	Osteichthyes *Engraulis enchrasicolus*	Dissection and visual identification with stereomicroscope	Microplastic	14%	Compa *et al.*, 2018
	Osteichthyes *Sardina pilchardus*			15%	
Mediterranean sea (Ebro Delta Natural park - Spain and Po Estuary Natural park - Italy)	Mollusca *Mitylus galloprovincialis*	Acid digestion using HNO_3 and HCl and visual identification with stereomicroscope	Microplastic	Average: 0.18 $\pm$ 0.15 item/individual	Vandermeersch *et al.*, 2015
Central Mediterranean sea (Strait of Messina, Italy)	Osteichthyes *Trachinotus ovatus*	Dissection and visual identification with stereomicroscope	Macro- and microplastic	24%	Battaglia *et al.*, 2016
Central Mediterranean sea (Strait of Sicily and Strait of MessinaItaly)	Osteichthyes *Diaphus metopoclampus*	Dissection and visual identification with stereomicroscope	Microplastic	0%	Romeo *et al.*, 2016
	Osteichthyes *Electrona risso*			6%	
	Osteichthyes *Hygophum benoiti*			7%	
	Osteichthyes *Myctophum punctatum*			4%	
Central Mediterranean sea (Eolian archipelago and Strait of Messina, Italy)	Osteichthyes *Thunnus alalunga*	Dissection and visual identification with stereomicroscope	Macro- and microplastic	13%	Romeo *et al.*, 2015
	Osteichthyes *Thunnus thynnus*			32%	
	Osteichthyes *Xiphias gladius*			12%	
Adriatic sea and North eastern Ionian sea (Italy, Slovenia and Croatia)	Osteichthyes *Chelidonichthys lucerna*	Dissection and visual identification by stereomicroscope /Dissection, digestion with 30% H_2O_2 and visual identification with stereomicroscope	Macro- and microplastic	0%	Anastasopoulou *et al.*, 2018
	Osteichthyes *Citharus linguatula*			2%	
	Osteichthyes *Chelon auratus*			95%	
	Osteichthyes *Mullus barbatus*			11%	
	Osteichthyes *Mullus surmuletus*			55%	
	Osteichthyes *Pagellus erythrinus*			20%	
	Osteichthyes *Sardina pilchardus*			26%	
	Osteichthyes *Scomber japonicus*			43%	

	Osteichthyes *Solea solea*			7%	
	Osteichthyes *Sparus aurata*			100%	
	Osteichthyes *Trachurus mediterraneus*			0%	
	Osteichthyes *Trachurus picturatus*			0%	
	Osteichthyes *Trachurus trachurus*			24%	
Eastern Ionian sea (Greece)	Chondrichthyes *Scyliorhinus canicula*	Dissection and visual identification with stereomicroscope	Plastic	0%	Anastatopolou *et al.*, 2013
	Chondrichthyes *Pteroplatytrygon violacea*		Macroplastic	50%	
	Chondrichthyes *Centrophorus granulosus*		Plastic	0%	
	Chondrichthyes *Etmopterus spinax*		Macroplastic	6%	
	Chondrichthyes *Squalus acanthias*		Plastic	0%	
	Chondrichthyes *Squalus blainville*		Macroplastic	1%	
	Chondrichthyes *Raja clavata*		Plastic	0%	
	Chondrichthyes *Raja oxyrinchus*			0%	
	Chondrichthyes *Galeus melastomus*		Macroplastic	3%	
	Osteichthyes *Brama brama*		Microplastic	0%	
	Osteichthyes *Conger conger*			0%	
	Osteichthyes *Epigonus telescopus*			0%	
	Osteichthyes *Helicolenus dactylopterus*			0%	
	Osteichthyes *Lepidopus caudatus*			0%	
	Osteichthyes *Merluccius merluccius*			0%	
	Osteichthyes *Micromesistius poutassou*			0%	

	Osteichthyes *Molva macrophthalma*			0%	
	Osteichthyes *Mora moro*			0%	
	Osteichthyes *Nettastoma melanorum*			0%	
	Osteichthyes *Pagellus bogareaveo*			2%	
	Osteichthyes *Phycis blennoides*			0%	
	Osteichthyes *Polyprion americanus*			0%	
	Osteichthyes *Scedophilus ovalis*			0%	
	Osteichthyes *Scorpaena elongata*			0%	
	Osteichthyes *Sudis hyalina*			0%	
	Osteichthyes *Xiphias gladius*			0%	
Eastern Ionian sea (Greece)	Mollusca Mitylus galloprovincialis	Dissection, digestion with 30% H2O2 and visual identification with stereomicroscope	Microplastic	47%	Digka *et al.*, 2016
Eastern Mediterranean sea (Israel, Palmahim National Park and Ashqelon National Park)	Ascidiacea *Microcosmus exasperatus*	Dissection, digestion with 10% KOH and visual identification with stereomicroscope	Microplastic and Phthalate esters as plastic tracers	n.a.	Vered *et al.*, 2019

Table 2.5. *Literature review of studies focusing on marine litter colonization published from 2013 to 2018.*

Study Area	Habitat	Taxa	Method	Occurrence %	Litter size	Reference
Malta island (Malta MPAs)	Benthic	Cnidaria	ROV survey at depths from 250 to 400 m. Two video transects were carried out on slope substrata, for a total of 4 hours and 50 minutes of video footage and 4000 m^2 of surveyed area	N/A	Macro-litter	*Deidun et al., 2014*
Northern Adriatic Sea (SIC-Off the coast of Chioggia)	Benthic	Cnidaria	ROV survey 4 areas, 17 dive, and each dive	Litter-fauna interactions were with most of the debris (65.7%)	Macro-litter	*Melli et al., 2017*
Central Mediterranean sea (EBSA Ecologically or Biologically Significant Area)	Benthic	Hydroids, algae and ophiuroids	19 ROV transects; range of deep: 20-220 m	The epibiontic colonization of debris items 9.28%	Macro-litter	*Consoli et al., 2018*
North Western Mediterranean Sea (Egadi Archipelago positioned between the strait of Sicily and the Tyrrhenian Sea)	Benthic	Sertulariid hydroids and Cidaridae sea urchins	17 video transects, 8 areas, range of deep: 30-270 m	Frequency (%) of colonization by macro-benthic invertebrates 69.3%	Macro-litter	*Angiolilo et al., 2015*
Central western Mediterranean (Pelagos Sanctuary SPAMI Tavolara - Punta coda Cavallo MPA)	Benthic	Serpulidae, (Sertulariidae), tunicate, bryozoans and zoanthids.	29 ROV dives, with 1–4 dives per site. A total of 1.3 km^2 of useful footage of hard bottoms has been acquired during the surveys, which allowed obtaining ca. 4200 independent photo sampling units	The epibiontic colonization of litter items was also high: 65%	Macro-litter	*Cau et al., 2017*
Western Mediterranean Sea (Pelagos Sanctuary SPAMI)	Surface and water column	Foraminifera, benthic macroalgae, hydrozoan, gastropods, bryozoans	Manta plankton net with a mesh size of 150 µm	19.3 individuals per 100 cm^2	Micro and macro-plastics	*Jorissen, 2014*
North-western Mediterranean (French continental margin) Pelagos Sanctuary SPAMI	Benthic	Polychaetes, Mollusca, Cnidaria	ROV; 101 video films; length explored 83 km; range of deep: 60-800 m	N/A	Macro-litter	*Fabri et al. 2014*

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3. Marine litter monitoring methods: gaps and needs

Taking stock of the results and findings of the Plastic Busters MPAs Deliverable 3.2.1 entitled 'State-of-the-art methods to monitor marine litter and its impacts on biodiversity' and the discussions held during the technical workshop on marine Litter monitoring held in November 2018 (Deliverable 3.4.1), the following gaps were identified with regards to marine litter monitoring methods:

3.1 Marine litter on beaches

Macro-litter

- The beach macro-litter monitoring protocols applied in the Mediterranean differ in terms of sampling units (size and positioning), frequency and timing of the surveys, size limits and classes of litter items surveyed, classification list and quantification units.
- The most frequently used method described by Galgani et al. (2013) in the MSFD TG10 'Guidance on Monitoring of Marine Litter in European Seas' needs to be further enhanced in order to ensure that litter items collected are classified in a consistent and comprehensive way that will facilitate the identification of the related sources and will allow the assessment of the effectiveness of the marine litter measures put in place.

Micro-litter

- The deliverable produced in the framework of the BASEMAN JPI Oceans project, addresses existing needs in terms of sampling and samples analysis for micro-litter monitoring in beach sediments.
- One of the main issues that need to be addressed is the lack of quality assurance/quality control (QA/QC) instruments. No organisations to-date offer proficiency training or testing in microplastics samples processing, no certified reference materials are available, no accreditation certificates have been issued and some procedures in use have not yet been validated.

3.2 Marine litter on the sea surface

Macro-litter

- The deployed floating litter monitoring methodologies in the Mediterranean for macro-litter are not harmonized, thus leading to incomparable results. The applied methodologies and protocols differ in the sampling approaches such as differences in the observation platforms, the type of surveys and the observation or sampling conditions and tools, the litter items classification lists.
- For the sea surface macro-litter monitoring there is a clear need to define a protocol that defines the optimum observation parameters such as observation height, observation width, observation distance, observation angle and vessel speed, as well as parameters related to the marine litter properties such as detection limit for observed litter size classes, classification list, item properties to be recorded (size, buoyancy, color, etc.). Furthermore, a harmonization is needed with regards to adequate sample size in different areas (coastal

waters, high sea areas) per season and per survey The Interreg Med MEDSEALITTER project is expected to play an instrumental role in terms of harmonizing all aforementioned parameters and defining suitable protocols that can be adapted (if needed) and tested within the Plastic Busters MPAs testing activities.

Micro-litter

- Sampling methods for sea surface floating micro-litter are more or less harmonized as in all studies surface towed nets are used with a mesh size opening of 330μm. Nevertheless some works report lower mesh size of the nets used, while differences exist regarding the vessel speed and the use of a flow meters for recording the water volume sampled.
- Another important issue which leads to the inconsistency of results is the variability in the analytical methodology used by each laboratory, especially regarding the pre-processing of the samples. Intercalibration exercises and the use of prepared reference samples are highly recommended, in addition to the spectroscopic identification of the polymer materials. Such an exercise took place within the MEDCIS-DG ENV project, where several of the Plastic Busters MPAs partners participated. It is desirable to build upon this experience in testing activities of the Plastic Busters MPAs.

3.3 Marine litter on the seafloor

Macro-litter

- Monitoring the marine environment for the presence of seafloor marine litter is a necessary part of assessing the extent and possible impact. However, it is important to use consistent and reliable methods of sampling and sample characterisation (e.g. number, size, shape, mass and type of material to gain the greatest benefit). For seafloor monitoring, general guidelines and protocols have been already described in the refernce documents of the MSFD TG ML and the UN Environment/MAP IMAP related documents, while supporting strategies were also described and published recently, with more details, in a dedicated UN report.
- The type of seafloor litter survey to be selected depends on the objectives of the assessment and on the magnitude of the pollution on the seafloor. Various protocols are available, including a tutorial from the project DeFishGear, to be used for regular monitoring in MPAs supporting harmonized approaches. Templates for recording data, also MSFD and UN Environment/MAP compatible are available.
- One identified need that has to be addressed is the need for improving the evaluation of the surface area investigated by seafloor litter surveys in order to express results more meaningfully in items per m^2 or km^2 and thus replace the units commonly in use (Items/Km distance), when sampling with Submersibles/ROVs. Generally, the methods already in use in many countries are to some extent harmonized at the basin scale (MEDITS project), including in protected areas. Monitoring activities could be operated on regular basis by deploying ROVs or through diving (scuba/snorkelling), in areas with rocky substrate. Opportunistic approaches such as regular surveys for biodiversity in MPAs has a great potential. Additional records of litter and interactions with marine organisms will provide sufficient information for measuring the indicator 10DC4 that has become mandatory within MSFD. This approach is well adapted for MPAs that are regularly recording data on biodiversity (abundance, distribution). Remote operated vehicles could represent useful data collection devices for

long term monitoring but, because of the necessary means, through opportunistic approaches only. All monitoring methods, either deploying ROV or diving, generally result in underwater observations or footage that are georeferenced. For monitoring purposes, a specific sampling scheme has to be defined (length of transects, distance above the seafloor for adequate observation/image resolution, etc.). In particular, the locations should be chosen according to strict criteria, based on the level of the available knowledge for the area, for example, a good understanding of fishing activities. Once collected, protocols for image annotation and analysis need to be defined. While some basic guidance has been already elaborated (e.g. within the Interreg Med AMARe project), a more formal protocol is needed, including a data recording sheet for observations, considering the typical sea floor categories.

Microlitter

- Regarding the methodology followed for the sampling and the analysis of subtidal sediments, there are a lot of publications worldwide. The common principal of almost all the studies concerning the separation of the microplastics from the sediment is the density separation by flotation. The solution, however, used for the flotation varies between studies (NaCl, NaI, $ZnCl_2$ etc.). The harmonized protocol for monitoring microplastics in sediments published in the framework of the BASEMAN JPI Oceans project addresses this methodological gap.
- Furthermore, the contamination of the samples during the analytical procedure is a very important factor that influences the quality of the results. As for the beach sediments, there is still lack of quality assurance/quality control (QA/QC) of the analyses since no inter-laboratory exercise has been organised yet.

3.4 Marine litter in rivers/river outflows

- There are no harmonized methodologies for providing quantitative data for comparable assessments of riverine litter. Applied methodologies differ in the targeted environmental compartment, litter size fraction and the technology used. This field is largely under research and thus related monitoring protocols need to be developed.

3.5 Marine litter in biota

- While several studies in Mediterranean MPAs have focused on the interactions between marine biota and marine litter, with different levels of information being gathered (e.g. on the types of interaction, on the interactions with different species, etc.), comparisons cannot be made due to the variety of methodological approaches being used; thus hampering down the effective consolidation of knowledge on the actual impacts. Thus there is an evident need for harmonizing the different monitoring approaches. Certain interactions might need greater efforts in order to harmonize the protocols with which they are investigated (e.g. the effect of litter colonization), while others might need lesser efforts to do so (e.g. the ingestion of marine litter by fishes). Concerning microlitter ingestion analytical methodologies (i.e. for microlitter in beach and seafloor sediments, for floating microliter), there is a need for quality assurance/quality control (QA/QC) of the analyses and planning of inter calibration exercises.

- Most of the studies consider the occurrence of marine litter in marine organisms, which can provide only indications on the physical impact of the ingested material. Thus, there is an imperative to establish a monitoring approach that considers linking the detection of the ingested marine litter to the physical and toxicological effects. Furthermore, in order to fully understand the impacts of marine litter in the marine ecosystems, a multi-species approach is needed considering factors such as the diversity of species (benthic, pelagic, etc.) and feeding habits (detritus-eaters, suspension-eaters, omnivores, carnivores), the diverse types of marine litter, the different marine litter size classes.
- With regards to the indicator species, currently, *Caretta caretta* has been proposed as a candidate biondicator species to evaluate the impact of marine litter in biota. The selection of a series of bioindicator organisms (in addition to the candidate IMAP indicator and D10 MSFD) in order to monitor various trophic levels, various geographical areas and different habitats is needed. The biondicator species should be selected both in terms of different "habitat" and different "home range" in order to provide greater ecological and spatial coverage of the investigated impact on biodiversity.

THE PLASTIC BUSTERS MPAs PARTNERSHIP