# State-of-the-art methods to monitor marine litter and its impacts on biodiversity

PREPARED BY

## THE INTERREG MED
## PLASTIC BUSTERS MPAS PROJECT

---

https://plasticbustersmpas.interreg-med.eu

## Document Information

| | |
|---|---|
| **Document** | D3.2.1 Report on the state-of-the-art methods to monitor ML and its impacts on biodiversity |
| **Version:** | V03 |
| **Date:** | 01/10/2018 |
| **Authors:** | Vlachogianni, Th., Fossi M.C., Anastasopoulou, A., Alomar, C., Aparicio, A., Baini, M., Caliani, I., Campani, T., Casini, S., Consoli, P., Deudero, S., Galgani, F., Kaberi H., Panti, C., E. Romeo, T., Tsangaris, C., Zeri, C. |

## Document Information

This report (Deliverable 3.2.1) provides an overview of the state-of-the-art methods to monitor marine litter and its impacts on biodiversity including endangered species.

## Approvals

| Date | Partner |
|---|---|
| 1/10/2018 | Th. Vlachogianni/MIO-ECSDE (Task Leader) |
| 1/10/2018 | Th. Vlachogianni/MIO-ECSDE (WP3 Leader)<br>M.C. Fossi/UNISI (Project Scientific Coordinator) |

## Document History

| Version | Date | Comments & Status | Author |
|---|---|---|---|
| V00 | 01/06/2018 | Draft outline | MIO-ECSDE, UNISI |
| V01 | 06/08/2018 | Consolidated draft texts | MIO-ECSDE, UNISI, HCMR, IEO, IFREMER, ISPRA |
| V02 | 25/09/2018 | Advanced/finalized texts | MIO-ECSDE, UNISI, IEO, ISPRA |
| V03 | 01/10/2018 | Finalized texts | MIO-ECSDE, UNISI, IFREMER, ISPRA |

## Citation

Vlachogianni, Th., Fossi M.C., Anastasopoulou, A., Alomar, C., Aparicio, A., Baini, M., Caliani, I., Campani, T., Casini, S., Consoli, P., Deudero, S., Galgani, F., Kaberi H., Panti, C., E. Romeo, T., Tsangaris, C., Zeri, C., 2018. State-of-the-art methods to monitor marine litter and its impacts on biodiversity. Interreg Med Plastic Busters MPAs project.

# Table of Contents

# 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 Develoment 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 present a summary review of the state-of-the-art methods to monitor marine litter (macro-litter and micro-litter) in all marine compartments (beach, sea surface, seafloor, biota) and its impacts on biodiversity. This report aims to take-stock of the marine litter monitoring methodologies applied in the Mediterranean region, without though overlooking any ground-breaking scientific advances made in the field, worldwide.

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 compiled summary report aims to establish a common understanding within the project partnership on the recent advances made with regards to marine litter monitoring in order to select the most mature, relevant and applicable protocols towards defining and testing a harmonized marine litter monitoring approach (Deliverables 3.3.1 and 3.3.2).

### 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 22:**

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. Monitoring marine litter on beaches

## 2.1. Macro-litter

Beach surveys for macro-litter (items > 2.5 cm) assessment are the most common mode of marine litter monitoring in the Mediterranean. A thorough screening of the literature revealed that most beach litter studies (published in peer-reviewed journals from 2013-2018) carried out in the Mediterranean focus on the collection and visual identification and classification of litter items found at a shoreline site. The protocols applied may differ in terms of sampling units (size and positioning), frequency and timing of the surveys, size limits and classes of litter items to be surveyed, classification list and quantification units (see Tab. 2.1).

Within the framework of the Marine Strategy Framework Directive, the Technical Group on Marine Litter (MSFD TG10) has developed guidelines on beach litter monitoring (Galgani et al., 2013). According to these guidelines the survey sites are selected taking into consideration the following criteria: they have a minimum length of 100 m; they are characterized by a low to moderate slope (~1.5-4.5 º); they have clear access to the sea (not blocked by breakwaters or jetties); they are accessible to survey teams throughout the year; they are ideally not subject to cleaning activities. Furthermore, the selected beaches are situated in the vicinity of ports or harbours, river mouths, coastal urban areas, tourism destinations; and in relatively remote areas. Surveys are carried out at intervals of three months in autumn (mid-September to mid-October), winter (mid-December to mid-January), spring (April) and summer (mid-June to mid-July). During the surveys, all macroscopic beach litter items larger than 2.5 cm in the longest dimension are collected and counted, ensuring the inclusion of caps, lids and cigarette butts. In each survey, the sampling unit used is a 100-metre stretch from the strandline to the back of the beach. The back of the beach is identified using coastal features such as the presence of vegetation, dunes, cliff base, road, fence or other anthropogenic structures such as seawalls (either piled boulders or concrete structures). Two (2) sections of a 100-metre stretch on the same beach are monitored, separated at least by a distance of 50m. All items found on the sampling unit are collected and are categorized in accordance with the 'MSFD TG10 Master List of Categories of Litter Items'. Results are expressed as counts of litter items per square meter ($m^2$) or counts of litter items per 100-metre stretch of beach. It should be noted, that according to the Decision EC/2017/848 on the 'Assessment of criteria and methodological standards for assessing the GES' the unit of counts/100 m is recommended for criterion D10C1 'Litter on coastline'.

Within the framework of the Barcelona Convention a guidance document for beach litter monitoring has been developed. According to this document the approach for selecting the survey site is the same as the one suggested by the MSFD TG10. Furthermore, the recommended sampling unit and the frequency and timing of the surveys follow the MSFD TG10 approach. The same applies for the quantification units and the size classes to be surveyed, however regarding the classification list there are some very few small differences. Indicative examples are the following: the categories of plastic drink bottles ≤0.5 l (G7) and plastic drink bottles ≥0.5 l (G8) in the MEDPOL Beach Litter Survey Form have been merged into one category entitled plastic drink bottles (G7/G8); similarly the plastic caps and lids (G21) and the plastic rings from bottle caps and lids (G24) have been merged into one category entitled plastic caps and lids, including rings from bottle caps/lids (G21/24). In total 7 such merges/differentiations have been identified.

***Table 2.1.*** *Literature review on beach macro-litter studies published in peer-reviewed journals from 2013-2018.*

| **Location** | **Sampling unit** | **Frequency & timing** | **Size classes** | **Classification list** | **Quantification units** | **Reference** |
|---|---|---|---|---|---|---|
| Slovenia | 50-m transect | 24 h timeframe | ≥2 cm | UNEP/IOC Litter classification list | items/m2 | *Laglbauer et al., 2013* |
| Tyrrhenian coast, Italy | 2m*2m ($4m^2$) plots | April-May | ≥2.5 cm | MSFD TG10 Masterlist | N/A | *Poeta et al., 2016* |
| North-western Adriatic coast, Italy | 50-m transect | May-June | ≥2 cm | UNEP/IOC Litter classification list | items/$m^2$ | *Munari et al., 2016* |
| Tyrrhenian coast, Italy | 100-m transect | Every 3 months | ≥2.5 cm | MSFD TG10 Masterlist | items/m | *Poeta et al., 2016* |
| Israel | 100-m transect | Seasonal (surveys were an average of 55 days apart) | ≥2.5 cm | UNEP/IOC Litter classification list | items/$100m^2$ | *Pasternak et al., 2017* |
| Israel | 10-m transect | April-July (every 2 weeks) | ≥ 2 cm | MSFD TG10 Masterlist | items/$m^2$ | *Portman and Brennan, 2017* |
| Mediterranean coastline, Morocco | 25-m transect | Seasonal | – | Material type | Weight | *Alshawafi et al., 2017* |
| Corfu, Greece | 100-m transect | Every 15 ± 5 days | ≥2.5 cm | MSFD TG10 Masterlist | items/100 m;<br>kg/100 m | *Prevenios et al., 2018* |
| Ulcinj, Montenegro | 2m*2m ($4m^2$) plots | May | ≥2.5 cm | MSFD TG10 Masterlist | N/A | *Šilc et al.,2018* |
| Pelagos Sanctuary, Italy | 100-m transect | Seasonal | ≥2.5 cm | OSPAR List | items/$100m^2$ | *Giovacchini et al., 2018* |
| Mediterranean coastline, Morocco | 100-m transect | November-December | ≥2.5 cm | UNEP List | items/100 m;<br>items/$m^2$;<br>gr/$m^2$; | *Maziane et al., 2018* |
| Adriatic and Ionian Seas, all countries | 100-m transect | Every 3 months | ≥2.5 cm | MSFD TG10 Masterlist | items/100m;<br>items/$m^2$ | *Vlachogianni et al., 2018* |

Several InterregMed modular projects and other projects have undertaken beach litter monitoring activities using different approaches as shown in Table 2.2. The vast majority of these initiatives are applying the MSFD TG10 and the MEDPOL guidelines for beach litter monitoring.

NGOs efforts provide a very important contribution to beach litter monitoring data in the Mediterranean with the vast majority of the NGOs active on the issue (i.e. LEGABIENTE, MAREVIVO, MEDSOS, MIO-ECSDE, HELMEPA, SURFRIDER, etc.) applying the MSFD TG10 methodology for beach litter monitoring.

Regarding the MSFD monitoring programmes of the Members States it seems that most of the Member States have adopted the MSFD TG10 methodology.

***Table 2.2.*** *Beach macro-litter monitoring undertaken by Interreg Med modular projects and other projects/initiatives.*

| **Project/Initiative** | **Sampling unit** | **Frequency & timing** | **Size classes** | **Classification list** | **Quantification units** |
|---|---|---|---|---|---|
| Interreg Med ACT4LITTER | 100-m transect | Every 3 months | ≥ 2.5 cm | MSFD TG10 Masterlist | items/100m; items/m$^2$ |
| Interreg Med BLUEISLANDS | 100-m transect | Monthly | ≥ 2.5 cm | OSPAR List | items/100m; items/m$^2$ |
| Interreg-BALKANMED Meltemi | 100-m transect | Every 3 months | ≥ 2.5 cm | MSFD TG10 Masterlist | items/100m; items/m$^2$ |
| EU SWIM-H2020 SM (Algeria, Egypt, Morocco) | 100-m transect | Every 3 months | ≥ 2.5 cm | MSFD TG10 Masterlist | items/100m; items/m$^2$ |
| UN Environment Programme/MAP Marine Litter-MED | 100-m transect | Every 3 months | ≥ 2.5 cm | MEDPOL LIST | items/100m; items/m$^2$ |
| IPA Adriatic DeFishGear | 100-m transect | Every 3 months | ≥ 2.5 cm | MSFD TG10 Masterlist | items/100m; items/m$^2$ |
| EEA Marine Litter Watch | 100-m transect | Every 3 months | ≥ 2.5 cm | MSFD TG10 Masterlist | items/100m |

Last but not least, despite their relatively recent emergence, drones are increasingly being deployed within research efforts, including beach litter monitoring. The scientific literature review yielded one study undertaken in the Mediterranean focusing on beach litter monitoring through aerial imagery (Deidun et al., 2018). The authors perceive this approach as a well-suited one to address the monitoring needs of MPAs managers, enabling the regular and exhaustive monitoring of long stretches of coastline within the confines of the same MPAs. However, the study pinpoints also the possible constraints imposed by data protection considerations, which have resulted in many countries adopting restrictions on drone flights in some areas (including beaches).

### 2.2. Micro-litter

Microplastics comprise a very heterogeneous assemblage of pieces that vary in size, shape, colour, specific density, polymer type, and other characteristics. For meaningful comparisons and to answer the specific questions and to test hypotheses through monitoring, it is important to define methodological criteria to quantify such metrics as for e.g. the abundance, distribution and composition of microplastics and to ensure sampling effort is sufficient to detect the effects of interest. Microplastics, commonly defined as plastic particles smaller than 5mm, are of particular concern in recent years because of their prevalence in the ocean and potential ingestion by marine organisms. Occurrence and distribution of microplastics to the global marine environment include both primary sources (derived from hand and facial cleansers, cosmetic preparations, scrubbers in air-blasting, and production waste from plastic processing plants) and secondary sources (derived from fragmentation of larger plastics as a result of physical and chemical effects). In general, large amounts of plastics end up as marine debris as a result of insufficient treatment capacity, accidental inputs, littering, illegal dumping and coastal human activities. Larger plastic items (meso-and macro-plastics) enter a beach or ocean and undergo mechanical, photo (oxidative) and/ or biological degradation, which breaks them into progressively smaller plastic fragments that eventually become undetectable to the naked eye.

The setup of standardized protocols to quantify particle density is needed in order to compare studies across time (i.e., seasonal and evolving trends) and space (i.e., regional and biogeographic variation).

Within the framework of the Marine Strategy Framework Directive, the Technical Group on Marine Litter (MSFD TG10) has developed guidelines on beach litter monitoring (Galgani et al., 2013) and generally recommends to: (a) quantify microplastics in the size range 20 µm to 5 mm, (b) categorize them according to their physical characteristics including size, shape and colour, (c) obtain information on polymer type, since this can help identify potential sources and pathways, (d) categorize to size bins with a minimum level of resolution of approximately 100 µm (20-100 µm, 101-200 µm, 201- 300 µm etc), (e) all particles in the range 20-100 µm to be subjected to further analysis to confirm identity (e.g. using FT-IR), (f) for particles in the size range 0.1 - 5mm a proportion (for example 10%) of the material in each size class, up to a maximum of 50 items per year or sampling occasion whichever is the least frequent) of the items considered to be microplastics be subjected to further analysis to confirm identity (e.g. using FT-IR). The latter step is important in order to 1) ensure quality control of visual identification and 2) gain information on the relative abundance of different polymer types which can be used to help identify potential sources and pathways leading to the accumulation of microplastics. Regarding the sampling frequency, it is recommended that microparticles in beaches are sampled at the same time as macro-litter on beaches, or in parallel with any other routine intertidal monitoring (e.g. for chemical contaminants, biota). However, detailed power analyses is required in order to determine the adequate number and timing of sampling events for detecting the levels of change (effect size) from the background/natural variability.

More specifically Galgani et al. (2013) recommend that microplastics should be monitored at the front of the shore (strandline) and where available on sandy shores (0.1 – 0.0125 mm diameter), separate samples should be collected to monitor each of two size classes of debris (1-5mm and 20 µm – 1 mm), samples should be collected from the upper 5cm of the beach sediment, a minimum of five replicate samples should be collected starting from the strandline, each replicate should be separated by at least 5 m, while replicates would be distributed in a stratified random manner so as to be representative of an entire beach or a specific section of a beach. Regarding large microplastics (1 – 5mm), they should be sampled using an extension of the protocol for meso debris (5-25mm) by stacking together the 5mm sieve with a further metal sieve of 1mm mesh to achieve volume reduction in the field. Small microplastics (20 µm – 1 mm) should be collected from the top 5 cm of sand using a metal spoon (suggest 15 ml) standardising sampling by volume and collecting approximately 250 ml of sediment. For the extraction of the microplastics they recommend the density separation using a concentrated saline NaCl solution (1.2 g/ml).

The NOAA Marine Debris Program has published in 2015 a more detailed manual with recommendations for extracting and quantifying in the laboratory synthetic particles in beach sediments (Masura et al., 2015). The plastic debris analyzed by this method is considered microplastic and ranges in size from 5 mm to 0.3 mm. The recommended procedure is summarized as follows: weigh 400 g of wet sediment, dry in an oven at 90°C overnight or until sample dryness, determine the dry sample weight, extraction with lithium metatungstate solution (density 1.6 g/mL), hand stir for several minutes, leave to settle, remove any visible solids >5mm with forceps, filter the floating material through a 0.3 mm sieve, oven dry the collected material at 90 $^{o}$C for 24 hours or longer, determine the mass of the dried solids and in case there is a lot of organic material, use the Wet Peroxide Oxidation. Final density separation with 5 M NaCl in a separating funnel, allow solids to settle overnight, visually inspect settled solids for any microplastics and discard, collect floating solids in a clean 0.3 mm custom sieve, allow the sieve to air dry while loosely covered with aluminum foil for 24 hours. Microscopic examination of the solids under a dissecting microscope at 40X magnification, weigh the mass of the microplastics and analysis of microplastics using Fourier-Transform Infrared Spectroscopy.

***Table 2.2.*** *Literature review on beach micro-litter studies published in peer-reviewed journals from 2013 and 2018 (ND: not described).*

| Location | Size | Sampling | | | Extraction | | | | | Identification | Reference |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **Beach zone** | **n** | **Depth (cm)** | **Drying duration/ Temp** | **Extraction process** | **Stirring time/ speed** | **Settling time** | **Repeat extractions** | | |
| Slovenian coast | 250µm-5mm | Entire beach | 3 | 5 | 24 h/60 °C | 1.2 kg/L NaCl | 2 min manually | 30 min | 2 | Visual (microscope) | *Laglbauer et al., 2014* |
| Slovenian coast | 250 µm-5mm | Sublittoral zone | 3 | ND | 24 h/60 °C | 1.2 kg/L NaCl | 2 min manually | 30 min | 2 | Visual (microscope) | *Laglbauer et al., 2014* |
| North-western Adriatic coast, Italy | ≤5mm | High tide mark | 6 | 5 | 48 h/50 °C | Optical | - | - | - | FTIR-ATR | *Munari et al., 2017* |
| Mediterranean coastline, Morocco | 1.25-4.75 mm | Entire beach | 3 | 5 | 1 h/65 °C | Optical | - | - | - | - | *Alshawafi et al., 2017* |
| Northern coast of Crete isl. | ≥2mm | Entire beach | 12-18 | 10 | - | Optical | - | - | - | - | *Karkanorachaki et al., 2018* |
| Mediterranean Sea (Esp, Fr, It, Gr, Tr, Is) | 0.3- 5mm | High tide line | 5 | 5 | 48 h/60 °C | 1.2 kg/L NaCl | 2 min/ 900 rpm | 8 h | 3 | Visual (microscope) and Raman spectroscopy | *Lots et al., 2017* |
| Northern Tunisian coast | ≥1mm | ND | 3 | 2-3 | air | 1.2 kg/L NaCl | 5 min manually | ND | ND | FTIR-ATR | *Abidli et al., 2018* |
| Kea isl., Aegean Sea | 1-2 mm | Upper beach | 3-4 | 3 | ND | 1.2 kg/L NaCl | ND | ND | ND | FTIR-ATR | *Kaberi et al., 2013* |
| Kea isl., Aegean Sea | 2-4 mm | Upper beach | 3-4 | 3 | ND | Optical | - | - | - | Visual and FTIR-ATR | *Kaberi et al., 2013* |
| Samos isl., Greece | ≥1.2 µm | Beach and sublittoral zone | 27 | 0-5,<br>5-10,<br>10-15 cm | ND | 1.2 kg/L NaCl | ND | ND | ND | ND | *De Ruijter et al., 2018* |

Several, partly different, methods of extracting microplastics from beach sediments are described in the literature. Besides the use of NaCl, recommended by Galgani et al (2013), several researchers use solutions (e.g. NaI, $ZnCl_2$) denser than NaCl in order to separate more dense microplastics. An elutriation column which facilitates the separation of microplastic particles from the sediment particles is also used by some researchers (e.g. Hengstmann et al., 2018; Kedzierski et al., 2016 & 2018, etc.). A new, totally different, approach in separating microplastics from environmental samples is based on their electrostatic behaviour (Felsing et al., 2018).

Differentiation from the recommended sampling of surface beach sediments is reported by Turra et al., 2014 in Brazil; Chubarenko et al., 2018 in the Baltic Sea and De Ruijter et al., 2018 in Greece, which tried a three-dimensional distribution of microplastics on sandy beaches.

Regarding, the polymer identification, microplastics can be easily identified as such either using infrared spectroscopy [FTIR] in the ATR mode or Raman spectroscopy. The latter is a relatively easier and faster semi-automated technique. Most Raman instruments are able to match the sample spectra to a spectral library to identify the specific polymer. Raman spectroscopy does not work well with the smaller or dark-colored microplastics and FTIR microscopy may have to be used. An emerging, faster technique for identification of MPs is hyperspectral imaging that yields false-color near infrared images of the particles. Advantage of this technique is that multiple samples can be imaged and identified automatically, simultaneously. Visual identification of MPs despite its simplicity is prone to serious error (Andrady, 2017 and references therein).

A review of the literature on microplastics in beach sediments for the years from 2013 to 2018 showed that the majority of studies examined the spatial distribution (abundance, mass, type, and/or size) of microplastics. Out of at least 35 publications reporting results worldwide, only 8 refer to the Mediterranean region (Tab. 2.2).

There is a lack of quality assurance/quality control (QA/QC) instruments available: e.g. no organisations yet offer proficiency training or testing, there have been no inter-laboratory studies, no certified reference materials are available, no standardized sampling and analysis protocols have been published, no accreditation certificates have been issued and some procedures in use have not yet been validated. Approaches for QA/QC will therefore be very useful for evaluating sources of variability and error and increasing confidence in the data collected. Currently, an inter-calibration exercise is running in the framework of MEDCIS project.

### 2.3. References

Abidli Sami, Joana C. Antunes, Joana L. Ferreira, Youssef Lahbib, Paula Sobral, Najoua Trigui El Menif, 2018. Microplastics in sediments from the littoral zone of the north Tunisian coast (Mediterranean Sea). Estuarine, Coastal and Shelf Science, 205, 1-9.

Alshawafi, A., Analla, M., Alwashali, E., Aksissou, M., 2017. Assessment of marine debris on the coastal wetland of Martil in the North-East of Morocco. Marine Pollution Bulletin, 117, 302–310.

Andrady Anthony L., 2017. The plastic in microplastics: A review. Marine Pollution Bulletin, 119, 12–22.

Chubarenko I.P., E.E. Esiukova, A.V. Bagaev, M.A. Bagaeva, A.N. Grave, 2018. Three-dimensional distribution of anthropogenic microparticles in the body of sandy beaches. Science of the Total Environment, 628–629, 1340–1351.

De Ruijter V.N., Costa V., Miliou A., 2018. Assessment of microplastics distribution in shallow marine sediments in Samos Island, Greece. Book of Abstracts, 12th Panhellenic Symposium of Oceanography & Fisheries, Corfu, Greece, p.216.

Deidun, A., Gaucia, A., Lagorio, S., Galgani, F., 2018. Optimising beached litter monitoring protocols through aerial imagery. Marine Pollution Bulletin, 131, 212–217.

Felsing Stefanie, Christian Kochleus, Sebastian Buchinger, Nicole Brennholt, Friederike Stock, Georg Reifferscheid, 2018. A new approach in separating microplastics from environmental samples based on their electrostatic behaviour. Environmental Pollution, 234, 20-28.

Galgani, F., Hanke, G., Werner, S., Oosterbaan, L., Nilsson, P., Fleet, D., Kinsey, S., Thompson, R.C., Van Franeker, J., Vlachogianni, T., Scoullos, M., Mira Veiga, J., Palatinus, A., Matiddi, M., Maes, T., Korpinen, S., Budziak, A., Leslie, H., Gago, J., Liebezeit, G., 2013. Guidance on Monitoring of Marine Litter in European Seas. Scientific and Technical Research series, Report EUR 26113 EN.

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. Marine Pollution Bulletin, 130, 140–152.

Hengstmann, E., Tamminga, M., vom Bruch, C., Fischer, E.K., 2018. Microplastic in beach sediments of the Isle of Rügen (Baltic Sea) - implementing a novel glass elutriation column. Marine Pollution Bulletin, 126, 263–274.

Kaberi, H., Tsangaris, C., Zeri, C., Mousdis, G., Papadopoulos, A., Streftaris, N., 2013. Microplastics along the shoreline of a Greek island (Kea isl., Aegean Sea): types and densities in relation to beach orientation, characteristics and proximity to sources. In: Proc. 4th Int. Conf. Environ. Manag. Eng. Plan. Econ. SECOTOX Conf. Mykonos island, Greece. June 24–28, 2013, pp. 197–202.

Kedzierski Mikaël, Véronique Le Tilly, Patrick Bourseaua, Guy Césarc, Olivier Sire, Stéphane Bruzaud, 2018. Microplastics elutriation system. Part B: Insight of the next generation. Marine Pollution Bulletin, 133, 9–17.

Kedzierski, M., Le Tilly, V., Bourseau, P., Bellegou, H., Cesar, G., Sire, O., Bruzaud, S., 2016. Microplastics elutriation from sandy sediments: a granulometric approach. Marine Pollution Bulletin, 107, 315–323.

Laglbauer Betty J.L., Rita Melo Franco-Santos, Miguel Andreu-Cazenave, Lisa Brunelli, Maria Papadatou, Andreja Palatinus, Mateja Grego, Tim Deprez, 2014. Macrodebris and microplastics from beaches in Slovenia. Marine Pollution Bulletin, 89, 356–366.

Lots Froukje A.E., Paul Behrens, Martina G. Vijver, Alice A. Horton, Thijs Bosker, 2017. A large-scale investigation of microplastic contamination: Abundance and characteristics of microplastics in European beach sediment. Marine Pollution Bulletin, 123, 219–226.

Masura, J., et al. 2015. Laboratory methods for the analysis of microplastics in the marine environment: recommendations for quantifying synthetic particles in waters and sediments. NOAA Technical Memorandum NOS-OR&R-48.

Maziane, F., Nachite, D., Anfuso, G., 2018. Artificial polymer materials debris characteristics along the Moroccan Mediterranean coast. Marine Pollution Bulletin, 128, 1–7.

Munari, C., Corbau, C., Simeoni, U., Mistri, M., 2016. Marine litter on Mediterranean shores: analysis of composition, spatial distribution and sources in north-western Adriatic beaches. Waste Manage. 49, 483–490.

Pasternak, G., Zviely, D., Ribic, C.A., Ariel, A., Spanier, E., 2017. Sources, composition and spatial distribution of marine debris along the Mediterranean coast of Israel. Marine Pollution Bulletin, 114, 1036–1045.

Poeta, G., Battisti, C., Bazzichetto, M., Acosta, A.T.R., 2016. The cotton buds beach: Marine litter assessment along the Tyrrhenian coast of central Italy following the marine strategy framework directive criteria. Mar. Pollut. Bull. 113 (1-2), 266-270.

Poeta, G., Conti, L., Malavasi, M., Battisti, C., Acosta, A., 2016. Beach litter occurrence in sandy littorals: The potential role of urban areas, rivers and beach users in central Italy. Estuarine, Coastal and Shelf Science, 181, 231-237.

Portman, M.E., Brennan, R.E., 2017. Marine litter from beach-based sources: Case study of an Eastern Mediterranean coastal town. Waste Management, 69, 535–544.

Prevenios M., Zeri C., Tsangaris C., Liubartseva S., Fakiris E., Papatheodorou G., 2018. Beach litter dynamics on Mediterranean coasts: Distinguishing sources and pathways. Marine Pollution Bulletin, 129, 448–457.

Šilc, U., Küzmiča, F., Caković, D., Stešević., D., 2018. Beach litter along various sand dune habitats in the southern Adriatic (E Mediterranean). Marine Pollution Bulletin, 128, 353–360.

Turra Alexander, Aruana B. Manzano, Rodolfo Jasao S. Dias, Michel M. Mahiques, Lucas Barbosa, Danilo Balthazar-Silva & Fabiana T. Moreira, 2014. Three-dimensional distribution of plastic pellets in sandy beaches: shifting paradigms. Scientific Reports, 4, 4435. DOI: 10.1038/srep04435.

Vlachogianni, Th., Fortibuoni, T., Ronchi, F., Zeri, Ch., Mazziotti, C., Tutman, P., Varezić, D.B., Palatinus, A., Trdan, S., 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. Marine Pollution Bulletin, 131(A), 745–756.

# 3. Monitoring marine litter on the sea surface

In the last five years (from 2013 to 2018) a total of 30 studies focusing on marine litter on the sea-surface in the Mediterranean Sea have been published. The present chapter provides an overview of the methods and protocols used in the Mediterranean in order to monitor marine litter floating on the sea surface. Depending on the litter size class monitored, methodologies vary; from visual observations aboard for macro-litter to surveys that make use of nets with different mesh-sizes for sampling micro-litter.

## 3.1. Macro-litter

For macro-litter, visual observation is the most common methodology used and relies on competent and dedicated observers. Direct visual observations need fewer resources, but are fraught with potential biases linked to differences in litter detectability due to observation conditions, litter size classes and observation platforms as well as due to the potential fatigue of the observer. Some of these factors are not so determinant when it comes to automatic recording of floating litter, which has been used in more recent applications and is provided by recording systems specifically set to acquire images from ships, aircrafts or drones. Apart from the 'traditional' RGB cameras, the use of thermic cameras and multi-spectral cameras is also being experimented for automated marine monitoring (Bryson and Williams 2015).

Floating macro-litter surveys have been conducted in several studies in the Mediterranean. These visual surveys have been carried out from different types of vessels (Table 3.1), ranging from small- or medium-sized boats (Di-Méglio and Campana, 2017; Zeri et al., 2018) to large ships (Ryan, 2013), including platforms of opportunity as ferries and cargo ships (ISPRA, 2015; Sà et al., 2016, Arcangeli et al., 2018). In many cases, these surveys are carried out simultaneously with other research surveys such as cetacean observations (Di-Méglio and Campana, 2017).

Observations are generally conducted only from the side of the ship with the best viewing conditions, because variable detection rates depend on the sea state, light conditions and the characteristics of floating objects (Galgani et al., 2013a). In some studies, only those conducted in sea state ≤ 2 Beaufort are taken into account (Di-Méglio and Campana, 2017). Observers scan the sea surface from the deck at a distance from the sea level which may vary according to the type of vessel. For example, sightings from research and sailing vessels are done closer to sea level (~ 5m, Suaria and Aliani, 2014 and ~ 3m, Di-Méglio and Campana, 2017), while distance to sea surface increases in passengers ferries (17–25 m, Arcangeli et al., 2018). These observations can be conducted either through naked eye (Vlachogianni et al., 2017; Zeri et al., 2018) or binoculars (Arcangeli et al., 2018) with different observation periods varying from 30 minutes to 1hour 30 minutes (Suaria and Aliani, 2014, Arcangeli et al., 2018). These visual surveys are tedious for observers, thus reducing the sighting time and increasing the number of replicates (transects) can help to reduce sampling error. Performed transects can vary in time and distance conducted. For example, transects of 30 minutes with a research vessel can have a mean length of 9.21 km (Suaria and Aliani, 2014) while transects conducted from a ferry can have a length of 65 km (Arcangeli et al., 2018). Even though the latter transects have a duration of 1.5 hours, the travelled distance is not three times larger than the 30 minutes transects performed in Suaria and Aliani, 2014.

Macrolitter density in the sea surface has been seen to vary according to the speed of the vessels (Vlachogianni et al., 2017), thus it is important to take into account this parameter. As for neuston net sampling towing speed has a low variation; 2-3.5 knots (Cózar et al., 2015, Ruiz-Orejón et al., 2016), vessels used in visual surveys have a more broad speed range: 6 – 25 knots (Di-Méglio and Campana, 2017, Arcangeli et al., 2018). In general, small boats can cover coastal waters, usually travelling at slow speed and detecting all items with at least one dimension bigger than 2.5 cm by naked eye (e.g.; Di-Méglio and Campana, 2017). The increase of observation height and vessel speed corresponds to a loss of ability to detect small- sized items. Large vessels, on the other hand, can survey large open sea areas and provide data on larger size classes (>20 cm), which are considered adequate indicators for describing spatial patterns over a larger scale (Sà et al., 2016; Arcangeli et al., 2018).

During ship surveys, automated photographs of the sea surface during daylight can be obtained through cameras applied on the bow of the platforms of opportunity such as commercial vessels or cruises (e.g. SeaLitterCAM, Galgani et al., 2013b). The recognition analysis is performed afterwards on the video/images acquired. A standard image analysis includes the characterization of pixels to distinguish water vs floating items, and the evaluation of items discriminability using linear discriminant analyses. Several algorithms for automated image analysis and object detection have been developed and proposed, based on the characterization of pixels and the analysis of colour and shape of objects (e.g. Maire et al., 2013). In this situation, the bias is linked to observation conditions and the post-processing recognition of images. Ship-based, drone and aerial surveys techniques have been tested through field experiments.

At a large monitoring program scale, aerial surveys from aircrafts to estimate the amounts of litter at sea and to locate areas of higher aggregations of floating litter are developed (Lecke-Mitchell & Unger et al. 2014). Aircraft surveys cover large areas but can detect only larger classes of items (i.e. the smallest size limit for aerial detection is ca. 30–40 cm). Aerial surveys are considered valuable for detecting spatial differences in abundance, but the high costs of these surveys prevent from a large replication to monitor changes over time (Galgani et al., 2013a;). Surveys are designed based on the line transect distance sampling technique, although aircraft cruise condition, such as speed and height, can make it difficult to take accurate measures. From aircraft also strip transects are used, especially when a multi-thematic monitoring is performed (e.g. French SAMM monitoring program, Laran et al., 2017). From aircrafts both visual observation and automatic detection techniques can be applied.

Automated recording of floating objects can be obtained through a variety of recording systems applied on Unmanned Aerial Vehicles (UAV) or other remote controlled devices, such as drones that can be used to monitor the presence of marine litter at different spatial scales in the sea surface. The use of these devices presents some advantages when compared to traditional visual techniques: human error of visual surveys is reduced; human risk (for pilots and observers) is reduced, while at the same time survey effort can be increased; the images are recorded permanently allowing subsequent statistic analysis (Bryson and Williams, 2015). The use of UAV for marine monitoring has seen a rapid development in recent years for the floating litter monitoring (e.g. in the INTERREG-MED project MEDSEALITTER), but also for the marine mammal and other marine fauna monitoring (e.g. Adame et al., 2017; Hodgson et al., 2013). Two main categories of UAV can be used for marine monitoring: fixed-wing drones and multicopteres. For all the techniques or platforms, two methods are generally applied to define the area to be monitored, fixed width transects and distance sampling. The first method is applied for density estimation and assumes that all litter is detected within a pre-defined distance from the observer, considering a conservative strip width based on preliminary measures. The distance sampling method assumes to estimate the perpendicular distance to each item to compensate for decreasing detection rate with increasing distance from the observer; separate detection curves should be estimated for different sea states (Ryan, 2013; Suaria & Aliani, 2014). The main constraint of both methods is the accurate definition of the monitored strip width or of the distance and angle between the observer and the items, measures that can be obtained with tools such as an inclinometer, range finder “stick” (Ryan, 2013) and laser range finder.

## 3.2. Micro-litter

To date the monitoring programs proposed by the MSFD TG10 contain the more appropriate methodology for monitoring floating micro-litter, in which the manta trawl net is the main equipment requirement. At the sea surface, micro–litter is typically sampled by surface towing plankton nets with mesh ranging from 0.1 to 0.5 mm, which captures particles limited to the size of the net aperture. Net tow sampling efforts typically capture marine litter particles smaller than 10 mm in size. The vast majority of observations since the 1970s have been made using plankton nets, with broadly similar sampling methodologies but variable reporting units (particle count per area or volume, or mass per area or volume). However, recently the most relevant characteristics of the sampling nets used are the mesh size and the opening area of the net. Mesh sizes ranges from 200 µm to 780 µm, with a majority of the studies (83%) ranging from 200 µm to 333 µm (Table 2). The manta nets is used with a variability of mesh sizes nets (330, 333, 500, 780 µm). The net aperture for rectangular openings of the nets (sea surface) ranged from 0.6 to 0.25 m. The towed speed ranges between 1 to 3.4 knots. It is important to highlight the need to use a net with same mesh size in order to obtain comparable data amongst sampling areas. A source of error for the quantification and abundance of micro-litter and microplastics on particular on the sea surface is the towing speed which needs a coordinated approach in order to reduce variability related to different studies.

Different methods and approaches for the separation of floating micro-litter from plankton and other organic matter are reported in literature. In most studies, samples previously stored (e.g. in formaldehyde solution) are directly examined and sorted under a stereomicroscope. In some studies, instead, samples are sieved and then washed with distilled or ultrapure water in order to remove large litter (Faur et al., 2015; Güven et al., 2017; Schmidt et al., 2017). Collingnon *et al.,* 2014 and Pedrotti *et al.,* 2016 have selected floating debris from the supernatant. A different approach is also reported by Gündoğdu and Çevik, 2017. In this study, the microplastics separation was performed in 5 stages: wet sieving, drying, wet peroxide oxidation, density separation and microscopic examination.

Generally, each sampled litter is characterized and classified by colour, shape and size. Concerning the shape, different methods have been reported to classify the debris shapes. In most studies, different shape categories or a master list of different types of plastic was developed after initial screening of the collected material.

Although the FT-IR spectroscopy technique is considered the most accurate technique for the polymers identification, in most of studies visual identification of micro-particles has been carried out.

***Table 3.1****. Literature review on floating macro-litter studies with visual observation published in peer-reviewed journals from 2013-2018.*

| Location | Type of vessel used | Vessel speed | Transect duration | Observation height | Observation width | Wind speed | Detection limit | Litter items classification list | Quantification units | References |
|---|---|---|---|---|---|---|---|---|---|---|
| **Adriatic Sea, Central and Western Mediterranean** | Research vessel | 10 knots | 0.5 hr | 5 m | - | < 5 Beaufort | > 2 cm | styrofoam / plastic / others | Items/km$^2$ | *Suaria and Aliani, 2014* |
| **Northern Mediterranean (Liguro-Provençal basin)** | Sailing vessel | 6 knots | - | 3 m | 50 m each side | < 2 Beaufort | > 1 cm | styrofoam / plastic / others | Items/km$^2$ | *Di-Méglio and Campana, 2017* |
| **Ligurian Sea, Sardinian-Balearic basin, Bonifacio Strait, Central Tyrrhenian Sea, Sicilian-Sardinian Channels, Adriatic Sea, Ionian Sea** | Ferry | 19–25 knots | 1.5 hr | 17–25 m | 25 - 100 m | < 2 Beaufort | > 20 cm | MSFD TG10 Masterlist | Items/km$^2$ | *Arcangeli et al., 2018* |
| **Western Mediterranean Sea** | Ferry | 19–25 knots | 1.5 hr | 17–25 m | 25 - 100 m | < 2 Beaufort | > 20 cm | MSFD TG10 Masterlist | Items/km$^2$ | *Campana et al., 2018* |
| **Northern Mediterranean, Pelagos Sanctuary** | Research vessel | 3 - 4 knots | 0.5 hr | - | 20 m each side | - | > 2.5 cm | MSFD TG10 Masterlist | Items/m$^2$ | *Fossi et al., 2017* |
| **Mediterranean French Exclusive Economic Zone extended to Pelagos sanctuary** | Aerial survey | 90 knots | - | 183 m | - | - | - | Macrodebris and Fishing debris | Total items | *Darmon et al., 2017* |
| **Coastal Adriatic waters** | Small vessel | 2-3 knots | 1 hr | 1-3.2 m | 6 – 8 m | 1 - 2 Beaufort | > 2.5 cm | MSFD TG10 Masterlist | Items/km$^2$ | *Vlachogianni et al., 2017; Zeri et al., 2018* |
| **Adriatic-Ionian waters** | Ferry | 26-27 knots | 1 hr | 25 m | 50 m<br>75 m<br>100 m | 1.5 Beaufort | > 20 cm | MSFD TG10 Masterlist | Items/km$^2$ | *Vlachogianni et al., 2017; Zeri et al., 2018* |
| **Adriatic Sea** | Research vessel | 10 knots | 0.5 hr | 5 m | - | < 20 knots | > 2.5 cm | - | Items/km$^2$ | *Carlson et al., 2017* |

***Table 3.2.*** *Literature review on floating micro-litter studies published in peer-reviewed journals from 2013-2018.*

| Location | Sampling | | | | | | Identification | References |
|---|---|---|---|---|---|---|---|---|
| | **Compartment** | **Net** | **Mesh size** | **Mouth** | **Vessel speed** | **Time** | | |
| **North Western Mediterranean Sea<br>Tuscan coast** | Sea surface/Water column | Surface samples: Manta-net<br>Vertical hauls: WP2 plankton net | Manta trawl net: 330 μm<br>WP2 net: 200 μm | Manta trawl: 0.5 × 0.25 m<br>WP2: 0.57 m diameter | Manta trawl: 2 – 3 knots<br>WP2: 0 knots | Manta trawl: 20 min<br>WP2: up to 100 m | FTIR | *Baini et al., 2018* |
| **Western Mediterranean Sea<br>PelagosSanctuary** | Sea surface | Manta-net | 330 μm | 0.5 × 0.25 m | 3 – 4 knots | 30 min | FTIR | *Fossi et al., 2017* |
| **Western Mediterranean SeaGulf of Lion** | Sea surface | Manta-net | 780 μm | 0.5 x 0.15 m | 2.5 knots | 20 min | Visual (microscope) | *Schmidt et al., 2017* |
| **Aegean-Levantine Sea, Turkish** | Sea surface | Manta-net | 333 μm | 0.4 x 0.2 m | - | - | FTIR | *Güven et al., 2017* |
| **Aegean-Levantine Sea** | Sea surface | Manta-net | 333 μm | 0.2 × 0.6 m | 2 knots | 15 min | Visual (microscope) | *van der Hal et al., 2017* |
| **Western Mediterranean Sea (Ligurian Sea)** | Sea surface | Neuston net | 200 μm | 0.6 x 0.2 m | 2.5 knots | 60 min | FTIR | *Pedrotti et al., 2016* |
| **Western Mediterranean Sea and Adriatic Sea** | Sea surface | Neuston net | 200 μm | 1 × 0.5 m | 1.5 – 2 knots | 5 min | FTIR-ATR | *Suaria et al., 2016* |
| **Whole Mediterranean** | Sea surface | Manta-net | 333 μm | 0.6 x 0.25 m | 3.13 knots | 15 - 30 min | Visual (microscope) | *Ruiz-Orejón et al., 2016* |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Adriatic Sea** | Sea surface | Neuston net | 300 µm | 0.6 × 0.15 m | 3 knots | 20 min | Chemical analysis | Gajšt et al., 2016 |
| **Western Mediterranean Sea** | Sea surface | Neuston net | 200 µm | 0.6 x 0.25 m | 1.5 knots | 20 min | Visual (microscope) | Fossi et al., 2016 |
| **Aegean-Levantine Sea Turkey** | Sea surface | Manta-net | 333 µm | 0.6 × 0.25 m | 2 knots | 20 min | Visual (microscope) | Gündoğdu and Çevik, 2017 |
| **Western Mediterranean Sea AsinaraNational Park PelagosSanctuary** | Sea surface | WP2 | 200 µm | 57 cm diameter | 0.772 m/s | 20 min | Visual (microscope) | Panti et al., 2015 |
| **Whole Mediterranean** | Sea surface | Neuston net | 200 µm | 1.0 × 0.5 m | 2 – 3 knots | 15 min | Visual (microscope) | Cózar et al., 2015 |
| **Western Mediterranean Sea** | Sea surface | Manta-net | 330 µm | 0.6 × 0.15 m | 1.4 m/s | 45 - 90 min | Visual (microscope) | Faure et al., 2015 |
| **Western Mediterranean Sea (Corsica)** | Sea surface | WP2 0.2 mm | 200 µm | 0.6 x 0.25 m | 2.5 km/h | 20 min | Visual (microscope) | Collignon et al., 2014 |
| **Western Mediterranean Sea (Sardinian coast)** | Sea surface | Manta-net | 500 µm | - | 2 knots | 20 min | Visual (microscope) | de Lucia et al., 2014 |
| **Adriatic and Ionian Seas** | Sea surface | Manta-net | 330 µm. | 0.6 × 0.24 m | < 3 knots | 30 min | Visual (stereomicroscope); ATR-FTIR spectroscopy | Zeri et al., 2018 |

### 3.3. References

Adame, K., Pardo, M. A., Salvadeo, C., Beier, E., Elorriaga-Verplancken, F., 2017. Detectability and categorization of California sea lions using an unmanned aerial vehicle.Marine Mammal Science. DOI: 10.1111/mms.12403.

Alomar, C and Deudero, S. 2017. Evidence of microplastic ingestion in the shark Galeusmelastomus Rafinesque, 1810 in the continental shelf off the western Mediterranean Sea. Environmental Pollution, 223: 223-229. DOI: 10.1016/j.envpol.2017.01.015

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. Marine Pollution Bulletin.

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. Marine Pollution Bulletin, 133, pp. 543-552.

Balderson, S., Martin, L., 2015. Environmental impacts and causation of “beached” Drifting Fish Aggregating Devices around Seychelles Islands: a preliminary report on data collected by Island Conservation Society (No. IOTC–2015–WPEB11–39 1). Island Conservation Society, Mahe,Seychelles

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.Marine pollution bulletin, 109(1), 55-60.

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

Bryson, M., Williams, S., 2015. Review of Unmanned Aerial Systems (UAS) for Marine Surveys. Australian center for Field Robotics, University of Sidney.

Campana, I., Angeletti, D., Crosti, R., Di Miccoli, V., &Arcangeli, A., 2018. Seasonal patterns of floating macro-litter across the Western Mediterranean Sea: a potential threat for cetacean species. RendicontiLincei. ScienzeFisiche e Naturali, 1-15.

Campanale et al., 2017.Visual sightings of floating debris in the Mediterranean Sea.Conference Paper ·Oct 2017; SETAC EUROPE 27th Annual Meeting.

Carlson DF, Suaria G, Aliani S, et al. Combining Litter Observations with a Regional Ocean Model to Identify Sources and Sinks of Floating Debris in a Semi-enclosed Basin: The Adriatic Sea. Front Mar Sci. 2017;4(April):1-16.

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). Marine Pollution Bulletin, 79(1-2), 293-298.

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. PLoSONE 10 (4), 1–12.

Darmon G, Miaud C, Claro F, Doremus G, Galgani F. Risk assessment reveals high exposure of sea turtles to marine debris in French Mediterranean and metropolitan Atlantic waters. Deep Res Part II Top Stud Oceanogr. 2017; 141(August): 319-328. doi:10.1016/j.dsr2.2016.07.005.

Davies T., Curnick D., Barde J., Emmanuel Chassot E. 2017. Potential environmental impacts caused by beaching of drifting fish aggregating devices and identification of management solutions and uncertainties. IOTC-2017-WGFAD01-08 Rev_1 (1-18)

De Lucia, G.A., Caliani, I., Marra, S., Camedda, A., Coppa, S., Alcaro, L., et al., 2014. Amount and distribution of neustonic micro-plastic off the Western Sardinian coast (Central-Western Mediterranean Sea). Mar. Environ. Res. 100, 10–16.

Deudero, S and Alomar, C. 2015. Mediterranean marine biodiversity under threat: reviewing influence of marine litter on species. Marine Pollution Bulletin, Volume 98, Issues 1–2, 15 September 2015, Pages 58–68. doi: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. Marine Pollution Bulletin 118, 155-166.

Directive (EU) 2015/720 of the European Parliament and of the Council of 29 April 2015 amending Directive 94/62/EC as regards reducing the consumption of lightweight plastic carrier bags.

Directive (EU) 2017/845 of 17 May 2017 amending Directive 2008/56/EC of the European Parliament and of the Council as regards the indicative lists of elements to be taken into account for the preparation of marine strategies.

Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. Official Journal L 327 , 22/12/2000 P. 0001 – 0073.

Directive, A. (1994).94/62/EC on packaging and packaging waste. Available on the Internet: http://europa. eu. int/scadplus/leg/en/lvb/l21207. htm. Cited, 10, 02-05.

Faur, 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 (16), 12190–12197.

Fossi M.C., Romeo T., Panti C., Baini M., 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. Frontiers in Marine Sciences, doi: 10.3389/fmars.2017.00167, 4 (167).

Fossi, M. C., Marsili, L., Baini, M., Giannetti, M., Coppola, D., Guerranti, C., Galgani, F., Hanke, G., Werner, S. D. V. L., & De Vrees, L., 2013a.Marine litter within the European marine strategy framework directive. ICES Journal of Marine Science, 70(6), 1055-1064.

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). Marine Pollution Bulletin,64(11), 2374-2379

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., UrbánRamírez, 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.

Gajšt, T., Bizjak, T., Palatinus, A., Liubartseva, S., Kržan, A., 2016. Sea surface microplastics in Slovenian part of the northern Adriatic. Mar. Pollut. Bull. 113, 392–399.

Galgani, F., Hanke, G., Werner, S. D. V. L., & De Vrees, L., 2013a. Marine litter within the European marine strategy framework directive. ICES Journal of Marine Science, 70(6), 1055-1064.

Galgani, F., Hervé, G., Carlon, R., 2013b. Wavegliding for marine litter.Rapport Commission International MerMéditerranée 40, 306.

Gündoğdu, S., Çevik, C., 2017. Micro- and mesoplastics in northeast Levantine coast of Turkey: the preliminary results from surface samples. Mar. Pollut. Bull. 118, 341–347. https://doi.org/10.1016/j.marpolbul.2017.03.002.

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.

Hodgson, A., Kelly, N., Peel, D., 2013. Unmanned Aerial Vehicles (UAVs) for surveying marine fauna: A dugong case study. PLoS ONE 8(11): e79556.

Hollman, P. C., Bouwmeester, H., & Peters, R. J. B., 2013.Microplastics in aquatic food chain: sources, measurement, occurrence and potential health risks (No. 2013.003). Rikilt-Institute of Food Safety.

ISPRA, 2015. Technical Annex II Marine litter & Marine macro-fauna Protocol for the agreement 'Fixed Line Transect using ferries as platform of observation for monitoring cetacean populations' Protocol for monitoring by vessel of floating marine macro-litter and marine macro-fauna along a fixed transect width. ISPRA, Rome, 10 pp.

Jambeck, J. R., 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. Science 347(6223), 768-771.

Kalogerakis, N., Kiparissis, S., Yiantzi, E., Perraki, T., Kalogerakis, G.C., Fodelianakis, S., Fava, F., Psillakis, E. 2014. PCBs and PAHs on plastic pellets and microplastics collected on the coastline of the island of Crete in eastern Mediterranean sea - Monitoring and fate. Proceedings of the 37th AMOP Technical Seminar on Environmental Contamination and Response, pp. 175-183.

Laran S., Pettex E., Authier M., Blanck A., David L., Doremus G., Falchetto H., Monestiez P., Van Canneyt O.&Ridoux V., 2017. Seasonal distribution and abundance of cetaceans within French waters-Part I: The North-Western Mediterranean, including the Pelagos sanctuary. Deep Sea Research Part II.

Liubartseva, S., Coppini, G., Lecci, R., Clementi, E., 2018.Tracking plastics in the Mediterranean: 2D Lagrangian model. Mar. Pollut. Bull. 129, 151–162. https://doi.org/10.1016/j.marpolbul.2018.02.019.

Maire, F., Mejias, L., Hodgson, A., Duclos, G., 2013.Proceedings of the 2013 IEEE/RSJ International Conference on Intelligent Robots and Systems. November 3-7, 2013. Tokyo, Japan.

MAP/UNEP, 2001. Litter Management in coastal zones of the Mediterranean Basin - Analysis of the questionnaire and proposals for guidelines. Document UNEP(DEC)/MED WG.183/4.

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. Environmental Chemistry, 12 (5), pp. 618-626.

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

Rochman, C.M., Hoh, E., Kurobe, T., Teh, S.J., 2013a. Ingested plastic transferscontaminantsto fish and induces hepatic stress. Nat. Sci. Rep. 3, 3263. http:// dx.doi.org/10.1038/srep03263

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.

Ryan, P. G., 2013. A simple technique for counting marine debris at sea reveals steep litter gradients between the Straits of Malacca and the Bay of Bengal. Marine Pollution Bulletin 69(1), 128-136.

Sá, S., Bastos-Santos, J., Araújo, H., Ferreira, M., Duro, V., Alves, F., Panta-Ferreira, B., Nicolau, L., Eira, C., Vingada, J., 2016.Spatial distribution of floating marine debris in offshore continental Portuguese waters, Marine Pollution Bulletin 104, 269–278.

Schmidt N., Thibault D., Galgani F., Paluselli A., Sempéré R. 2017. Occurrence of microplastics in surface waters of the Gulf of Lion (NW Mediterranean Sea). Progress in Oceanography, https://doi.org/10.1016/j.pocean.2017.11.010

Suaria, G., Aliani, S., 2014.Floating debris in the Mediterranean. Marine Pollution Bulletin 86 (1-2), 494-504.

Suaria, G., Avio, C.G., Lattin, G.L., Regoli, F., Aliani, S., 2015.Neustonicmicroplastics in the Southern Adriatic Sea.Preliminary results.Micro 2015. In: Seminar of the Defishgear Project, Abstract Book (Piran), 42.

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. Nature Sci. Rep. 6, 37551.http://dx.doi.org/10.1038/srep37551.

Thiel, M., Hinojosa, I.A., Miranda, L., Pantoja, J.F., Rivadeneira, M.M., Vásquez, N., 2013. Anthropogenic marine debris in the coastal environment: a multi-year comparison between coastal waters and local shores. Mar. Pollut. Bull. 71, 307–316.

UNEP, 2015.Marine Litter Assessment in the Mediterranean.UNEP/MAP Athens, 45 pp.

UNEP/MAP Regional Plan for Marine litter Management in the Mediterranean (UNEP(DEPI)/MED IG.21/09), Athens,2013.

Unger, B., Herr, H., Gilles, A., Siebert, U., 2014. Evaluation of spatio-temporal distribution patterns of marine debris in the SCI Sylt Outer Reef. 28th Conference of the European Cetacean Society, Liège (Belgium).

van der Hal, N., Ariel, A., Angel, D.L. 2017. Exceptionally high abundances of microplastics in the oligotrophic Israeli Mediterranean coastal waters. Marine Pollution Bulletin, 116 (1-2), pp. 151-155.

Veiga, J.M., Fleet, D., Kinsey, S., Nilsson, P., Vlachogianni, T., Werner, S., Galgani, F., Thompson, R.C., Dagevos, J., Gago, J., Sobral, P., Cronin, R., 2016.Identifying Sources of Marine Litter.MSFD GES TG Marine Litter Thematic Report; JRC Technical Report; EUR 28309.

Viršek, M. K., Lovšin, M. N., Koren, Š., Kržan, A., &Peterlin, M., 2017. Microplastics as a vector for the transport of the bacterial fish pathogen species Aeromonassalmonicida. Mar Pollut Bull, 125(1-2): 301-309.

Vlachogianni, Th., Zeri, Ch., Ronchi, F., Fortibuoni, T., Anastasopoulou, A., 2017. Marine Litter Assessment in the Adriatic and Ionian Seas. IPA-Adriatic DeFishGear Project, MIO-ECSDE, HCMR and ISPRA. pp. 180 (ISBN: 978-960-6793-25-7).Zambianchi, E., Trani, M., Falco, P., 2017.Lagrangian transport of marine litter in the Mediterranean Sea. Front. Environ. Sci. 5. https://doi.org/10.3389/fenvs.2017.00005.

Zampoukas, N., Palialexis, A., Duffek, A., Graveland, J., Giorgi, G., Hagebro, C., Hanke, G., Korpinen, S., Tasker, M., Tornero, V., Abaza, V., Battaglia, P., Caparis, M., Dekeling, R., Frias Vega, M., Haarich, M., Katsanevakis, S., Klein, H., Krzyminski, W., Laamanen, M., Le Gac, J.C., Leppanen, J.M., Lips, U., Maes, T., Magaletti, E., Malcolm, S., Marques, J.M., Mihail, O., Moxon, R., O'Brien, C., Panagiotidis, P., Penna, M., Piroddi, C., Probst, W.N., Raicevich, S., Trabucco, B., Tunesi, L., Van der Graaf, S., Weiss, A., Wernersson, A.S., Zevenboom, W., 2014. Technical guidance on monitoring for the Marine Strategy Framework Directive. JRC Scientific and Policy Report. Scientific and Technical Research series, Report EUR 26499.

Zeri, C., Adamopoulou, A., Bojanic Varezic, D., Fortibuoni, T., Kovac Virsek, M., Krzan, A., Mandic, M., Mazziotti, C., Palatinus, A., Peterlin, M., Prvan, M., Ronchi, F., Siljic, J., Tutman, P., Vlachogianni, Th., 2018. Floating plastics in Adriatic waters (Mediterranean Sea): From the macro- to the micro-scale. Marine Pollution Bulletin, 136, 341-350.

# 4. Monitoring marine litter on the sea floor

## 4.1. Macro-litter

Less than 20 studies were conducted since 2013 (see Table 4.1) in the Mediterranean Sea, and very few covered extensive geographic areas or considerable depths. Today, much of the existing data on seafloor marine litter comes from trawl surveys conducted through the experimental trawl fishery vessels (e.g. the MEDITS project).

Simple protocols based on existing trawling surveys, and on diving and video imagery are the most applied approaches when others such as acoustic remain experimental. The most common approaches to evaluate sea-floor litter use opportunistic sampling. This type of sampling is usually coupled with fisheries surveys and programmes focusing on biodiversity monitoring, since methods for determining seafloor litter distributions (e.g. trawling, diving, video) are similar to those used for benthic biodiversity monitoring and assessment. The use of submersibles or Remotely Operated Vehicles (ROVs) is another applied approach for deep sea areas although this requires expensive equipment.

Monitoring programmes for demersal fish stocks, undertaken as part of the Mediterranean International Bottom Trawl Surveys, operate at large regional scale and provide data using a harmonized protocol, which may provide a consistent support for monitoring litter at regional sea scale on a regular basis. Within the European MEDITS a protocol for marine litter assessments using trawling programmes has been developed. This protocol harmonizes the steps and procedures needed to provide an accurate methodology applicable for MSFD monitoring (facilitating the evaluation of sources, trends, data analysis, etc.)

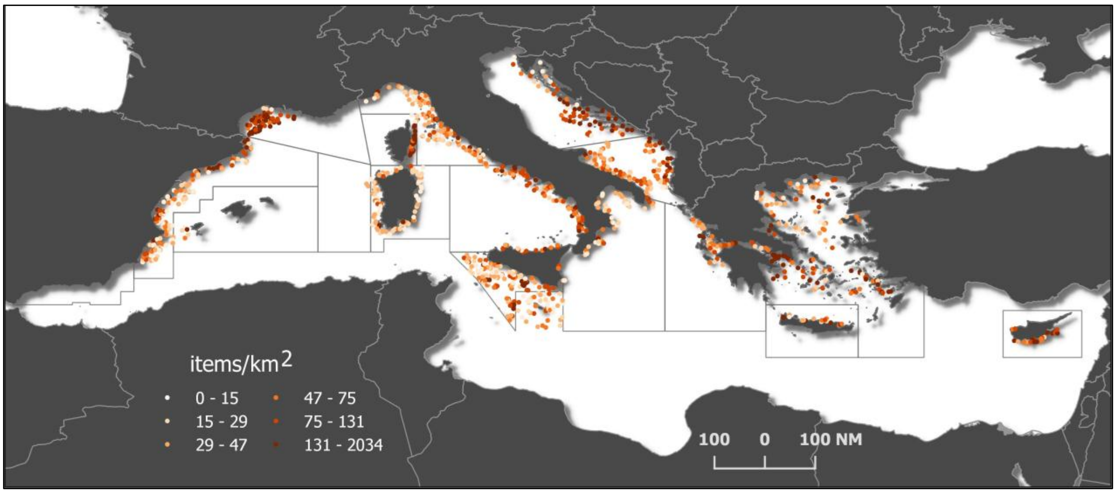

***Figure 3.1.*** *Harmonized monitoring of plastic litter in the northern Mediterranean Sea performed by 12 institutions in countries. Data was collected in 1279 surveyed stations sampled during fish stocks assessment cruises (MEDITS project) using the same protocol and trawl net. Results are expressed as plastic densities (items per* $Km^2$*). (Spedicato et al., 2018)*

For shallow waters, information is obtained from on-going monitoring of benthic species during camera surveys, and through regular diving activities (Galgani et al., 2013) usually coupled with regular surveys on biodiversity (marine reserve, offshore platforms, etc.), since methods for determining seafloor litter distributions (e.g. trawling, diving, video) are similar to those used for benthic biodiversity assessments.

Trawling (otter or beam trawl) is an efficient method for large scale evaluation and monitoring of seafloor litter. It enables the control of mesh sizes and opening width (Galgani et al., 2013). However, sampling restrictions in rocky areas (incompatible with trawling) may lead to underestimation of the quantities present. Pole trawling, working deeper in the sediment and down to the abyssal plain where only soft bottom occur, provide more consistent evaluations. Amongst the advantages, monitoring programmes for demersal fish stocks operate at large regional scale and provide data using a harmonized protocol, which may provide a consistent support for monitoring.

If pole trawling may support locally some experiments in the deep sea, only some countries will have the possibility to use submersibles or Remotely Operated Vehicles (ROVs). This will be done giving however priority to coastal canyons and through an opportunistic approach in order to limit costs. This approach is of great use for non-accessible areas such as areas far beyond the commonly used fishing grounds (sandy bottoms) and the continental shelf and extend the assessment of marine litter in bathyal environments, reaching the maximum depths from the Mediterranean Sea. Optical surveys can provide a precise geolocalisation of items as well as additional information about the local environmental and the biological setting, but only for visible macroscopic litter. Image-based surveys, with non-contiguous images, does not capture however the full range of the heterogeneity of litter distribution.

Several studies have analysed still images, a sort of sub-sample of video surveys (Tubau et al., 2013, Pham et al., 2014), whereas other have analysed a continuous video (Pham et al., 2014) or a combination of still and video images (Fabri et al., 2014). In any case, quantification of the area sampled or observed is an issue as an estimation of the absolute size of an image or the evaluation of the surface requires lasers or precise information about the position and attitude of the vehicle and the camera.

ROVs are adequate tools to monitor specifically the interactions between litter and marine organisms (Angiolillo et al. 2015; Bo et al., 2013, 2014; Consoli et al., 2018; Melli et al., 2017). For litter, ALDFG may represent almost 100% of total debris, especially in fishing grounds and fishing lines represent a large part of entanglement records (Consoli et al., 2018). Marine Strategy Framework Directive for European waters and the UNEP/MAP regional plan that have both included entanglement in the list of impacts may monitor harm through this approach.

Typically, monitoring of marine litter through fish stock assessment cruises is based on the record of different types of items that are categorized using the MSFD sea floor litter template. 29 different categories, derived from the MSFD master list (Galgani et al., 2013) enable to characterize some specific types such as single use plastics and fishing related litter. As a counterpart, ROV surveys are usually not categorizing items following the MSFD recommendations, since more experimental and more often dedicated to studies on biodiversity. Nevertheless, surveys are often recording individual items with highly relevant associated information such as pictures, interaction with organisms or associated substrate.

It must be noted that the interpretation of results from monitoring marine litter on the sea floor is made difficult because the ageing of plastics at depth is not known and the presence of debris on the sea floor may have begun before specific scientific investigations started in the 1990s. Due to the persistence of certain litter materials, the monitoring of litter on the sea floor must consider accumulation processes of past decades and does not permit to evaluate fluxes as cleaning is often not possible. Timescales of observation should therefore be adapted, requiring multiannual frequencies for deep sea floor surveys.

Reporting units are depending on the method/approach used. Sampling through diving or imagery (ROV) will use items/ units of distance (100 m, 1 km) whereas trawling will enable units to be reported by surface (items/ha or km$^2$).

***Table 4.1.*** *Literature review on seafloor macro-litter studies published in peer-reviewed journals from 2013-2018.*

| Location | Habitat | Sampling | Classification list | units | Depth | References |
|---|---|---|---|---|---|---|
| Santa Maria di Leuca, Italy | Shelf/slopes | 10 transects, Towed Camera | N/A | Items/10 $m^2$ | 386–1179 m | *D' Onghia et al., 2017* |
| Western Mediterranean Sea (Sardinia Island, Italy) | Continental shelves/slope | GOC73 experimental bottom trawl, 18.4 km2 covered experimental trawl MEDITS | N/A | Items/$km^2$ | 0-800 | *Alvito et al., 2018* |
| Thyrenian Sea | rocky bottoms/ slope | 69 video transects, 26 areas, 6.03 km2 | N/A | Items/$km^2$ | 30–300 m | *Angiolilo et al., 2015* |
| Ligurian and Tyrrhenian seas (western Mediterranean Sea) | Rocky banks | 69 video transects, 4 sites, 2930 m2 samples | N/A | Items/100 $m^2$ | 70 - 280 m | *Bo et al., 2014* |
| Ligurian and Tyrrhenian seas (western Mediterranean Sea) | Hard bottom, rocky surface | ROV, 7 sites, 500 m long linear video transect, 1000 m2 sample | N/A | Items/m of survey | 50 – 150 m | *Cánovas-Molina et al., 2016* |
| Sardinia (Central Western Mediterranean) | canyons | 17 sites, 29 ROV surveys (1-4 dives per site) | N/A | Items/ $m^2$ | 100 – 480 m | *Cau et al., 2017* |
| Greece(Gulf of Kuşadası) | continental shelf | Trawl | N/A | Items/$km^2$ | 9 -210 m | *Cerim et al, 2014* |
| Greece (Gulf of Güllük) | continental shelf | Trawl | N/A | Items/$km^2$ | 9 -210 m | *Cerim et al, 2014* |
| Greece (Gulf of Gökova) | continental shelf | Trawl | N/A | Items/$km^2$ | 9 -210 m | *Cerim et al, 2014* |
| Greece (Marmaris) | continental shelf | Trawl | N/A | Items/$km^2$ | 9 -210 m | *Cerim et al, 2014* |
| Sicily | rocky banks | 19 ROV transect, | N/A | Items/$km^2$ | 20-200 | *Consoli et al. 2018* |
| North eastern Mediterranean, Mersin Bay (Turkey) | continental shelf | 132 hauls, demersal trawls | N/A | Kg/ha | 19 – 178 m | *Eryasar et al, 2014* |
| Mediterranean sea | Bathyal/abyssal | 292 tows, Otter/agasiz trawl, 12 mm mesh | N/A | Kg/$km^2$ | 900-3000m | *Ramirez-Llodra 2013* |
| France-Mediterranean | slopes | 17 canyons, 101 ROV dives | N/A | Items/km survey | 80-700m | *Fabri et al., 2014* |
| Straits of Sicily, Italy | Soft bottoms | 120 hauls | N/A | Items/$km^2$ | 10-800 | *Fiorentino et al., 2015* |
| Western Mediterranean Sea (Gulf of Alicante) | continental shelf/slope | 886 hauls, otter trawl (237 km2) | N/A | Items/$km^2$ | 50–700 m | *García-Rivera et al, 2017* |
| Mediterranean, Southern France | shelves & canyons | 90 sites (trawls, 0.045 km2/tow, 20 mm mesh) | N/A | Items/$km^2$ | 0-800 m | *Gerigny et al., 2018* |
| Turkey ( Antalaya) | Soft bottoms/ bathyal | Trawl, 32 stations, 24 mm mesh, 0.27- 0.82 km2/haul | N/A | Items/$km^2$ | 200-800m | *Guven et al., 2013* |
| Greece (Saronikos, Patras, Echinades) | Soft bottoms | 69 hauls , 10,9 km2, 50 mm mesh | N/A | Items/$km^2$ | 20-350 | *Ioakemidis et al., 2014* |
| Greece (saronikos) | Rocky/slopes | ROV, 0.007 km2 | N/A | Items/$km^2$ | 94-115 | *Ioakemidis et al., 2015* |

| | | | | | | |
|---|---|---|---|---|---|---|
| Greece (Argolikos) | Soft bottoms | 8 hauls, 0.8 km2 | N/A | Items/km$^2$ | 150-750 | *Ioakemidis, 2015* |
| North-western Mediterranean Sea | Canyon | ROV, 21 immersions, 41 hours 20 minutes | N/A | % of site with litter | 79 – 401 m | *Lastras et al., 2016* |
| Morocco (from Cap Boujdor to Cap Blanc) | Continental shelves/slope | 100 hauls, scientific trawling survey, 47,541 km2 | N/A | Kg/km$^2$ | 10-266 m | *Loulad et al, 2017* |
| Sud-east Adriatic Sea | Mixed bottom (hard and soft) | SCUBA DIVERS, 47 DIVE, 0,02 km2 | N/A | Items/1000 m$^2$ | 0-40 | *Macic et al., 2017* |
| North-western Mediterranean Sea (Blanes, Gulf of Valencia, Eivissa Channel) | Canyons, seamounts and landslides | ROV, 7 transects, 30 h video | N/A | Recroded items | 60 – 800 m | *Mecho et al., 2017* |
| Adriatic Sea, Italy | Mixed bottom (hard and soft) | 17 ROV transects, 19.5 km of total lenght | N/A | Items/100 m$^2$ | 21-23 | *Melli et al., 2016* |
| North western Adriatic Sea | Rocky outcrops bottom | 2 expeditions with oceanographic vessel, ROV; 17 transectes | N/A | Items/100 m2 | 15-40 m | *Melli et al., 2017* |
| Maltese islands | continental shelf | 44 hauls, Otter Trawl | N/A | Items/km$^2$ | 49-697 m | *Misfud et al, 2013* |
| North and central Adriatic Sea | Soft bottoms | rapido trawl at 67 hauls, 1.9 km2 | N/A | Items/km$^2$ | 0-100 | *Pasquini et al., 2016* |
| Central Mediterranean Sea (Calabrian slope) | Continental slope | Trawl,18.9 ha covered), 4 samples | N/A | Kg/ha | 1400 m (mean) | *Pham et al., 2014* |
| Western Mediterranean Sea | Continental slope | Trawl, 56 ha covered, 8 samples | N/A | Kg/ha | 1500 m (mean) | *Pham et al., 2014* |
| Eastern Mediterranean Sea (Crete-Rhodes Ridge) | Continental slope | Trawl, 37.9 ha covered, 8 samples | N/A | Kg/ha | 1500 m (mean) | *Pham et al., 2014* |
| North-western Mediterranean Sea (Blanes) | Continental slope | Trawl, 407 ha covered, 94 samples | N/A | Kg/ha | 1387 m (mean) | *Pham et al., 2014* |
| North-western Mediterranean Sea (Gulf of Lion) | Continental shelf | Trawl, 276.4 ha covered, 52 samples | N/A | Items/ha | 85 m (mean) | *Pham et al., 2014* |
| North-western Mediterranean Sea (Blanes) | Canyons | ROV, 2 ha covered, 4 samples<br>Trawl, 33.9 ha covered, 13 samples | N/A | Items/ha | 1496 m (mean ROV)<br>1431 m (mean Trawl) | Pham et al., 2014 |
| North-western Mediterranean Sea (Gulf of Lion) | Canyons | Trawl, 126.5 ha covered, 11 samples | N/A | Items/ha | 510 m (mean) | *Pham et al., 2014* |
| Western Mediterranean Sea (Algero-Balearic Basin) | Deep Basin | Trawl, 16 ha covered, 3 samples | N/A | Kg/ha | 2883 m (mean) | *Pham et al., 2014* |
| Eastern Mediterranean Sea (Crete-Rhodes Ridge) | Deep Basin | Trawl, 2.8 ha covered, 2 samples | N/A | Kg/ha | 3000 m (mean) | *Pham et al., 2014* |
| Central Mediterranean Sea (Calabrian Basin) | Deep Basin | Trawl, 12.5 ha covered, 3 samples | N/A | Kg/ha | 2967 m (mean) | *Pham et al., 2014* |
| Thyrenian sea | Fishing grounds | 6 x 1.5 ha samples, trawl, 10mm mesh | N/A | Items/km$^2$ | 40-80m | *Sanchez et al., 2013* |

| | | | | | | |
|---|---|---|---|---|---|---|
| Spain-Mediterranean | Fishing grounds | 6 x 1.5 ha samples, trawl, 10mm mesh | N/A | Items/km$^2$ | 40-80m | *Sanchez et al., 2013* |
| Northern and central Adriatic Sea | shelves | 67 hauls (beam trawl), 4.3 km2 | N/A | Items/km$^2$ | 0-100 | *Strafella et al., 2015* |
| NW Mediterranean sea | Canyons | ROV, 26 dives | N/A | Items/km$^2$ | 140-1730 | *Tubau et al., 2015* |
| Adriatic and Ionian Seas | Mixed bottom (hard and soft) | 121 hauls with several mesh size | MSFD TG10 Masterlist | Items/km$^2$ | 10-281 | *Vlachogianni et al 2017* |
| Adriatic Sea | Mixed bottom (hard and soft) | 38 visual transects by SCUBA divers and snorkeking | MSFD TG10 Masterlist | Items/ 100 m$^2$ | 3-24 | *Vlachogianni et al 2017* |

### 4.2. Micro-litter

Most of the reviewed studies focus on quantifying the micro-plastics abundance in the marine environment. Sediment sampling can require significantly more effort and resources because of the depth. The observed variations in environmental samples are largely due to many factors, including a large diversity in the type and size of particles, the locations examined (e.g. proximity to sources), the sample matrix, the patchy distribution of microplastics and sampling conditions (Alomar et al., 2016).

Because of land-based emissions, including wastewater treatment plants (WWTPs), rivers, urban and industrial coastal centres, the selection of sampling site will have a significant impact of the abundance and type of plastic surveyed. General sampling strategies are based on bulk or volume reduced, depending on the type of microplastic accumulation being investigated, using box corer or sediment grab, and considering the importance of the upper layer with largest percentage found near surface.

The most common approach is to extract plastic particles from sediment using a density separation based on the difference in density between plastic and sediment particles, typically by agitating the sediment sample in concentrated salt solutions (Van Cauwenberghe et al., 2013) to obtain higher densities. These modifications result in an increased extraction efficiency for high-density micro-plastics such as polyvinylchloride or polyethylene terephthalate.

Visual examination is the most common method used to assess size and quantities of microplastics, although it can have a relatively high error rate (Gesamp, 2016). Various imaging approaches, such as zooscan may be practical for the visualization or counting of microplastic particles, with the potential to enable large numbers of samples to be analysed rapidly. Methods that identify the type (pellets, filaments, plastic films, granules, etc.), shape (cylindrical, sheets, etc.), state (eroded, broken, etc.) and colour must be used on regular basis, when electron microscopy may provide higher resolution but cannot be used to determine polymer type.

For the identification of polymers, calculation of specific density, Fourrier Transform-Infra Red (FTIR), attenuated total reflectance (ATR) Fourrier Transform-Infra Red or “deep Raman” spectroscopy, pyrolysis-gas chromatography-mass spectrometry (Pyr-GC-MS), Scanning Electron Microscopy – Energy-Dispersive X-ray Spectroscopy (SEM-EDS), Focal Plane Array-Based Reflectance Micro-FT-IR Imaging, FT-IR /imaging and thermogravimetry (TGA) have been applied, each of them with advantages or limitations (Van Cauwenberghe et al. 2013; Rocha-Santos and Duarte, 2015, Gesamp, 2016). Selecting suitable and comparable quantification and identification methods for microplastics is crucial for evaluating concentrations of and risks due to microplastic pollution. The most appropriate methods remain to be determined for further harmonization and use on regular basis, understanding that visual identification alone is inappropriate for studies on particles below 100 µm (Gesamp, 2016).

Further research on methods needs to consider sampling design in terms of (i) the number and the size of replicates, (ii) the spatial area and the frequency of sampling, (iii) the method used for sampling (i.e. type of core for sediment samples), and (iv) methods used for identification of micro-plastics. There is also a need to mitigate airborne contamination.

Studies sampling an area (using quadrants) will often report abundances per unit of surface ($m^2$). If bulk samples from the surface to a specific depth are taken, the reporting unit is $m^3$. Detailed information on the density of the sediment is required. Additionally, within studies reporting weight, a distinction must be made among those reporting wet (sediment) weight and those reporting dry weight. This adds to the constraints of converting from weight to volume units, or vice versa. Sediment samples from different locations or even different zones on one beach have different water content. Therefore, some authors choose to express micro-plastic abundance per sediment as dry weight to eliminate this variable (Van Cauwenberghe et al., 2013; Vianello et al., 2013).

***Table 4.2.*** *Literature review on microplastics in deep sediments related studies published in peer-reviewed journals from 2013 to 2018.*

| **Location** | **Sampling** | **Depth** | **Laboratory analysis** | **References** |
|---|---|---|---|---|
| **32"22.90 N 31"43.130 E** | 25 $cm^2$ Core sampling, 1-5mm | 1176-4848 | Density (NaCl) separation, visual counts, 4 categories (fibres, pellets, films, spherical) | *Van cauwernberghe et al., 2013* |
| **NW basin, canyons & slope** | Canyons/slopes/abyssal plain, ROV/core sampling, 0.32-5mm | 300-3500 | Density (NaCl) separation, visual counts, fibers & particles separation, FTIR analysis | *Woodall et al., 2014* |
| **Eolian Islands** | Undisturbed sediment (5 cm depth) collected by scientific scuba divers, using wide mouth glass jars | 30 | Surface sediment, sieving, visual observation, MSFD categories (5) | *Fastelli et al., 2016* |
| **Malta** | 0.1-m2 van Veen grab at eight sampling stations | 4-22 | Density (NaCl) separation, visual counts, 3 categories (fibrous, rounded and irregular) | *Romeo et al., 2015* |
| **Croatia** | sediment collected by scientific scuba divers, using wide mouth glass jars in 10 sites. Three replicates for each site | 3-15 | Density (NaCl) separation of sieved fractions, MSFD categories (5) | *Blǎsković et al., 2017* |
| **Balearic Islands** | Superficial core sampling (0-3.5 cm) with scuba diving, 1- 5 mm | 8-10 m | Density (NaCl) separation of sieved fractions, MSFD categories (5) | *Alomar et al., 2016* |

## 4.3. References

Alomar, C., Estarellas, F., Deudero, S., 2016. Microplastics in the Mediterranean Sea: Deposition in coastal shallow sediments, spatial variation and preferential grain size. Marine Environmental Research 115, 1-10.

Alvito A., Bellodi, A., Cau, A., Moccia, D., Mulas, A., Palmas, F., Pesci, P., Follesa, M. C., 2018. Amount and distribution of benthic marine litter along Sardinian fishing grounds (CW Mediterranean Sea). Waste Management 75: 131-140.

Andrady, A. L., 2015. Persistence of plastic litter in the oceans. In M. Bergmann., L. Gutow, M. Klages (Eds.), Marine anthropogenic litter (pp. 57–72). Berlin, Springer.

Angiolillo M, Lorenzo B, Farcomeni A, Bo M, Bavestrello G, Santangelo G, Cau A, Mastascusa V, Cau A, Sacco F, Canese S, 2015. Distribution and assessment of marine debris in the deep Tyrrhenian Sea (NW Mediterranean Sea, Italy). Mar Pollut Bull 92:149–159

Blăsković, A., Fastelli, P., Čižmek, H., Guerranti, C., Renzi, M., 2017. Plastic litter in sediments from the Croatian marine protected area of the natural park of Telaščica bay (Adriatic Sea). Mar. Pollut. Bull. 114, 583–586.

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.

Cánovas-Molina, A., Montefalcone, M., Bavestrello, G., Cau, A., Bianchi, C.N., Morri, C., Canese, S., Bo, M. 2016. A new ecological index for the status of mesophotic megabenthic assemblages in the Mediterranean based on ROV photography and video footage. Continental Shelf Research, 121, pp. 13-20

Cau, A., Alvito, A., Moccia, D., Canese, S., Pusceddu, A., Rita, C., Angiolillo, M., Follesa, M.C., 2017. Submarine canyons along the upper Sardinian slope (Central Western Mediterranean) as repositories for derelict fishing gears. Marine Pollution Bulletin, 123 (1-2), pp. 357-36.

Cerim, H., Filiz, H., Gülşahin, A. and Erdem, M., 2014. Marine Litter: Composition in Eastern Aegean Coasts. Open Access Library Journal, 1: e573.

Consoli P., F. Andaloro, C. Altobelli, P. Battaglia, S. Campagnuolo, S. Canese, L. Castriota, T. Cillari, M. Falautano, C. Peda, P. Perzia, P. Sinopoli, P. Vivona, G. Scotti, V. Esposito, F. Galgani, T. Romeo, 2018. 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. Environmental Pollution, 236, 405-415.

D'Onghia G., C. Calculli, F. Capezzuto, R. Carlucci, A. Carluccio, A. GrehanA. Indennidate, P. Maiorano, F. Mastrototaro, A. Pollice, T. Russo, A. Savini, L. Sion, A. Tursi, 2017. Anthropogenic impact in the Santa Maria di Leuca cold-water coral province (Mediterranean Sea): Observations and conservation straits. Deep Sea Research Part II: Topical Studies in Oceanography 145, 87-101.

Eryasar AR, Ozbilgin H, Gucu AC, Sakinan S, 2014. Marine debris in bottom trawl cages and their effects on the selectivity grids in the north eastern Mediterranean. Mar Pollut Bull 81:80–84.http://dx.doi.org/10.1016/j.marpolbul.2014.02.017

Fabri, M.C., Pedel, L., Beuck, L., Galgani, F., Hebbeln, D., Freiwald, A., 2014. Megafauna of vulnerable marine ecosystems in French mediterranean submarine canyons: spatial distribution and anthropogenic impacts. Deep-Sea Res. Part II-Top. Stud. Oceanogr. 104 (0), 184–207.

Fastelli P, Blašković A, Bernardi G, Romeo T, Čižmek H, Andaloro F, Russo GF, Guerranti C, Renzi M. 2016. Plastic litter in sediments from a marine area likely to become protected (Aeolian Archipelago's islands, Tyrrhenian Sea).Marine Pollution Bulletin; 113, 1; 526-529.

Fiorentino, F., Gancitano, V., Giusto, G.B., Massi, D. Sinacori, G., Titone, A., Vinci, A., Garofalo, G., 2015. Marine litter on trawlable bottoms of the Strait of Sicily. Biol. Mar. Medit. 22 (1), 225-228.

García-Rivera S, Sánchez Lizaso JL, Bellido Millán JM, 2017: Composition, spatial distribution and sources of macro-marine litter on the Gulf of Alicante seafloor (Spanish Mediterranean). Mar Pollut Bull 121:249–259, 2017.

Gerigny O., M. Brun, M.C Fabri, C. Tomasino, A. Jadaud and F. Galgani, 2018. Seafloor litter in the Mediterranean sea: quantities, distribution and typology in the French marine waters. Marine Pollution Bulletin, Special Volume 6th IMDC, *Submitted.*

GESAMP, 2016. Sources, fate and effects of microplastics in the marine environment: part two of a global assessment. (Kershaw, P.J., and Rochman, C.M., eds). (IMO/FAO/UNESCO-

IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection). Rep. Stud. GESAMP No. 93, 220 p.

Guven O, Gu¨lyavuz H, Deval MC, 2013. Benthic debris accumulation in bathyal grounds in the Antalya Bay, Eastern Mediterranean. Turk J Fish Aquat Sci 13:43–49

Ioakeimidis C, F Galgani & G Papatheodorou, 2017. Occurrence of Marine Litter in the Marine Environment: A World Panorama of Floating and Seafloor Plastics. In H. Takada and H.K. Karapanagioti (eds.), Hazardous Chemicals Associated with Plastics in the Marine Environment, Hdb Env Chem, DOI 10.1007/698_2017_22, ©Springer International, Publishing AG 2017, 29 pages

Ioakeimidis C, Papatheodorou G, Fermeli G, Streftaris N, Papathanassiou E, 2015. Use of ROV for assessing marine litter on the seafloor of Saronikos Gulf (Greece): a way to fill data gaps and deliver environmental education. SpringerPlus 4:463

Ioakeimidis C, Zeri C, Kaberi H, Galatchi M, Antoniadis K, Streftaris N, Galgani F, Papathanassiou E, Papatheodorou G, 2014. A comparative study of marine litter on the seafloor of coastal areas in the Eastern Mediterranean and Black Seas. Mar Pollut Bull 89: 296–304

Lastras G, Canals M, Ballesteros E, Gili J-M, Sanchez-Vidal A. 2016. Cold-Water Corals and Anthropogenic Impacts in La Fonera Submarine Canyon Head, Northwestern Mediterranean Sea. PLoS ONE 11(5): e0155729. https://doi.org/10.1371/journal.pone.0155729

Lopez-Lopez, L., González-Irusta, J., Punzon, A., Serrano, A., 2017. Benthic litter distribution on circalittoral and deep sea bottoms of the southern Bay of Biscay: Analysis of potential drivers. Continental Shelf Research, 144, 112–119

Loulad S, Houssa R, Rhinane H, Boumaaz A, Benazzouz A, 2017. Spatial distribution of marine debris on the seafloor of Moroccan waters. Mar Pollut Bull 124, 303-313.

Macic, V., Mandic, M., Pestoric, B., Gacic, Z., & Paunovic, M., 2017. First assessment of marine litter in shallow south-east Adriatic Sea. Fresen. Environ. Bull. 26 (7), 4834–4840.

Mecho, A., Aguzzi, J., De Mol, B., Lastras, G., Ramirez-Llodra, E., Bahamon, N., Company, J.B., Canals, M. 2017. Visual faunistic exploration of geomorphological human-impacted deep-sea areas of the north-western Mediterranean Sea. Journal of the Marine Biological Association of the United Kingdom, pp. 1-12.

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 (2), 821-830.

Misfud R, Dimech M, Schembri PJ, 2013. Marine litter from circalittoral and deeper bottoms off the Maltese islands (Central Mediterranean). Mediterr Mar Sci 14:298–308, 2013.

Pasquini, G., Ronchi, F., Strafella, P., Scarcella, G., Fortibuoni, T., 2016. Seabed litter composition, distribution and sources in the Northern and Central Adriatic Sea (Mediterranean). Waste Manage. 58, 41-51.

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., Isidro, E., Jones, D.O., Lastras, G., Morato, T., Gomes-Pereira, J.N., Purser, A., Stewart, H., Tojeira, 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.

Pham, C.K., Ramirez-Llodra, E., Alt, C.H.S., 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., Tojeira, 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), art. no. e95839,

Ramirez-Llodra E, De Mol B, Company JB, Coll M, Sarda` F, 2013. Effects of natural and anthropogenic processes in the distribution of marine litter in the deep Mediterranean Sea. Prog Oceanogr 118:273–287.

Rocha-Santos, T. and A. C. Duarte, 2015. A critical overview of the analytical approaches to the occurrence, the fate and the behavior of microplastics in the environment. TrAC Trends in Analytical Chemistry, 65: 47-53.

Romeo, T., D'Alessandro, M., Esposito, V., Scotti, G., Berto, D., Formalewicz, M., Noventa, S., Giuliani, S., Macchia, S., Sartori, D., Mazzola, A., Andaloro, F., Giacobbe, S., Deidun, A., Renzi, M., 2015b. Environmental quality assessment of Grand Harbour (Valletta, Maltese Islands): a case study of a busy harbour in the Central Mediterranean Sea. Environ. Monit. Assess. 187, 4950–4953.

Sánchez P., Masó M., Sáez R.,.De Juan S., Muntadas A., M. Demestre, 2013. Baseline study of the istribution of marine debris on soft-bottom habitats associated with trawling grounds in the northernMediterranean. Scientia Marina 77(2), 247-255, Barcelona (Spain) ISSN: 0214-8358

Spedicato M.T., W. Zupa, P. Carbonara, A. Esteban, F. Fiorentino, M.C. Follesa, A. Cau, F. Galgani, C. Garcia, A. Jadaud, C. Ioakeimidis, I.Isajlovic, G. Lazarakis, E. Lefkaditou, G. Lembo, M. Mandic, P. Maiorano3, R. Micallef, M. Sartini, F. Serena, I. Thasitis, 2018. Spatial distribution of marine macro-litter on the seafloor in the northern Mediterranean Sea: the MEDITS initiative. Scientia marina, *In press*

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

Tubau, X., Canals, M., Lastras, G., Rayo, X., Rivera, J., Amblas, D., 2015. Marine litter on thefloor of deep submarine canyons of the Northwestern Mediterranean Sea: the role of hydrodynamic processes. Prog. Oceanogr. 134 (0), 379–403.

UNEP, 2015. Marine Litter Assessment in the Mediterranean, UNEP/MAP, Athens, 2015, 45 pages. ISBN No: 978-92-807-3564-2

Van Cauwenberghe, L., et al., 2013. Microplastic pollution in deep-sea sediments. Environ Pollut 182: 495-499.

Vianello, A., et al., 2013. Microplastic particles in sediments of Lagoon of Venice, Italy: First observations on occurrence, spatial patterns and identification. Estuarine, Coastal and Shelf Science 130: 54-61.

Vlachogianni, Th., Anastasopoulou, A., Fortibuoni, T., Ronchi, F., Zeri, Ch., 2017. Marine Litter Assessment in the Adriatic and Ionian Seas. IPA-Adriatic DeFishGear Project, MIO-ECSDE, HCMR and ISPRA. pp. 168 (ISBN: 978-960-6793-25-7).

Woodall, L. C., et al., 2014. The deep sea is a major sink for microplastic debris. R Soc Open Sci 1(4): 140317.

# 5. Monitoring marine litter in rivers/river outflows

## 5.1. Macro-, meso- and micro-litter

Riverine litter refers to litter present in rivers and on riverbanks. The rivers act as pathways which collect litter from run-off and direct input, transporting it towards the aquatic and marine environment. Litter may also remain in the river catchment, to possibly be released at a later date in its entirety or after physical degradation.

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.

As reported in 2016 by González et al., different monitoring methods are used in two environmental compartments: river water bodies and riverbanks. For a river water body, the river water surface can be monitored by visual observation and image acquisition, while collection methodologies of the water column include the use of retaining structures and sampling using grids, nets and filtration systems (with different mesh sizes and openings ) at different water depths. Riverbank monitoring comprises the observation and eventual collection of litter items and sediment samples from the riverbanks.

***Table 5.1.*** *Main methodologies for monitoring litter by size categories in different compartments of a river (González et al., 2016).*

| Size category | River water body | River bank |
|---|---|---|
| MACRO | Visual observation | Visual observation + Collection |
| | Automated image acquisition systems | |
| | Retaining structures (e.g. dams) | |
| | Riverbed and bottom nets | |
| | Booms / floats | |
| MESO | Booms / floats | Visual observation + Collection |
| | Manta trawl / net | |
| MICRO | Manta trawl / net | Sediment samples |
| | Pumps | |

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 first method used a manta net (mesh size 330 μm), especially modified for monitoring in rivers. The second applied the pump-mantanet method, where water was pumped through the manta-net, providing results on litter in suspension; and the third method used a sampler that contained two metal nets (for surface and suspension sampling) with a mesh size of 3.2 mm.

The mantanet and the pump-mantanet samples were treated in the same analytical way. The samples were cleaned with the use of sieves. Microlitter was first removed from the samples using stereomicroscopes and micro tweezers. Particles were dried and put in closed petri dishes for image analysis and chemical analysis. Each particle was photographed under the microscope. Chemical analysis from the categories fragments and pellets of particles was performed using Near Infrared spectroscopy (NIR). Each particle was categorized according to the MSFD TG 10 Master List.

The analysis of the samples obtained with the third method was performed using visual identification. Each particle was first categorized according to the MSFD TG 10 Master List, and after that its size and colour (by the MSFD TG 10 Master List) was also determined.

## 5.2. References

González, D., Hanke, G., Tweehuysen, G., Bellert, B., Holzhauer, M., Palatinus, A., Hohenblum, P., Oosterbaan, L. 2016. Riverine Litter Monitoring - Options and Recommendations. MSFD GES TG Marine Litter Thematic Report; JRC Technical Report; EUR 28307.

Van der Wal, M., van der Meulen, M., Tweehuysen, G., Peterlin, M., Palatinus, A., Kovač-Viršek, M., Coscia, L., Kržan, A., 2015. SFRA0025: Identification and Assessment of Riverine Input of (Marine) Litter. Eunomia Research & Consulting Ltd.

# 6. Monitoring marine litter in biota

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 through e.g. abrasion of coral reefs from fishing gear and disruption of species colonies, reduced oxygenation or 'smothering' of species communities as well as through release and diffusion of toxic compounds that can potentially lead to bio-accumulation and bio-magnification of toxics.

Among the different interactions, ingestion is the most studied impact (some 40 papers have been published in the last 5 years) and several methodological approaches have been developed and adopted according to the species and the litter size classes investigated. On the contrary, entanglement and coverage/colonization are far less studied (some 20 papers for each type of harm have been published). Fewer studies have actually focused on the impact of marine litter on organisms inhabiting Mediterranean pelagic and coastal MPAs, and different methodologies are applied to detect the possible effects related to the interaction with marine litter.

## 6.1. Ingestion

The ingestion of marine litter has been reported worldwide in various organisms ranging from invertebrates to vertebrates, including endangered species (Kühn et al., 2015; Werner et al., 2016; Wright et al., 2013). This phenomenon can be explained in different ways: marine organisms may ingest litter deliberately because of their resemblance to prey (Campani et al., 2013; Cole et al., 2011; Romeo et al., 2016; Wright et al., 2013) or accidentally while feeding on their prey, e.g. by filter feeding (Fossi et al., 2014, Fossi et al 2016) or hunting on shoals (Battaglia et al., 2016; Romeo et al., 2015), or as a result of secondary ingestion (debris already ingested by prey). Depending on the litter size and on the species, marine litter particles may be excreted or accumulated in the gastrointestinal tract, and cause physical and mechanical damages, such as abrasion and obstruction of gastrointestinal tract, inflammation, blockage of feeding appendages or filters, (Cole et al., 2011; Li et al., 2016; Pedà et al., 2016; Wright et al., 2013) or malnutrition and pseudo-satiation resulting in a reduced food intake (Kühn et al., 2015; Romeo et al., 2017). In cases where the gastrointestinal tract becomes completely blocked or severely damaged, marine litter ingestion may lead directly to the death of the organism (Werner et al., 2016). Moreover, the ingestion of plastic debris represents a serious hazard for vertically migrant micronekton due to the buoyancy of this material, which may hinder their movements in the water column (Romeo et al. 2016).

Marine litter, in particular plastic litter, may also represent a direct and indirect vehicle for the introduction of chemical substances into the marine biota and the food-web, although this issue is still debated (Koelmans et al., 2016), with potential impacts on human health (Barboza et al., 2018). These chemicals could become available for organisms (Rios et al., 2007; Rochman et al., 2014) and can enter cells, chemically interacting with biological molecules and causing endocrine system disruptions (Teuten et al., 2009). Therefore biological consequences such as oestrogenic effects (Sonnenschein and Soto, 1998) and reduction of testosterone production (Foster, 2006) might occur at species level. Additionally, the sub-lethal and chronic effects of these chemicals could likely compromise populations and communities and have long term consequences.

### 6.1.1. Species investigated

From 2013 to 2018, more than 40 papers on the incidence of marine litter ingestion in marine organisms in the Mediterranean basin have been published. Most of the research was carried out on the Western Mediterranean Sea, whereas the Ionian Sea and the Central Mediterranean Sea, the Adriatic Sea, and the Aegean Levantine Sea were less investigated. Over the same period, in these studies litter ingestion has been investigated on 94 Mediterranean species, belonging to different taxonomic groups including invertebrates, fish, sea turtles, seabirds and marine mammals (Figure 6.1 and Table 6.1).

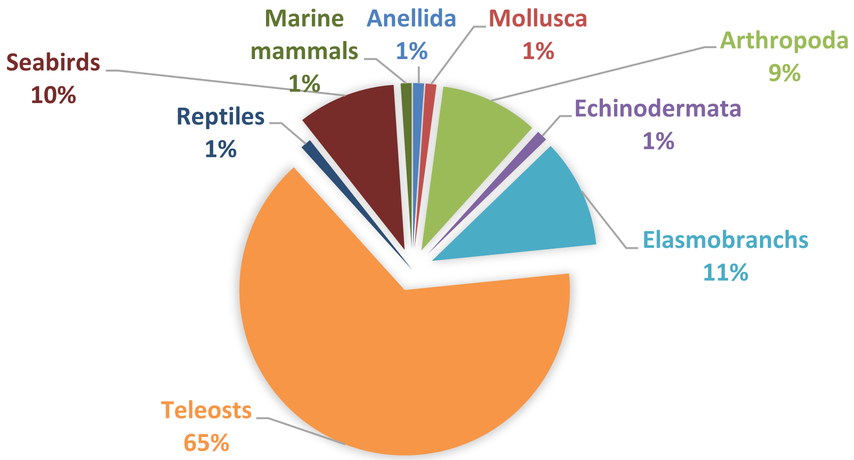

***Figure 6.1.*** *Percentage of species investigated among different taxa from 2013 to 2018 on marine litter ingestion in the Mediterranean Sea.*

Among these in only 74 out of 94 species the ingestion of marine litter items has been documented. While fish species represent the majority of the affected species (60%), also a considerable number of endangered species have been reported to ingest marine litter. All Mediterranean turtles (*Caretta caretta*, *Chelonia mydas* and *Dermochelys coriacea)* and some marine mammals (*Physeter microcephalus, Balaenoptera physalus, Tursiops truncatus, Grampus griseus and Stenella coeruleoalba*) were found to be affected by debris ingestion in published studies. Most studies on these endangered species dealt with stranded individuals. Marine litter ingestion in seabirds is a well-documented phenomenon on a global scale, as reported by Laist (1997) and Kühn et al. (2015), whereas the presence of marine debris in several bird species belonging to the Procellariiformes, Suliformes and Charadriiformes orders in the Mediterranean basin has been studied only by Codina-García et al. (2013) (Table 6.1). The few studies available on marine litter ingestion by marine invertebrates (Alomar et al., 2016; Digka et al., 2018; Fossi et al., 2014; Gusmão et al., 2016; Remy et al., 2015; Vandermeersch et al., 2015) investigated several species belonging to the Annelids, Crustaceans, Echinoderms and Molluscs (Table 6.1).

Understanding fully the impact of litter on marine organisms is a challenging task and its one of the main aim of the Plastic Busters MPAS project. In order to compare results of different areas and to adopt targeted management strategies and measures, it is very important to assess the impacts of litter on marine organisms via the use of a common approach (Fossi et al. 2018), linking the detection of the ingested marine litter to the physical and toxicological effects. In this sense, bioindicator species at regional and local scale with both ecological and commercial interest should be considered (Fossi et al., 2018).

***Table 6.1.*** *Overview of published studies investigating the potential ingestion of marine litter by different species* in the Mediterranean area (2013-2018), divided by taxa.*

*(a) Elasmobranchs*

| Taxa | Species | Habitat | Method | Litter size | Polymer id | Reference |
|---|---|---|---|---|---|---|
| **Elasmobranchs** | *Galeus melastomus* | demersal | Dissection and stereomicroscope | Macro | No | *Anastasopoulou et al. , 2013* |
| | | | Dissection and stereomicroscope | Micro | No | *Cartes et al., 2016* |
| | | | Dissection and stereomicroscope | Micro | Yes | *Alomar & Deudero, 2017* |
| **Elasmobranchs** | *Scyliorhinus canicula* | demersal | Dissection and stereomicroscope | Macro | No | *Anastasopoulou et al., 2013* |
| **Elasmobranchs** | *Pteroplatytrygon violacea* | pelagic | Dissection and stereomicroscope | Macro | No | *Anastasopoulou et al., 2013* |
| **Elasmobranchs** | *Raja clavata* | demersal | Dissection and stereomicroscope | Macro | No | *Anastasopoulou et al., 2013* |
| **Elasmobranchs** | *Raja oxyrinchus* | demersal | Dissection and stereomicroscope | Macro | No | *Anastasopoulou et al., 2013* |
| **Elasmobranchs** | *Centrophorus granulosus* | demersal | Dissection and stereomicroscope | Macro | No | *Anastasopoulou et al., 2013* |
| **Elasmobranchs** | *Centroscymnus coelolepis* | demersal | Dissection and stereomicroscope | Micro | No | *Cartes et al., 2016* |
| **Elasmobranchs** | | | | | | |
| **Elasmobranchs** | *Etmopterus spinax* | demersal | Dissection and stereomicroscope | Macro | No | *Anastasopoulou et al., 2013* |
| **Elasmobranchs** | | | Dissection and stereomicroscope | Micro | No | *Cartes et al., 2016* |
| **Elasmobranchs** | *Squalus acanthias* | demersal | Dissection, digestion (15% H2O2) and observation using stereomicroscope | Micro | Yes | *Avio et al., 2015* |
| **Elasmobranchs** | | | Dissection and stereomicroscope | Macro | No | *Anastasopoulou et al., 2013* |
| **Elasmobranchs** | *Squalus blainville* | demersal | Dissection and stereomicroscope | Macro | No | *Anastasopoulou et al., 2013* |

---

* *In only 74 out of 94 species the ingestion of marine litter items has been documented.*

*(b) Teleosts*

| Taxa | *Species* | Habitat | Method | Litter size | Polymer id | Reference |
|---|---|---|---|---|---|---|
| **Teleosts** | *Sudis hyalina* | Mesopelagic | Dissection and stereomicroscope | Macro | No | *Anastasopoulou et al., 2013* |
| **Teleosts** | *Conger conger* | Demersal | Dissection and stereomicroscope | Macro | No | *Anastasopoulou et al., 2013* |
| **Teleosts** | *Nettastoma melanurum* | Demersal | Dissection and stereomicroscope | Macro | No | *Anastasopoulou et al., 2013* |
| **Teleosts** | | | Dissection and stereomicroscope | Micro | No | *Cartes et al., 2016* |
| **Teleosts** | *Saurida undosquamis* | Demersal | Dissection, digestion (35% hydrogen peroxide) and stereomicroscope | Micro | No | *Güven et al., 2017* |
| **Teleosts** | *Engraulis encrasicolus* | Pelagic | Dissection and stereomicroscope | Micro | Yes | *Compa et al., 2018* |
| | | | Dissection, digestion (NaClO) and stereomicroscope | Micro | Yes | *Collard et al., 2015* |
| **Teleosts** | *Sardina pilchardus* | Pelagic | Dissection, digestion (15% H2O2) and stereomicroscope | Micro | Yes | *Avio et al., 2015* |
| | | | Dissection, digestion (35% hydrogen peroxide) and stereomicroscope | Micro | No | *Güven et al., 2017* |
| | | | Dissection and stereomicroscope/Dissection, digestion (30% H2O2) and stereomicroscope | Macro/Micro | No | *Anastasopoulou et al., 2013* |
| | | | Dissection, digestion (30% H2O2) and observation using stereomicroscope | Micro | Yes | *Digka et al., 2016* |
| | | | Dissection and stereomicroscope | Micro | Yes | *Compa et al., 2018* |
| **Teleosts** | *Merluccius merluccius* | Benthopelagic | Dissection, digestion (15% H2O2) and stereomicroscope | Micro | Yes | *Avio et al., 2015* |
| **Teleosts** | | | Dissection and stereomicroscope | Macro | No | *Anastasopoulou et al., 2013* |
| **Teleosts** | *Micromesistius poutassou* | Benthopelagic | Dissection and stereomicroscope | Macro | No | |
| **Teleosts** | *Molva macrophthalma* | Demersal | Dissection and stereomicroscope | Macro | No | |
| **Teleosts** | *Mora moro* | Demersal | Dissection and stereomicroscope | Macro | No | *Anastasopoulou et al., 2013* |
| **Teleosts** | | | Dissection and stereomicroscope | Micro | No | *Cartes et al., 2016* |
| **Teleosts** | *Phycis blennoides* | Demersal | Dissection and stereomicroscope | Micro | No | *Cartes et al., 2017* |
| **Teleosts** | *Phycis blennoides* | Demersal | Dissection and stereomicroscope | Macro | No | *Anastasopoulou et al., 2013* |
| **Teleosts** | *Trachyrincus scabrus* | Demersal | Dissection and stereomicroscope | Macro | No | *Cartes et al., 2016* |
| **Teleosts** | *Diaphus metopoclampus* | Benthopelagic | Dissection and stereomicroscope | Micro/ Macro | No | *Romeo et al., 2016* |
| **Teleosts** | *Electrona risso* | Mesopelagic | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Teleosts** | *Hygophum benoiti* | Mesopelagic | | | | |
| **Teleosts** | *Myctophum punctatum* | Mesopelagic | | | | |
| **Teleosts** | *Alepocephalus rostratus* | Demersal | Dissection and stereomicroscope | Micro | No | *Cartes et al., 2016* |
| **Teleosts** | *Cataetyx laticeps* | Demersal | | | | |
| **Teleosts** | *Argyrosomus regius* | Benthopelagic | Dissection, digestion (35% hydrogen peroxide) and stereomicroscope | Micro | No | *Güven et al., 2017* |
| **Teleosts** | *Boops boops* | Benthopelagic | Dissection and stereomicroscope | Micro | No | *Nadal et al., 2016* |
| **Teleosts** | *Brama brama* | Pelagic | Dissection and stereomicroscope | Macro | No | *Anastasopoulou et al., 2013* |
| **Teleosts** | *Caranx crysos* | Pelagic | Dissection, digestion (35% hydrogen peroxide) and stereomicroscope | Micro | No | *Güven et al., 2017* |
| **Teleosts** | *Dentex dentex* | Benthopelagic | Dissection, digestion (35% hydrogen peroxide) and stereomicroscope | Micro | No | *Güven et al., 2018* |
| **Teleosts** | *Dentex gibbosus* | Benthopelagic | Dissection, digestion (35% hydrogen peroxide) and stereomicroscope | Micro | No | *Güven et al., 2019* |
| **Teleosts** | *Diplodus annularis* | Demersal | Dissection, digestion (35% hydrogen peroxide) and stereomicroscope | Micro | No | *Güven et al., 2020* |
| **Teleosts** | *Epigonus telescopus* | Benthopelagic | Dissection and stereomicroscope | Macro | No | *Anastasopoulou et al., 2013* |
| **Teleosts** | *Lepidopus caudatus* | Pelagic | Dissection and stereomicroscope | Macro | No | *Anastasopoulou et al., 2013* |
| **Teleosts** | *Lithognathus mormyrus* | Demersal | Dissection, digestion (35% hydrogen peroxide) and stereomicroscope | Micro | No | *Güven et al., 2017* |
| **Teleosts** | *Liza aurata* | Benthopelagic | Dissection, digestion (35% hydrogen peroxide) and stereomicroscope | Micro | No | *Güven et al., 2018* |
| **Teleosts** | *Mullus barbatus* | Demersal | Dissection, digestion (35% hydrogen peroxide) and stereomicroscope | Micro | No | *Güven et al., 2019* |
| | | | Dissection, digestion (15% H2O2) and stereomicroscope | Micro | Yes | *Avio et al., 2015* |
| | | | Dissection, digestion (NaOH) and observation using stereomicroscope | Micro | No | *Bellas et al., 2016* |
| | | | Dissection and stereomicroscope/Dissection, digestion (30% H2O2) and stereomicroscope | Macro/Micro | No | *Anastasopoulou et al., 2018* |
| | | | Dissection, digestion (30% H2O2) and stereomicroscope | Micro | Yes | *Digka et al., 2018* |
| **Teleosts** | *Mullus surmuletus* | Demersal | Dissection and stereomicroscope | Micro | Yes | *Alomar et al., 2017* |
| | | | Dissection, digestion (35% hydrogen peroxide) and stereomicroscope | Micro | No | *Güven et al., 2017* |
| **Teleosts** | *Nemipterus randalli* | Demersal | Dissection, digestion (35% hydrogen peroxide) and stereomicroscope | Micro | No | *Güven et al., 2018* |
| **Teleosts** | *Pagellus acarne* | Benthopelagic | Dissection, digestion (35% hydrogen peroxide) and stereomicroscope | Micro | No | *Güven et al., 2019* |
| **Teleosts** | *Pagellus bogaraveo* | Benthopelagic | Dissection and observation using stereomicroscope | Macro | No | *Anastasopoulou et al., 2013* |
| **Teleosts** | *Pagellus erythrinus* | Demersal | Dissection, digestion (35% hydrogen peroxide) and stereomicroscope | Micro | No | *Güven et al., 2017* |
| | | | Dissection and stereomicroscope/Dissection, | Macro/Micro | No | *Anastasopoulou et al., 2018* |

|  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|
|  |  |  | digestion (30% H2O2) and stereomicroscope |  |  |  |
|  |  |  | Dissection, digestion (30% H2O2) and observation using stereomicroscope | Micro | Yes | *Digka et al., 2018* |
| **Teleosts** | *Pagrus pagrus* | Demersal | Dissection, digestion (35% hydrogen peroxide) and stereomicroscope | Micro | No | *Güven et al., 2017* |
| **Teleosts** | *Pelates quadrilineatus* | Demersal | Dissection, digestion (35% hydrogen peroxide) and stereomicroscope | Micro | No | *Güven et al., 2018* |
| **Teleosts** | *Polyprion americanus* | Pelagic / demersal | Dissection and observation using stereomicroscope | Macro | No | *Anastasopoulou et al., 2013* |
| **Teleosts** | *Pomadasys incisus* | Demersal | Dissection, digestion (35% hydrogen peroxide) and stereomicroscope | Micro | No | *Güven et al. 2017* |
| **Teleosts** | *Schedophilus ovalis* | Pelagic /bathipelagic | Dissection and stereomicroscope | Macro | No | *Anastasopoulou et al., 2013* |
| **Teleosts** | *Sciaena umbra* | Demersal | Dissection, digestion (35% hydrogen peroxide) and stereomicroscope | Micro | No | *Güven et al., 2017* |
| **Teleosts** | *Scomber japonicus* | Pelagic | Dissection, digestion (35% hydrogen peroxide) and stereomicroscope | Micro | No | *Güven et al., 2018* |
|  |  |  | Dissection and stereomicroscope/Dissection, digestion (30% H2O2) and stereomicroscope | Macro/Micro | No | *Anastasopoulou et al., 2018* |
| **Teleosts** | *Serranus cabrilla* | Demersal | Dissection, digestion (35% hydrogen peroxide) and stereomicroscope | Micro | No | *Güven et al., 2017* |
| **Teleosts** | *Siganus luridus* | Demersal | Dissection, digestion (35% hydrogen peroxide) and stereomicroscope | Micro | No | *Güven et al., 2018* |
| **Teleosts** | *Sparus aurata* | Demersal | Dissection, digestion (35% hydrogen peroxide) and stereomicroscope | Micro | No | *Güven et al., 2019* |
| **Teleosts** | *Thunnus alalunga* | Pelagic | Dissection and stereomicroscope | Micro/Macro | No | *Romeo et al., 2015* |
| **Teleosts** | *Thunnus thynnus* | Pelagic | Dissection and stereomicroscope | Micro/Macro | No | *Romeo et al., 2016* |
| **Teleosts** | *Trachinotus ovatus* | Pelagic | Dissection and stereomicroscope | Micro | No | *Battaglia et al., 2016* |
| **Teleosts** | *Trachurus mediterraneus* | Pelagic | Dissection, digestion (35% hydrogen peroxide) and stereomicroscope | Micro | No | *Güven et al., 2017* |
| **Teleosts** | *Trachurus trachurus* | Pelagic | Dissection and stereomicroscope/Dissection, digestion (30% H2O2) and stereomicroscope | Macro/Micro | No | *Anastasopoulou et al., 2018* |
| **Teleosts** | *Umbrina cirrosa* | Demersal | Dissection, digestion (35% hydrogen peroxide) and stereomicroscope | Micro | No | *Güven et al., 2017* |
| **Teleosts** | *Upeneus moluccensis* | Demersal | Dissection, digestion (35% hydrogen peroxide) and stereomicroscope | Micro | No | *Güven et al., 2018* |
| **Teleosts** | *Upeneus pori* | Demersal | Dissection, digestion (35% hydrogen peroxide) and stereomicroscope | Micro | No | *Güven et al., 2019* |
| **Teleosts** | *Xiphias gladius* | Pelagic | Dissection and stereomicroscope | Micro/ Macro | No | *Romeo et al., 2015* |
| **Teleosts** | *Xiphias gladius* | Pelagic | Dissection and stereomicroscope | Macro | No | *Anastasopoulou et al.,2013* |
| **Teleosts** | *Citharus linguatula* | Benthic | Dissection and stereomicroscope/Dissection, digestion (30% H2O2) andstereomicroscope | Macro/Micro | No | *Anastasopoulou et al., 2018* |
| **Teleosts** | *Solea solea* | Benthic | Dissection and stereomicroscope/Dissection, digestion (30% H2O2) andstereomicroscope | Macro/Micro | No | *Anastasopoulou et al., 2019* |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | Dissection, digestion (10% KOH), separation (NaI) and stereomicroscope | Micro | Yes | *Pellini et al., 2018* |
| **Teleosts** | *Chelidonichthys lucerna* | Benthic | Dissection, digestion (15% $H_2O_2$) and stereomicroscope | Micro | Yes | *Avio et al., 2015* |
| **Teleosts** | *Helicolenus dactylopterus* | Benthic | Dissection and stereomicroscope | Macro | No | *Anastasopoulou et al., 2013* |
| **Teleosts** | *Scorpaena elongata* | | | | No | |
| **Teleosts** | *Trigla lucerna* | Benthic | Dissection, digestion (35% hydrogen peroxide) and stereomicroscope | Micro | No | *Güven et al., 2017* |
| **Teleosts** | *Lagocephalus spadiceus* | Benthopelagic | Dissection, digestion (35% hydrogen peroxide) and stereomicroscope | Micro | No | *Güven et al., 2018* |

### *(c) Arthropoda, Mollusca, Echinodermata, Anellida*

| **Taxa** | **Species** | **Habitat** | **Method** | **Litter size** | **Polymer id** | **Reference** |
|---|---|---|---|---|---|---|
| **Arthropoda** | *Gammarella fucicola* | benthic | Microscopic slides | Micro | No | *Remy et al., 2015* |
| | *Gammarus aequicauda* | benthic | Microscopic slides | Micro | No | *Remy et al., 2016* |
| | *Melita hergensis* | benthic | Microscopic slides | Micro | No | *Remy et al., 2017* |
| | *Nototropis guttatus* | benthic | Microscopic slides | Micro | No | *Remy et al., 2018* |
| | *Nebalia strausi* | benthic | Microscopic slides | Micro | No | *Remy et al., 2019* |
| | *Palaemon xiphias* | benthic | Microscopic slides | Micro | No | *Remy et al., 2020* |
| | *Liocarcinus navigator* | benthic | Microscopic slides | Micro | No | *Remy et al., 2021* |
| | *Athanas nitescens* | benthic | Microscopic slides | Micro | No | *Remy et al., 2022* |
| | *Galathea intermedia* | benthic | Microscopic slides | Micro | No | *Remy et al., 2023* |
| **Mollusca** | *Mytilus galloprovincialis* | benthic | Digestion ($HNO_3$:$HClO_4$ (4:1 v:v)/69% nitric acid) and stereomicroscope | Micro | No | *Vandermeersch et al., 2015* |
| | | | Dissection, digestion (30% $H_2O_2$) and stereomicroscope | Micro | Yes | *Digka et al., 2018* |
| **Echinodermata** | *Holothuria forskali* | benthic | Dissection and observation of fecal pellet with stereomicroscope | Micro | No | *Alomar et al., 2016* |
| **Anellida** | *Saccocirrus papillocercus* | benthic | Dissection and stereomicroscope | Micro | Yes | *Gusmão et al., 2016* |

### *(d) Reptiles, Seabirds, Marine Mammals*

| Taxa | *Species* | Habitat | Method | Litter size | Polymer id | Reference |
|---|---|---|---|---|---|---|
| **Reptiles** | *Caretta caretta* | benthopelagic | Dissection and visual inspection adopting the MSFD protocol | Micro/ Macro | No | *Campani et al., 2013* |
| | | | Dissection or fecal pellets and visual inspection adopting the MSFD protocol | Micro/ Macro | No | *Camedda et al., 2014* |
| | | | Dissection and visual inspection adopting the MSFD protocol | Micro/ Macro | No | *Matiddi et al., 2017* |
| **Seabirds** | *Calonectris diomedea* | | Dissection and visual inspection | Micro/ Macro | No | *Codina-García, 2013* |
| **Seabirds** | *Puffinus yelkouan* | | Dissection and visual inspection | Micro/ Macro | No | *Codina-García, 2013* |
| **Seabirds** | *Puffinus mauretanicus* | | Dissection and visual inspection | Micro/ Macro | No | *Codina-García, 2013* |
| **Seabirds** | *Morus bassanus* | | Dissection and visual inspection | Micro/ Macro | No | *Codina-García, 2013* |
| **Seabirds** | *Ichthyaetus audouinii* | | Dissection and visual inspection | Micro/ Macro | No | *Codina-García, 2013* |
| **Seabirds** | *Larus michahellis* | | Dissection and visual inspection | Micro/ Macro | No | *Codina-García, 2013* |
| **Seabirds** | *Ichthyaetus melanocephalus* | | Dissection and visual inspection | Micro/ Macro | No | *Codina-García, 2013* |
| **Seabirds** | *Rissa tridactyla* | | Dissection and visual inspection | Micro/ Macro | No | *Codina-García, 2013* |
| **Seabirds** | *Stercorarius skua* | | Dissection and visual inspection | Micro/ Macro | No | *Codina-García, 2013* |
| **Marine mammals** | *Physeter macrocephalus* | pelagic | Dissection and visual inspection | Macro | No | *de Stephanis et al., 2013* |

### 6.1.2. Sampling strategies

The sampling strategies applied within the reviewed studies vary according to the taxonomic group, the size and conservation status of the species. If the specimens are protected, threatened or endangered, special permits are required for transport and necropsy and it is advantageous to involve regional or national networks to maximize sample retrieval. Dead sea turtles, seabirds and marine mammals can be collected from beaches or at sea from accidental mortalities such as victims of longline fishing (by catch) or of boat collisions. Regarding living specimens, faecal pellets can be collected, using a non-invasive tecnique, in rescue facilities.

On the other hand, monitoring activities on commercially harvested species are simple; specimens and samples can be easily become available through fishing activities. Invertebrates and fish can be obtained from existing active monitoring programs, for example the MEDITS program, or from ad hoc monitoring campaigns using fishing vessels. For reliable information on changes (or stability) in quantities of ingested litter and their effect, a statistical sample size is recommended, as well as continuous sampling in order to collect the background information needed to define 'good environmental status' and to evaluate possible temporal trends.

### 6.1.3. An overview of the biological samples investigated

Analysis of ingested litter has been carried out in many different marine organisms. The methodologies applied vary according to the species, the size of the organisms and the desired analytical level. Generally, the whole organism is analyzed when small invertebrates/fish species are being investigated, whereas for larger organisms the grastrointestinal (GI) tract or feacal pellets are the main target tissues and samples to be analyzed. Currently, only two studies in the Mediterranean report the analysis of faecal pellets in sea turtles (Camedda et al., 2014) and holoturians (Alomar et al., 2016) (Table 6.1).

### 6.1.4. Litter size classes examined

Regarding the size of the marine litter investigated, over 60% of the published papers investigated the presence of microlitter, also due to the small size of the species investigated (Figure 6.2). It is important to underline that some studies have not taken into account the size of the particles in the characterization of ingested marine litter (Table 6.1).

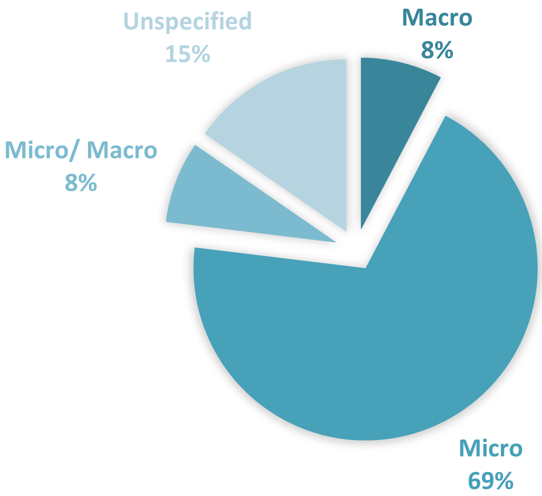

***Figure 6.2.*** *Percentage of studies per litter size classes from 2013 to 2018 on marine litter ingestion in the Mediterranean Sea.*

### 6.1.5. An overview of the methods for sample processing

#### *6.1.5.1. Macrolitter*

In recent years, several Mediterranean initiatives and projects such as the DeFishGear, INDICIT, and MEDSEALITTER have worked towards the improvement and harmonization of protocols aiming to detect marine litter in biota. These protocols are based on the guidelines produced by the MSFD task group on marine litter. The IPA-Adriatic DeFishGear project elaborated a protocol for the detection of marine litter in fish species (Vlachogianni et al., 2017). The INDICIT project has produced a protocol for monitoring the impacts of marine litter on sea turtles (stomach contents, faecal pellets). MEDSEALITTER is also working on protocols for monitoring marine litter in sea turtles and fish species.

In the reviewed studies the protocols for monitoring ingested litter in different marine species such as seabirds, sea turtles, fish etc., are based on the MSFD TG 10 Guidelines (Galgani et al., 2013). More specifically:

- The protocol for seabirds is based on tool “10.2.1_T1 - Fulmar” and tool “10.2.1_T2 - Shearwater” which follow the OSPAR Ecological Quality Objective (EcoQO). However, only a single study by Codina-Garcia in 2013 reported the occurrence of marine litter ingestion in 9 species of Mediterranean seabirds, using visual inspection of the seabirds’ stomach content.
- Regarding sea turtles, the protocol is based on the tool “10.2.1_T3 - Sea turtle” which also includes the protocol for sampling litter excreted by live sea turtles (fecal pellet analysis). Several studies on loggerhead sea turtle *Caretta caretta* in the Mediterranean Sea have been performed (Campani et al., 2013; Camedda et al., 2014; Matiddi et al., 2018). Each report and study applied the MSFD monitoring protocol for sea turtles. According to the MSFD, the stomach content of stranded *Caretta caretta* should also be used as indicator to measure the trends of marine litter and regional differences.
- Since fishes are among the most studied organisms, different methods have been adopted to evaluate litter ingestion. A review of the available methods for the detection of litter in fishes and invertebrates has been recently published by FAO (Lusher et al 2017a). It was found that marine litter ingestion by smaller fish and invertebrate species is detected via the use of chemicals (basic or acid compounds or oxidizing agents) or enzymes to digest the organic matter. The resulting solution is then filtered and the particles observed under a stereomicroscope and analysed by (micro) Fourier Transform Infrared Spectroscopy (µ) FTIR or Raman Spectroscopy to identify the chemical nature of the particles .

#### *6.1.5.2. Microlitter*

Analysing microplastics ingested by biota is a challenging task, and for this reason an increasing number of techniques have been developed in recent years (Lusher et al., 2017a, 2017b). Of the currently existing methods, the ones most widely tested are listed in Table 6.1. For each method, the costs, the strengths and weaknesses, and its applicability to the organism being studied should be carefully considered. Here we report in detail the most commonly used method.

The gastro-intestinal tract or the whole organism should be rinsed with deionised filtered (0.45 µm) water to avoid contamination of the sample. To degrade organic matter, the sample can be digested using a 10% KOH solution at 60°C for 12 hours (Rochman et al., 2015), or 15%, 30% or 35% $H_2O_2$ is also often used (Avio et al., 2015, Anastasopoulou et al., 2018, Guven et al., 2017) or using enzymatic digestion (Cole et al., 2014) (see Table 6.1). After digestion of the organic matter, the solution obtained is filtered onto glass fibre filters and the residual inorganic particles analysed though a stereomicroscope. Such particles (e.g. non-digested tissue particles, inorganic residues, microplastics, etc.) are characterised by their type, size, and colour, and then weighted and subdivided into different categories. Given the difficulty to visually identify marine microlitter, further analyses are used to confirm the isolated particle. Such analyses can be performed by spectroscopy (FT-IR or Raman) or by the "hot needle" technique, if spectroscopy instruments were not available. Spectroscopic analysis is also used to determine the nature of the polymer making-up the particles, to better define the nature and source of contamination in the bioindicator species.

More recently, selective fluorescent staining using Nile Red is being applied as a rapid-screening approach to detect and quantify microplastics in environmental samples (Erni-Cassola et al., 2017; Maes et al., 2017) and could potentially be applied to biota samples as this technique allows for fluorescent particles, of up to a few micrometers, to be identified and counted as well as offering the possibility of plastic categorization based on surface polarity characterization of the identified particles (Maes et al., 2017). During all the analytical procedures, particular attention must be paid to the prevention of possible environmental contamination due to the ubiquitous nature of certain types of particles (e.g. synthetic fibres). For this reason, together with the samples, it is recommended to include experimental blanks. Moreover, all analytical procedures should be carried out with glass material where possible, minimizing the use of plastic laboratory material and in closed areas with little ventilation and air circulation for example from air conditioners.

### 6.1.6. An overview of the litter classification lists

The most frequently used marine litter items classification list in the reviewed studies is the MSFD TG10 list.

### 6.1.7. An overview of the quantification indices and units

The results of the analysis of marine litter ingestion are expressed as follows:

- Presence/absence of litter in the whole organism/GI tract/faecal pellets;
- Occurrence (%) of individuals that have ingested marine litter among a subpopulation/population/species;
- The abundance (N) and weight (g) of marine litter (macro- and micro-litter) ingested per individual (N items/individual; g/individual) as a total and per category of litter.

### 6.1.8. Monitoring the impacts of marine litter on biota

Recent studies in the different regions of the Mediterranean basin suggest that some areas are affected by high concentrations of marine litter including microplastics and plastic additives (phthalates), representing a potential risk to biodiversity (Darmon et al., 2017; Fossi et al., 2014, 2016, 2017). The impact of marine litter and its interactions with Mediterranean marine organisms were reviewed by Deudero and Alomar (2015), who identified almost 134 species affected by marine litter in total. According to the most recent review, marine litter ingestion has been documented for 91 Mediterranean species, belonging to different taxonomic groups including invertebrates, fish, sea turtles, seabirds and marine mammals (Fossi et al., 2018). Ingestion of marine litter and contaminants associated to it can produce physiological effects in biota which can be assessed through the analysis of specific biomarkers.

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. In addition to the physical harm, there is growing concern regarding the chemical hazards related to the ingestion of marine litter. Plastic additives (e.g. PBDEs and phthalates) can directly leach from plastic debris, leading to the accumulation within marine organisms of chemicals such as persistent, bioaccumulating and toxic (PBT) substances; these substances can also be adsorbed and transported by marine litter. Some studies have examined the possible link between the chemical effects of plastic ingestion and the risk of bioaccumulation along the trophic web. For instance, Fossi et al. (2014, 2016) and Baini et al. (2017) detected levels of phthalates and organochlorines in specimens of *Euphausia krohnii*, muscle samples of basking shark *Cetorhinus maximus* and in blubber samples of four cetaceans: fin whale

*Balaenoptera physalus*, bottlenose dolphin *Tursiops truncatus*, Risso's dolphin *Grampus griseus* and striped dolphin *Stenella coeruleoalba*. The levels of these toxic chemicals in species tissues have been used as possible tracers of exposure to plastic ingestion and can be used as baseline data to monitor impacts caused by marine litter in species. As mentioned in the previous section, if these chemicals become bioavailable, they can penetrate cells and chemically interact with biologically important molecules. This may cause adverse effects at different levels of biological organisation, from molecular level to tissue level, including liver toxicity (Avio et al., 2015b; Rochman et al., 2013), alterations of gene expression (Karami et al., 2017; Sleight et al., 2017), genotoxic effect (Avio et al., 2015a), endocrine disruption (Rochman et al., 2014; Teuten et al., 2009) and histological alterations (Avio et al., 2015a, 2015b; Pedà et al., 2016). However, most of these effects have been shown in laboratory studies and very few are available from field studies and particularly on Mediterranean organisms (Avio et al., 2015b).

A more comprehensive evaluation of the actual ecotoxicological risk for the bioindicator species, associated with the presence of marine litter in the Mediterranean area, can be performed using a set of extremely sensitive diagnostic and prognostic methodologies, the so-called biomarkers. Such methodologies are based on an evaluation of the “response” - at the organism, population or community level - induced by a source of environmental chemical stress. To evaluate the possible effects on the bioindicator species, from molecular to cellular level, a set of biomarkers can be applied that integrate the data obtained from the detection of marine litter and the plastic tracers with a more complex ecotoxicological evaluation of the health status of the selected bioindicator organisms (Fossi et al., 2018) (Table 6.2).

***Table 6.2.*** *Overview of published studies on marine litter impact on biota in the Mediterranean area (2013-2018) divided by taxa.*

| **Taxa** | **Species** | **Impact related to marine litter ingestion** | **Method** | **Reference** |
|---|---|---|---|---|
| Mollusca | *Mytilus galloprovincialis* | Biological effects | genotoxic effect<br>histological alterations | *Avio et al., 2015b* |
| Arthropoda | *Euphausia krohnii* | Transfer of chemical | phthalates | *Fossi et al. 2014* |
| Elasmobranchs | *Cetorhinus maximus* | Transfer of chemical | phthalates | *Fossi et al. 2014* |
| Teleosts | *Mullus surmuletus* | Biological effects | oxidative stress | *Alomar et al. 2017* |
| Marine mammals | *Balaenoptera physalus* | Transfer of chemical | phthalates | *Fossi et al. 2014* |
| | | | | *Baini et al. (2017)* |
| Marine mammals | *Tursiops truncatus* | Transfer of chemical | phthalates | *Baini et al. (2017)* |
| Marine mammals | *Grampus griseus* | Transfer of chemical | phthalates | *Baini et al. (2017)* |
| Marine mammals | *Stenella coerulealba* | Transfer of chemical | phthalates | *Baini et al. (2017)* |

Physical and ecotoxicological effects strictly related to marine litter and, in particular, to plastics can be directly addressed in very few cases; therefore an integrated approach, linking the detection of ingested marine litter with the physical and toxicological effects related to the ingestion of contaminated plastic litter, is needed.

One of these very few studies in the field have carried out the following 3-fold approach. The application of the threefold approach can elucidate not only the rate of ingestion among the different bioindicators, but also the multiple sub-lethal stresses that marine litter ingestion can cause in the short and long term. Each of the three investigation tools that make up the threefold approach can be applied independently or simultaneously to the selected bioindicator species (Fossi et al 2018).

The methodology to monitor the impacts of marine litter on marine organisms can be performed following three different scales of approach, depending on the target organism (Fossi et al., 2018):

i. analysis of the gastro-intestinal content in vertebrates/invertebrates in order to evaluate the marine litter ingested by organisms. Information on the degree to which the biota ingests marine litter (including microplastics) is essential in order to determine and monitor threshold levels.

ii. quantitative and qualitative analysis of plastic additives (eg. phthalates and PBDEs) and PBT compounds in the tissues (for example muscle) of bioindicators, used as “plastic tracers”. (Rochman et al., 2015, Baini et al. 2017).

iii. analysis of the effects based on biomarker responses at different biological levels (from gene/protein expression variations to histological alterations). Assessing the undesirable biological responses related to the ingestion of marine litter and the accumulation of plastic associated compounds is crucial to evaluating the extent of the threat of marine litter and plastic ingestion to marine organisms (Fossi et al. 2016; Pedà et al., 2016; Avio et al., 2015b).

### 6.2. Entanglement

According to UNEP (2016), entanglement incidents in marine debris lead to wounds or death, with a declining order of species affected per taxon, for 192 species of invertebrate, 89 species of fish, 83 species of bird, 38 species of mammal and all species of sea turtle. In the Mediterranean there is a general lack of data on the interaction of marine organisms with litter through entanglements. Entanglement or more generally interactions between litter and marine organisms, has been described for cetaceans, pinnipeds, marine turtles, birds, fishes including sharks and for many invertebrates both and coastal and deep sea areas (Galgani et al., 1996; Cedrian, 2008; Rodriguez et al., 2013; Bo et al., 2014; Tubau et al., 2015; Colmenero et al., 2017). As recent work has shown, lost gear or litter in general can also harm benthic organisms and habitats, including deep sea Mediterranean species like sponges, gorgonians, or certain cold water corals (Pham et al., 2014; Fabri et al., 2014, Consoli et al., 2018).

The incidence of entanglement can vary strongly according to region, to the fishing activities with which animals interact and more generally to the presence of litter. Generally, the factors that may contribute to organisms being entangled in, or strangled by, abandoned fishing gear or litter include the presence of organisms in or near the nets, and water turbidity, making the litter and gear less visible. For cetaceans, whose ability to detect nets by echolocation is fundamental (Kühn et al., 2015), ambient noise can hide or distort the echoes produced by fishing gear. In vertebrates, fasting is one of the frequent consequences of entanglement, as well as the impossibility of moving and thus escaping from predators. Entanglement also leads to wounds susceptible to secondary infections and sometimes amputation after constriction (NOAA, 2014).

Benthic organisms can also be caught in lost traps or other litter items. Typically, crabs, octopus, fishes and many small invertebrates are taken in traps on the seabed and die, an impact that is very common in some Mediterranean countries where fishermen are considering octopuses as target species.

In the deep Mediterranean Sea, interactions between litter and marine organisms were suggested years ago, using manned or unmanned submersibles, including ROVs (Galgani et al., 2016; Pham et al., 2014), which also showed that debris may be used as a habitat by some macro-invertebrates. Because of their morphology, gorgonians, some sponges, corals and colonial zoanthids might be more susceptible to this kind of damage, as observed by Angiolillo et al. (2015) and Consoli et al. (2018). In contrast, entanglement in derelict fishing gear has been reported to cause severe damage in corals (Octocorallia and Hexacorallia), gorgonians and sponges with evident signs of tissue damage and epibiosis of fouling organisms (see Oliveira et al., 2015 for review). Recently, during a survey carried out in the central Mediterranean Sea, Consoli et al. (2018) found a total of 16 species (15 cnidarians and 1 sponge) showing some form of interaction (Entanglement/Coverage and or damage) with litter and 12 of these were species of conservation concern according to international directives and agreements. Moreover, they observed a significant positive relationship between the number of litter-fauna interactions and the mean litter density: this latter indicator could thus be considered a good tool for the monitoring of marine litter impact on benthic communities.

### 6.3. Marine litter as transport vector and habitat

Marine litter (macro- and micro-plastic) can represent a transport vector or a habitat for marine organisms found on the sea surface or on the seafloor.

The hydrophobic surface of plastic marine debris stimulates microbial colonization, producing what is referred to as "microbial reefs," where they may contribute to the self- breakdown or degradation of plastics (Dussud and Ghiglione, 2014). Plastic when in seawater rapidly develops (after 1 week) a biofilm that includes primary producers, consumers, predators and decomposers (Watnick and Kolter, 2000) that encourages the attachment of larger organisms that use chemical and/or physical characteristics as a cue to settle (Zardus et al., 2008; Hadfield et al., 2014).

Since 2013, some 20 scientific peer-reviewed papers investigated the colonization and coverage of litter in the Mediterranean Sea (Table 6.3). Only four papers have studied, in terms of presence and effects, the colonization of floating micro- and macro- litter by different phyla of organisms, while others investigated the presence of litter coverage and colonization in the deep-sea.

*Floating litter*

The papers that investigate the colonization of the floating litter have collected the litter with manta trawls or visual sightings (Cabezas et al., 2013; Dussud et al., 2018; Virsek et al., 2017; Tutman et al., 2017). Traditional methods such as microbiology and microscopy (SEM, TEM), and DNA isolation (e.g. RT- PCR, PCR) techniques were used to investigate the species that use the litter as transport vector or habitat.

The floating macro-litter became habitat for dispersal rafting of some species such as benthic foraminiferal species (*R. concinna*; Jorissen, 2014) amphipods *(C.andreae*; Cabezas et al., 2013) and crabs (*Liocarcinus navigator*, *Planes minutus*; Tutman et al. 2017). Microorganisms have also shown to be capable of rafting through microplastics spreading up their natural dispersion process. Virsek et al. (2017) have identified 28 bacterial species on the microplastics particles, including pathogenic fish bacteria (*Aeromonas spp.*) and hydrocarbon-degrading bacterial species. Dussud et al. (2018) revealed for the first time a clear niche partitioning between free-living, organic particle-attached, and plastic marine debris (PMD). The authors observed that PMD represent favorable environments for a large number of species, with significantly higher evenness compared to free living and organic fraction particle. The PMD fraction was dominated by Cyanobacteria (40.8%, mainly Pleurocapsa sp.)

and Alphaproteobacteria (32.2%, mainly Roseobacter sp.). Moreover, the biofilms covered between 0 and 3.5% of the surface area, revealing a rather patchy covering of the PMD.

*Deep-sea litter*

The full extent of effects that litter has on deep-sea habitats and their fauna is still poorly understood, but several impacts have been documented in different studies (Gündoğdu et al. 2017; Ioakemidis et al., 2015; Ramirez-Llodra et al. 2013). Most of the studies on deep-sea litter have used semi-quantitative sampling gears (e.g. trawls, dredges; bottom trawl; Gündoğdu et al., 2017; Ramirez-Llodra et al., 2013) to collect the samples. More recently, the development and increasing utilization of Remotely Operated Vehicles (ROVs) has allowed investigating the coverage and colonization of different species on deep-sea litter, limiting the damage to the benthos in slopes and rocky bottoms. Moreover, ROV is less complicated than submersible and generally cheaper (Deidun et al., 2015; Cau et al., 2017; Consoli et al., 2018; Melli et al., 2017; Ioakemidis et al., 2015; Lastras et al., 2016; Fabri et al., 2014; Bo et al., 2014; Taviani et al., 2017). All the researchers have used image analysis, captured by ROV, for the identification of species that inhabit and coverage the litter.

Nine papers studied the presence of fauna on litter in the Mediterranean deep-sea. Some authors have investigated the presence of megafauna-colonized litter on sea bed, either comparing total biomass of megafauna with total litter weight (Ramirez-Llodra et al. 2013) or recording the different fouling species (Gündoğdu et al. 2017; Ioakeimidis et al., 2015). One of the most important impacts of litter highlighted on soft bottom environment of the Mediterranean Sea is the alteration of the texture of the seabed (artificial hardgrounds) and thus the change of the structure of benthic communities (Gündoğdu et al., 2017; Ioakemidis et al., 2015; Melli et al., 2017).

Derelict nets and longlines, often entangled around rocky obstacles and under tension, form an artificial substrate which is preferentially colonized by the serpulid policheate, ramified hydroids (Sertulariidae), encrusting sponges, colonial tunicate, bryozoans and zoanthids, and occasionally by *Neopycnodonte cochlear* and scleractinian corals (Cau et al. 2017; Fabri et al 2014).

The presence of the epibiosis on the tissue damage of coral colonies inflicted by entanglement and discarded long lines covered by hydroids is observed by Bo et al. (2014) and Deidun et al. (2015). Litter-fauna interactions were investigated, with 70% of the items presenting high fouling levels, and epibionts coverage on 50-100% of their surface. Interactions and usage, as substrate or refuge, of the litter by megafauna (asteroids, holothurians, crustaceans and demersal fish) is also observed (Cau et al. 2017; Melli et al., 2017).

Nowadays, specific protocols to evaluate the effect of litter colonization and coverage are not available.

***Table 6.3.*** *Overview of the methodological approaches used to investigate marine litter colonization and coverage in the Mediterranean area (2013-2018).*

| Location | Habitat | Taxa | Method | Occurrence % | Litter size | Reference |
|---|---|---|---|---|---|---|
| Sardinia Channel | Benthic | Cnidaria | Four ROV dives were carried out for a total length of ca. 1700 m. Taxonomic identification was based on still image and analysis through the robotic arm of the ROV, together with a grab sample to gain information on composition of all | n.a. | Macro-litter | *Taviani et al., 2017* |

|  |  |  |  |  |  |  |
|---|---|---|---|---|---|---|
|  |  |  | aspects of coral rubble |  |  |  |
| Malta island | Benthic | Cnidaria | ROV survey was at depths ranging between 250 and 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 seabed area | n.a. | Macro-litter | *Deidun et al., 2014* |
| Northern Adriatic Sea | 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* |
| The Saronikos Gulf | Benthic and pelagic | Polychaeta, Echiura, Gastropoda, Ascidia, Bivalvia, Serpulidae, Anthozoa Rhodophycea, Gastropoda, Echinodermata Rhodophycea, Anthozoa, Echinoedea, Asteroidea | ROV survey, 1 area, 2 dive | n.a. | Macro-litter | *Ioakeimidis et al., 2015* |
| Central western Mediterranean | 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* |
| North-western Mediterranean (Andalusian coasts) | Beach and benthic | Arthropoda | Manual collection; genomic DNA | n.a. | Macro-litter | *Cabezas et al., 2013* |
| Western Mediterranean Sea | Surface and water column | Bacteria | 32 manta trawls (mesh size 333 µm); DNA extracted from 72 randomly sorted PMD (recently introduced plastic marine debris) from each the 32 stations and from all the 3 mm- and 0.2 mm-pore size filters from surrounding seawater. Between 2 and 4 plastic fragments (filament, | Most of the samples were highly colonized by bacteria | Micro and macro-plastics (2.8 - 23.8 mm) | *Dussud et al., 2018* |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | pellet or sheet, size > 2 mm) were extracted separately at each station; RT-PCR | | | |
| Western Mediterranean Sea (Tyrrhenian Sea) | Benthic | Epibionted species (not identified) | ROV with range 70-280 m of deep; 940 video frames corresponding to almost 3000 m$^2$ of analyzed surface | n.a. | Micro e macro-litter | *Bo et al., 2014* |
| Western Mediterranean Sea (Bastia, Corsica, France) | 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* |
| Western Mediterranean;Central Mediterranean; Eastern Mediterranean | Benthic | Megafauna | Otter-trawl Maireta system (OTMS) and an Agassiz trawl. OTMS 40 mm mesh size, covered by an outer net 12 mm in mesh size and a total net length of 25 m; range of speed 2.6-2.8 knots. Agassiz trawl: 12 mm net mesh size, a 2.5 m horizontal opening and 1.2 m vertical opening; 2.0 knots. 6 transects; samples taken at 900, 1050, 1200, 1350, 1500, 1750, 2000, 2250 and 2700 m | n.a. | Micro e macro-litter | *Ramirez-Llodra et al., 2013* |
| North-western Mediterranean | Benthic | Cnidaria | 21 ROV (equipped with three video camera) immersions; 420 Gb of video images were recorded (41 h 20 min); 7914 positions; range of depths: 79-401 m | n.a. | Macro-litter | *Lastras et al., 2016* |
| North-western Mediterranean (French continental margin) | 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* |
| South Adriatic Sea | Benthic | Arthropoda | Visual sightings of floating objects in the open in an area with a depth of 1226 m | n.a. | Macro-litter | *Tutman et al., 2017* |
| Slovenian coast-North Adriatic sea | Benthic and pelagic | Bacteria | Manta net with 308 µm pore size, the boat speed was approx. 2.5 knots and the time of sampling was 30 min. Transects were approx. 1.3 nm long. Used only fragments, due other | n.a. | Micro-plastics | *Viršek et al., 2017* |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | higher abundance and good surface to volume ratio, which enables effective growth to bacterial biofilms | | | |
| East Mediterranean Sea | Benthic | Cnidaria, Crustacea, Mollusca, Echinodermata Tunicata, Pisces | Bottom trawl net (4.5 m length; 0.8 m mouth; 20 mm mesh size). The trawl net was towed for 20 min. All macro-plastics were taken and placed into cold storage containers, and quickly separated to determine the species | Number of species in each sampling station | Macro-plastics | *Gündoğdu et al., 2017* |

## 6.4. References

Alomar, C., Estarellas, F., Deudero, S., 2016. Microplastics in the Mediterranean Sea: Deposition in coastal shallow sediments, spatial variation and preferential grain size. Mar. Environ. Res. 115, 1–10. https://doi.org/10.1016/j.marenvres.2016.01.005.

Alomar, C., Deudero, S., 2017. Evidence of microplastic ingestion in the shark Galeus melastomus Rafinesque, 1810 in the continental shelf off the western Mediterranean Sea. Environmental Pollution. https://doi.org/10.1016/j.envpol.2017.01.015.

Alomar, C., Sureda, A., Capó, X., Guijarro, B., Tejada, S., Deudero, S., 2017. Microplastic ingestion by Mullus surmuletus Linnaeus, 1758 fish and its potential for causing oxidative stress. Environmental Research 159, 135–142. https://doi.org/10.1016/j.envres.2017.07.043.

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 Research Part I: Oceanographic Research Papers 74, 11–13. https://doi.org/10.1016/j.dsr.2012.12.008

Anastasopoulou, A., Mytilineou, Ch., 2015. Methodology for monitoring macro-litter in biota. Output of DeFishGear Project. http://www.defishgear.net/project/main-linesof-activities.

Anastasopoulou, A., Viršek, M. C., Varezić, D. B., Digka, N., Fortibuoni, T., Koren, Š., Mandić, M., Mytilineou, C., Pešić, A., Ronchi, F., Šiljić, J., Torre, M., Tsangaris, C., Tutman, P., 2018. Assessment on marine litter ingested by fish in the Adriatic and NE Ionian Sea macro-region (Mediterranean), Marine Pollution Bulletin 133, 841-851. https://doi.org/10.1016/j.marpolbul.2018.06.050

Angiolillo M., B. diLorenzo, A. Farcomeni, M. Bo, G. Bavestrello, G. Santangelo, A. Cau, V. Mastascusa, A. Cau, F. Sacco, S. Canese (2015) Distribution and assessment of marine debris in the deep Tyrrhenian Sea (NW Mediterranean Sea, Italy), Mar. Pollut. Bull., 92, pp. 149-159

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

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

Avio, C.G., Cardelli, L.R., Gorbi, S., Pellegrini, D., Regoli, F., 2017. Microplastics pollution after the removal of the Costa Concordia wreck: first evidences from a biomonitoring case study. Environmental Pollution 227, 207–214. http://dx.doi.org/10.1016/j.envpol.2017.04.066

Baini M., Pedà C., Panti C., Caliani I., Casini S., Leonzio C., Fossi M.C., 2017. Bioindicator selection in the strategies for monitoring marine litter in the Mediterranean Sea. Realided with the contribution of several institutions: Università di Siena SDSN, Un Environment/Mediterranean Action Plan (Greece), Istituto Superiore per la Protezione e la Ricerca Ambientale (ISPRA) (Italy), Museum National D'Histoire Naturelle (France), Institut Francais de Recherche pour l'Exploitation de la Mer (IFREMER) (France), Hellenic Centre for Marine Research (HCMR) (Greece).

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., Vethaak, A. D., Lavorante, B. R., Lundebye, A. K., & Guilhermino, L. (2018). Marine microplastic debris: An emerging issue for food security, food safety and human health. Marine Pollution Bulletin, 133, 336-348.

Barnes D.K.A. (2002) Invasions by marine life on plastic debris. Nature 416, 808–809. doi.org/10.1038/416808a.

Barnes D.K., Galgani F., Thompson R.C., M. Barlaz (2009) Accumulation and fragmentation of plastic debris in global environments. Philosophical Transactions of the Royal Society of London B: Biological Sciences 364 (1526), 1985–1998.

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). Italian Journal of Zoology 83, 121–129. https://doi.org/10.1080/11250003.2015.1114157.

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. Marine Pollution Bulletin 109, 55–60. https://doi.org/10.1016/j.marpolbul.2016.06.026.

Bo M., S. Bava, S. Canese, M. Angiolillo, R. Cattaneo-Vietti, G. Bavestrello (2014). Fishing impact on deep Mediterranean rocky habitats as revealed by ROV investigation. Biological Conservation. 171. 167–176. 10. 1016/j. biocon. 2014. 01. 011.

Cabezas M.P., Navarro-Barranco C., Ros M., J.M. Guerra-García (2013) Long-distance dispersal, low connectivity and molecular evidence of a new cryptic species in the obligate rafter Caprella andreae Mayer, 1890 (Crustacea: Amphipoda: Caprellidae). Helgol. Mar. Res., 67, 483–497 doi 10.1007/s10152-012-0337-9.

Camedda, A., Marra, S., Matiddi, M., Massaro, G., Coppa, S., Perilli, A., Ruiu, A., Briguglio, P., de Lucia, G.A., 2014. Interaction between loggerhead sea turtles (*Caretta caretta*) and marine litter in Sardinia (Western Mediterranean Sea). Marine Environmental Research 100, 25–32. https://doi.org/10.1016/j.marenvres.2013.12.004

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). Marine Pollution Bulletin 74, 225–230. https://doi.org/10.1016/j.marpolbul.2013.06.053.

Cartes, J.E., Soler-Membrives, A., Stefanescu, C., Lombarte, A., Carrassón, M., 2016. Contributions of allochthonous inputs of food to the diets of benthopelagic fish over the northwest Mediterranean slope (to 2300m). Deep Sea Research Part I: Oceanographic Research Papers 109, 123–136. https://doi.org/10.1016/j.dsr.2015.11.001.

Cau A., Alvito A., Moccia D., Canese S.,, Pusceddu A., Rita C., Angiolillo M., M.C. Follesa (2017) Submarine canyons along the upper Sardinian slope (Central Western Mediterranean) as repositories for derelict fishing gears. Mar. Poll. Bul., 123, 357–364

Cedrian D. (2008) Seals-fisheries interactions in the Mediterranean monk seal (*Monachus monachus*): related mortality, mitigating measures and comparison to dolphin-fisheries interactions. Transversal Working Group on by catch/incidental catches. O Headquarters, Rome (Italy), 15-16 September 2008

Claro F., Pham C., A. Liria Loza, M. Bradai, A. Camedda, O. Chaieb, G.Darmon, A. de Lucia, H. Attia El Hili, E. Kaberi, Y.Kaska, M. Matiddi, C. Monzon-Arguelo, P. Ostiategui, L. Paramio O. Revuelta, C. Silvestri, D. Sozbilen, J. Tòmas, C. Tsangaris, M. Vale, F. Vandeperre, C. Miaud (2018) State of the art: Entanglement with marine debris by biota. Implementation of the indicator of marine litter on sea turtles and biota in regional seas conventions and MSFD areas. Report of the European project INDICIT (indicit-europa.eu), 54 pages, in press.

Codina-García, M., Militão, T., Moreno, J., González-Solís, J., 2013. Plastic debris in Mediterranean seabirds. Marine Pollution Bulletin 77, 220–226. https://doi.org/10.1016/j.marpolbul.2013.10.002.

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

Cole, M., Webb, H., Lindeque, P.K., Fileman, E.S., Halsband, C., Galloway, T.S., 2014. Isolation of microplastics in biota-rich seawater samples and marine organisms. Scientific Reports 4. https://doi.org/10.1038/srep04528.

Collard, F., Gilbert, B., Eppe, G., Parmentier, E., Das, K., 2015. Detection of Anthropogenic Particles in Fish Stomachs: An Isolation Method Adapted to Identification by Raman Spectroscopy. Archives of Environmental Contamination and Toxicology 69, 331–339. https://doi.org/10.1007/s00244-015-0221-0.

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. Marine Pollution Bulletin 128, 89–96. https://doi.org/10.1016/j.marpolbul.2018.01.009.

Consoli P., F. Andaloro, C. Altobelli, P. Battaglia, S. Campagnuolo, S. Canese, L. Castriota, T. Cillari, M. Falautano, C. Peda, P. Perzia, P. Sinopoli, P. Vivona, G. Scotti, V. Esposito, F. Galgani, T. Romeo (2018) 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, Env. Poll. 236, 405-415. doi: 10.1016/j.envpol.2018.01.097.

Colmenero A., C.Barría, E.Broglio, S.García-Barcelona (2017) Plastic debris straps on threatened blue. shark Prionace glauca. Mar. Poll. Bull., 115 (1-2), 436-438.

Darmon, G., Miaud, C., Claro, F., Doremus, G., Galgani, F., 2017. Risk assessment reveals high exposure of sea turtles to marine debris in French Mediterranean and metropolitan Atlantic waters. Deep Sea Research Part II: Topical Studies in Oceanography 141, 319–328. https://doi.org/10.1016/j.dsr2.2016.07.005.

Deidun A., Andaloro F., Bavestrello G., Canese S., Consoli P., Micallef A., Romeo T., M. Bo (2015) First characterisation of a Leiopathes glaberrima (Cnidaria: Anthozoa: Antipatharia) forest in Maltese exploited fishing grounds. Italian Journal of Zoology, 2015, 82 (2), 271–280, doi 10.1080/11250003.2014.986544 de Stephanis, R., Giménez, J., Carpinelli, E., Gutierrez-Exposito, C., Cañadas, A., 2013. As main meal for sperm whales: Plastics debris. Marine Pollution Bulletin 69, 206–214. https://doi.org/10.1016/j.marpolbul.2013.01.033.

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

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

Dussud C., J.F. Ghiglione (2014) Bacterial degradation of synthetic plastics. In: Briand, F. (Ed.), Marine litter in the Mediterranean and Black Seas, vol. 46. CIESM Publisher, Monaco, pp. 43-48.

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., J.F. Ghiglione (2018) Evidence of niche partitioning among bacteria living on plastics, organic particles and surrounding seawaters. Env. Poll. 236, 807- 816

Eich A., Mildenberger T., Laforsch C., M. Weber (2015) Biofilm and diatom succession on polyethylene (PE) and biodegradable plastic bags in two marine habitats: early signs of degradation in the pelagic and benthic zone? PLoS ONE 10, e0137201. doi:10.1371/journal.pone.0137201.

Erni-Cassola, G., Gibson, M. I., Thompson, R. C., & Christie-Oleza, J. A. (2017). Lost, but found with Nile red: a novel method for detecting and quantifying small microplastics (1 mm to 20 μm) in environmental samples. Environmental science & technology, 51(23), 13641-13648.

Fabri M., I.Pedel, L.Beuck, F.Galgani, D.Hebbeln, A.Freiwald (2014). Megafauna of vulnerable marine ecosystems in French mediterranean submarine canyons: Spatial distribution and anthropogenic impacts. Deep-Sea Res. II, 104, 184–207.

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*). Marine Environmental Research 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. Environmental Pollution 209, 68–78. https://doi.org/10.1016/j.envpol.2015.11.022

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. Frontiers in Marine Science 4. https://doi.org/10.3389/fmars.2017.00167.

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. Environmental Pollution 237, 1023–1040. https://doi.org/10.1016/j.envpol.2017.11.019.

Galgani G. (2015) Marine litter, future prospects for research. Frontiers in Marine Science, 2, 87, 1-5.

Galgani F., T. Romeo (2018) 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. Environmental Pollution, 236, 405-415. http://doi. org/10. 1016/j. envpol. 2018. 01. 097.

Galil S. (2007) Seeing Red: Alien species along the Mediterranean coast of Israel Bella. Aquatic Invasions, 2, 4, 281-312. DOI 10.3391/ai.2007.2.4.2.

Gregory M. (2009) Environmental implications of plastic debris in marine settings— entanglement, ingestion, smothering, hangers-on, hitch-hiking and alien invasions. Philos Trans R Soc Lond B Biol Sci. 2009 Jul 27; 364(1526): 2013–2025. doi: 10. 1098/rstb. 2008. 0265

Gündoğdu S., Çevik C., S. Karaca (2017) Fouling assemblage of benthic plastic debris collected from Mersin Bay, NE Levantine coast of Turkey, Mar. Poll. Bull. 124, 147–154.

Gusmão, F., Domenico, M.D., Amaral, A.C.Z., Martínez, A., Gonzalez, B.C., Worsaae, K., Ivar do Sul, J.A., Cunha Lana, P. da, 2016. In situ ingestion of microfibres by meiofauna from sandy beaches. Environmental Pollution 216, 584–590. https://doi.org/10.1016/j.envpol.2016.06.015.

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. Environmental Pollution 223, 286–294. https://doi.org/10.1016/j.envpol.2017.01.025.

Krueger-Hadfield S.A., Balestreri C. Schroeder J.S., Highfield A.C., Hélaouët P., Allum J., Moate R., Lohbeck K.T., Riebesell U., Reusch T.B.H., Rickaby R.E.M., Young J., Brownlee C., DC Schroeder (2014) Genotyping an Emiliania huxleyi (Prymnesiophyceae) bloom event in the North Sea reveals evidence of asexual reproduction. Biogeosciences 11: 5215-5234

Ioakeimidis C., Papatheodorou G., Fermeli G., Streftaris N., E. Papathanassiou (2015) Use of ROV for assessing marine litter on the seafloor of Saronikos Gulf (Greece): a way to fill data gaps and deliver environmental education. SpringerPlus 4, 463 DOI 10.1186/s40064-015-1248-4.

F.J. Jorissenn (2014) Colonization by the benthic foraminifer Rosalina (Tretomphalus) concinna of Mediterranean drifting plastics. CIESM Workshop Monographs 46, 87-95.

Karami, A., Groman, D.B., Wilson, S.P., Ismail, P., Neela, V.K., 2017. Biomarker responses in zebrafish (*Danio rerio*) larvae exposed to pristine low-density polyethylene fragments. Environmental Pollution 223, 466–475. https://doi.org/10.1016/j.envpol.2017.01.047.

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

Kühn S., E. Bravo Rebolledo, J. Franeker (2015) Deleterious Effects of Litter on Marine Life M. Bergmann, L. Gutow, M. Klages (Eds.), Marine Anthropogenic Litter. Springer International Publishing, Cham (2015), pp. 75-116

Laist, D.W., 1997. Impacts of Marine Debris: Entanglement of Marine Life in Marine Debris Including a Comprehensive List of Species with Entanglement and Ingestion Records, in: Coe, J.M., Rogers, D.B. (Eds.), Marine Debris. Springer New York, pp. 99–139.

Lastras G., Canals M., Ballesteros E., Gili J., Sanchez-Vidal A. (2016) Cold-Water Corals and Anthropogenic Impacts in La Fonera Submarine Canyon Head, Northwestern Mediterranean Sea. PLoS ONE 11(5): e0155729. doi:10.1371/journal.pone.0155729.

Li, W.C., Tse, H.F., Fok, L., 2016. Plastic waste in the marine environment: A review of sources, occurrence and effects. Science of The Total Environment 566–567, 333–349. https://doi.org/10.1016/j.scitotenv.2016.05.084

Lusher, A.L., Hollman, P.C.H., Mendoza-Hill, J.J., 2017a. 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 No. 615. FAO, Rome, Italy.

Lusher, A.L., Welden, N.A., Sobral, P., Cole, M., 2017b. Sampling, isolating and identifying microplastics ingested by fish and invertebrates. Anal. Methods 9, 1346–1360. https://doi.org/10.1039/C6AY02415G

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. Environmental Pollution 232, 467–476. https://doi.org/10.1016/j.envpol.2017.09.070.

Maes, T., Jessop, R., Wellner, N., Haupt, K., & Mayes, A. G. (2017). A rapid-screening approach to detect and quantify microplastics based on fluorescent tagging with Nile Red. Scientific Reports, 7, 44501

Matiddi, M., Hochsheid, S., Camedda, A., Baini, M., Cocumelli, C., Serena, F., Tomassetti, P., Travaglini, A., Marra, S., Campani, T., Scholl, F., Mancusi, C., Amato, E., Briguglio, P., Maffucci, F., Fossi, M.C., Bentivegna, F., de Lucia, G.A., 2017. Loggerhead sea turtles (*Caretta caretta*): A target species for monitoring litter ingested by marine organisms in the Mediterranean Sea. Environmental Pollution 230, 199–209. https://doi.org/10.1016/j.envpol.2017.06.054.

Melli V., M. Angiollillo, S. Canese, O. Giovanardi, S. Querin, T. Fortibuoni, 2017. The first assessment of marine debris in a Site of Community Importance in the northwestern Adriatic Sea (Mediterranean Sea). Marine Pollution Bulletin, 114, 2, 821-830, https://doi. org/10. 1016/j. marpolbul. 2016. 11. 012

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. Environmental Pollution 214, 517–523. https://doi.org/10.1016/j.envpol.2016.04.054

NOAA (National Oceanic and Atmospheric Administration Marine Debris Program.) (2014) Report on the Entanglement of Marine Species in Marine Debris with an Emphasis on Species in the United States. Silver Spring, MD. 28 pp

Oliveira F., P. Monteiro, L. Bentes, N. Henriques, R. Aguilar, J. Gonçalves (2015) Marine litter in the upper São Vicente submarine canyon (SW Portugal): Abundance, distribution, composition and fauna interactions. Marine Pollution Bulletin, 97, 1-2, 401-407, https:// doi. org/10. 1016/j. Mar. Pol. Bul. 2015. 05. 060

Pauli N., Petermann J.S., Lott C., M. Weber (2017) Macrofouling communities and the degradation of plastic bags in the sea: an in situ experiment. R. Soc. open sci. 4: 170549. http://dx.doi.org/10.1098/rsos.170549.

Pedà, C., Caccamo, L., Fossi, M.C., Gai, F., Andaloro, F., Genovese, L., Perdichizzi, A., Romeo, T., Maricchiolo, G., 2016. Intestinal alterations in European sea bass Dicentrarchus labrax (Linnaeus, 1758) exposed to microplastics: Preliminary results. Environmental Pollution 212, 251–256. https://doi.org/10.1016/j.envpol.2016.01.083

Pellini, G., Gomiero, A., Fortibuoni, T., Ferrà, C., Grati, F., Tassetti, N., Polidori, P., Fabi, G., Scarcella, G., 2018. Characterization of microplastic litter in the gastrointestinal tract of Solea solea from the Adriatic Sea. Environmental Pollution 234, 943–952. https://doi.org/10.1016/j.envpol.2017.12.038.

Pham C., E. Ramirez-Llodra, C. Alt, T. Amaro, M. Bergmann, M. Canals, J. Company, J. Davies, G. Duineveld, F. Galgani, K. Howell, V. Huvenne, E. Isidro, D. Jones, G. Lastras, T. Morato, J. Gomes Pereira, A. Purser, H. Stewart, I. Tojeira, X. Tubau, D. Van Rooij, P. Tyler (2014) Litter Distribution and Density in European Seas, from the Shelves to Deep Basins, Plos One, 9, p. e95839

RAC/SPA (Regional Activity Center for Specially Protected Areas Protocol- Barcelona convention) (2017) Defining the most representative species for IMPA common indicator 18. RAC/SPA, Tunis , UNEP Document (WG 439 Inf.12, 2017). 37 pages

Ramirez-Llodra E., De Mol B., Company J.B., Coll M., F. Sardà (2013) Effects of natural and anthropogenic processes in the distribution of marine litter in the deep Mediterranean Sea. Progress in Oceanography, 118, 273–287.

Remy, F., Collard, F., Gilbert, B., Compère, P., Eppe, G., Lepoint, G., 2015. When Microplastic Is Not Plastic: The Ingestion of Artificial Cellulose Fibers by Macrofauna Living in Seagrass Macrophytodetritus. Environmental Science & Technology 49, 11158–11166. https://doi.org/10.1021/acs.est.5b02005.

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.

Rochman, C.M., Hoh, E., Kurobe, T., Teh, S.J., 2013. Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. Scientific Reports 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.

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. Scientific Reports 5, 14340. https://doi.org/10.1038/srep14340.

Rodríguez B., J. Bécares, A.Rodríguez, J.Manuel Arcos (2013) Incidence of entanglements with marine debris by northern gannets (Morus bassanus) in the non-breeding grounds. Mar. Poll. Bull., 75, 259–263.

Romeo, T., Battaglia, P., 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. Poll. Bull. 95, 358–361. https://doi.org/10.1016/j.marpolbul.2015.04.048

Romeo, T., Pedà, C., Fossi, M.C., Andaloro, F., Battaglia, P., 2016. First record of plastic debris in the stomach of Mediterranean lanternfishes. Acta Adriatica 57, 115–124.

Sleight, V.A., Bakir, A., Thompson, R.C., Henry, T.B., 2017. Assessment of microplastic-sorbed contaminant bioavailability through analysis of biomarker gene expression in larval zebrafish. Marine Pollution Bulletin 116, 291–297. https://doi.org/10.1016/j.marpolbul.2016.12.055

Sonnenschein, C., Soto, A.M., 1998. An updated review of environmental estrogen and androgen mimics and antagonists. J. Steriod Biochem. Mol. Biol. 65, 143–150.

Taviani M., Angeletti L., Canese S., Cannas R., Cardone F., Cau A., Cau A.B., Follesa M.C., Marchese F., Montagna P., C.Tessarolo (2017) The "Sardinian cold-water coral province" in the context of the Mediterranean coral ecosystems. Deep-Sea Res. II, 145, 61–78.

Teuten, E.L., Saquing, J.M., Knappe, D.R.U., Barlaz, M.A., Jonsson, S., Bjorn, A., Rowland, S.J., Thompson, R.C., Galloway, T.S., Yamashita, R., Ochi, D., Watanuki, Y., Moore, C., Viet, P.H., Tana, T.S., Prudente, M., Boonyatumanond, R., Zakaria, M.P., Akkhavong, K., Ogata, Y., Hirai, H., Iwasa, S., Mizukawa, K., Hagino, Y., Imamura, A., Saha, M., Takada, H., 2009. Transport and release of chemicals from plastics to the environment and to wildlife. Philosophical Transactions of the Royal Society B: Biological Sciences 364, 2027–2045. https://doi.org/10.1098/rstb.2008.0284.

Thompson R.C., Olsen Y., Mitchell R.P., Davis A., Rowland S.J., John A.W.G., McGonigle D., A.E. Russell (2004) Lost at sea: where is all the plastic? Science, 304, 838.

Thompson R.C., Moore C.J., vomSaal F.S., S.H. Swan (2009) Plastics, the environment and human health: current consensus and future trends. Philos. Trans. R. Soc. B 364, 2153–2166.

Tubau X., M.Canals, G.Lastras, X.Rayo, J.Rivera, D.Amblas (2015) Marine litter on the floor of deep submarine canyons of the Northwestern Mediterranean Sea: The role of hydrodynamic processes. Progr. in Oceanog., 134, 379-403.

Tutman P., Kapiris K., Kirinčić M., A. Pallaoro (2017) Floating marine litter as a raft for drifting voyages for Planes minutus (Crustacea: Decapoda: Grapsidae) and Liocarcinus navigator (Crustacea: Decapoda: Polybiidae). Mar. Poll. Bull., 120, 217–221.

UNEP (2016a) Marine plastic debris and microplastics – Global lessons and research to inspire action and guide policy change. United Nations Environment Programme, Nairobi, 192 p.

Vandermeersch, G., Van Cauwenberghe, L., Janssen, C.R., Marques, A., Granby, K., Fait, G., Kotterman, M.J.J., Diogène, J., Bekaert, K., Robbens, J., Devriese, L., 2015. A critical view on microplastic quantification in aquatic organisms. Environmental Research 143, Part B, 46–55. https://doi.org/10.1016/j.envres.2015.07.016

van Franeker, J.A., Blaize, C., Danielsen, J., Fairclough, K., Gollan, J., Guse, N., Hansen, P.-L., Heubeck, M., Jensen, J.-K., Le Guillou, G., Olsen, B., Olsen, K.-O., Pedersen, J., Stienen, E.W.M., Turner, D.M., 2011. Monitoring plastic ingestion by the northern fulmar Fulmarus glacialis in the North Sea. Environmental Pollution 159, 2609–2615. https://doi.org/10.1016/j.envpol.2011.06.008.

Viršek M.K., Lovšin M.N., Koren Š., Kržan A., M. Peterlin (2017) Microplastics as a vector for the transport of the bacterial fish pathogen species Aeromonas salmonicida. Mar. Poll. Bull., 125, 301–309.

Vlachogianni, T., Anastasopoulou, A., Fortibuoni, T., Ronchi, F., Zeri, C., 2017. Marine Litter Assessment in the Adriatic and Ionian Seas. IPA-Adriatic DeFishGear Project, MIO-ECSDE, HCMR and ISPRA.

Watnick P., R. Kolter J Bacteriol. (2000) Biofilm, City of microbes. 182(10), 2675–2679.

Werner, S., Budziak, A., Franeker, J. van, Galgani, F., Hanke, G., Maes, T., Matiddi, M., Nilsson, P., Oosterbaan, L., Priestland, E., Thompson, R., Veiga, J., Vlachogianni, T., 2016. Harm caused by Marine Litter: MSFD GES TG Marine Litter - thematic report.

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

Zardus J.D., Nedved B.T., Huang Y., Tran C., M.G. Hadfield (2008) Microbial biofilms facilitate adhesion in biofouling invertebrates. Biol. Bull. 214, 91–98. doi:10.2307/25066663.

# 7. List of acronyms

| | |
|---|---|
| **ALDFG** | Abandoned, lost, discarded fishing gear |
| **AUT** | Agricultural University of Tirana |
| **D10** | Descriptor 10 (Marine Litter) |
| **DeFishGear** | Derelict Fishing Gear Management System in the Adriatic Region |
| **EC** | European Commission |
| **EcAp** | Ecosystem Approach |
| **EU** | European Union |
| **FAO** | Food and Agriculture Organization of the United Nations |
| **FTIR** | Fourier-transform infrared spectroscopy |
| **FTIR-ATR** | Fourier-transform infrared spectroscopy - Attenuated Total Reflectance |
| **GES** | Good Environmental Status |
| **GI** | Gastrointestinal |
| **IMAP** | Integrated Monitoring and Assessment PrOGRAMME |
| **IPA** | Instrument for Pre-accession Assistance |
| **ISPRA** | Italian National Institute for Environmental Protection and Research |
| **MAP** | Mediterranean Action Plan |
| **MEDPOL** | Mediterranean Pollution Monitoring Programme |
| **MIO-ECSDE** | Mediterranean Information Office for Environment, Culture and Sustainable Development |
| **MPAs** | Marine Protected Areas |
| **MSFD** | Marine Strategy Framework Directive |
| **MSFD TG10** | MSFD Technical Sub-Group on Marine Litter |
| **NGO** | Non-Governmental Organisation |
| **NOOA** | Marine Debris Monitoring and Assessment |
| **OSPAR** | Convention for the Protection of the Marine Environment of the North-East Atlantic |
| **QA** | Quality assurance |
| **QC** | Quality control |
| **ROVs** | Remotely operated vehicles |
| **S.D.** | Standard Deviation |
| **UNEP** | United Nations Environment Programme |
| **WWTP’s** | Waste Water Treatment Plants |

## THE PLASTIC BUSTERS MPAs PARTNERSHIP