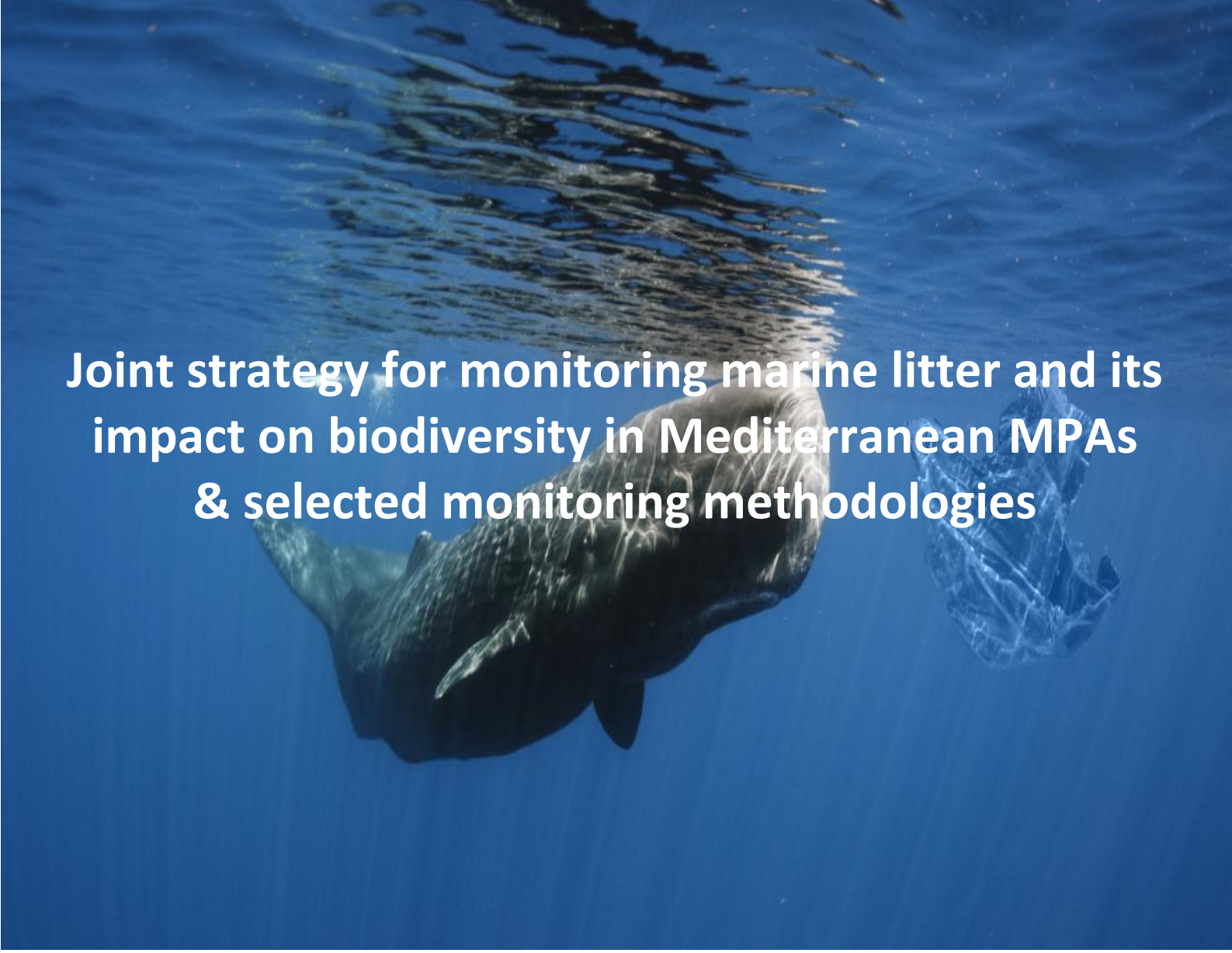

Joint strategy for monitoring marine litter and its impact on biodiversity in Mediterranean MPAs & selected monitoring methodologies

PREPARED BY

THE INTERREG MED
PLASTIC BUSTERS MPAs PROJECT

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https://plasticbustersmpas.interreg-med.eu

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This document (Deliverable 3.3.1) describes the key elements of the PlasticBusters MPAs strategy for monitoring marine litter and its impact on biodiversity in Mediterranean MPAs as well as a brief description of the selected monitoring methodologies.

Approvals

Date	Partner
24/04/2019	M.C. Fossi/UNISI (Project Scientific Coordinator) Th. Vlachogianni/MIO-ECSDE (WP3 Leader)

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Contents

1 Introduction

1.1 PlasticBusters MPAs in a nutshell

PlasticBusters MPAs is a 4-year-long InterregMed-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.

PlasticBusters MPAs consolidates Mediterranean efforts against marine litter by:

- Assessing the impacts of marine litter on biodiversity in MPAs and identifying marine litter 'hotspot' areas;
- 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 (SDSN Med). This initiative frames the priority actions needed to tackle marine litter in the Mediterranean basin and was labelled under the Union for the Mediterranean (UfM) in 2016, gathering the political support of 43 Euro-Mediterranean countries.

1.2 Aim and scope of this report

The overarching aim of this report is to describe the PlasticBusters MPAs harmonized monitoring methodology to detect the impact of marine litter on Mediterranean ecosystems and particularly on marine biodiversity, including endangered species inhabiting pelagic and coastal MPAs (cetaceans, sea turtles, birds, endangered sharks, etc.). The realization of this document involved more than 20 regional marine litter experts to collectively define the key elements of a joint strategy for marine litter monitoring in Mediterranean MPAs that is consistent with the recent work of the EU MSFD Technical Group on marine litter and the Barcelona Convention CORMON group. This report also ensures that all project partners that will be involved in the phase of testing of the harmonized marine litter monitoring approach are coordinated and share the same knowledge and capacities. To this end, a technical workshop was organized in Bonifacio in November 2018, which was dedicated to discussing and developing the PlasticBusters MPAs harmonized marine litter monitoring approach for Mediterranean MPAs. This technical meeting provided the opportunity for exchange of information, experiences and know-how related to the harmonized monitoring methodology to detect the impacts of marine litter on Mediterranean ecosystems and marine biodiversity, including endangered species inhabiting pelagic and coastal MPAs. The meeting was also attended by a number of experts involved in other marine-litter-related InterregMED projects (i.e., MedSEALitter, Act4Litter, AMARE) as well as other relevant initiatives (INDICIT) and important institutions at the Mediterranean level (UNEP/MAP, ACCOBAMS, D10 TG MSFD).
This report – which constitutes Del. 3.3.1 entitled 'Joint strategy for monitoring ML and its impacts on biodiversity in Med MPAs' – draws from the discussion held at the above-mentioned technical workshop (D.3.4.1) and on the deliverable Del. 3.2.2 entitled 'Diagnostic report on knowledge gaps and needs for marine litter and marine litter monitoring in Med MPAs' and will serve as the basis for the creation of a toolkit (D.3.3.2 - Toolkit for monitoring Ml and its impact on biodiversity in Med

MPAs) to be used by MPAs in monitoring marine litter and its impact on the biodiversity and ecosystems within and outside the MPAs (Fig.1).

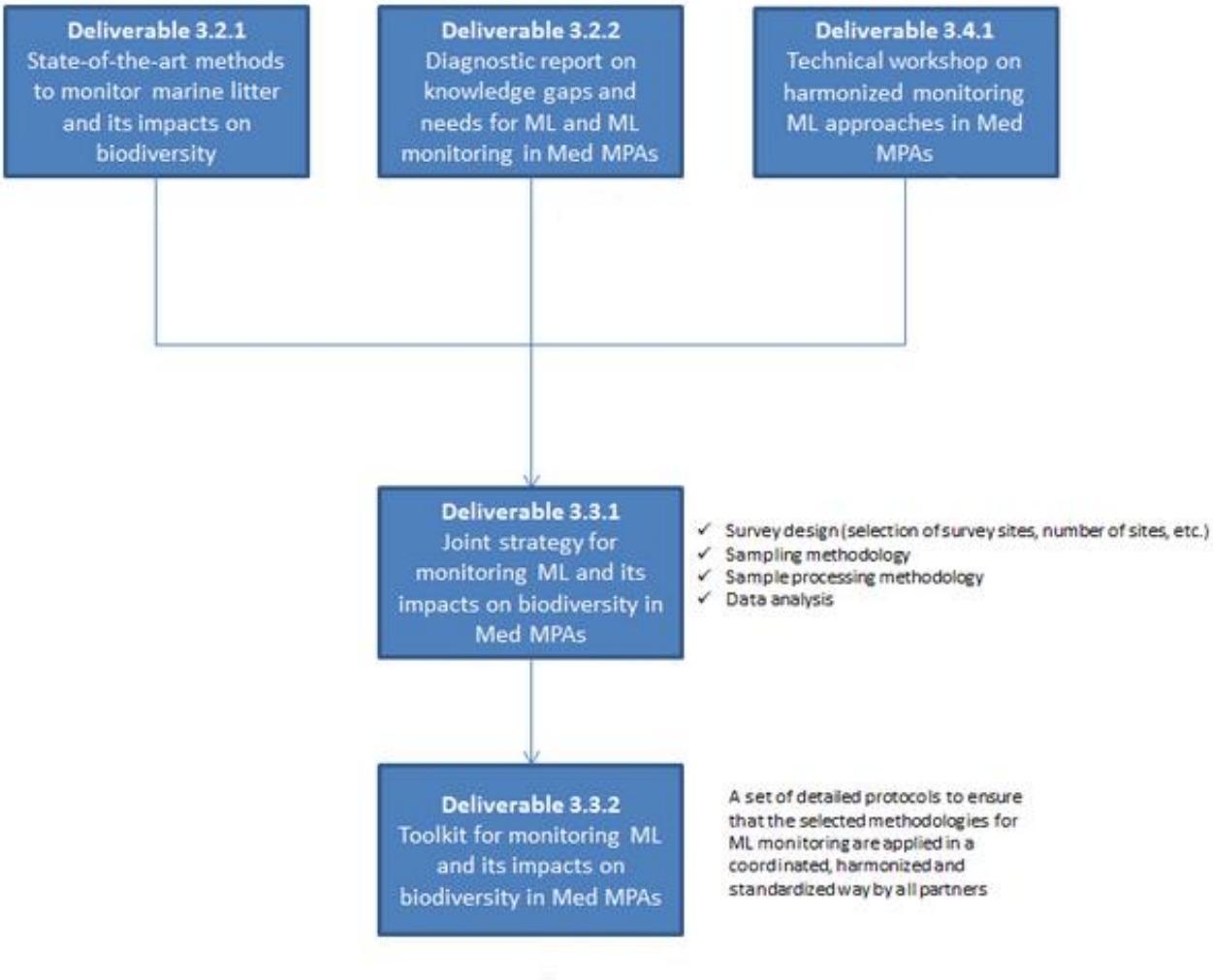

Figure 1. Overview of the PlasticBusters MPAs WP3 deliverables and their interconnections.

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 refers to items larger than 25 mm in the longest dimension, mesolitter to items between 5 mm to 25 mm, and microlitter to items ranging from 1 µm to 5 mm. This latter size class is sometime further broken down into large microlitter ranging from 1 mm to 5 mm and microplastic, from 1 µm to 1 mm in size.

The main legislative frameworks related to marine litter monitoring are the EU Marine Strategy Framework Directive – MSFD (2008/56/EC, 2010/477/EC, 2017/848/EC) and the Barcelona Convention Ecosystem Approach (COP19 IMAP Decision IG.22/7, UNEP/MED WG.450/3, June 2018) (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

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

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 Joint strategy for monitoring marine litter and its impact on biodiversity in Mediterranean MPAs: the Plastic Busters approach

2.1 The Plastic Buster approach

The studying phase (WP3) of the project addresses the need for defining a harmonized and coordinated response to marine litter in Mediterranean MPAs, by identifying and consolidating approaches for the monitoring and mitigation of marine litter impacts in Mediterranean MPAs. Plastic Busters MPAs deploys the multidisciplinary strategy and common framework of action developed within the Plastic Busters initiative led by UNISI SDSN-MED.

The main findings expected by the application of the Plastic Busters approach are:

a) DIAGNOSTIC PHASE: development of effective methodologies to diagnose the marine litter (including microplastics) presence and impacts on biodiversity inhabiting Mediterranean MPAs (Del. 3.3.1), including the identification of Marine Litter hotspots (Del. 3.5.1).

b) MITIGATION PHASE: development of efficient tailor-made surveillance, prevention and mitigation measures in Med MPAs (Del.3.6.1) designed according to the diagnostic actions carried out during the monitoring phase. Identification of a series of measures to prevent and mitigate the impacts of marine litter in the hotspot areas, while capitalizing on previous projects results (e.g. Interreg MED ACT4LITTER, MEDSEALITTER, DG-ENV INDICIT, IPA-Adriatic DeFishGear, etc.).

The main steps and methodologies in the development of the DIAGNOSTIC PHASE of the Plastic Busters approach are (Fig.2):

(i) development of models for the identification of marine litter “hotspot” areas in Mediterranean MPAs (Del. 3.5.1);

(ii) identification, finetuning and/or elaboration of monitoring protocols to assess the presence and impacts of macro- and microplastics in pelagic and coastal Mediterranean MPA environments in a harmonized way in the following compartments:

- on beaches
- on the sea surface
- on the sea floor
- in rivers

(iii) identification, finetuning and/or elaboration of monitoring protocols to detect the impact of marine litter on Mediterranean marine biodiversity inhabiting pelagic and coastal MPAs in a harmonized way. In detail:

a) Monitoring marine litter presence and impact in commercially harvested species (invertebrates and vertebrates).

b) Monitoring marine litter presence and impact in endangered species (cetaceans, sea turtles, sea birds, etc.).

(iv) development of harmonized GIS system and risk analysis in hotspots and control areas (in and outside MPAs).

It is important to underline that the methodologies described in the present deliverable will be validated and tested during the Testing phase of the project (WP4) and the final version of this

integrated and harmonized methodological approach will be completed during the Transferring phase (WP5).

The final aim of the application of this approach will be to support MPA managers in their efforts to achieve the conservation goals set in their MPAs (WP6). Furthermore, these results will facilitate effective policymaking at local, national and regional levels with regards to the prevention, reduction and removal of marine litter in Mediterranean MPAs, within the framework of the EU MSFD and the Barcelona Convention Regional Plan for Marine Litter Management in the Mediterranean (Fig. 2).

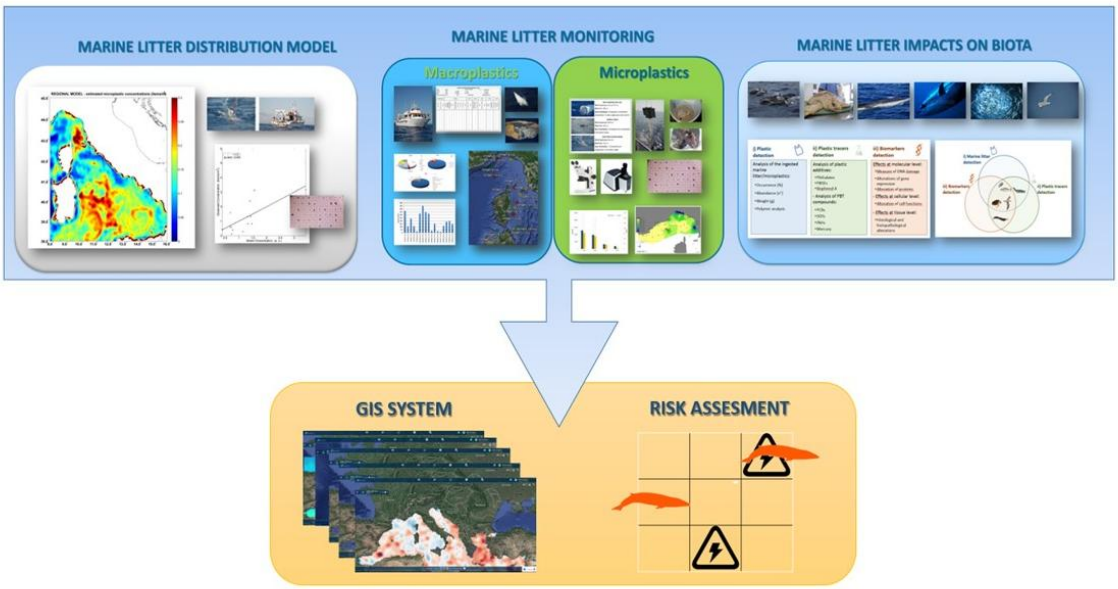

Figure 2. *Main steps and methodologies of the diagnostic phase of the Plastic Busters approach.*

2.2 The application of the Plastic Buster approach in the Mediterranean MPAs

The Plastic Buster approach will be applied in the Testing phase (WP4) of the Plastic Busters MPAs InterregMed Project to tackle the problem of the quantitative and qualitative diagnosis of the impacts of marine litter in Mediterranean MPAs and on marine biodiversity and the related issue of the protection of biodiversity and ecosystems in pelagic and coastal MPAs through targeted prevention and mitigation actions. The main expected findings of the application of this harmonized approach, described in this deliverable, are:

1. a comprehensive assessment of the quantities, status, composition and impacts of marine litter on marine species in the pelagic and coastal MPAs;
2. a hotspot analysis mapping marine litter accumulation areas based on both ocean currents and convergence areas, and state-of-the-art modelling and satellite-based tools;
3. a marine litter forecasting model predicting the transport patterns and marine litter accumulation areas, to design targeted mitigation actions in Mediterranean MPAs.

The testing of the harmonized monitoring approach and the MPA-specific measures will be made in close collaboration with MPA managers and multiple stakeholders groups through a participatory approach. Marine litter surveys will be carried out in pelagic and coastal MPAs (e.g. Pelagos Sanctuary, Tuscan Archipelago, Zakynthos National Marine Park, Parque Nacional Del Archipiélago

De Cabrera) in close collaboration between the partner MPA managers and the partners with strong competences on marine litter monitoring (ISPRA, UNISI, IFREMER, IEO, HCMR, MIO-ECSDE).

The harmonized monitoring protocols are described in detail in the thematic chapters subdivided in the following methodological sections:

a. Survey design: this section describes how the survey will be planned in the different MPAs according to MPAs extension
b. Sampling methodology: this section describes how the sampling should be performed in all the environmental compartaments as well as biota and the methodologies to be used
c. Sample processing methodology: this section describes how the samples should be processed and how data should be analysed and reported.

The monitoring methodologies described in this deliverable have been developed to be tailor-made according to the extension of the Marine Protected Areas to be monitored. Whenever necessary, these methodologies have been described for each of the three types of protected areas completed in the project:

a) **Large pelagic and coastal areas** (SPAMI, EBSA): SPAMI Pelagos Sanctuary

b) **Medium scale MPAs**: Tuscan Archipelago

c) **Small scale MPAs**: Cabrera, Zakynthos

The end goal is to develop a monitoring strategy, which could be adapted to other Mediterranean MPAs on the basis of their extension and characteristics – including MPAs/SPAMIs (Specially Protected Areas of Mediterranean) and EBSAs (Ecological or Biologically Significant Areas) – during the transferring phase of the project.

3 Monitoring marine litter on beaches

3.1 Macro-Litter methodology

3.1.1 Survey design

Site selection

The sites to be monitored should be selected randomly but taking into consideration certain criteria (Galgani et al., 2013). The selected beaches should be located in Mediterranean coastal and marine protected areas and should be situated (wherever applicable):

- ✓ In the vicinity of ports or harbors;
- ✓ In the vicinity of river mouths;
- ✓ In the vicinity of coastal urban areas;
- ✓ In the vicinity of tourism destinations;
- ✓ In relatively remote areas.

In addition, the selected beaches should:

- ✓ Have a minimum length of 100m;
- ✓ Be characterized by a low to moderate slope (~1.5-4.5º), which excludes very shallow tidal mudflat areas;
- ✓ Have clear access to the sea (not blocked by breakwaters or jetties);
- ✓ Be accessible to survey teams throughout the year;
- ✓ Ideally not be subject to cleaning activities. In case they are subjected to litter collection activities, the timing of non-survey related beach cleaning must be known so that litter flux rates (the amount of litter accumulation per unit time) can be determined.

The surveys should not pose any threat to endangered or protected species and their habitats, such as sea turtles, sea birds or shore birds, marine mammals or sensitive beach vegetation.

In each site selection, these criteria should be followed as closely as possible. However, when making the final selection of the beaches to be monitored the surveyors can use their expert judgment and experience related to the coastal area and marine litter situation in their respective country.

Frequency and timing of surveys

At least four surveys should be carried out, one per each season of the year. The proposed survey periods are:

1. Autumn: mid-September-mid October
2. Winter: mid-December-mid January
3. Spring: April
4. Summer: mid-June-mid July

3.1.2 Sampling Methodology

Sampling unit

The sampling unit should be a 100-meter stretch of beach along the strandline and reaching to the back of the beach (see figure 1). The back of the beach needs to be explicitly 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) sampling units (100-meter stretches) on the same beach should be monitored. They should be separated at least by a 50-meter stretch. The same sampling units should be monitored for all subsequent surveys.

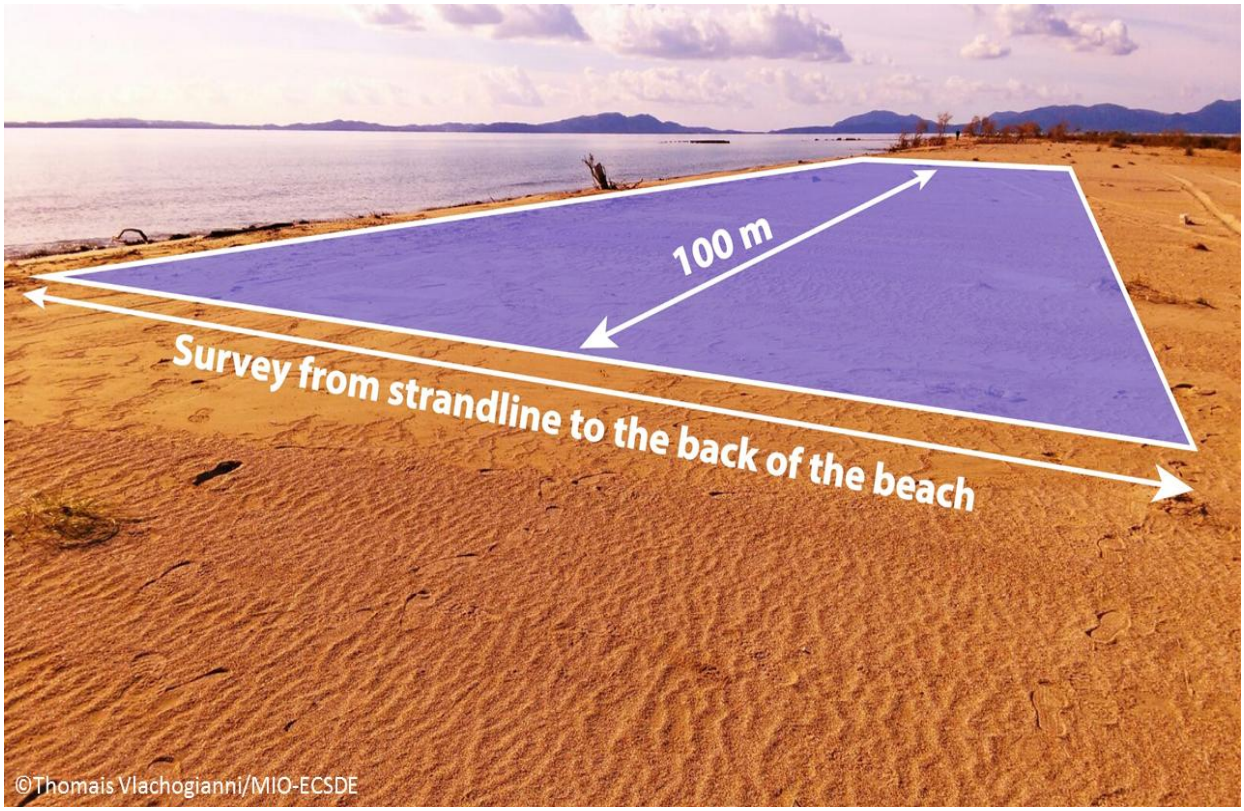

Figure 3. *The sampling unit for beach macro-litter monitoring.*

Litter size limits to be surveyed

There are no upper size-limits for litter items to be recorded on beaches. But in order to ensure the inclusion of caps, lids, cigarette butts and other similar items in the quantification of beach litter, items as small as 2.5 cm in the longest dimension have to be recorded.

3.1.3 Sample Processing Methodology

Items found on the sampling unit will be classified by type according to the MSFD Technical Group on Marine Litter Joint List of Litter Categories (Fleet et al., 2019) (Annex 1) and the associated manual for the application of the list. During the survey, all litter items should be sorted by 'category type' and then removed from the beach.

3.2 Micro-Litter methodology

A great variety of methods concerning both the sampling and laboratory analysis are applied in studies investigating plastic abundances on beaches. In addition, investigation of microlitter in beach sediments is time consuming, technically demanding and require specialist equipment and training and thus specific survey guidelines have not been included in the Regional Seas Convention (RSC)'s monitoring programmes.

During the project workshop that took place in Bonifacio in November 2018 (Del 3.4.1), prior to the drafting of this deliverable, it was decided to consider only the large microplastics (1-5 mm fraction of microplastics), as this separation procedure is simple and can be efficiently applied by stakeholders.

3.2.1 Survey design

Site selection

The site selection and the timing for the microlitter monitoring should closely follow the strategy for monitoring macrolitter based primarily on the TG10 guidelines (Galgani et al, 2013). Microlitter should be monitored in the same transects used for macrolitter and during the same four seasonal macrolitter surveys wherever possible. The partners should choose sandy beaches following the criteria set for the macrolitter surveys, where macro- and micro-litter will be studied seasonally.

3.2.2 Sampling Methodology

Sampling unit

The most recent and detailed protocol was published in the framework of the BASEMAN JPI Oceans project (Frias et al, 2018), based basically on the guidelines of the TG10 (Galgani et al, 2013).

More specifically: the sampling area should be defined by marking out a 100 m transect in width, parallel to the water edge (sea), using a measuring tape and taking note of the GPS coordinates on each side of the transect. This transect will define the sampling area i.e. from the shoreline (low tide) to above the strand line (accumulation zone Collect a minimum of 3 samples along three transects vertical to the high tide line. Make sure the area between the two high tide lines is surveyed. Mark the sampling unit (30 x 30 cm or 50 x 50 cm or 1x1 m) using the measuring tape or a quadrat and record the GPS coordinates of each unit. Collect the top 3-5 cm of sediment using a metal shovel or similar. Note the volume of the sample.

Litter size limits to be surveyed

In case that only the large microplastics (1-5 mm) will be separated, sieving of sediment samples *in situ* through two metallic sieves with 1 mm and 5 mm mesh size is an effective method of reducing the sample volume. During sieving, remove large or obviously non-plastic items, e.g. shells, leaves, twigs, etc. If the beach sediments are wet and difficult to pass through the 1 mm sieve, use seawater to help the sieving. Otherwise, take the samples to the laboratory stored in glass jars or zip-lock bags.

Take the opportunity to count also the mesoplastics in the material retained on the 5 mm sieve. Since particles larger than 2.5 cm are collected in the macrolitter surveys, here it is suggested to also count mesolitter particles ranging between 5 mm and 2.5 cm.

3.2.3 Sample Processing Methodology

In case the sediment samples are sieved on the beach, the material retained on the sieves is to be transferred to the lab and dried in the oven for 24h or kept in a desiccator.

Identification

Concerning the separation of microplastics from sediment - the most important step of the analysis - sieving is implemented for large microplastics (1-5 mm) while floatation is used for small microplatics (<1 mm) due to density differences between plastic and sediment particles. Different high density salt solutions have been applied for this purpose. The size of particles also differs between studies; nevertheless, the widely accepted size categories are the following: >5 mm-2.5 cm: mesoplastics, >1 mm-5 mm large microparticles, >1 µm-1 mm: small microplastics and <1 µm: nanoplatics.

Visual identification of the type of microplastics described in peer-reviewed publications and the categories suggested are the following: 1. Pellet, 2. Fragment, 3. Fibre, 4. Film, 5. Rope and filaments, 6. Microbeads, 7. Styrofoam (expanded polystyrene-PS), 8. Rubber. Microbeads and rubber are rarely recorded in environmental samples.

The most common colours identified are the following: 1. Black, 2. Blue, 3. White, 4. Transparent, 5. Red, 6. Green, 7. Multicolour, 8. Other.

For the identification of the polymer type, it is recommended to use ATR-FTIR spectrometer or Raman spectroscopy. Note that visual identification is not deemed adequate and it shall not replace chemical analysis.

All steps shall be conducted using 100% cotton labcoats and precautions are to be taken to avoid cross-contamination (e.g. airborne fibers).

Reporting units

The proposed reporting units for microplastics retrieved from sediment samples are:

1. no. MPs per area (# particles m^{-2})
2. no. MPs per volume (# particles m^{-3})
3. no. MPs per mass (# particles kg^{-1} dry sediment). In this case the weight of the sediment sample is needed or the density of the sediment
4. mass of MP per area (g MP m^{-2})
5. mass of MP per volume (g MP cm^{-3})

Environmental variables

The essential environmental variables to be considered during the processing of the sample are:

1. Type of beach sediment, determined by granulometry, 2. Wind speed and direction, 3. Beach slope, 4. Amount of macro- and meso- marine litter, 5. Proximity to anthropogenic sources, 6. Proximity to river streams and/or estuaries, 7. Proximity to beach infrastructures (e.g. cafes, restaurants, nightclubs). Note that these variables are also collected by the macrolitter surveyors.

Forms to collect data while sampling beach sediments and observation datasheets for the lab will be distributed to the partners willing to study microplastics on beaches.

3.3 References

Frias et al., (2018). Standardised protocol for monitoring microplastics in sediments. JPI-Oceans BASEMAN project.

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.

Fleet et al., 2019. Marine Litter Categories. Guidance for monitoring of macro-litter. Scientific and Technical Research series, Report EUR EN.

4 Monitoring marine litter in sea surface

4.1 Survey design

Site selection

4.1.1 Macrolitter and microlitter

Simultaneous monitoring of floating litter (macro-, meso- and micro-litter) and the presence of biota (marine mammals, sea turtles, seabirds, etc.) should be conducted to assess the overlap between the occurrence of plastic and biota in the area monitored. These simultaneous observations are intended to contribute to the development of future "risk maps".

Following the MEDSEALITTER approach, the methodologies for monitoring marine litter on the sea surface have been elaborated according to the size of the different Marine Protected Areas to be studied (large, medium and small).

4.1.2 Large Pelagic Areas (SPAMI, EBSA)

A different spatial distribution is to be applied according to the size of the MPAs being studied. In the case of large pelagic areas, such as the SPAMI Pelagos Sanctuary (one of selected area of the Testing phase), the testing design should cover an area of at least 40-50 nautical miles (nm) for each sampling day. The survey design shall be adapted using the output of the forecastig model (Del. 3.5.1), which identifies hot/cold spot areas. The outcome of the field observations (the analysis of the samples and surveys) shall be used to validate the forecasting model.

The survey should be carried out in a single season (late spring/summer) by a standardized protocol, depending on the vessel used and the area monitored.

A proposed scheme of the activity is shown in Fig. 4.

The monitoring of the biota shall be performed during this sampling phase as further described in paragraph 4.2.3.

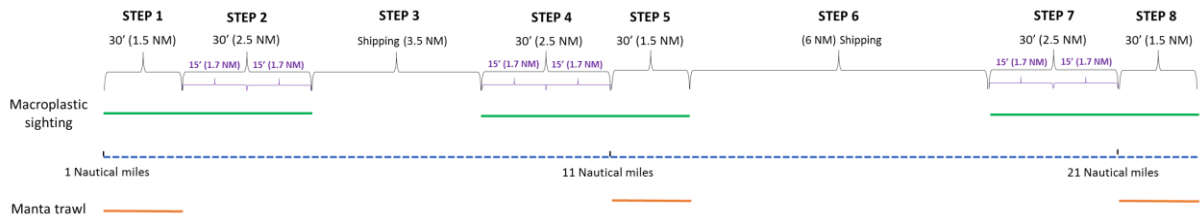

Figure 4. *Large Pelagic Areas survey design. Green line: Macrolitter monitoring for 30 minutes at 5-6 knots; Dotted blue line: biota observation and monitoring; Orange line: manta trawl, 30 minutes at 2.5 knots.*

4.1.3 Medium Scale MPAs

When surveying floating marine litter in medium scale MPAs (e.g. PNAT MPAs, Capraia and Giglio Islands), a total of 8 transects should be carried out from the coast up to 3 miles offshore, following the simultaneous monitoring methodology. For larger islands (e.g. PNAT MPAs, Elba Island), a total of 12 transects should be performed. Specific transects should be also carried out within the large distance bewtween the islands and the mainland coasts (e.g., from the islands of the Tuscan National Park (PNAT) to the Pelagos Sanctuary and *viceversa*). Every 10 miles, a visual transect of 30 minutes at a speed of 3 knots should be carried out.

4.1.4 Small Scale MPAs

Given that weather conditions and hydrodynamics vary according to the orientation of the island and the presence of small inlets, short surveys are suggested in the nearshore and offshore regions at different locations. In each location two sites are to be sampled, which are parallel to the coast: one site at < 1 km nearshore and the other at 2 km offshore. The survey is to be carried out in a single season (late spring/summer). In the case of Cabrera National Park (one of selected area of the Testing phase), for instance (see Figure 5), five sampling locations have been selected according to environmental and anthropogenic characteristics of the small MPAs.

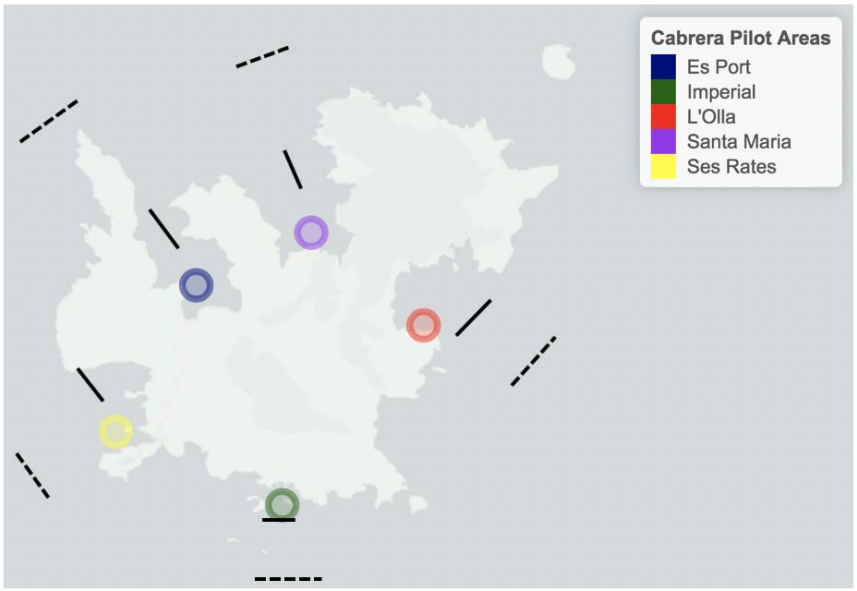

Figure 5. *Example of proposed sea surface sampling at Cabrera National Park. Bold lines indicate survey trawls 250 m off the coast and dotted lines surveys located 1 nm off the coastline.*

4.2 Sampling methodology

4.2.1 Floating macrolitter

Sampling unit

The observations are to be made from the bow of the boat and only in optimum sea and weather conditions (Visibility: good and very good; Beaufort Sea state: between 0 and 2).

Two different observers should alternate every 30 min to avoid fatigue. In small coastal areas, where a lower number of transects and explored areas are to be carried out, a single observer might be used for visual monitoring.

The observation width will be estimated according to the observation height of the vessel used in each MPA.

Litter size limits to be surveyed and categories

In surveying floating litter, the categories agreed by the TG on marine litter of the MSFD (updated with the latest Joint List released by the EU) shall be followed (Annex 1). Identification and categorization of items is to be organized by material (Artificial polymer material, Processed/Worked wood, Metal, Cloth/Textile, Paper/Cardboard, and Rubber).

The following size classes should be considered (2.5 cm-5 cm; 5 cm-10 cm; 10 cm-20 cm; 20 cm-30 cm; 30 cm-50 cm; 50 cm - 100 cm; >100 cm) specifying whether single or aggregate objects are surveyed, and always taking into account the observation height, i.e. the ability to detect small sized items.

4.2.2 Microlitter

Sampling procedure

Microlitter sampling should be performed using a manta-net, mesh size of 330-335 µm, equipped with a flow meter. The manta-net should be towed for 30 min at a constant vessel speed < 3 knots and both start and end position should be recorded with GPS. All tows are conducted from the ship's side and beyond the ship's wake. After completion of each tow, the net should be washed thoroughly with filtered seawater to collect all particles in the cod-end. The sample collected in the cod-end should then be rinsed with seawater on a 300 µm metallic sieve and transferred in glass jars. The samples should be stored in 70% ethanol solution for further analysis and a limited number kept frozen to perform chemical analysis.

4.2.3 Biota monitoring protocol

Biota monitoring should be perfomed, simultaneously with the monitoring of floating litter (Figure 4), by observing and recording the presence of large marine biota. The process is characterized by two phases:

Transiting phase (Off Effort): this phase is defined as the displacement of the vessel from the port to the starting point of the first marine litter transect and back to the port from the ending point of last marine litter transect. During the transiting phase, GPS recording track of vessel should always be on (opportunistic sightings might occur during this phase).

Searching phase (On Effort): "On Effort" starts with the starting point of the first marine litter transect and ends at the last point of the last marine litter transect. Once "on effort", the observer team starts to work. The observer team is composed of at least 3 observers positioned in elevated platforms to scan for cetaceans plus 1 more person in charge of data-acquistion. The presence of 1-2 extra observers is strongly recommended to guarantee shifts for resting. During the "on effort" phase, observers will be looking for biota with binoculars.

Environmental data shall be recorded every 30 minutes.

In case of cetacean sighting, the following information must be communicated:

- Time
- Species
- Minimum / Maximum / Best number of individuals
- Animal bearing (using the compass in the binoculars) (A°)
- Vessel heading (using the compass in the binoculars) (B°)
- Distance from the sighting (using the graticule in the bino) (C)
- Notes: presence of juveniles, peculiar behaviours, etc

A°, B° and C should be recorded at the first detection. If a sighting (cetacean or associated species) is approached, this should be noted in the notes. Real position of sighting shall be then recorded as soon as the sighted animal(s) is reached.

4.3 Sample processing

4.3.1 Macrolitter

Identification and reporting units

Data is to be reported on a paper sheet and/or a portable computer/tablet when possible. The density (D) of macrolitter should be calculated using the formula D=N/(W×L) (Hinojosa and Thiel, 2009) where N is the number of observed plastic debris, W is the maximum distance perpendicular to the transect and L is the total length (in km) of the transect.

4.3.2 Microlitter

Sample processing and size classification

Once in the lab, the manta-trawl samples are to be processed as follows (Fig. 6) (Zeri et al., 2018):

- Wet Sieving – Separation into 3 size classes using 5 mm, 1 mm, and 0.3 mm stainless steel mesh sieves. Accordingly, microlitter is classified in three size classes: Small Microlitter SML (300 µm – 1 mm), Large Microlitters LML (1 mm-5 mm), mesolitter (5 mm-25 mm).
- Mesolitter (5 mm-25 mm) and LML (1 mm – 5 mm): Visually inspect the sample on the sieve and transfer only plastics in pre-weighted Petri dish. Dry at 60°C and weigh to determine the mass of mesoplastics.
- SML (0.3 mm-1 mm): Collect the sample from the sieve with deionised water and filter through pre-weighed GF/C filters. Dry the filters at 60°C for 24 hours and weigh. Determine the mass of small microlitter particles (SML mass). In case of high natural organic matter content in the samples, a step of peroxide digestion precedes filtration. Collect the digested material with deionised water and continue with filtration, drying and mass determination.
- Sorting: Sort separated size classes in the categories set by TGML-EmodNet categories and transfer into pre-weighed Petri dishes. For LMLs and SMLs, examination is done under stereomicroscope together with a digital camera and a software.
- Weigh the Petri dishes and determine the mass of each category.
- For 10% of the selected items, identify the polymer type using a spectrometer (FT-IR spectrophotometer or Raman spectroscropy).
- Contamination Control: all surfaces should be clean. The glassware is to be rinsed thoroughly with purified water. Samples are to be covered with foil paper during the analysis. Petri dishes are to be covered with glass lids during observation under stereomicroscope. Procedural blank samples should be used in all steps and items similar to those found in blank samples excluded, as they should be considered airborne contamination.
- Samples are to be kept in Petri dishes for long-term storage.

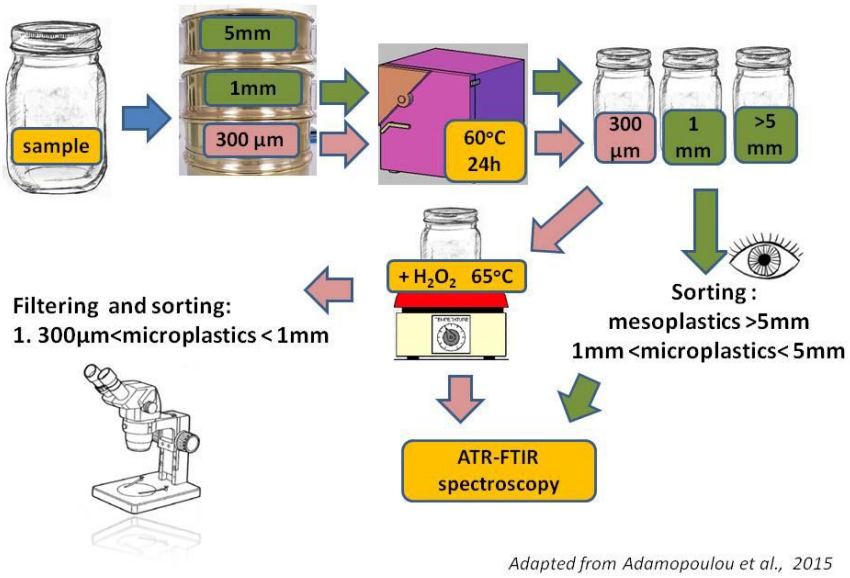

Figure 6. *Schematic represantation of the various steps of processing floating meso- & microlitter samples.*

4.3.3 Biota data

Data obtained during the biota monitoring is to be used in post-processing to compute real position of sighting (fundamental information when cetaceans sighted are not approached) and effective width of sampled area. Data is to be then integrated into the model together with macro-, meso- and micro-litter results (see chapter 9 “Risk Assessment”).

4.4 References

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5 Monitoring marine litter on the sea floor

The monitoring of marine litter on the seafloor will be performed by means of visual census methods, which are to be tested in the project MPAs at different depths:

A) Ultra-deep areas and ***Deep sea areas*** (eg. SPAMI - Pelagos Sanctuary): data should be collected from ROV (Remotely Operated Vehicles) footages. However, since a standardized method is not yet available, results should be collected using different methodologies and then compared in order to derive an agreed final Plastic Busters MPAs protocol.

B) Coastal Areas: building on previous projects and studies, a protocol to be developed by Plastic Busters MPA partner should be tested by Scuba diving, ROV and, if possible, by citizen science in all the MPAs involved in the project.

5.1 Macro-litter methodology

5.1.1 Survey design

Site selection

Sites should be selected to ensure that they:

- ✓ Comprise areas with uniform substrate (ideally sand/silt bottom);
- ✓ Consider areas that might accumulate litter;
- ✓ Avoid areas of risk (presence of munitions) and sensitive areas;
- ✓ Do not exert impacts on any endangered or protected species.

Sites should be chosen following a dual approach: (i) selecting sites that meet certain criteria (e.g. are close to ports, river mouths, cities, etc.); (ii) choosing randomly from a large number of sites.

Frequency and timing of survey

At least two surveys, one in autumn and one in spring should be carried out. The proposed survey periods are:

- ✓ Autumn: mid-September to mid-October
- ✓ Spring: April

Shoud surveys have to be implemented in the summertime, from the period from mid-June to mid-July should be preferred.

5.1.2 Sampling methodology

Sampling unit

SCUBA

The survey area is defined by the transect width and length. The line transects are defined with a nylon line, marked every 5 meters with resistant paints, that is deployed using a diving reel while SCUBA diving. Distances should be determined either by laying out a 100 m tape measure or alternatively by laying a 100 m length of weighted rope across the bottom. The start and end point of each transect should be identified with marker buoys and recorded using a GPS.

The length of the line transects could vary between 50 and 100 m while that of the width between 4 and 8 m, depending on the depth, the depth gradient, the turbidity, the habitat complexity and the litter density (see table below).

ROV

The sampling unit is defined by the ROV transect width and length. The surveyed area is calculated by multiplying the transect length with the visual field (width) of the ROV video. The visual field is estimated by the laser pointers scale in the video images or from the ROV altitude.

Monitoring of seafloor marine litter in medium scale MPAs, such as for instance in the PNAT MPAs (e.g. Capraia Island), should be performed with ROVs, as illustrated in Figure. 7.

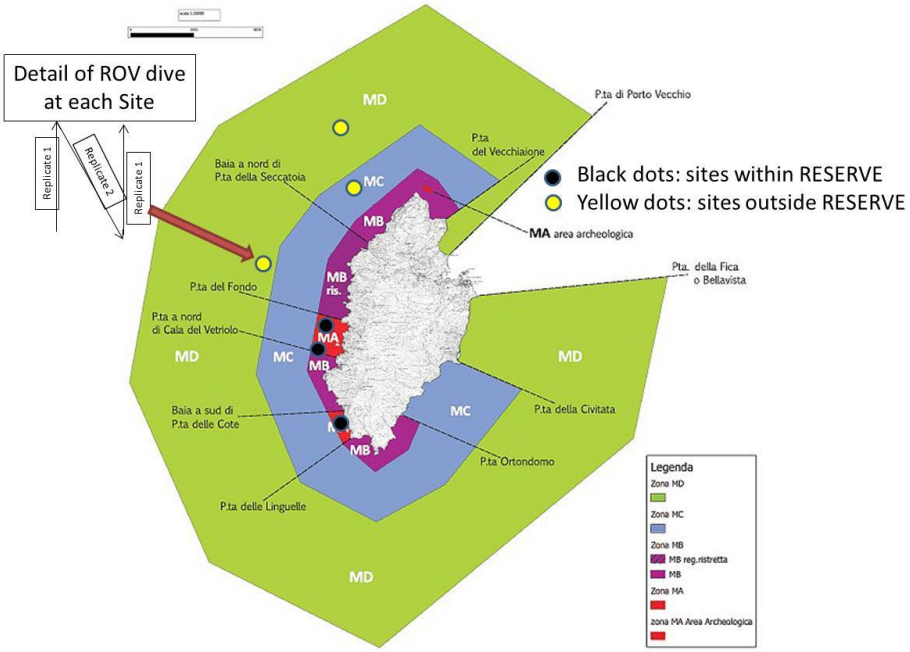

Figure 7. *ROV sample design in Capraia Island.*

Transects start at a minimum depth of 40-50 m and should be at least 100 m long. The three replicates may be performed seamless: starting from 40-50 m depth, the ROV is to descend along the bottom making the first transect, then it rises up again to the aforementioned bathymetry and descend again (see Fig.7). The path should therefore have a zig-zag shape and be composed of three transects that should be divided during data processing. Time is expected to be saved by avoiding the edge of the ROV.

ROV should be equipped with:

- high definition video camera
- laser beams at known distance, to use as a metric scale
- underwater acoustic tracking position system, to provide a detailed geographical and depth position of the ROV

Citizen Science

This optional and complementary activity is intended to involve recreational divers as volunteers in a Citizen Science (CS) initiative: recreational divers can remove (or just count) marine litter and report data on the types, quantities and locations of litter collected during recreational dives inside the MPA. Recreational divers should be trained so that they can collect and separate debris items according to the same master list used for ROV survey and SCUBA diving surveys performed by scientific research divers.

This CS initiative allows overcoming economic constraints on research divers' data collection and it is expected to be highly successful at collecting a very large amount of data in a short period of time.

5.1.3 Sample Processing Methodology

SCUBA

When conducting underwater visual surveys, lighter litter items should be collected (while larger items should just be marked), brought ashore and entered in the 'Seafloor Litter Monitoring Sheet for shallow waters'. Moreover, digital photos should be taken for all items with an underwater camera and, once identified, entered in the 'Benthic Litter Monitoring Sheet'. On the sheet, each type of item shall be given a unique identification number. Unknown litter, or items that are not on the survey sheet, should be noted in the appropriate "other item box". A short description of the item should then be included on the survey sheet.

The unit in which litter should be recorded is number of items and it should be expressed as counts of litter items per 100 meter square (litter items/100m^2).

ROV

All litter items observed from each video transect should be entered on the 'Seafloor Litter Monitoring Sheet'. The identification and correct categorization of litter items should be facilitated by photos. On the sheet, each type of item shall be given a unique identification number. Unknown litter or items that are not on the survey sheet should be noted in the appropriate "other item box". A short description of the item should then be included on the survey sheet.

The unit in which marine litter should be recorded is the number of items and it should be expressed as counts of litter items per 100 square meters (litter items 100 m^{-2}). When it is not possible to estimate the surveyed area (e.g. when lasers are not available), the unit in which marine litter could be expressed is items per 100 meters (items 100 m^{-1}).

5.2 References

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6 Monitoring marine litter in rivers

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 and be possibly 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.

Given that many Mediterranean coastal and marine protected areas are located in the vicinity of river outflows into the Sea, it is considered of high importance to monitor marine litter in the river water bodies. In this respect, a harmonized monitoring of floating macro-litter by visual observation is to be piloted in selected areas in line with the approached described by González et al., (2016).

6.1 Macro-litter monitoring methodology

6.1.1 Survey design

Site selection

The sites to be monitored should be located in the vicinity of the river mouth, which can provide a solid indication (unless there are significant sinks, e.g. in the estuary) of the cumulative amount of litter that is released in the coastal and marine environment. As estuaries are highly complex systems, sampling should be done upstream to facilitate data acquisition and interpretation. Likewise, in tidal environments, a monitoring site should be chosen that is not subject to the influence of tidal currents on the observed litter. The selected sites should be characterized by an undisturbed linear flow.

Frequency and timing of surveys

At least four surveys should be carried out, one per season of the year, apart from the rivers outflows on large pelagic areas which will be performed in one season only. The proposed survey periods are:

1. Autumn: mid-September-mid October
2. Winter: mid-December-mid January
3. Spring: April
4. Summer: mid-June-mid July

6.1.2 Sampling methodology

Survey area

The visual observations should be done from an elevated monitoring site, ideally a bridge or any other available structure allowing appropriate viewing for identification of floating items bigger than 2.5 cm. The height of the selected observation site (vertical distance between observer's eyes and river surface) should allow detection of litter items down to 2.5 cm (lower limit for macro litter), but use of binoculars could help with identification if necessary. Each monitoring session should last 30–60 min. It is recommended to perform the observations facing upstream to have an unobstructed view of the arriving water surface. Observers will have to select the appropriate time of day for monitoring, considering light conditions (e.g., to reduce light reflections or shades). Definition of the observation track width (section where the observer focuses for identification of items) will allow estimation of litter fluxes in relation to the river section total width (distance between the two

margins at the monitoring site). In addition, the river surface water speed is also considered for surface flux calculation.

Litter size limits to be surveyed

Litter items in the size range of 2.5 cm (in the longest dimension) to 50 cm should be monitored and reported. However, it is recommended to also record items larger than 50 cm in order to understand the relevance of larger than 50 cm items in the statistical evaluation of data. Given that visual observation will not permit the exact measuring of object sizes, the following size range classes should be reported for each recorded litter item:

A. 2.5 cm-5 cm
B. 5 cm-10 cm
C. 10 cm-20 cm
D. 20 cm-30 cm
E. 30 cm-50 cm
F. > 50 cm

6.1.3 Sample Processing Methodology

Items found in the sampling unit should be classified by type according to the MSFD TG ML updated marine litter items category list (Fleet et al., 2019) (see Annex 1).

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.

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7 Monitoring marine litter in biota

7.1 Monitoring marine litter ingestion and impacts on biota: the PlasticBusters MPAs monitoring approach

This paragraph describes methodological approaches for monitoring marine litter in MPAs and marine litter hotspots, with the end objective to establish the impact of marine litter on endangered (cetaceans, sea turtles, seabirds) and commercially harvested species (invertebrates and fish).

The main monitoring activities described are:

a. sentinel species selection and sampling in MPAs;
b. endangered species survey and sampling in MPAs (eg. cetaceans sampling, samples from stranded and hospitalized turtles);
c. ecotoxicological investigation of the impact of marine litter (POPs, plastic additives, biomarkers) in target species;
d. detection of marine litter impact on fishery resources;
e. final assessment of marine litter impacts on marine species in MPAs;
f. risk analysis in hotspots and MPAs (through the merging of modeling and field data).

Assessing the impact of litter on marine organisms is a challenging task. Physical and ecotoxicological effects strictly related to marine litter and, in particular, to plastics can be directly addressed in just few cases thus calling for an integrated approach. The impact of litter on marine organisms should be assessed using a multi-tier monitoring approach, proposed within PlasticBusters MPAs, which links marine litter ingestion detection with the physical and toxicological effects related to the ingestion of contaminated plastic litter.

The proposed monitoring approach – defined as the *threefold monitoring approach* – relies on the following three kinds of data (Fig. 8):

I. analysis of the gastro intestinal content in vertebrates/invertebrates (or of the whole organism, in the case of small invertebrates) to evaluate the marine litter ingested by the selected species, with a particular focus on plastics and microplastics. This analysis must focus on assessing the occurrence (%) of individuals that have ingested marine litter, the abundance (n°) of marine litter ingested per individual, the weight (g) of marine litter ingested as a total and per category of litter, the colour of litter items, as well as the polymer characterization of the plastic litter and microplastics ingested by the different individuals/species analysed. Information on the extent to which marine biota ingests marine litter (including microplastics) is essential to determine and monitor threshold levels to define 'good environmental status' (GES) for marine litter and plastic pollution (as recommended by the EU MSFD and other regional and international regulations such as, specifically: Descriptor 10-MSFD, EO 10 and IMAP Common indicator 24-IMAP). The development of robust legislation relies on toxicological studies with ecological relevance, requiring an accurate measure of marine litter and microplastic loads in organisms in the field. As such, it is essential that researchers are able to accurately isolate, identify, quantify, and characterize debris assumed by the biota.
II. quantitative and qualitative analyses of plastic additives (e.g., phthalates and polybrominated diphenyl ethers-PBDEs) and Persistent, Bioaccumulative and Toxic (PBT) compounds in the tissues of bioindicators, used as "plastic tracers". The detection of plastic additives and PBT compounds that can migrate from plastic litter to the tissues of organisms could represent the degree of accumulation of compounds related to the ingested plastic litter and the causes of its putative ecotoxicological effects;
III. analysis of the effects of marine litter and additives based on biomarker responses at different biological levels (from gene/protein expression variations to histological

alterations). Assessing the undesirable biological responses (alteration of a set of biomarkers by the measurement of endpoints) to the ingestion of marine litter and the accumulation of plastic associated compounds is crucial; this allows understanding and evaluating the extent of the threat of marine litter and plastic ingestion to marine organisms at individual and, ultimately, at population level.

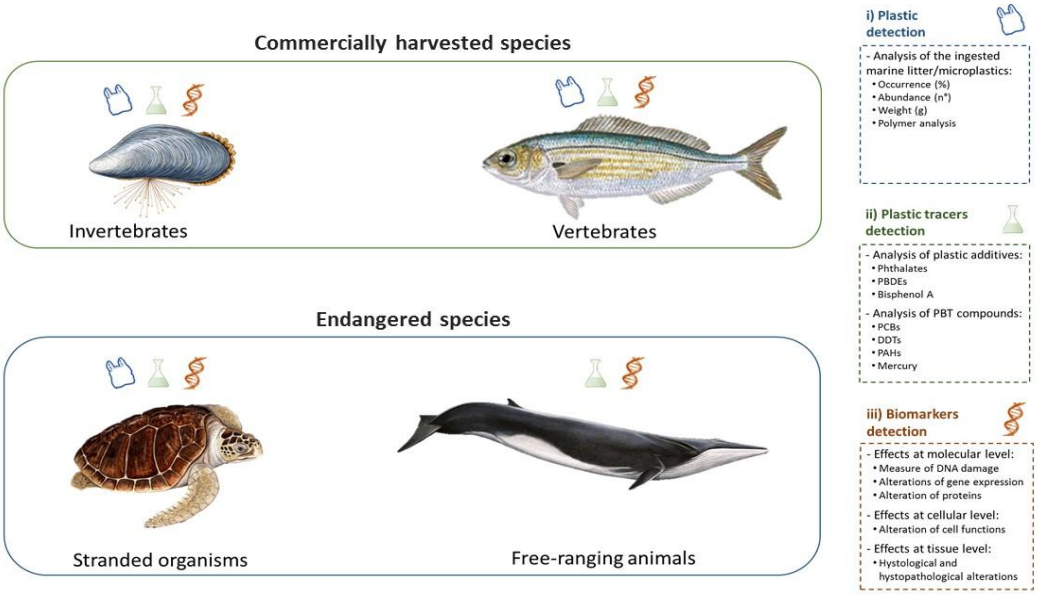

***Figure 8**. The threefold monitoring approach proposed in the PlasticBusters MPAs project.*

It is more difficult to determine the chemical harm related to plastic ingestion and to ascertain related sub-lethal impacts. 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.

7.2 Species selection

The selection of sentinel species to monitor the impact of marine litter on Mediterranean fauna is crucial for the assessment of this environmental threat. It is essential for the development of standardized sampling methods and harmonized protocols for the establishment of a consistent regional approach for the Mediterranean basin, as foreseen by the PlasticBusters MPAs project. The selection of sentinel species has to meet specific criteria and respond to the need of monitoring different habitats in MPAs (from coastal areas to offshore, from benthic environments to pelagic waters) at different spatial scales (Fossi et al. 2018) (Figure 9).

According to available data on the interaction of marine litter with Mediterranean marine organisms and the criteria for selecting sentinel species, different sentinel species are proposed here as bio-indicators of the presence of marine litter and its effects in different ecological compartments. The species have been also selected on the basis of table 2:

a. Home range: local scale, small-scale (FAO Geographical subareas), medium-scale (Mediterranean UN Environment/MAP sub-regions) and Mediterranean Basin scale;
b. Habitat: sea surface, coastal waters, open waters, seafloor, coastline and beach;
c. Occurrence of ingestion of marine litter;

d. Distribution in the target Mediterranean MPAs.

In table 2, the selected species for each habitat and ecological compartment have been reported. Green text is used for the target species that should be analysed in all MPAs while blue text is used for the suggested auxiliary (or secondary) species.

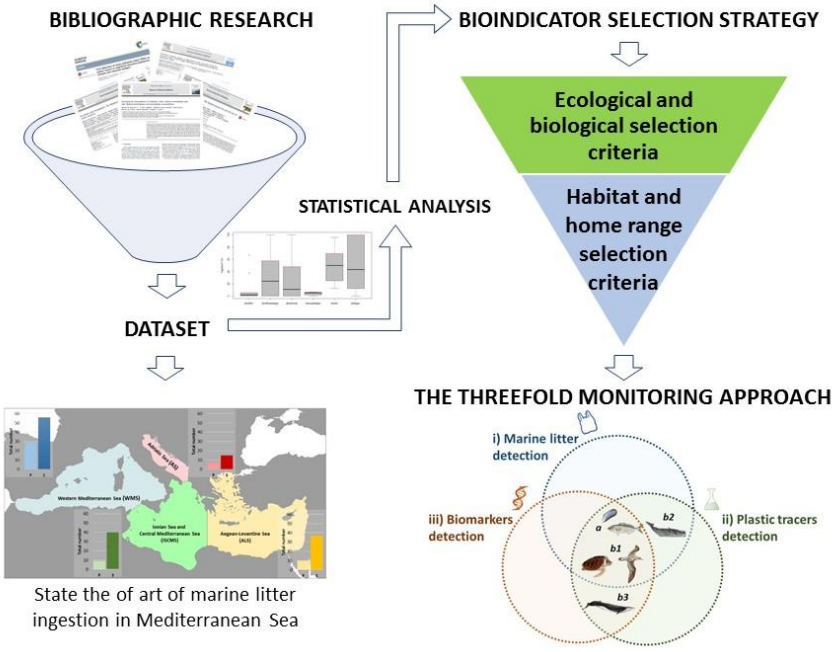

Figure 9. *Selection strategy of sentinel species to monitor the impact of marine litter on Mediterranean biota.*

Sentinel species are subdivided in two categories: a) **commercially harvested species**; and b) **endangered species** (free ranging and stranded marine mammals, hospitalized and stranded sea turtles).

The application of the three categories of monitoring techniques (Figure 10) – that is i) Marine litter ingested detection, ii) Plastic tracers detection, and iii) Biomarkers detection in the sentinel species – requires varying degrees of expertise, ranging from techniques easily applicable by the majority of partners/institutions involved (marine litter ingested detection), to the most specialized and complex one, such as the estimation of ecotoxicological effects (plastic tracers and biomarker analysis). The gradient of expertise is described for the four tipologies of organisms: a) commercially harvested species, b) stranded endangered species, c) hospitalized endangered species, d) free-ranging endangered species (Figure 10).

Table 2. Sentinel species selected in relation to habitat and home range (***Target species – Secondary species)***

	SEA SURFACE	COASTAL WATERS	OPEN WATERS	SEAFLOOR	COAST LINE AND BEACH SEDIMENT
BASIN SCALE *(Mediterranean Sea)*	*Calonectris diomedea* *Puffinus spp.*	*Calonectris diomedea* *Puffinus spp.*	***Caretta caretta*** *Balaenoptera physalus* *Physeter macrocephalus* *Xiphias gladius* *Thunnus thynnus* *Chelonia mydas* *Dermochelys coriacea*		
MEDIUM-SCALE *(Mediterranean UN Environment/MAP sub-regions)*			***Caretta caretta*** *Thunnus alalunga* *Coryphaena hippurus* *Euthynnus alletteratus* *Stenella coeruleoalba* *Ziphius cavirostris*		
SMALL-SCALE *(FAO GSA)*	Isopods *Jellyfish*	***Boops boops*** *Trachinotus ovatus*	***Engraulis encrasicolus*** ***Sardina pilchardus*** *Trachurus sp.* *Sardinella aurita* Myctophids	***Mullus surmuletus*** ***Diplodus spp.*** *Mullus barbatus* *Pagellus sp.* *Lithognathus mormyrus* *Galeus melastomus* *Merluccius merluccius*	
LOCAL SCALE				***Paracentrotus lividus***	**Decapods *(Pachygrapsus marmoratus)*** *Mytilus galloprovincialis (wild or in cages)*

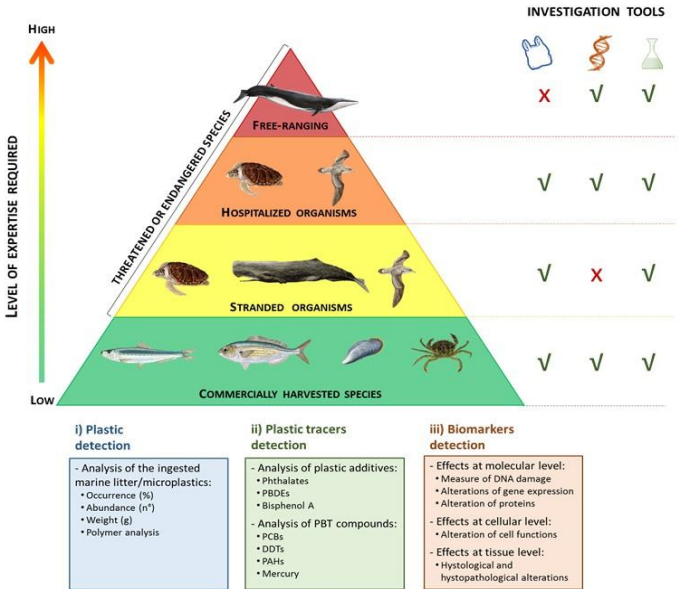

Figure 10. *Approach for monitoring marine litter ingestion and impact in Mediterranean biota as adopted by the PlasticBusters MPAs project. Blue plastic bag: marine litter detection; DNA double helix: Biomarker detection; Green flask: contaminants (plastic tracers) detection.*

7.3 INGESTION

7.3.1 COMMERCIALLY HARVESTED SPECIES

Different species (including commercially harvested fish and invertebrates) are proposed as sentinels of the presence of marine litter. Factsheets for the selected species can be found in Annex 2.

Invertebrates

7.3.1.1 Survey design

Sampling approaches

Marine invertebrate species such as mussels, crabs, sea urchins and isopods should be sampled according to one of the following approaches:

- Marine invertebrates are collected in the study area;
- Marine invertebrates are collected in other adjacent areas with similar conditions and are translocated in the study area with the use of metal cages. They are sampled preferably after one to three months.
- Marine invertebrates are purchased by local fishermen active in the study area.

It is recommended to record the following information for each sampling site:

- **Climate variables**: Sea temperature, mean wave height, maximum wave height, mean wave period, wave direction.
- **Environmental variables:** Sediment granulometry, nutrients, turbidity, chlorophyll-a, salinity.
- **Habitat Characteristics :** Habitat type, habitat composition.
- **Coastline morphology:** Beach, cliffs, estuaries, closed bay, open bay, creeks.
- **Anthropogenic variables:** Anchoring allowance, diving, sewage input, fishing activities, presence of fishing gear, poaching.
- **Protection status:** Protection level, protection status, number of years before/after the establishment of protection status.

For specimens purchased from fishermen, the following information should be recorded: date and time of capture, name of boat(s) and fishing gear used, sampling depth. If possible, the latitude and longitude of each point where the species were captured should be recorded. If this is not possible, the area where the species were captured could be extrapolated from the Automatic Identification System (AIS).

Frequency and timing of surveys

Marine invertebrates should be sampled at least once per year, preferably between May and September or according to the species availability.

7.3.1.2 Sampling Methodology

Sample size

The minimum number of specimens sampled per sampling site for all the above sampling approaches is reported here below:

- Mussels: 30 specimens
- Crabs: 5 specimens
- Sea urchins: 5 specimens
- Isopods: 5 pools of 10 specimens (collected with the manta sampling)

Tissues collection

To perform litter, contaminant and biomarker analysis, tissues should be removed from living organisms. Alternatively, if performing only litter and contaminant analysis, tissues can be dissected from animals frozen at -20°C. Before specimens' dissection, the following information should be recorded: name of the species; weight of each individual with or without epiphytes (not applicable for mussels); length and width of each individual; any visible deformation; standard identification code of the animal written on the label.

Dead organisms

Dissect the specimen in order to obtain the targeted tissues or transfer the specimen to the laboratory in ice containers. Once in the laboratory, precede either with the dissection of tissues for contaminant analysis or store the specimen at -20°C until its dissection for microplastics and contaminant analysis. In case the specimens were stored at -20°C, thaw them at room temperature.

Alive organisms

Extract the different tissues, freeze them in liquid nitrogen in cryo vials and stored them at -80°C or in dry ice for biomarker and contaminant analysis. Each tissue should be stored in aluminium foil or cryovial/eppendorf and must be labelled with a unique ID for each animal.

If the dissection of different tissues is not possible, the whole organism should be used for litter, biomarkers and contaminant analysis.

For the methodological paragraph, see paragraph 7.3.5.

Litter size limits to be surveyed

Litter size classes to be surveyed depend on the size of the investigated invertebrate. Usually for mussels and small size invertebrates, only microplastics are ingested and can be detected. Litter items larger than 30 µm in their longest dimension can be detected using the protocol described for microplastics and macroplastics analysis.

Fish

7.3.1.3 Survey design

Sampling approaches

Survey design should follow already developed protocols, adapted to each region (*Interreg Med MEDSEALITTER, D10 MSFD, IPA-Adriatic DeFishGear projects*).

The monitoring of marine litter in fish should be carried out focusing on commercial species and species of ecological interest.

Fish species should be sampled following one of the following approaches depending on the type of analysis to be performed:

- For the analysis of litter and associated contaminants, fish species (dead) can be purchased by local fishermen active in the study area.
- For the analysis of litter, associated contaminant and biomarker analysis, fish species (alive) should be collected in the study area via a dedicated sampling campaign;

It is recommended to record the following information for each sampling site:

- **Climate variables**: Sea temperature, mean wave height, maximum wave height, mean wave period, wave direction, etc.

- **Environmental variables:** Sediment granulometry, nutrients, turbidity, chlorophyll-a, salinity, etc.
- **Habitat Characteristics :** Habitat type and composition, etc.
- **Coastline morphology:** Beach, cliffs, estuaries, closed bay, open bay, creeks, etc.
- **Anthropogenic variables:** Anchoring allowance, diving, sewage input, fishing activities, presence of fishing gear, poaching, etc.
- **Protection status:** level and status protection, number of years before/after the establishment of protection status, etc.

Frequency and timing of surveys

Sampling should be undertaken at least once per year, preferably between September and October.

7.3.1.4 Sampling Methodology

Sample size

A minimum of 30 individuals per fish species should be sampled at each site.

Tissues collection

To perform litter, contaminants and biomarker analysis, tissues should be removed from living organisms. Alternatively, if performing only litter and contaminant analysis, tissues can be removed from animals frozen at -20°C. Before the dissection, the following information should be recorded for each fish sample (dead or alive): date and time of capture, name of sampling location, name of boat(s) providing samples, sampling gear, latitude and longitude of each point where species are captured, sampling depth, and sample size.

Immediately after sampling, rinse the fish and label the fish samples with a unique ID for each individual. Before the dissection of the fish, record the morphometric and morphological data of each specimen. It is recommended to perform the removal of tissues in the laboratory and under controlled conditions to avoid airborne contamination.

Dead organisms

Transport the sampled species to the laboratory in ice containers and proceed with the dissection or store at -20 °C until dissection for litter analysis and contaminant analysis. Before dissection, thaw fish in the laboratory (if previously stored at -20°C) at room temperature.

Collect muscle and liver for contaminant analysis and gastro intestinal (GI) tract for litter analysis. Each tissue should be labelled with a unique ID and stored in aluminium foils at -20°C.

Alive organisms

While aboard the sampling vessels, keep the sampled live animals in seawater with oxygenators, and transport the animals to the laboratory for dissection. Alternatively, animals can be dissected on board. Before dissection, anaesthetise the animals following the related guidelines from each competent authority for country.

Collect the bood to obtain blood smears and centrifuge a blood aliquot to obtain plasma samples. Extract liver, kidney, gills, muscle, bile and freeze them in liquid nitrogen in cryo vials for storage at -80°C or in dry ice for biomarker analysis. Extract the gastro intestinal tract and store it at -20°C for litter analysis. Each tissue should be stored in aluminium foil or cryovial/eppendorf and shall be labelled with a unique ID for each animal.

If the dissection of the fish is carried out on board, transport the tissue samples for biomarker analysis to the laboratory in liquid nitrogen (or dry ice) and the samples for litter and contaminants analysis in ice containers.

For the methodological paragraph, see paragraph 7.3.5.

Litter size limits to be surveyed

Litter items larger than 1 mm can be classified following the steps described below for macrolitter detection.

Litter items larger than 30 µm can be classified following the steps described below for microlitter detection.

7.3.2 STRANDED ENDANGERED SPECIES

7.3.2.1 Survey design

The monitoring approach adopted in the PlasticBuster MPAs project is intended to allow performing, for the first time, a deep risk assessment ranging from the individual to the species-wide level, including the evaluation of possible toxic effects (Figure 9). Given *Caretta caretta* wide distribution in the Mediterranean Sea, and its use in both the MSFD D10 monitoring program and IMAP indicators, the loggerhead sea turtle (*Caretta caretta*) is proposed as open waters target species. We also propose another sea turtle species (*Chelonia mydas, Dermochelys coriacea*), two seabird species (Calonectris diomedea and Puffinus spp) and several cetaceans species (e.g. *Balaenoptera physalus, Physeter macrocephalus, Stenella coeruleoalba and Ziphius cavirostri*s) as secondary sentinel species (see table 2).

The detailed description of the species being monitored is reported in the Annex 2.

Sampling approach

Monitoring activities on protected species require special permits for specimen transport and necropsy and it is thus advantageous to involve regional or national networks to maximize sample retrieval. Dead sea turtles, seabirds and marine mammals should be collected from beaches or at sea from accidental mortalities. Stranded individuals should be necropsied upon their discovery, because their freezing may affect the characterization of the health status. Should this not be possible, the carcass should be frozen at -20°C. The tissue handling and collection procedure should be carried out only by authorized personnel and in strict accordance with the relevant national and international guidelines and permits.

7.3.2.2 Sampling Methodology

Sea turtles

The protocol for stranded sea turtles is based on UNEP/MAP document (2019). Morphologic parameters and total body weight should be measured, and the cause of death noted. The sex of the specimens should be determined visually, where possible. An aliquot of fat, muscle, kidney, liver and caprapace scutes should be collected and kept in aluminium foil at -20°C for the evaluation of contaminant levels (Table 3). The gastro intestinal (GI) tract should be isolated for the marine litter detection as described in the paragraph 7.3.5.

Seabirds

Sampling should follow the MSDF TG10 Guidelines (Galgani et al., 2013). Data on age, sex, morphologic parameters and possible cause of death should be noted. An aliquot of fat, muscle, liver and kidney should be collected and kept in aluminium foil at -20°C for the evaluation of contaminant levels (Table 3). The GI tract should be isolated as described in the paragraph 7.3.5.

Marine Mammals

Small stranded marine mammals should be transported to an authorized centre for necropsy; for large animals, dissection is usually done directly at the stranding site by authorized institutions. Before the necropsy is carried out, morphometric measurements, sex and possible cause of death should be noted. An aliquot of blubber, muscle, liver, kidney and brain should be collected for contaminant analysis (Table 3). The content of the GI should be isolated (as described in the paragraph 7.3.5.) and examined to determine the diet of the animal and for analyzing ingested marine litter, including plastic.

7.3.2.3 Sample Processing Methodology

For the methodological description, see paragraph 7.3.5.

7.3.3 HOSPITALIZED ENDANGERED SPECIES

7.3.3.1 Survey design

Surveys on hospitalized live specimens of sea turtles (*Caretta caretta, Chelonia mydas, Dermochelys coriacea*) and seabirds (*Calonectris diomedea*, *Puffinus spp*) shall be carried out in the rescue centers located inside the MPAs or in neighboring areas, to guarantee the applicability of this methodology.

7.3.3.2 Sampling Methodology

Sea Turtles

Alive sea turtles hospitalized in rescue centers should be manually removed from water for the 30 min sampling period. The cares and procedures carried out on the rescued turtles during the entire rehabilitation period should be performed in accordance with routine veterinary practices and guidelines for the conservation and rehabilitation of marine turtles. The collection of biological tissues such as blood, capapace and skin biopsy must be made with the support of the centres' veterinary while faeces can be collected by the volunteers or the operators of the rescue centres. Each tissue, stored in aluminium foil or Eppendorf, must be labelled with a unique ID for each individual. All the biological samples extracted will be used for biomarker and chemical analyses.

Seabirds

The sampling of alive seabirds hospitalized in rescue centers should be made by authorized personnel. Biological tissues (blood, oil gland secretion, faeces) must be collected, processed and immediately stored in liquid nitrogen or dry ice. Each tissue must be stored in aluminum foil and labeled. All the biological samples collected are to be used for biomarker and chemical analyses.

7.3.3.3 Sample Processing Methodology

For the methodological description, see paragraph 7.3.5.

7.3.4 FREE-RANGING ENDANGERED SPECIES

7.3.4.1 Survey design

Sea birds

Sampling of alive seabirds can be conducted in seabird colonies (free-ranging animals) by authorized personell. In seabird colonies, nests can be difficult to access. Safety requirements for boating, climbing and hiking should be followed. In some risky conditions, despite protocols being simple, only experts should be asked to take samples. Moreover, seabird welfare and safety should be a priority for coordinators and operators, and unnecessary stress to birds should be avoided. Some precautions, such as cover bird head, avoid noise, exclude from sampling nests in unfavourable conditions, and fast sampling procedures should be considered case by case.

Marine mammals

Marine mammals (cetaceans) surveys should be carried out along with the marine litter and microplastic sea surface sampling (see survey design and biota protocol). Simultaneous monitoring (in particular in pelagic areas such as the Pelagos Sanctuary) of floating macro-litter and biota presence should be conducted. This simultaneous observation (which capitalizes efforts in terms of time/energy) should contribute to the development of future "risk maps" (see details in paragraph 4.2.3 and chapter 9).

Skin biopsies (epidermis and dermis/blubber) from free-ranging dolphins (e.g., *Tursiops truncatus,* and *Stenella coeruleoalba*) can be obtained using an aluminium pole armed with biopsy tips. Skin biopsies from large odontocete (*Physeter macrocephalus*) or mysticete (i.e., *Balaenoptera physalus*) species can be obtained with a crossbow or airgun and darts armed with tips. To avoid the possibility

of infection, the bolt tip needs to be sterilised before use. Biopsy samples can be sampled between the dorsal fin and the upper part of the caudal peduncle upon approaching the animal at a suitable distance and speed, as specifically permitted for the species.

7.3.4.2 Sampling Methodology

Sea birds

Blood, oil gland secretion, faeces and abandoned eggs must be collected, processed and immediately stored in liquid nitrogen or dry ice. Each tissue must be stored in aluminum foil and labeled. All the biological samples collected are to be used for biomarker and chemical analyses.

Marine mammals

The skin biopsy (epidermis and dermis/blubber) needs to be stored immediately in the proper conditions required for intended analyses. Common storage conditions include: frozen, as it is, in liquid nitrogen, dry ice, or -80 °C and -20 °C freezers, or stored either cold or at room temperature in cell medium, buffer, or specific reagents. All the samples are to be used for biomarker and chemical analysis.

7.3.4.3 Sample Processing Methodology

For the methodological description, paragraph see 7.3.5.

7.3.5 SAMPLE PROCESSING METHODOLOGY: THE THREEFOLD MONITORING APPROACH FOR BIOTA

The application of the threefold approach (Figure 13) can elucidate both the rate of ingestion among the different sentinel species, and the multiple sub-lethal stresses that marine litter ingestion can cause in the short and long term (Fossi et al 2018). Each of the three investigation tools (i-ii-iii) that make up the threefold approach can be applied independently or simultaneously to the selected sentinel species, which are a) commercially harvested species; and b) endangered species.

a) For commercial species, for instance mussels and fish, it is possible to detect the occurrence and rate of marine litter ingestion, and to quantify the potential contaminants accumulation and their relative biological effects (eg. genotoxicity biomarkers, lysosomal stability, lipid peroxidation); b) For protected species (e.g. sea turtles, seabirds or marine mammals) the approach will depend on whether the organisms have been found dead (e.g. stranded or bycatch) or if a free-ranging organism has been sampled non invasively: b1) in hospitalized organisms and stranded organisms (2-3 h after death), it is possible to detect the occurrence of marine litter ingestion, and to quantify the accumulation of possible contaminants and their biological effects (biomarker responses); b2) in stranded organisms (not in a good state of conservation), analysis of contaminants and gastro intestinal content (with a particular focus on plastics and microplastics) can be carried out; b3) an indirect approach can be used for free-ranging animals: the levels of plastic additives, PBT compounds and biological effects can be measured to evaluate the exposure to marine litter, for example using a skin biopsy taken from free-ranging cetaceans.

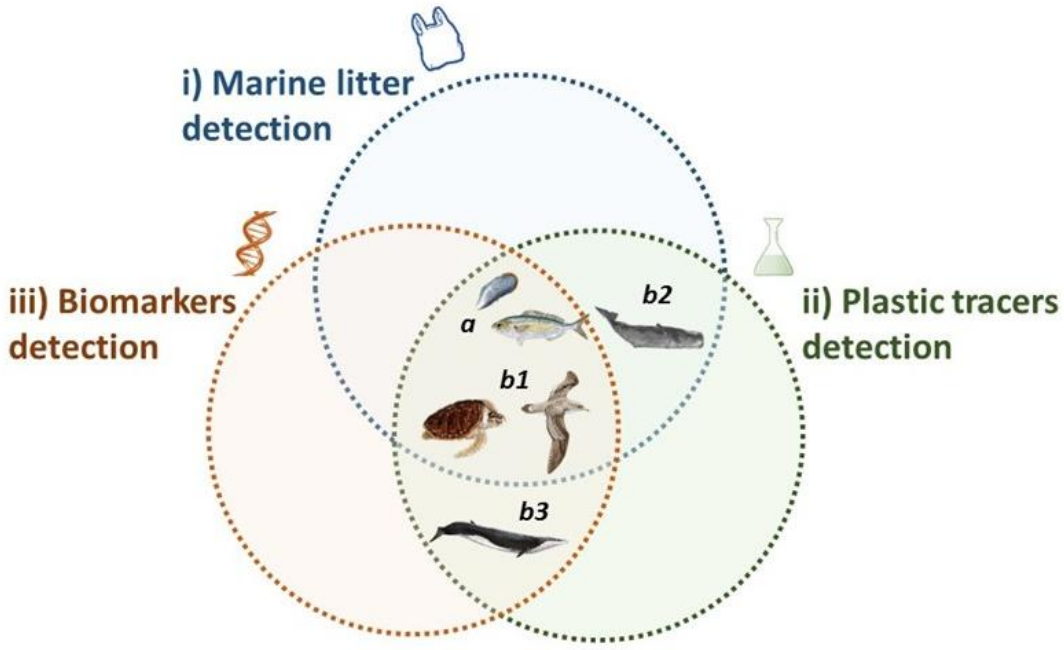

Figure 13. *Threefold monitorning approach in commercial and endangered species.*

i) Marine Litter - Plastic Detection

Sampling methodologies follow already developed protocols (*INDICIT, MEDSEALITTER, UNEP/MAP, D10 MSFD, IPA-Adriatic DeFishGear documents*) although adapted depending of whether commercial species, stranded organisms, hospitalized organisms or free-ranging species are surveyed.

MACROPLASTIC DETECTION

For the detection of macroplastics and macrolitter items in marineorganisms, when dealing with dead animals, the gastro intestinal tract should be used, while when dealing with live animals, faeces and regurgitates collected in the field or in rescue centers should be used.

Once at the laboratory, biological samples should be firstly digested and then sorted and identified under a stereomicroscope.

Invertebrates

Usually for mussels and small size invertebrates, only microplastics are ingested and can be detected (see following section).

Fish

For litter analysis and classification, the following procedure is used:

- Place GI (stomach and intestine) in a glass petri dish or beaker, weigh and rinse the GI with purified water. Be careful to annotate the fish ID in each petri-dish/beaker.
- Place a filter paper in a petri dish (blank sample) in the working area during fish dissection to test airborne contamination.
- Cut open the stomach and intestine, remove stomach and intestine contents and weigh them separately; then sort prey or litter items into separate categories under a stereomicroscope.
- Measure and weight the litter items found and classify them according to the Joint List of Litter Categories of the MSFD Technical Group on Marine Litter (Annex 1).

Endangered species

Sort prey and/or litter items into separate categories under a stereomicroscope, taking care of recording their weight. Then measure the size of litter items and classify them according to the Joint List of Litter Categories of the MSFD Technical Group on Marine Litter (Annex 1).

Data analysis

In addition, the following parameters should be recorded:

- For all categories (litter and other elements), the dry mass (grams, precision 0.01 g) of each category: dry the sample at room temperature for 24h minimum or in a stove at 35°C for 12h.
- For litter categories only:
    - the number of fragments in each category; a fragment is a piece of litter that can be identified.
    - the number of items in each category; an item is a set of fragments that seem to originate from the same piece of litter.
- For the plastic litter categories only: the total number of plastic fragments per colour category, with specifics as follow:
        - Total number of white-transparent plastic fragments;
        - Total number of dark coloured plastic fragments (black, blue, dark green…);
        - Total number of light coloured plastic fragments (cream, yellow, pink, light green…).
- Analyse at least 10% of the detected microplastics by FTIR (Fourier Transform Infrared Spectroscopy) or Raman spectroscopy to determine the polymer composition and confirm the polymer origin of the detected particles.

MICROPLASTIC DETECTION

Invertebrates and fish

The protocols developed in the framework of the DeFishGear (Tsangaris et al., 2015) and MEDSEALITTER projects are to be used for the detection of microplastics in invertebrates and fish. Both protocols are based on the MSFD TG 10 Guidelines (Galgani et al., 2013). The following sample processing methodology is proposed to be used in PlasticBusters MPAs for fish and invertebrate samples.

For microplastic extraction, the organic matter samples should be digested 15% H_2O_2 or 10% KOH, heated on a hot plate at 60 °C and filtered under vacuum on fiber glass filters. The filters should then be examined under a stereomicroscope for the quantification and characterization of microplastics.

The microplastics particles should be photographed, counted and categorized according to maximum length, color, and type, following the MSFD TG 10 Masterlist (Galgani et al., 2013) (see Annex 1). FTIR (Fourier Transform Infrared Spectroscopy) or Raman spectroscopy should be used to determine the polymer composition and confirm the polymer origin of the detected particles in at least 10% of the detected microplastics.

Precautions against contamination are essential during all steps of the sample processing due to the ubiquitous nature of certain types of microplastics, such as synthetic fibers, that can contaminate the samples. Procedural blank samples should be used throughout the entire sample processing. During the analyses procedure, two glass petri dishes should be placed at each side of the stereomicroscope and checked for microplastics before and after each sample. A 100% cotton laboratory coat shall be worn at all times during the procedure.

Recovery of microplastics by the applied extraction procedure should be tested on fish tissue samples enriched with a specific number (e.g., 10 particles/sample) of different plastic particles of known polymer type and size (positive controls, minimum number). The number of particles detected after the processing of these samples as described above, should be used to calculate % recovery of microplastics.

Endangered species

Examine the filter in the Petri dish under a stereomicroscope for particles resembling microplastics. Cover the filter with glass lids during observation not to contaminate the sample. Photograph, count and record the type, colour and maximum length of microplastic particles using image analysis software. Categorize microplastic particles according to the Joint List of Litter Categories of the MSFD Technical Group on Marine Litter (Annex 1). Analyse at least 10% of the detected microplastics by FTIR (Fourier Transform Infrared Spectroscopy) or Raman spectroscopy to determine the polymer composition and confirm the polymer origin of the detected particles.

Reporting units

For each individual an assessment is made of:

1. Frequency of **occurrence** (%) of ingested macro and microplastics for each species, calculated as the percentage of the individuals examined with ingested macro and microplastics.
2. **Abundance** (N) of macro and microplastics ingested per individual (average number of items/individual) for each species, calculated as a total and per category. Since currently there are inconsistencies in the literature in reporting abundance of ingested litter, it is recommended to report average number of items per individual considering both all individuals examined and only individuals found with ingested macro and litter.
3. The **percentage of the individuals affected** in relation with the individuals of the whole sample examined (all species).

Data analysis

For each organism the following data on litter ingested is to be reported:

1. Characteristics of the litter found (colour, shape) in each specimen.
2. Number, length, weight and nature of the polymer (10%) of the items examined for each species.
3. Recovery rate of microplastics.

ii) Plastic tracers Detection

Because of their physical and chemical properties, marine plastic debris are associated with a 'cocktail of chemicals', including those that are ingredients of the plastic material (plastic additives) itself and those absorbed from the marine environment (e.g. persistent, bioaccumulative and toxic substances-PBTs) (Rochman, 2015). Assessing hazards associated with plastic in aquatic habitats requires knowledge regarding potentially exposed organisms, the exposure concentrations, and the types of polymers comprising the debris.

Plastic additives

During the production of plastics, several additives (e.g. flame retardants, stabilizers, plasticizer) are included to provide plastics with certain characteristics (e.g., flexibility, strength and colour; Lithner et al., 2011). Such chemical products have been proven to be harmful and may be released during all the stages of the life cycle of a plastic product, from production to use, and up to disposal (Halden, 2010; Lithner et al., 2011; Oehlmann et al., 2009; Papaleo et al., 2011; Teuten et al., 2009).

The compounds to be detected in different tissues/fluids are:

- *Phthalates:* is a group of chemicals widely used as additives to make plastics more flexible and harder to break; they can interfere with endocrine system.
- *Bisphenol A*: used in the production of polycarbonate, can have endocrine disrupting effects (Crain et al., 2007; Halden, 2010; Oehlmann et al., 2009) and the styrene and polyvinyl chloride monomer, used in the production of polystyrene and polyvinyl chloride (PVC), can be carcinogenic and/or mutagenic (Lithner et al., 2011; Papaleo et al., 2011; Xu et al., 2004).
- *Polybrominated diphenyl ethers:* they belong to the group of brominated flame retardants (BFRs), which are used in various polymeric materials such as plastic parts, resins, textiles, and other substrates to reduce their fire hazards (BSEF; Król et al. 2012).

Persistent, bioaccumulative and toxic substances (PBTs)

In addition to the plastic additives that may leach from plastic when resealed into the marine environment, plastics tend to adsorb in their surface persistent bioaccumulative and toxic substances (PBTs) (e.g. organochlorine compounds OCs, PAHs and PBDEs - see Endo et al., 2005; Heskett et al., 2012; Hirai et al., 2011; Mato et al., 2001; Ogata et al., 2009; Rios et al., 2010) and metals (e.g., lead, copper and cadmium) (Ashton et al., 2010; Holmes et al., 2014; Rochman et al., 2014) that are present in the seawater.

Depending on the compounds and the tissue to be analysed, different methods should be applied to detect the presence of plastic-related contaminants in the sentinel species; details on the specific method to be used depending on the chemical compound and the tissue are reported in Table 3.

Table 3. *Tissues and methods to be used to detect plastic- related contaminants in biota.*

	CHEMICAL COMPOUND	TISSUE/SAMPLE	ANALYTICAL METHOD
PLASTIC ADDITIVES	**Phthalates**	Fat, blubber, muscle, liver, whole organism	Baini et al. (2017), Fossi et al. (2016); (Savoca et al., 2018)
		Blood	Takatori et al. (2004)
		Oil gland secretion	Hardesty et al. (2015)
	Bisphenol A	Muscle, whole organism	Ballesteros-Gòmez et al. (2009)
		Fat, blubber	Xue and Kannan (2016)
		Blood	Cobellis et al. (2009)
	Polybrominated diphenyl ethers	Fat, blubber, muscle, whole organism, liver, blood	Muñoz-Arnanz et al. (2016)
SORBED CONTAMINANTS	**Polycyclic aromatic hydrocarbons**	Fat, blubber, muscle, whole organism, liver, blood	Marsili et al. (2001)
	Organochlorine contaminants	Fat, blubber, muscle, whole organism, liver, blood	Marsili and Focardi (1997)
	Mercury	Blood, muscle, whole organism, kidney, liver, skin	Correa et al. (2013)

iii) Biomarkers/effects detection

The toxicological effects associated with the presence of marine litter and related contaminants can be evaluated using a set of diagnostic and prognostic methodologies, by biomarkers. This approach is to foresee, and hence mitigate, negative outcomes at the ecological level. In order to evaluate the possible effects of plastics litter on sentinel species - ranging from molecular to cellular levels - a set of biomarkers can be applied to the sentinel species. These results can then be integrated with data obtained from plastic tracers' detection and marine litter quantification to provide a more comprehensive evaluation of the ecotoxicological health status of the analysed biota. A non exhaustive list of potential biomarkers techniques applicable to different systematic groups is reported in Table 4.

The biomarkers have been selected on the basis of the level of biological responses and in relation to the main effects related to marine litter/microplastics ingestion. The selected biomarkers can diagnose the different impacts related to: a) physical damages/effects of marine litter, b) exposure to/effect of chemical tracers, and c) exposure to/effect of adsorbed chemicals.

Different endpoints have been selected based on the experimental exposure of model species to microplastics and associated contaminants and on the basis of further validation on field monitoring.

In addition to the tests to be performed on the species sampled in the MPAs, further and more comprehensive analysis could be performed such as the multi-diagnostic approach on tissue collected from endangered species using a non lethal approach, such as skin biopsy of marine mammals and sea turtles (Figure 14).

For the species for which enough background information is available on transcriptome/proteome and metabolome, an "omic" approach (e.g., transcriptomics, proteomics or metabolomic) could also be used. However, such info is likely lacking when a monitoring approach in the field has to be performed.

Table 4. *End-points measured in invertebrates (i) and vertebrates (v) by the biomarker approach.*

EFFECT	TISSUE	TEST
GENOTOXICITY	Hemolymph, digestive gland (i)	Comet assay (Avio et al., 2015) (*) Mn test (Avio et al., 2015) (*)
	Blood (v)	Comet assay (Molino et al., 2019) (*) Mn test (Bolognesi et al., 2006) ENA assay (Casini et al., 2018); (Pacheco and Santos, 1997)
OXIDATIVE STRESS	Digestive gland (i)	LPO, CAT, SOD, GST, GSH, GR, GPX (Avio et al., 2015) (*)
	Liver, kidney, gill (v)	CAT, GST, LPO, GPX, GR, GSH (Yu et al., 2018) (*)
	Plasma, skin (v)	LPO (Fossi et al., 2016), Casini et al., 2018) CAT (Fossi et al., 2013)
IMMUNOTOXICITY	Digestive gland (i)	CASP, TRAF, Transcriptomics (Avio et al., 2015; Sussarellu et al., 2016) (*)
	Blood (v)	Total and differential white blood cells (WBC) count (Casal and Orós, 2007; Davis et al., 2008; Caliani et al., 2019) H:L ratio (Caliani et al., 2019) Respiratory burst (Secombes, 1990; Caliani et al., 2019) TAS assay (Miller et al., 1993; Caliani et al., 2019) Lisozyme enzyme (Keller et al., 2006; Caliani et al., 2019) casp8, casp9, TRAF (Karami et al. 2017; Mathieu-Denoncourt et al., 2015) (*)
REPRODUCTION	Gonads (i)	Gamete Quality and Larval Development (Sussarellu et al., 2016) (*)
	Plasma, Gonads (v)	CYP17A, CYP19, ERs, VTG, StAR (Mathieu-Denoncourt et al., 2015) (*) Vitellogenin (Fossi et al., 2004)
	Plasma, skin (v)	Vitellogenin (Herbst et al., 2003) CYP17A, CYP19, ERs, VTG, StAR (Mathieu-Denoncourt et al., 2015; Panti et al., 2011) (*)
HISTOPATHOLOGY INFLAMMATION AND MORPHOLOGY	Digestive gland (i)	Histopathology, histology (Avio et al., 2015) (*)
	Liver, kidney, gill (v)	Histopathology,histology (Pedà et al. 2016; Karami et al. 2017; Batel et al., 2018) (*)
XENOBIOTIC METABOLISM AND BIOTRANSFORMATION	Digestive gland, whole organism (i) Liver, blood, bile (v)	Porphyrins (Grandchamp et al. 1980; Guerranti et al. 2014) (*) Bile metabolites (Oliveira et al 2013) (*) EROD (Zhang et al., 2019) (*)
	Blood, skin, excreta, liver (v)	CYP1A; AHR, CYP3A (Fossi et al. 2014, Panti et al. 2011; Rochman et al., 2013) (*) Porphyrins (Guerranti et al., 2014) (*)
NEUROTOXICITY	Whole organisms, muscle (i)	AChE activity (Magni et al., 2018) (*)
	Brain, muscle, plasma (v)	AChE, BChE (Barboza et al., 2018) (*)
CELLULAR STRESS	Whole organisms, muscle, hemolymph (i)	Lysosomal membrane stability-LMS (Canesi et al 2015) (*) IDH (Oliveira et al., 2013) (*)
	Blood, skin, liver, kidney (v)	PPARA, PPARG, HSP70, GPX, E2F1 (Mathieu-Denoncourt et al., 2015; Panti et al., 2011) (*) Gamma glutamyl transferase (GGT) (Nematdoost Haghi and Banaee, 2017) (*) Cortisol and corticosterone (Flower et al., 2015) LDH (Nematdoost Haghi and Banaee, 2017) (*)

(*) effects detected after laboratory or field exposure with MPs or Plastic Tracers

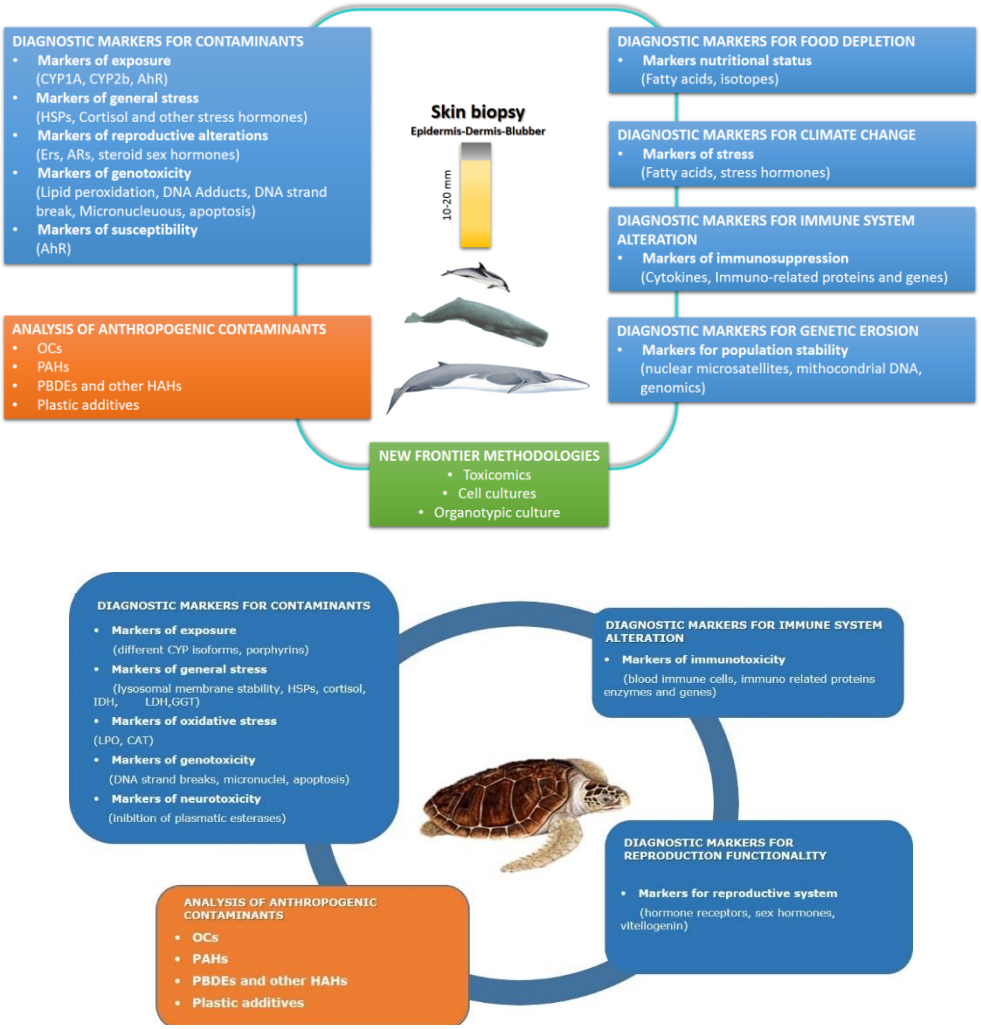

Figure 14. *A three-fold approach to detect the marine litter presence and impacts on alive sea turtles and cetaceans.*

Final remark on the application of the threefold Plastic Busters monitoring strategy to monitor ingestion impact on MPAs Biodiversity

The application of the threefold approach (Fig. 13), proposed here as a novelty by the Plastic Busters consortium, can elucidate the rate of ingestion among the different sentinel species as well as the multiple sub-lethal stresses that marine litter ingestion can cause in the short and long term (Fossi et al 2018). Each of the three investigation tools that make up the threefold approach can be applied independently or simultaneously to the selected sentinel species.

In conclusion, the application of the three categories of monitoring techniques in the selected species requires a different degree of expertise (as shown in Figure 10) for the four categories of organisms considered: (a) commercial species, (b) stranded endangered species, (c) hospitalized endangered species, and (d) free-ranging endangered species.

To harmonize monitoring activities on biota in all the MPAs covered by WP4 and WP5 of the Plastic Busters MPAs project, at least the first phase of the threefold monitoring strategy (analysis of ingested marine litter), should be applied by all partners in the various selected sentinel species.

7.4 Entanglement and coverage

7.4.1 Survey design

The interactions between debris and benthic invertebrates should be evaluated as part of the seafloor litter monitoring.

Monitoring activities could be operated on regular basis by ROVs or through diving by using the same survey design described in paragraph 5.1.1.

Endangered species

Data assessing the impact of entanglement on endangered individuals and populations would be significant for assessing their population health and trends.

Several species of epibenthic invertebrates are listed as endangered/vulnerable by various international directives and agreements and are thus considered as target species in this protocol. Considering that the proposed method for monitoring endangered species should only rely on imaging techniques, there is no regulatory constraints in using these species as indicator species. In line with the project INDICIT (INDICIT consortium, 2018a, b), data related to pressures on these taxa should be of particular interest for assessing the conservation state and trends of populations/ habitats, as well as the efficiency of conservation measures for species.

7.4.2 Sampling methodology

The sampling methodology proposed to assess entanglement and coverage of marine litter relies on images' acquisition through visual census techniques carried out by ROVs (Remotely Operated Vehicle) and SCUBA diving (see chapter 5.1.2). It is proposed to assess entanglement and coverage opportunistically, in relation to the assessment of seafloor litter abundance.

7.4.3 Sample processing methodology

Once collected, protocols for image annotation and analysis should build on the protocol derived from the AMARE project and be updated within the PlasticBusters MPAs project. An important constraint, which has no simple solution, lies in determining how the occurrence of entanglement is linked to the number of litter items. For instance, a high occurrence of entangled invertebrates in an area might not be caused by separate litter items, but by a single longline several kilometers long.

7.5 References

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8 GIS (Geographic Information System)

Global Positioning System (GPS) should be used to geolocate data collected during all sampling stages of the scientific surveys. To ensure homogeneity in the collection of GPS data, the same datum and coordinate system should be maintained. As such, the use of WGS 84 (EPSG:4326) as a coordinate system across all the partner regions is suggested within the Plastic Busters MPAs project, to ensure uniformity in data projections and reduce projection error across regions, thus increasing accuracy. Then, each region will be able to project to a metric system (such as a UTM) as needed (i.e. calculate distance to shoreline, transect length). All datafile sources need to include the appropriate metadata associated to the shapefile or the raster file developed. If there is any additional manipulation of the file (i.e. manual editing, clipping), a source file should be included providing an adequate protocol to its creation. All GIS data will be analysed using freely available QGIS or ArcGIS if partners have access.

8.1 GIS data

Each partner country may have a higher resolution coastline data, which they may wish to use. In addition, metadata for other shapefiles, such as the Marine Protected Area boundaries, will be added to the Plastic Busters MPAs repository.

8.2 Monitoring marine litter on beaches

Coordinates shall be recorded at the beginning and end of each transect and at each sampling location, consistently with the monitoring protocol (see chapter 3).

8.3 Monitoring marine litter on the sea surface

Coordinates shall be recorded at the beginning and at the end of each transect. Due to difficulties in conducting ship survey in a straight line, it is advisable to record the entire track of the survey with the best possible GPS resolution, according to battery and memory usage of the device, to then estimate the size of the surveyed area.

8.4 Monitoring marine litter on the seafloor

For seafloor surveys, a buoy should be released at the surface for a GPS data point to be collected at the survey location from the sea surface, from either a boat or upon immersion of the divers.

8.5 Monitoring marine litter in biota

It is important to determine the general location of where the biota samples are collected.

- For samples obtained from necropsies, it is necessary to identify the location where the individual was stranded.
- For samples from fisheries, whenever possible, it is necessary to acquire information about the fishing area where the samples were captured. This can be determined by speaking directly with the fisherman or by the remote tracking of the fishing vessels through their transmitted AIS (Automatic Identification System) data.
- For samples recovered from the sea floor (i.e. crabs), a buoy should be released at the surface for a GPS data points to be collected at the survey location from the sea surface from either a boat or upon immersion of the divers.

9 Risk assessment

Most environmental risks are spatially and temporally limited. For marine litter, risk assessment should indicate where and when harm may occur. This is not only defined by the potential encounter of marine organisms with litter items, but also takes into account an assessment of the potential harmfulness of litter items, such as the nature and shape of litter (Fossi et al., 2017).

The assessment of environmental risks conceptually involves four stages including: (1) assessing the potential consequences after exposure to a particular level (hazard identification/ characterization); (2) assessment of the exposure (probability that a hazard will occur); (3) the characterization of the risk, combining hazard and exposure; and (4) the evaluation of uncertainties (Werner et al., 2016).

Risk assessment has been used for birds (Wilcox et al., 2015) and sea turtles (Schuyler et al., 2014), investigating whether plastic litter ingestion prevalence in marine turtles has changed over time, what types of litter are most commonly ingested, the geographic distribution of litter ingestion by marine turtles relative to global litter distribution, and which species are most likely to ingest litter and at what stage in their life.

In the Mediterranean, a study based on aerial surveys (Darmon et al., 2017) investigated the distribution of both litter and sea turtles, allowing to map the probabilities of sea turtles encountering floating litter, and identify the areas where such encounter could take place. Fossi et al. (2017) also investigated the possible overlap between microplastic, accumulation areas and fin whale feeding grounds in the Pelagos Sanctuary. The simulated microplastic distribution and modelled potential fin whale feeding habitats overlapped, contributing to the risk assessment of fin whale exposure to microplastics.

9.1 Strategy

The approach proposed within the Plastic Busters MPA project is the one used in the aforementioned studies (Darmon et al., 2017, Fossi et al, 2017), predicting the areas where species will likely be most affected by marine litter, allowing the definition of species-specific sensitive areas for ingestion probability, and providing the basic information to be used for the mapping of areas of higher sensitivity. Observed and predicted distribution, available as outputs from distribution models, of both litter (macro or microplastics) and species (ranging from plankton to large vertebrates), should be jointly used to obtain effective and reliable sensitivity and risk maps. The same approach could be used to predict areas where the risk of interactions occurs, with possible consequences for fish quality and associated risk, including for human consumption.

9.2 Survey design and data

Risk assessment studies within the Plastic Busters MPAs project are set to focus primarily on the Pelagos Sanctuary. Surface litter distribution should be modelled and compared with Plastic Busters MPAs data collected during monitoring surveys. Data on large vertebrates (birds, cetaceans and sea turtles) and fishes from aerial surveys (e.g., Accobams Survey Initiative, to be confirmed) could be analysed for their suitability (availability, number of observations, possible mapping of distribution, etc.) and the relevant data should be compiled for an evaluation of their geographical distribution. Once validated, the distribution of both marine litter and sampled species should be overlapped to identify areas of higher probability of exposure. Those areas – to be considered as high risk areas – should be analysed in details to provide recommendations for management.

The same approach should be applied to identify risk for demersal fishes: the availability of data on both marine litter and demersal fish species, as caught by trawling, should be checked from regular

fish stocks assessment cruises (MEDITS project). This should enable to evaluate the relevance of risk assessment for demersal fish stocks and better define gaps and perspectives.

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THE PLASTIC BUSTERS MPAs PARTNERSHIP

Annex 1 Masterlist of Marine Litter Items *(to be replaced by the MSFD TG ML Joint List)*

ARTIFICIAL POLYMER MATERIALS			
Code	**Items name**	**Item counts**	**Total**
G1	4/6-pack yokes, six-pack rings		
G3	Shopping bags, incl. pieces		
G4	Small plastic bags, e.g. freezer bags, including pieces		
G5	Plastic bag collective roll; what remains from rip-off plastic bags		
G7	Drink bottles <=0.5l		
G8	Drink bottles >0.5l		
G9	Cleaner/cleanser bottles & containers		
G10	Food containers incl. fast food containers		
G11	Beach use related cosmetic bottles and containers, e.g. Sunblocks		
G12	Other cosmetics bottles & containers		
G13	Other bottles & containers (drums)		
G14	Engine oil bottles & containers <50 cm		
G15	Engine oil bottles & containers > 50 cm		
G16	Jerry cans (square plastic containers with handle)		
G17	Injection gun containers		
G18	Crates and containers / baskets		
G19	Car parts		
G21	Plastic caps/lids from drinks		
G22	Plastic caps/lids from chemicals, detergents (non-food)		
G23	Plastic caps/lids unidentified		
G24	Plastic rings from bottle caps/lids		
G25	Tobacco pouches / plastic cigarette box packaging		
G26	Cigarette lighters		
G27	Cigarette butts and filters		
G28	Pens and pen lids		
G29	Combs/hair brushes/sunglasses		
G30	Crisps packets/sweets wrappers		
G31	Lolly sticks		
G32	Toys and party poppers		
G33	Cups and cup lids		
G34	Cutlery and trays		
G35	Straws and stirrers		
G36	Fertilizer/animal feed bags		
G37	Mesh vegetable bags		
G40	Gloves (washing up)		
G41	Gloves (industrial/professional rubber gloves)		
G42	Crab/lobster pots and tops		
G43	Tags (fishing and industry)		
G44	Octopus pots		
G45	Mussels nets, Oyster nets		
G46	Oyster trays (round from oyster cultures)		

G47	Plastic sheeting from mussel culture (Tahitians)		
G49	Rope (diameter more than 1cm)		
G50	String and cord (diameter less than 1cm)		
G53	Nets and pieces of net < 50 cm		
G54	Nets and pieces of net > 50 cm		
G56	Tangled nets/cord		
G57	Fish boxes - plastic		
G58	Fish boxes - expanded polystyrene		
G59	Fishing line/monofilament (angling)		
G60	Light sticks (tubes with fluid) incl. packaging		
G62	Floats for fishing nets		
G63	Buoys		
G64	Fenders		
G65	Buckets		
G66	Strapping bands		
G67	Sheets, industrial packaging, plastic sheeting		
G68	Fiberglass/fragments		
G69	Hard hats/Helmets		
G70	Shotgun cartridges		
G71	Shoes/sandals		
G72	Traffic cones		
G73	Foam sponge		
G79	Plastic pieces 2.5 cm > < 50cm		
G80	Plastic pieces > 50 cm		
G82	Polystyrene pieces 2.5 cm > < 50cm		
G83	Polystyrene pieces > 50 cm		
G84	CD, CD-boxes		
G85	Salt packaging		
G86	Fin trees (from fins for scuba diving)		
G87	Masking tape		
G88	Telephone (incl. parts)		
G89	Plastic construction waste		
G90	Plastic flower pots		
G91	Biomass holder from sewage treatment plants		
G92	Bait containers/packaging		
G93	Cable ties		
G95	Cotton bud sticks		
G96	Sanitary towels/panty liners/backing strips		
G97	Toilet fresheners		
G98	Diapers/nappies		
G99	Syringes/needles		
G100	Medical/Pharmaceuticals containers/tubes		
G101	Dog faeces bags		
G102	Flip-flops		
G124	Other plastic/polystyrene items (identifiable)		

RUBBER			
Code	**Items name**	**Item counts**	**Total**
G125	Balloons and balloon sticks		
G126	Balls		
G127	Rubber boots		
G128	Tyres and belts		
G129	Inner-tubes and rubber sheets		
G130	Wheels		
G131	Rubber bands (small, for kitchen/household/post use)		
G132	Bobbins (fishing)		
G133	Condoms (incl. packaging)		
G134	Other rubber pieces		

CLOTH/TEXTILE			
Code	**Items name**	**Item counts**	**Total**
G137	Clothing / rags (clothes, hats, towels)		
G138	Shoes and sandals (e.g. leather, cloth)		
G139	Backpacks & bags		
G140	Sacking (hessian)		
G141	Carpet & furnishing		
G142	Rope, string and nets		
G143	Sails, canvas		
G144	Tampons and tampon applicators		
G145	Other textiles (incl. rags)		

PAPER/CARDBOARD			
Code	**Items name**	**Item counts**	**Total**
G147	Paper bags		
G148	Cardboard (boxes & fragments)		
G150	Cartons/Tetrapack Milk		
G151	Cartons/Tetrapack (others)		
G152	Cigarette packets		
G153	Cups, food trays, food wrappers, drink containers		
G154	Newspapers & magazines		
G155	Tubes for fireworks		
G156	Paper fragments		
G158	Other paper items		

PROCESSED/WORKED WOOD			
Code	**Items name**	**Item counts**	**Total**
G159	Corks		
G160	Pallets		
G161	Processed timber		
G162	Crates		
G163	Crab/lobster pots		
G164	Fish boxes		
G165	Ice-cream sticks, chip forks, chopsticks, toothpicks		
G166	Paint brushes		
G167	Matches & fireworks		
G171	Other wood < 50 cm		
G172	Other wood > 50 cm		

METAL			
G174	Aerosol/Spray cans		
G175	Cans (beverage)		
G176	Cans (food)		
G177	Foil wrappers, aluminum foil		
G178	Bottle caps, lids & pull tabs		
G179	Disposable BBQs		
G180	Appliances (refrigerators, washers, etc.)		
G181	Tableware (plates, cups & cutlery)		
G182	Fishing related (weights, sinkers, lures, hooks)		
G184	Lobster/crab pots		
G186	Industrial scrap		
G187	Drums, e.g. oil		
G188	Other cans (< 4 L)		
G189	Gas bottles, drums & buckets (> 4 L)		
G190	Paint tins		
G191	Wire, wire mesh, barbed wire		
G193	Car parts / batteries		
G194	Cables		
G195	Household Batteries		
G198	Other metal pieces < 50 cm		
G199	Other metal pieces > 50 cm		

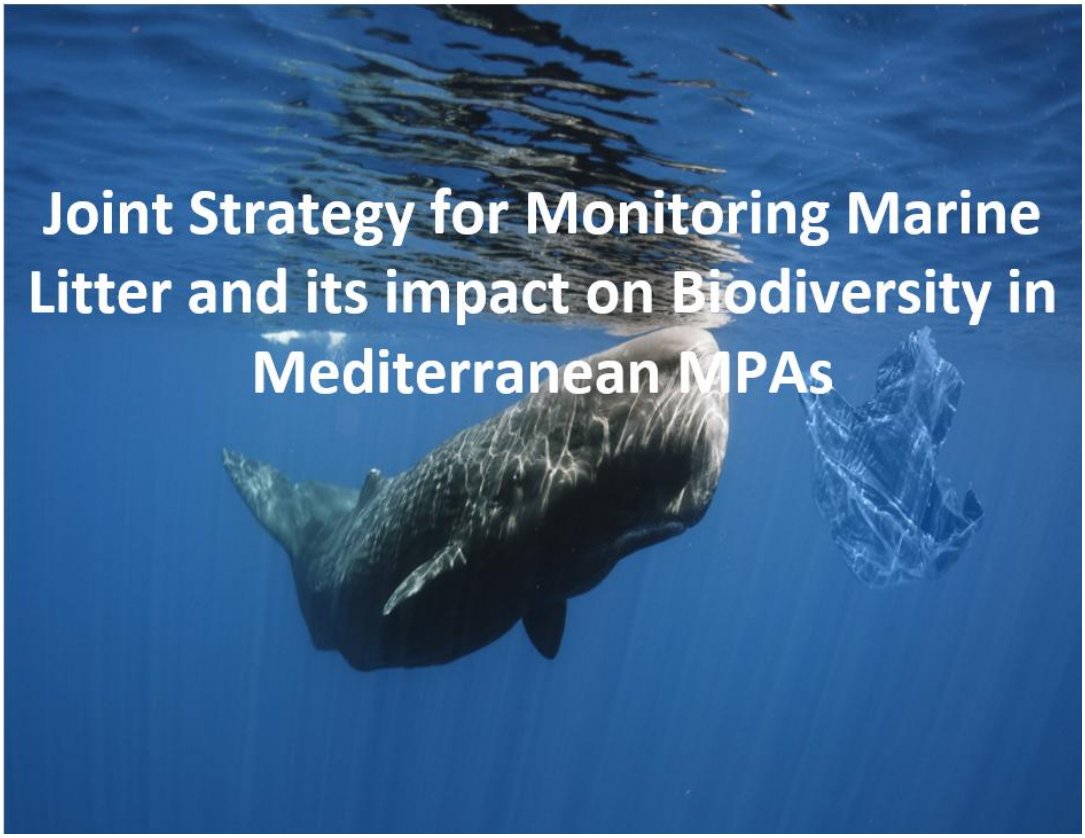
GLASS			
Code	**Items name**	**Item counts**	**Total**
G200	Bottles, (including pieces)		
G202	Light bulbs		
G208	Glass fragments >2.5 cm		
G210a	Other glass items		

CERAMICS			
Code	**Items name**	**Item counts**	**Total**
G204	Construction material (brick, cement, pipes)		
G207	Octopus pots		
G208	Ceramic fragments >2.5cm		
G210b	Other ceramic/potterys items		

SANITARY WASTE			
G95	Cotton bud sticks		
G96	Sanitary towels/ panty liners/ backing strips		
G97	Toilet fresheners		
G98	Diapers/nappies		
G133	Condoms (incl. packaging)		
G144	Tampons and tampon applicators		
	Other sanitary waste		

MEDICAL WASTE			
Code	**Items name**	**Item counts**	**Total**
G99	Syringes/needles		
G100	Medical/Pharmaceuticals containers/tubes		
G211	Other medical items (swabs, bandaging, adhesive plaster etc.)		

PARAFFIN/WAX PIECES			
Code	**Items name**	**Item counts**	**Total**
G213	Paraffin/wax		



Annex 2

Bioindicators fact sheets

Interreg Mediterranean
EUROPEAN UNION
PLASTIC BUSTERS MPAs

Invertebrates

Target species

For ***SEAFLOOR*** at ***LOCAL SCALE***

Paracentrotus lividus Lamarck, 1816

Source : https://sealifebase.ca

Common name: Stony sea urchin

Phylum: Echinodermata

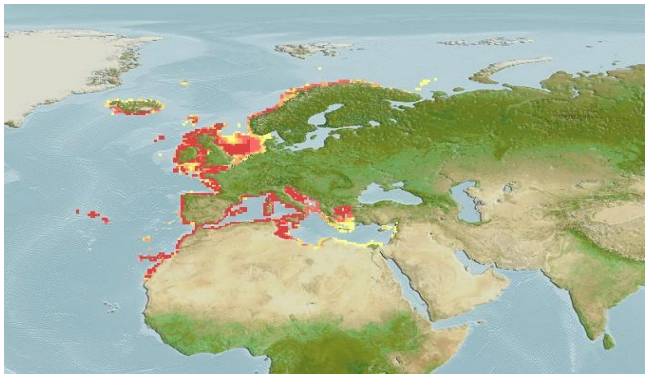
Class: Echinoidea

Order: Camarodonta

Family: Parechinidae

Geographical distribution: *P. lividus* is a common benthic echinoid distributed in the Eastern Atlantic and the Mediterranean Sea. It is considered one of the most ubiquitous echinoids in the Mediterranean sub littoral zone.

Habitat: *P. lividus* is usually found right below low water mark at depths of twenty metres and sometimes also in rock pools and in seagrass meadows.



Geographical distribution of *Paracentrotus lividus* (www.aquamaps.org)

Size and Lifespan: Max length 6.0 cm. Fertilization is external. Brooding is common, eggs are held either on the peristome, around the periproct or deep into the concavities on the petaloids. Life cycle: Embryos develop into planktotrophic larvae (echinoplateus) and live for several months before they sink to the bottom using their tube feet to adhere to the ground where they metamorphose into young urchins.

Feeding habits: *P. lividus* feeds on marine plants and animal material.

Commercial importance: The gonads are considered a delicacy in Lebanon, France, Italy, Spain, Malta, parts of Croatia (most notably on the island of Korčula), and to a lesser extent in Greece. The urchins have been harvested for export over a wider area including Croatia, Portugal and Ireland.

Protection: *P. lividus* is listed in Annex III of the SPA/BIO Protocol of the Barcelona Convention and in Annex III of Bern Convention.

Knowledge on plastic ingestion: Only experimental works have been done on *P. lividus* embryos through the leaching of chemicals (Martínez-Gómez et al., 2017; Messinetti et al., 2018). Microplastic ingestion was found to alter the postembryonic development and growth of *P. lividus* although not affecting their survival rate in these experimental works. Experimental works were also done by Torre et al. (2014) and Pinsino et al. (2017) to investigate the toxicity of polystyrene nanoparticles in *P. lividus* embryos**.**

Use as biological indicator in other projects: *P. lividus* has not been used as a bioindicator of microplastic ingestion in other projects.

Target species

For ***COAST LINE*** *and* ***BEACH SEDIMENT*** at **LOCAL SCALE**

Pachygrapsus marmoratus Fabricius, 1787

Source: https://sealifebase.ca

Common name: Marbled shore crab

Phylum: Arthropoda

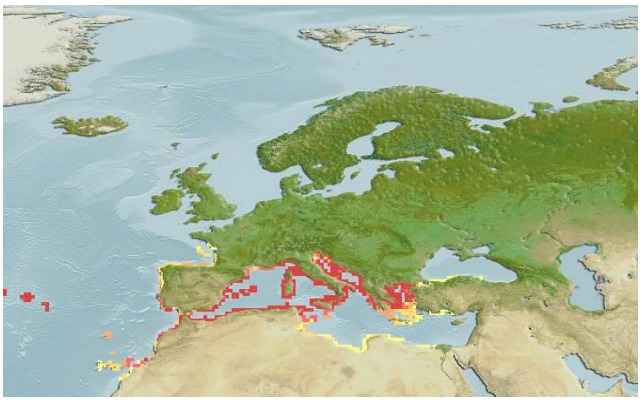
Class: Malacostraca

Order: Decapoda

Family: Grapsidae

Geographical distribution: *P. marmoratus* is found in the Eastern Atlantic and the Mediterranean Sea.

Habitat: *P. marmoratus* is an intertidal species. It is found on rocks from upper to middle shore, also in crevices in breakwaters, pier piles or similar habitats.



Geographical distribution of *Pachygrapsus marmoratus* (www.aquamaps.org)

Size and Lifespan: *P. marmoratus* has a shell length of up to 4 cm. It reproduces in July-August and has a life expectancy of 3-4 years.

Feeding habits: *P. marmoratus* is omnivorous. It actively searches for food in the intertidal zone and usually feeds on benthic invertebrates such as limpets, barnacles and mussels as well as algae.

Commercial importance: Not investigated due to lack of importance

Protection: No specific protection status.

Knowledge on plastic ingestion: There is no information on microplastic ingestion in *Pachygrapsus marmoratus*.

Use as biological indicator in other projects: *P. marmoratus* has not been used as a bioindicator of microplastic ingestion in other projects.

Secondary species

For ***COAST LINE*** *and* ***BEACH SEDIMENT*** at **LOCAL SCALE**

Maja squinado Herbst, 1788

Source: https://sealifebase.ca

Common name: Spinous spider crab

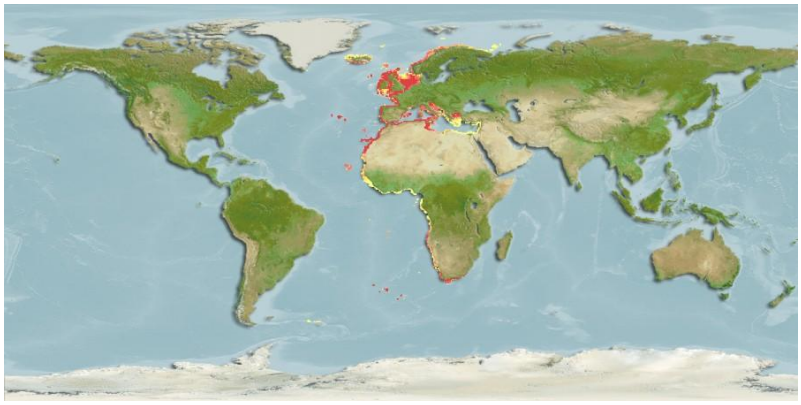
Phylum: Arthropoda

Class: Malacostraca

Order: Decapoda

Family: Majidae

Geographical distribution: *Maja squinado* is found in the Mediterranean Sea from Spain, north to Slovenia and east to Turkey. *Maja brachydactyla,* which was until recently considered to be the same species, is found in the northeast Atlantic Ocean from Morocco to Scotland.



Geographical distribution of *Maja squinado* (www.aquamaps.org)

Habitat: *M. squinado* is a benthic migratory species that can be found from the sublittoral area to depths of about 90 m on rocky bottoms. Juveniles inhabit shallow rocky and sandy areas while adults live in deeper areas.

Size and Lifespan: *M. squinado* presented a carapace length (CL) ranging between 22.5 and 87 mm.

Feeding habits: *M. squinado* feeds on macroalgae and benthic invertebrates.

Commercial importance: *M. squinado* fisheries are developed in Europe with a capture production of 6462 tonnes in 2016, according to FAO (2018). *M. squinado* populations in the Mediterranean Sea have drastically reduced in recent years by over-exploitation.

Protection: No specific protection status.

Knowledge on plastic ingestion: Microplastic ingestion has been reported in *M. squinado* from the Celtic Sea in 42.4% of the examined samples (Welden et al., 2018).

Use as biological indicator in other projects: *M. squinado* has not been used as a bioindicator of microplastic ingestion in other projects.

Secondary species

For ***COAST LINE*** *and* ***BEACH SEDIMENT*** at **LOCAL SCALE**

Mytilus galloprovincialis Lamarck, 1819

Source : https://sealifebase.ca

Common name: Mediterranean mussel

Phylum: Mollusca

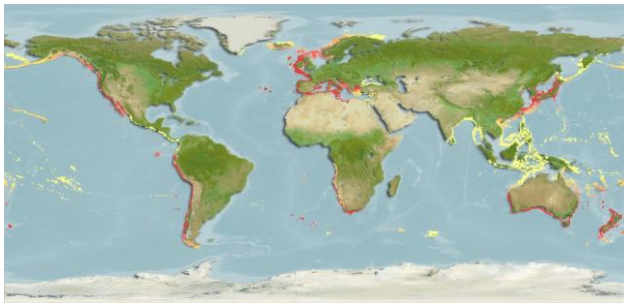
Class: Bivalvia

Order: Mytiloida

Family: Mytilidae

Geographical distribution: *M. galloprovincialis* is native in the Mediterranean Sea, Black Sea and Northeast Atlantic Ocean and introduced in the Arctic, Indian and Pacific Oceans

Habitat: *M. galloprovincialis* is a benthic species that lives on hard substrates from the intertidal zone to depths of 40 m. It is found attached by the byssus threads to rocks and piers within sheltered harbors and estuaries, and on rocky shores of open coasts. It often forms dense beds.



Geographical distribution of *Mytilus galloprovincialis* (www.aquamaps.org)

Feeding habits: Mussels are filter feeders, feeding on phytoplankton and detritus.

Commercial importance: *M. galloprovincialis* is among the most cultivated bivalves globally. In the Mediterranean Sea, it is mainly cultivated but also captured from natural beds. Global aquaculture production reported to FAO for 2016 is 105,332 tonnes (FAO, 2018) although this value does not include data from Spain and China, which produce substantial quantities of mussels but are reported to FAO under a different name category. Global capture production reported by FAO for 2016 is 1,068 tonnes (FAO, 2018).

Protection: No specific protection status.

Knowledge on plastic ingestion: Native, caged and cultivated mussels *M. galloprovincialis* in the Mediterranean Sea have been found to ingest microplastics (Vandermeersch et al., 2015, Avio et al., 2017, Digka et al., 2018) with occurrence of microplastic ingestion ranging from 10% to 47.5 %. Mussels *Mytilus edulis* from European, Chinese and Canadian waters have also been found to ingest microplastics (e.g. Mathalon and Hill 2014, Li et al 2016, Phuong et al. 2018). Both *Mytilus* sp have been shown to be affected by microplastic exposure in laboratory experiments (e.g. Green et al. 2019, Détrée and Gallardo-Escárate 2018).

Use as biological indicator in other projects: *Mytilus* sp have been used worldwide for decades as bioindicators of coastal pollution in mussel watch programs (Beyer et al. 2017). In the Mediterranean Sea, *M. galloprovincialis* has been used in the UNEP MAP MED POL programme as well as in various national and international projects. Caged mussels are often used (e.g. Mytilos, Mytimed projects), an approach that is useful for large geographical scale monitoring since mussels can be immersed at any location and/or depth. Mussels are among the indicators species proposed for monitoring marine litter ingestion by the Marine litter MED project (UNEP/MAP SPA/RAC, 2018) and have been used to assess microplastic ingestion in the Adriatic and N. Ionian Sea by the DeFishGear project (http://defishgear.net).

Secondary species

For ***SEA SURFACE*** at ***SMALL-SCALE***

Pelagia noctiluca Forsskål, 1775

Source: european-marine-life.org

Common name: Mauve stinger

Phylum: Cnidaria

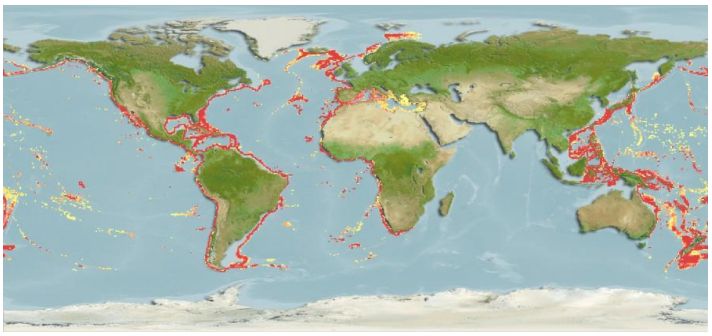
Class: Scyphozoa

Order: Semaeostomeae

Family: Pelagiidae

Geographical distribution: *P. noctiluca* is considered a semi-cosmopolitan species found in the Atlantic Ocean, the Mediterranean Sea and the Pacific Ocean.

Habitat: *P. noctiluca is* an open water jellyfish found predominantly in offshore areas and encroaching upon coastal seas when abundances are high and meteorological conditions drive it shoreward.



Geographical distribution of *Pelagia noctiluca* (www.aquamaps.org)

Size and Lifespan: *P. noctiluca* has a 60-90 mm bell diameter, as adult.

Feeding habits: *P. noctiluca* is considered an opportunistic feeder, feeding on a wide range of zooplankton prey. *P. noctiluca* is preyed upon by a wide range of vertebrate and invertebrate species including top predators ocean sunfish, loggerhead sea turtle, leatherback sea turtle, bluefin tuna, little tunny, spearfish and swordfish.

Commercial importance: *P. noctiluca* is not of commercial value. Its outbreaks in the Mediterranean Sea result in economic losses in the tourism industry.

Protection: No specific protection status.

Knowledge on plastic ingestion: Plastic ingestion has been documented in *P. noctiluca* from the Tyrrhenian Sea in 4 out of 20 specimens examined (20% of the examined specimens) (Macali et al. 2018).

Use as biological indicator in other projects: Mucus secreted by jellyfish including *P. noctiluca* has been found to bioaccumulate nanoparticles (Patwa et al., 2015). The GoJelly project (https://gojelly.eu) will use jellyfish mucus as main raw material to develop, test and promote a gelatinous solution to microplastic pollution by developing a microplastics filter for commercial and public use.

Fish

Target species

For ***COASTAL WATERS*** at ***SMALL SCALE***

Boops boops (Linnaeus, 1758)

Source: http://www.colapisci.it/

Common name: Bogue

Phylum: Chordata

Class: Actinopterygii

Order: Perciformes

Family: Sparidae

Geographical distribution: Eastern Atlantic (Norway to Angola, including the Canary Islands, Cape Verde and the Sao Tome-Principe Islands) and it is common from the Bay of Biscay to Gibraltar. It is also found in the Mediterranean and Black Sea.

Geographical distribution of *Boops boops* (www.fishbase.de)

Habitat: *B. boops* is a benthopelagic species, inhabiting a broad depth range distribution from 0 to 350 m, although most common in the 0 - 100 m range. It is found in coastal and pelagic waters on several bottoms (sand, mud, rocks and seaweeds). It is a gregarious species, ascending to the surface mainly at night.

Size and Lifespan: *B. boops* attains the maximum length of 40 cm, but the standard length more common is 20 cm. Its maximum age is 11 years.

Feeding habits: *B. boops* is an omnivorous species, feeding mainly on crustaceans and cnidaria. It is frequently associated with the seagrass meadows like *Posidonia oceanica* and *Cymodocea nodosa*. Trophic level: from 2.53 to 3.30.

Commercial importance: This fish is a commercial species caught in several Mediterranean fisheries. It is caught by gillnet, trammel net, combined gillnet-trammel net, purse seines, lampara net and bottom trawls.

Protection: It is listed as Least Concern in the IUCN Red List (Bizsel et al., 2011).

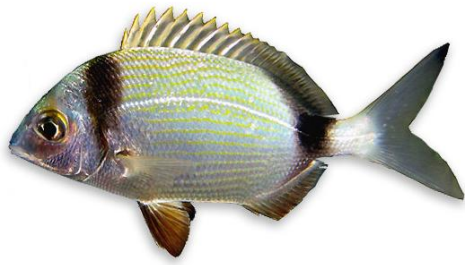
Knowledge on plastic ingestion: Plastic ingestion in *B. boops* is reported in the Mediterranean Sea and the occurrence of marine litter in the stomachs is about 61.9% (Nadal et al. 2016; Neves 2015).

Use as biological indicator in other projects: *B. boops* was used as bioindicator species for microplastics ingestion in the MEDSEALITTER project and INDICIT II project, as well as used as bioindicator species for monitoring chemical contaminants in the UNEP/MAP MED POL programme.

Target species

For ***SEAFLOOR*** at ***SMALL-SCALE***

Diplodus vulgaris (Geoffroy Saint-Hilaire, 1817)



Source: http://www.colapisci.it/

Common name: Common two-banded seabream

Phylum: Chordata

Class: Actinopterygii

Order: Perciformes

Family: Sparidae

Geographical distribution: Eastern Atlantic (Bay of Biscay to Cape Verde and the Canary Islands but also from Angola to South Africa), Mediterranean and Black Sea.

Habitat: *D. vulgaris* is a benthopelagic and oceanodromous species. This species inhabits rocky and sometimes sandy bottoms usually close to *Posidonia oceanica,* up to a maximum depth of 160 m, but more commonly in less than 50 m and sometimes in lagoons.

Geographical distribution of *Diplodus vulgaris* (www.fishbase.de)

Size and Lifespan: *D. vulgaris* attains the maximum length of 45 cm, as total length but is common to 22 cm. The maximum age recorded is 14 years old.

Feeding habits: *D. vulgaris* shows a generalist feeding behaviour. It is an omnivorous species feeding mainly on crustaceans, worms and molluscs. *D. vulgaris* as other *Diplodus* species can ingest mouthfuls of sediment together with prey during feeding activity. Trophic level: from 3.0 to 3.7.

Commercial importance: *D. vulgaris* is a species of high commercial value at larger sizes and represents an important species for small-scale fisheries in some Mediterranean areas. This species is also important for semi-industrial (in Sicily, the Adriatic and Egypt) and recreational fisheries. It is caught by gillnet, trammel net, combined gillnet-trammel net, traps hand lines and also by bottom longlines.

Protection: *D. vulgaris* is assessed as Least Concern in IUCN Red List (Bizsel et al., 2011).

Knowledge on plastic ingestion: There is no information on microplastic ingestion in *D. vulgaris*.

Use as biological indicator in other projects: *D. vulgaris* has not been used as a bioindicator of microplastic ingestion in other projects.

Target species

For ***OPEN WATERS*** at ***SMALL-SCALE***

Engraulis encrasicolus (Linnaeus, 1758)

Source: http://www.colapisci.it/

Common name: European anchovy

Phylum: Chordata

Class: Actinopterygii

Order: Clupeiformes

Family: Engraulidae

Geographical distribution: Eastern Atlantic (Norway to South Africa), Baltic Sea, Mediterranean Sea, Black and Azov Seas. Also recorded in the Canal and Gulf of Suez.

Habitat: *E. encrasicolus* is a pelagic and oceanodromous species, usually occurring in shallow waters (up to 50 m). It forms large schools. *E. encrasicolus* is a migratory and euryhaline species that can tolerate salinities of 5 - 41 ppt and in some areas it enters lagoons, estuaries and lakes, especially during spawning.

Geographical distribution of *Engraulis encrasicolus* (www.fishbase.de)

Size and Lifespan: *E. encrasicolus* is a small fish, attaining the maximum standard length of 20 cm, but is more common at 13.5 cm SL. The maximum age recorded is 5 years old.

Feeding habits: *E. encrasicolus* feeds on planktonic organisms, and zooplankton (mainly crustaceans) is considered to provide its major dietary input. European anchovy feeding is mainly diurnal, although nocturnal feeding activities have been reported. Filter-feeding and raptorial-feeding are the main feeding mechanisms in small and large European anchovy, respectively. Trophic level: from 3.40 to 3.50.

Commercial importance: This species has high commercial importance in the Eastern Central Atlantic, Mediterranean and the Black Seas. *E. encrasicolus* is a target species for purse seine, lampara net and pelagic trawl fisheries but it is also caught by gillnet. It is also used as bait in longline fishing.

Protection: Some stocks have been assessed by FAO-General Fisheries Commission for the Mediterranean (GFCM) SAC (Scientific Advisory Committee) and Sub-Committee on Stock Assessment (SCSA) in several GSA areas. In recent years, these stocks showed a high variability and only in a few cases decreasing trends were found. In the Mediterranean Sea, the minimum catch size by the GFCM is 9 cm (Reg. CE 1967/2006). In some cases there are national efforts to control the fisheries (e.g. time and area closures). It is assessed as Least Concern in the IUCN Red List.

Knowledge on plastic ingestion: Plastic ingestion in *E. encrasicolus* is reported in the Mediterranean Sea and the occurrence of marine litter in the stomachs is about 20.3% (Collard et al. 2015; Collard et al. 2017; Compa et al 2018).

Use as biological indicator in other projects: *E. encrasicolus* has not been used as a bioindicator of microplastic ingestion in other projects.

Target species

For ***SEAFLOOR*** at ***SMALL-SCALE***

Mullus surmuletus Linnaeus, 1758

Source:http://www.colapisci.it/

Common name: Striped Red Mullet

Phylum: Chordata

Class: Actinopterygii

Order: Perciformes

Family: Mullidae

Geographical distribution: Eastern Atlantic (Western Norway to Senegal including English Channel and the Canary Islands), Mediterranean and the Black Sea.

Geographical distribution of *Mullus surmuletus* (www.fishbase.de)

Habitat: *M. surmuletus* is a demersal and oceanodromous species. It usually occurs at a depth range of 5 – 409 m over sandy and muddy substrates, in particular the adults occur on broken and rough grounds although they are also found over sand and soft bottoms at depths less than 100 m.

Size and Lifespan: *M. surmuletus* attains the maximum length of 40 cm SL, but it is more common at 25 cm SL. The maximum age recorded is 11 years old.

Feeding habits: *M. surmuletus* feeds on benthic preys such as crustaceans (shrimps and amphipods), polychaetes, mollusks, and benthic fish. Diet varies seasonally and suggests a specialist feeding behaviour. Trophic level: from 3.2 to 3.6.

Commercial importance: *M. surmuletus* is an important commercial species and it represents a target species of small-scale and semi-industrial fisheries in some Mediterranean areas and northeast Atlantic. It is caught by gillnet, trammel net, combined gillnet-trammel net, bottom trawl.

Protection: It is listed as Data deficient for Europe in the IUCN Red List (Collette et al., 2015)

Knowledge on plastic ingestion: Plastic ingestion in *M. surmuletus* is reported in the Mediterranean Sea and the occurrence of marine litter in the stomachs is about 64.04% (Anastasopoulou et al. 2018; Güven et al. 2017).

Use as biological indicator in other projects: *M. surmuletus* was used as bioindicator species for macro and micro litter ingestion in the DeFishGear project and INDICIT II project. *Mullus sp.* It used in Italy as indicator in the MSFD monitoring program.

Target species

For ***OPEN WATERS*** at ***SMALL-SCALE***

Sardina pilchardus (Walbaum, 1792)

Source: http://www.colapisci.it/

Common name: European Pilchard

Phylum: Chordata

Class: Actinopterygii

Order: Clupeiformes

Family: Clupeidae

Geographical distribution: Northeast Atlantic (Iceland and North Sea southward to Bay de Gorée, Senegal), Mediterranean (common in the western part and in Adriatic Sea, rare in the eastern part), Sea of Marmara and Black Sea.

Habitat: *S. pilchardus* is a pelagic and oceanodromous species. This species occurs at depths between 10 and 100 m. *S. pilchardus* forms schools, usually at depths of 25 to 55 m or even 100 m during daytime, rising to 10 to 35 m at night. It is abundant in some zones, especially in upwelling areas. *S. pilchardus* is a migratory fish and is a 'cold water' species.

Geographical distribution of *Sardina pilchardus* (www.fishbase.de)

Size and Lifespan: *S. pilchardus* is a small fish, attaining the maximum length of 27.5 cm SL, but is more common at 20 cm. The maximum age recorded is 8 years old.

Feeding habits: *S. pilchardus* is an opportunistic species. This species can switch between non-selective filter feeding and selective predation. European pilchard is a planktivorous fish, and it feeds mainly on planktonic crustaceans but also on fish eggs, larvae and algae. Trophic level: from 3.1 to 3.2.

Commercial importance: This species is considered an important commercial resource. It is mainly caught by purse seines, lampara net and pelagic trawl.

Protection: *S. pilchardus* is assessed as Least Concern in IUCN Red List (Di Natale et al., 2011). Several stocks have been recently assessed by SCSA GFCM (Subcommittee of Stock Assessment for GFCM) in several GSA areas. In most cases, these stocks were considered fully exploited. The European Union (EU) has a minimum landing size adopted by EC countries in the Mediterranean Sea, which is 11 cm, or EU member states can convert this measurement into 55 specimens per kg (Reg.CE 1967/2006).

Knowledge on plastic ingestion: Plastic ingestion in *S. pilchardus* is reported in the Mediterranean Sea and the occurrence of marine litter in the stomachs is about 24.6% (Anastasopoulou et al. 2018; Avio et al. 2015; Compa et al 2018; Digka et al. 2018; Güven et al. 2017).

Use as biological indicator in other projects: *S. pilchardus* has not been used as a bioindicator of microplastic ingestion in other projects.

Secondary species

For ***OPEN WATERS*** at ***MEDIUM-SCALE***

Coryphaena hippurus Linnaeus, 1758

Source: http://www.colapisci.it/

Common name: Common dolphinfish

Phylum: Chordata

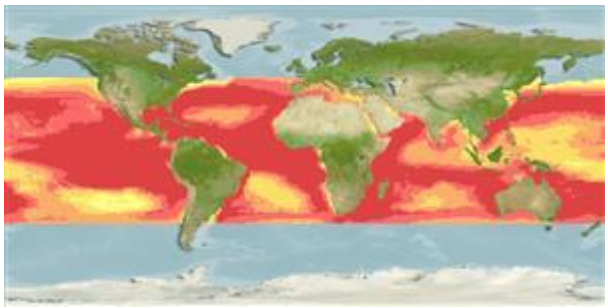
Class: Actinopterygii

Order: Perciformes

Family: Coryphaenidae

Geographical distribution: Atlantic, Indian and Pacific: in tropical and subtropical waters.

Habitat: C. hippurus is an epipelagic, oceanodromous, cosmopolitan species and highly migratory species distributed throughout the world's tropical and subtropical oceans in waters greater than 20°C.



Geographical distribution of *Coryphaen hippurus* (www.fishbase.de)

Size and Lifespan: *C. hippurus* is a large fish, attaining the maximum length of 210 cm TL, but it is common at 100 cm TL. The maximum age recorded is 4 years old.

Feeding habits: It is a top predator and its wide spectrum of prey items suggests a generalist feeding behaviour. It is an opportunistic and voracious pelagic predator forming schools. This predator feeds mainly on teleost fishes, zooplankton, crustaceans and cephalopods (in particular squids). The large proportion of epipelagic species in its diets indicates that this predator forages mainly in subsurface layers of oceanic waters. Cannibalism is an important aspect that concerns this species. Trophic level: 4.4.

Commercial importance: *C. hippurus* is a species of commercial value. It represents one of the most important target species for small-scale fisheries in several areas. It is caught by purse seines nets and drifting longline. In the Mediterranean, this species is caught with the help of fish aggregation devices (FADs) by the artisanal fishermen using surrounding nets. It is also an important target for recreational fishing.

Protection: It is listed in the Annex I of the 1982 Convention on the Law of the Sea. It is listed as Least Concern in the IUCN Red List (Yokes et al., 2011).

Knowledge on plastic ingestion: Plastic ingestion in *C. hippurus* is reported in the Mediterranean Sea and the occurrence of marine litter in the stomachs is about 14.3% (Deudero 1998; Deudero & Alomar 2015; Massuti et al.1998).

Use as biological indicator in other projects: *C. hippurus* has not been used as a bioindicator of marine litter and microplastic ingestion in other projects.

Secondary species

For ***OPEN WATERS*** at ***MEDIUM-SCALE***

Euthynnus alletteratus (Rafinesque, 1810)

Source: http://www.colapisci.it/

Common name: Little Tunny

Phylum: Chordata

Class: Actinopterygii

Order: Perciformes

Family: Scombridae

Geographical distribution: Mediterranean and Black Sea, Atlantic Ocean, in tropical and subtropical waters, including the Caribbean Sea and Gulf of Mexico.

Habitat: *E. alletteratus* is a pelagic and oceanodromous species found on the continental shelf or in coastal areas with swift currents and offshore islands. In the Mediterranean, it is also found far offshore. It usually occurs at a depth range of 1 - 150 m. It is a highly migratory species and often forms schools with other scombrid species.

Geographical distribution of *Euthynnus alletteratus* (www.fishbase.de)

Size and Lifespan: *E. alletteratus* attains the maximum length of 122 cm TL, but it is common at 80 cm TL. It can reach about 16 kg in weight. The maximum age the species can reaches is between 7 and 10 years old.

Feeding habits: It is an opportunistic and very voracious predator usually feeding on teleost fish (mainly clupeoids), but also on crustaceans, tunicates and cephalopods. Trophic level: 4.5.

Commercial importance: Little Tunny is a commercial species that is part of a multispecific fishery. This resource is exploited by commercial, artisanal and recreational fisheries as target species or accessory species. It is caught by purse seiners, mid-water trawlers, hand lines, surface drifting long-lines and gillnets. This species is also caught in association with fish aggregation devices (FADs).

Protection: It is listed in the Annex I (highly migratory species) of the 1982 Convention on the Law of the Sea. No management recommendations have been presented by ICCAT due to the lack of proper data, historical series and analyses. Although worldwide catches are relatively stable, there are likely regional declines. To date, this species is listed as Least Concern in the IUCN Red List (Collette & Heessen, 2015).

Knowledge on plastic ingestion: Plastic ingestion in *E. alletteratus* is reported and the occurrence of marine litter in the stomachs is about 3.4% (Falautano et al. 2007).

Use as biological indicator in other projects: *E. alletteratus* has not been used as a bioindicator of microplastic ingestion in other projects.

Secondary species

For ***SEAFLOOR*** at ***SMALL-SCALE***

Galeus melastomus Rafinesque, 1810

Source: http://www.colapisci.it/

Common name: Blackmouth catshark

Phylum: Chordata

Class: Elasmobranchii

Order: Carcharhiniformes

Family: Pentanchidae

Geographical distribution: Mediterranean Sea and Northeast Atlantic (Faeroe Islands and Trondheim, Norway southward to Senegal).

Habitat: *G. melastomus* is a demersal deep-water shark, occurring on the outer continental shelves and upper slopes usually at a depth range of 200 – 1200 m, but also found deeper.

Geographical distribution of *Galeus melastomus* (www.fishbase.de)

Size and Lifespan: *G. melastomus* is a small shark, attaining the maximum length of 90 cm TL in females and at least 61 in males. The maximum age is about 7 years old.

Feeding habits: *G. melastomus* is considered an active predator characterized by a generalist feeding behaviour. This species forages in mid-water depths, in the near bottom layer and also on the seabed. *G. melastomus* feeds mainly on bottom invertebrates, including crustaceans and cephalopods, but also on teleost as demersal and benthic fish. The diet of Blackmouth catshark includes also small pelagic fish (lanternfishes) and mesopelagic and pelagic cephalopods and other small elasmobranchs. Trophic level: from 3.7 to 4.3.

Commercial importance: *G. melastomus* does not have a commercial importance but it is a bycatch species in demersal trawl and longline fisheries and is generally discarded.

Protection: *G. melastomus* is listed as Least Concern in the IUCN Red List (Abella et al., 2016). The General Fisheries Commission for the Mediterranean (GFCM) banned bottom trawling below depths of 1,000 m in the Mediterranean Sea in February 2005 (recommendation GFCM/2005/1 on the management of certain fisheries exploiting demersal and deepwater species) and this came into force in September 2005. In European Commission waters, a combined total allowable catch (TAC) is set for a group of deep-water sharks, which includes *G. melastomus*.

Knowledge on plastic ingestion: Plastic ingestion in *G. melastomus* is reported in the Mediterranean Sea and the occurrence of marine litter in the stomachs is about 6.5% (Alomar & Deudero 2017; Anastasopoulou et al., 2013; Cartes et al., 2016; Carrassón et al., 1992; Madurell 2003).

Use as biological indicator in other projects: *G. melastomus* has not been used as a bioindicator of marine litter microplastic ingestion in other projects.

Secondary species

For ***SEAFLOOR*** at ***SMALL-SCALE***

Lithognathus mormyrus (Linnaeus, 1758)

Source:. Interreg Med Plastic Busters MPAs project.

Common name: Striped Seabream

Phylum: Chordata

Class: Actinopterygii

Order: Perciformes

Family: Sparidae

Geographical distribution: Eastern Atlantic (Bay of Biscay to Cape of Good Hope in South Africa; including the Canary and Cape Verde Islands and also Madeira Is.), Western Indian Ocean (southern Mozambique), Strait of Gibraltar and the Mediterranean Sea.

Geographical distribution of *Lithognathus mormyrus* (www.fishbase.de)

Habitat: *L. mormyrus* is a demersal species inhabiting on the shelf, over sandy and muddy bottoms as well as seagrass-beds and estuaries. This species is found at depths ranging from 0 to 80 m in the Mediterranean, but usually it occurs at depths between 10 - 20 m. It is a gregarious species, sometimes forming schools.

Size and Lifespan: *L. mormyrus* attains the maximum length of 55 cm TL, but it is common at 30 cm TL. The maximum age recorded is 11-12 years old.

Feeding habits: *L. mormyrus* is a carnivorous species feeding on worms, molluscs and small crustaceans. This species is an active seeking bottom feeder whose diet consists of diverse benthic groups, with wide range of size and morphology. Own to the feeding behaviour, detritus is usually found in the stomachs of *L. mormyrus*. Trophic level: from 3.3 to 3.5.

Commercial importance: *L. mormyrus* is a commercial species caught in some Mediterranean fisheries. In the Strait of Sicily, it represents an important target species of small-scale fisheries. It is caught by trammel net, gillnet and seine nets. It is also a target of recreational fishing.

Protection: It is assessed as Least Concern in IUCN Red List (Bizsel e al., 2011).

Knowledge on plastic ingestion: Plastic ingestion in *L. mormyrus* is reported in the Mediterranean Sea and the occurrence of marine litter in the stomachs is about 34.8% (Güven et al., 2017).

Use as biological indicator in other projects: *L. mormyrus* has not been used as a bioindicator of microplastic ingestion in other projects.

Secondary species

For ***SEAFLOOR*** at ***SMALL-SCALE***

Merluccius merluccius (Linnaeus, 1758)

Source: http://www.colapisci.it/

Common name: European hake

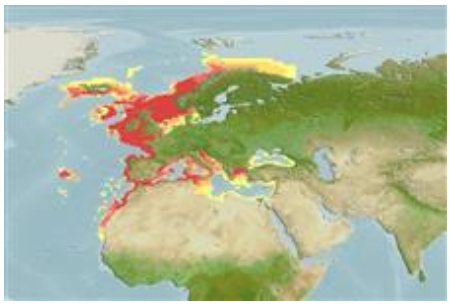
Phylum: Chordata

Class: Actinopterygii

Order: Gadiformes

Family: Merlucciidae

Geographical distribution: Eastern Atlantic (Norway and Iceland to Mauritania). It is also found in the Mediterranean Sea and along the southern coast of the Black Sea.



Geographical distribution of *Merluccius merluccius* (www.fishbase.de)

Habitat: *M. merluccius* is a demersal species, that has been found in depths ranging from 30 to 1075 m but it is usually found between 70 and 400 m. European hake prefers muddy bottoms, but can be also found in sandy bottoms. It lives close to the bottom during the day, but moves off-bottom to the water column at night.

Size and Lifespan: *M. merluccius* is a large fish, attaining the maximum length of 140 cm TL, but it is common at 45 cm TL. The maximum age recorded is 20 years old.

Feeding habits: *M. merluccius* is a bentho-pelagic active predator throughout its entire life. This species is able to feed on fast-moving pelagic preys in the water column. Moreover, it is able to carry out daily vertical migrations; in fact, adults live on the bottom during the day, but move off-bottom at night and feed mainly on fish (anchovies, clupeids, mesopelagic fish, gadiform fish) and squids. The young specimens feed on crustaceans (especially euphausiids and amphipods). In addition to circadian migrations, they perform also horizontal migrations as a consequence of searching for food. Size and seasonality are the factors that most influence hake diet. Cannibalism is an important aspect that concerns this species. Trophic level: from 3.8 to 4.5.

Commercial importance: *M. merluccius* is one of the most important Mediterranean commercial resources. This species is among the main target species of the demersal fisheries of the Mediterranean Sea. It is caught by gillnet, bottom longlines and bottom trawl fishery.

Protection: *M. mercluccius* is assessed as Vulnerable for the Mediterranean in the IUCN Red List (Di Natale et al., 2011). *M. merluccius* is a priority species for the General Fisheries Commission for the Mediterranean (GFCM). In Mediterranean region this species is considered overfished. It is regulated through fishing effort controls, selectivity, fishing closures, minimum landing size, etc. in the GSAs. There are also some national regulations regarding minimum landing size (for instance, the minimum landing size is 25 cm in Turkey, 20 cm in Morocco as well as in the EC regulation).

Use as biological indicator in other projects: *M. merluccius* has been used in Italy as indicator in the MSFD monitoring program.

Knowledge on plastic ingestion: Plastic ingestion in *M. merluccius* is reported in the Mediterranean Sea and the occurrence of marine litter in the stomachs is about 21.3% (Anastasopoulou et al. 2013; Avio et al. 2015; Giani et al., 2019).

Use as biological indicator in other projects: *M.merluccius* used in Italy as indicator in MSFD monitoring program.

Secondary species

For ***OPENWATERS*** at ***SMALL-SCALE***

Myctophidae

Source: http://www.colapisci.it/

Common name: lanternfish

Phylum: Chordata

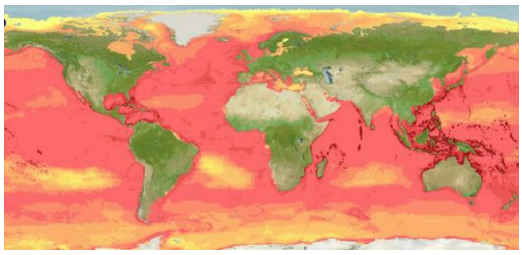
Class: Actinopterygii

Order: Myctophiformes

Family: Myctophidae

Geographical distribution: all Oceans, including the Mediterranean Sea.

Habitat: lanternfishes are pelagic species, occurring in mesopelagic and bathypelagic waters. They are important component of micronekton, forming large aggregations visible on acoustic sounders. This family includes vertically migrating species, able to carry out large depth excursions to reach epipelagic waters during night.



Geographical distribution of *Myctophidae* (www.fishbase.de)

Size and Lifespan: Lanternfishes are small teleosts, attaining the maximum length of about 12-15 cm in larger species. Their life span is short (1 year or less depending on the species), although recently their maximum age has been reassessed by a daily interpretations of otolith rings.

Feeding habits: Lanternfishes are key species in the pelagic trophic web of all oceans, including the Mediterranean Sea. They mainly feed crustaceans such as copepods and at larger sizes, amphipods and euphausiids. Small siphonophores, pteropod molluscs and small mesopelagic fish (e.g. *Cyclothone* spp) are also part of their diet. Since Myctophidae includes several species with different vertical migratory behaviour, their diet changes on the basis of different specializations and feeding strategy. Several lanternfishes perform diel vertical migrations, so assuring the energy transfer throughout the water column. Trophic level: from 3.2 to around 4.

Commercial importance: Myctophids usually have no commercial importance, although in several areas some attempts to exploit these abundant resources are in progress. However they comprise more than half of all deep-sea biomass and are a critical component of marine ecosystems worldwide, being key species in the trophic ecology of several important fish resources (such as tunas).

Protection: No specific protection status.

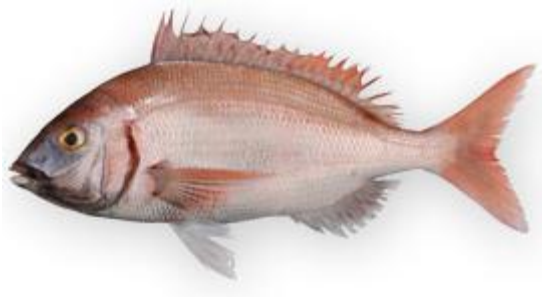
Knowledge on plastic ingestion: Plastic ingestion in lanternfishes has been already documented and the occurrence of marine litter in their stomachs reach in some cases up to 6.8% (i.e. *Hygophum benoiti;* Romeo et al. 2016) in the Mediterranean Sea. This percentage is higher in other oceans.

Use as biological indicator in other projects: Myctophids have not been used as a bioindicator of microplastic ingestion in other projects.

Secondary species

For ***SEAFLOOR*** at ***SMALL-SCALE***

Pagellus erythrinus (Linnaeus, 1758)



Source: http://www.colapisci.it/

Common name: Common pandora

Phylum: Chordata

Class: Actinopterygii

Order: Perciformes

Family: Sparidae

Geographical distribution: Eastern Atlantic (Norway to Guinea-Bissau, including Cape Verde, Madeira and the Canary Islands). It is also presents in the Mediterranean Sea and in the western Black Sea. Rarely, its presence has been recorded in Scandinavia.

Geographical distribution of *Pagellus erythrinus* (www.fishbase.de)

Habitat: *P. erythrinus* is a benthopelagic species. It can occur up to 300 m but commonly inhabits depths ranging from 20 to 100 m.
P. erythrinus lives on inshore waters, on different bottoms (rock, gravel, sand and mud). The young specimens can be found near the shore.

Size and Lifespan: *P. erythrinus* attains the maximum length of 60 cm SL, but it is more common at 25 cm SL. The maximum age recorded is 15 years old.

Feeding habits: This species feeds mainly on crustaceans, worms and other benthic invertebrates and small fishes. In particular, common pandora's feeds on invertebrates from epi- and endo-fauna: Polychaeta, Crustacea Decapoda, Lamellibranchiata and other groups of organisms such as Isopoda, Ophiuroida, Pisces and Cephalopoda. Trophic level: from 3.1 to 3.8.

Commercial importance: *P. erythrinus* is an important commercial resource in the Mediterranean region, being a target species of small-scale, industrial and recreational fisheries. It is caught by gillnet, trammel net, combined gillnet-trammel net, hand lines, bottom longlines and also by seine nets. In Canary Islands, this species is caught with traps.

Protection: It is assessed as Least Concern in the IUCN Red List (Bizsel et al., 2011).

Knowledge on plastic ingestion: Plastic ingestion in *P. erythrinus* is reported in the Mediterranean Sea and the occurrence of marine litter in the stomachs is about 30.2% (Anastasopoulou et al. 2018; Digka et al. 2018; Güven et al. 2017; Savoca et al., 2019).

Use as biological indicator in other projects: *P. erythrinus* was used as bioindicator species for macro and micro litter ingestion in the DeFishGear project.

Secondary species

For ***OPEN WATERS*** at ***SMALL-SCALE***

Sardinella aurita Valenciennes, 1847

Source: http://www.colapisci.it/

Common name: Round sardinella

Phylum: Chordata

Class: Actinopterygii

Order: Clupeiformes

Family: Clupeidae

Geographical distribution: Eastern (from Gibraltar southward to Saldanha Bay in South Africa) and Western Atlantic Ocean (from Cape Cod in USA to Argentina, including Bahamas, Antilles, Gulf of Mexico and the Caribbean coast), Mediterranean Sea and Black Sea.

Geographical distribution of *Sardinella aurita* (www.fishbase.de)

Habitat: *S. aurita is a* pelagic and oceanodromous species. This species usually occurs at a depth range of 0 - 350 m and forms schools in coastal waters from inshore to edge of shelf. It prefers waters with a temperature below 24°C. Strongly migratory species, often rising to surface at night.

Size and Lifespan: *S. aurita* attains the maximum length of 36 cm TL, but it is common at 25 cm. The maximum age recorded is 7 years old.

Feeding habits: It is an opportunistic feeder with trophic level that changes with body size, season, geographic area and upwelling intensity. It feeds mainly on zooplankton, in particular copepods. Juveniles also prey on phytoplankton. Trophic level: 3.4.

Commercial importance: This species is considered a commercial resource. It is caught by hand lines, purse seines and lampara net but it is also caught by bottom trawl fishery. It is widely used as bait.

Protection: *S. aurita* is listed as Least Concern in the IUCN red List (Di Natale et al., 2011).

Knowledge on plastic ingestion: There is no information on microplastic ingestion in *S. aurita*

Use as biological indicator in other projects: *S. aurita* has not been used as a bioindicator of microplastic ingestion in other projects.

Secondary species

For ***SEAFLOOR*** at ***SMALL-SCALE***

Spondyliosoma cantharus (Linnaeus, 1758)

Source: http://www.colapisci.it/

Common name: Black seabream

Phylum: Chordata

Class: Actinopterygii

Order: Perciformes

Family: Sparidae

Geographical distribution: Eastern Atlantic (Scandinavia to northern Namibia, including the Strait of Gibraltar, Madeira, Canary Islands, and Cape Verde), Mediterranean and the Black Sea.

Geographical distribution of *Spondyliosoma cantharus* (www.fishbase.de)

Habitat: *S. cantharus* is a benthopelagic and oceanodromous species. It inhabits the continental shelf, especially seagrass beds and rocky or sandy bottoms up to about 300 m depth, although it usually occurs between 10 m and 100 m. Juveniles are found in shallower water, to about 50 m depth and will remain inshore until about two to three years of age. It is a gregarious species, sometimes it forms large schools.

Size and Lifespan: *S. cantharus* attains the maximum length of 60 cm SL, but it is common at 30 cm. The maximum age recorded is about 14-15 years old.

Feeding habits: *S. cantharus* is omnivorous, feeding mainly on small invertebrates, especially crustaceans but also algae. Trophic level: 3.3 (calculated for small individuals).

Commercial importance: This species is locally important for fishery. In European waters, this species is exploited by recreational and commercial fishers. It is caught by hand lines, trammel net, gillnet, bottom long-line and pots.

Protection: This species is listed as Least Concern in the IUCN red list (Bizsel et al., 2011).

Knowledge on plastic ingestion: There is no information on microplastic ingestion in *S. cantharus*

Use as biological indicator in other projects: *S. cantharus* has not been used as a bioindicator of microplastic ingestion in other projects.

Secondary species

For **SEAFLOOR** at **SMALL-SCALE**

Thunnus alalunga (Bonnaterre, 1788)

Source: http://www.colapisci.it/

Common name: Albacore Tuna

Phylum: Chordata

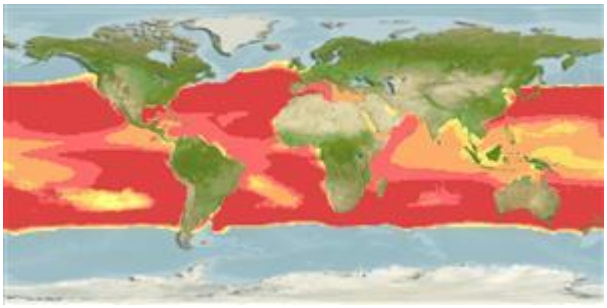
Class: Actinopterygii

Order: Perciformes

Family: Scombridae

Geographical distribution: Cosmopolitan, found in tropical and temperate waters of all oceans, including the Mediterranean Sea.

Habitat: *T. alalunga* is a pelagic-oceanic and oceanodromous species that seasonally migrates. It usually occurs at a depth range of 0 - 600 m. Albacore Tuna is abundant in surface waters between 15.6° to 19.4°C; and large albacore are also found in waters with a temperature ranging between 13.5° and 25.2°C. It is known to concentrate along thermal discontinuities. It is a highly migratory species. This species forms schools with other scombrid species and schools may be associated with floating objects



Geographical distribution of *Thunnus alalunga* (www.fishbase.de)

Size and Lifespan: *T. alalunga* attains the maximum length of 140 cm FL, but it is common at 100 cm. The maximum age recorded is 13 years old.

Feeding habits: The albacore forages in epipelagic and upper mesopelagic waters, down to a depth of 500 m. It is a pelagic carnivorous predator which feeds on teleost fish, particularly mesopelagic fish crustaceans and cephalopods. Trophic level: from 4.0 to 4.5.

Commercial importance: This species is one of the most important commercial tuna species exploited in Mediterranean. It is caught by drifting long-lines, purse seines and lines.

Protection: This species is listed as a highly migratory species in Annex I of the 1982 Convention on the Law of the Sea. The recommendations 17-05 and 16-05 by ICCAT establishing management measures for the stock of Mediterranean Albacore Tuna. It is listed as Least concern for the Mediterranean in the IUCN Red List (Di Natale et al., 2011), although a population decline globally of 37% has been estimated over the past 20 years (1987–2007) or three generation lengths.

Knowledge on plastic ingestion: Plastic ingestion in *T. alalunga* is reported in the Mediterranean Sea and the occurrence of marine litter in the stomachs is about 12.9% (Romeo et al. 2015).

Use as biological indicator in other projects: *T. alalunga* has not been used as a bioindicator of marine litter and microplastic ingestion in other projects.

Secondary species

For ***OPEN WATERS*** at ***BASIN SCALE***

Thunnus thynnus (Linnaeus, 1758)

Source: http://www.colapisci.it/

Common name: Atlantic bluefin tuna

Phylum: Chordata

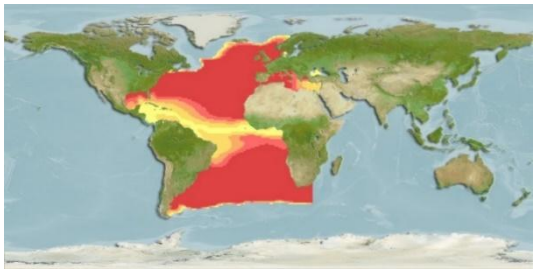
Class: Actinopterygii

Order: Perciformes

Family: Scombridae

Geographical distribution: Western (Labrador and Newfoundland to Gulf of Mexico, Caribbean Sea to Venezuela and Brazil) and Eastern Atlantic (Lofoten Islands off Norway to Canary Islands, Mauritania and South Africa), Mediterranean and the southern part of the Black Sea.

Habitat: *T. thynnus* is a pelagic, oceanodromous species, seasonally found close to shore. It can tolerate a wide range of temperatures. *T. thynnus* usually occurs at a depth range of 0 - 100 m, but is reported up to 500 m of depth. It is a highly migratory species.



Geographical distribution of *Thunnus thynnus* (www.fishbase.de)

Size and Lifespan: *T. thynnus* is a large fish, attaining the maximum length of 458 cm TL, but more common between 40 and 200 cm. The maximum age recorded is 32 years old.

Feeding habits: It is a top predator and its broad spectrum of prey items suggest a generalist feeding behaviour. It is an opportunistic predator which usually feeds on aggregated prey in schools or patches. It preys on small schooling fishes, cephalopods and shrimps. Juveniles prey mainly on zooplankton, small cephalopods and pelagic coastal fishes. Adults prey mainly on cephalopods and fishes. Recent papers showed the high importance of mesopelagic prey in the diet of *T. thynnus* in some key areas (feeding grounds of Strait of Messina, southern Turkey, Iceland Basin). Trophic level: from 4.1 to 4.5.

Commercial importance: This species is worldwide considered a valuable fishery resource. It is caught by purse seines, longlines, hook and lines, traps. It is also used for commercial fish farming in the Mediterranean Sea.

Protection: The importance of the Atlantic bluefin tuna has been emphasized at international level since the 1966, during the Conference of Plenipotentiaries (Rio de Janeiro, Brazil), which established the International Commission for the Conservation of Atlantic Tunas (ICCAT) and adopted the Convention for the Conservation of Atlantic Tunas. Fisheries quotas have been set up since 1982, and pluri-annual recovery action plans have been adopted by the ICCAT contracting parties since 2007. Several international institutions, commissions and conventions have focused their efforts on the conservation status of *T. thynnus* (i.e., GFCM of the FAO, Convention on Migratory Species of Wild Animals of Bonn in 1983, IUCN, CITES). It is listed in the Annex I of the 1982 Convention on the Law of the Sea as well as in the Annex III of the SPA/BIO Protocol of the Barcelona Convention. IUCN Red List Status: Endangered for the Mediterranean (Di Natale et al., 2011).

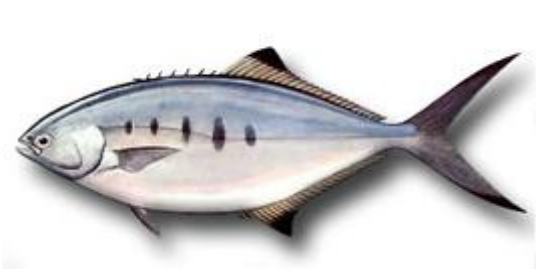
Knowledge on plastic ingestion: Plastic ingestion in *T. thynnus* is reported in the Mediterranean Sea and the occurrence of marine litter in the stomachs is about 12.7% (De la Serna et al., 2012; Karakulak et al., 2009; Romeo et al., 2015).

Use as biological indicator in other projects: *T. thynnus* has not been used as a bioindicator of marine litter and microplastic ingestion in other projects.

Secondary species

For ***COASTAL WATERS*** at ***SMALL-SCALE***

Trachinotus ovatus (Linnaeus, 1758)



Source: http://www.colapisci.it/

Common name: Pompano

Phylum: Chordata

Class: Actinopterygii

Order: Perciformes

Family: Carangidae

Geographical distribution: Eastern Atlantic (Bay of Biscay, British and Scandinavian waters to Angola) and Mediterranean Sea.

Habitat: *T. ovatus* is a pelagic-neritic and thermophilic species, forming schools. Adults are moderately common in shallow water in areas of surge. Juveniles occur in the surf-zone of sandy bottoms and this species lives in clear waters. *T. ovatus* usually occurs at a depth range of 50 - 200 m.

Geographical distribution of *Trachinotus ovatus* (www.fishbase.de)

Size and Lifespan: *T. ovatus* attains the maximum length of 70 cm TL, but it is common at 35 cm. The maximum age recorded is 4 years old.

Feeding habits: It is an opportunistic feeder, showing a particular voracity. Adults feed mainly on small crustaceans, molluscs and fishes. The presence of insects and some neustonic organisms (e.g. *Porpita porpita*) among prey suggests the behaviour of hunting just beyond the surface layer, making *T. ovatus* more vulnerable to the ingestion of floating plastic debris. Trophic level: 3.9.

Commercial importance: This species is of minor commercial importance and is gaining commercial interest in some small-scale fishery activities. It is a target of recreational fishing. It is caught by hand lines, gillnet, and surrounding nets.

Protection: It is assessed as Least Concern by the IUCN Red List (Di Natale et al., 2011).

Knowledge on plastic ingestion: Plastic ingestion in *T. ovatus* has been reported in the Mediterranean Sea and the occurrence of marine litter in the stomachs is about 24.4% (Battaglia et al. 2016).

Use as biological indicator in other projects: *T. ovatus* has not been used as a bioindicator of microplastic ingestion in other projects.

Secondary species

For ***OPEN WATERS*** at ***SMALL-SCALE***

Trachurus spp. (Trachurus mediterraneus, T. picturatus, T. trachurus)

Source: http://www.colapisci.it/

Common name: horse mackerels and blue jack mackerel

Phylum: Chordata

Class: Actinopterygii

Order: Perciformes

Family: Carangidae

Geographical distribution: Eastern Atlantic (Norway to South Africa), Mediterranean Sea, Marmara and Black seas, southern and western parts of the Azov Sea.

Habitat: *Trachurus* spp. are benthopelagic or pelagic and oceanodromous species. These species can be found in neritic zones and island shelves, banks and seamounts and also in upwelling areas. *Trachurus* spp. have a wide bathymetric distribution, from surface layers to deep waters, reaching up to 1050 m depth (i.e. *T. trachurus*). These species usually occur at a depth range of 0 – 400 m. *Trachurus* spp. are commonly migratory species and form schools.

Geographical distribution of *Trachurus spp.* (www.fishbase.de)

Size and Lifespan: *Trachurus spp.* attain a maximum total length of approximately 60 - 70 cm, but they are more common at 20 or 30 cm. The maximum age recorded is about 10-12 years in Mediterranean waters for larger species.

Feeding habits: *Trachurus* spp. are opportunistic predators feeding on fish (especially sardines and anchovies), cephalopods and small crustaceans. Some species prey mainly on planktonic and micronektonic organisms belonging to the mesopelagic and lower epipelagic environments (i.e. *Trachurus picturatus*). Trophic level: from 3.2 to 3.9.

Commercial importance: *Trachurus spp.* are commercial species in several areas of the Mediterranean Sea. These commercial resources are exploited by industrial and artisanal fisheries and some species also targeted by the recreational fisheries. They are caught by purse seines, lampara net, bottom longlines, small driftnets, set nets, bottom trawl, as well as hook and lines. In the western Mediterranean, juvenile of *Trachurus spp (T. picturatus)* are often captured around the FADs.

Protection: *Trachurus mediterraneus, T. picturatus* and *T. trachurus are* listed as Least Concern for the Mediterranean in the IUCN Red List (Herrera et al., 2015, Di Natale et al., 2011 and Di Natale et al., 2011, respectively).

Knowledge on plastic ingestion: Plastic ingestion in *Trachurus* spp. has been documented and the occurrence of marine litter in their stomachs reaches 62.1% (*Trachurus mediterraneus*) in the Mediterranean Sea (Anastasopoulou et al. 2018; Deudero 1998; Güven et al. 2017).

Use as biological indicator in other projects: *Trachurus* spp. have not been used as a bioindicator of microplastic ingestion in other projects.

Secondary species

For ***OPEN WATERS*** at ***BASIN SCALE***

Xiphias gladius Linnaeus, 1758

Source: http://www.colapisci.it/

Common name: Swordfish

Phylum: Chordata

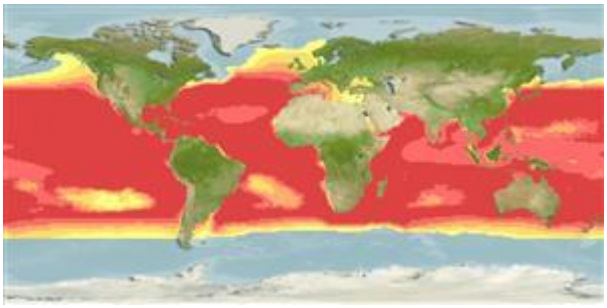
Class: Actinopterygii

Order: Perciformes

Family: Xiphiidae

Geographical distribution: Worldwide distributed in tropical, temperate and sometimes cold waters. It occurs in the Mediterranean Sea, the Sea of Marmara, the Black Sea and the Sea of Azov.

Habitat: *X. gladius* is a pelagic-oceanic and oceanodromous species that can be found in open waters. It inhabits a depth range of 0 to 2878 m but usually occurs at a depth range of 0 - 550 m. Although being an oceanic species, it is sometimes found in coastal waters. Generally, this species prefers temperatures ranging from 18°C to 22°C. Swordfish is a highly migratory species.



Geographical distribution of *Xiphias gladius* (www.fishbase.de)

Size and Lifespan: *X. gladius* is a large fish, attaining the maximum length of 455 cm FL, but it is common at 300 cm. The maximum age recorded is 15 years old.

Feeding habits: It is a top predator that uses his sword to kill its prey. It is an opportunistic predator and it can move to forage from the surface to the deep waters over a wide depth range. Swordfish feeds in deep water during the day and stay in the mixed layer at night. This species feeds mainly on cephalopods and fish. Trophic level: 4.5.

Commercial importance: *X. gla*dius is a highly important commercial species, which is caught by drifting longlines, harpoon and driftnets (now prohibited in EU waters). *X. gla*dius was also caught in tuna traps. It is also a target of recreational fisheries. In the Strait of Messina (Mediterranean Sea) *X. gla*dius is still caught by harpoon, using traditional boats, from late spring to summer.

Protection: This species is listed as a highly migratory species in Annex I of the 1982 Convention on the Law of the Sea. A multi-annual recovery plan for Mediterranean swordfish has been adopted by the ICCAT through the recommendations [13-04] and [16-05], which establish important management measures (Total Allowable Catch, fishing season closures, Minimum landing size) for the conservation status of *X. gladius.* It is listed as Least Concern in the IUCN Red List (Collette & Heessen, 2015).

Knowledge on plastic ingestion: Plastic ingestion in *X. gladius* is reported in the Mediterranean Sea and the occurrence of marine litter in the stomachs is about 12.3% (Anastasopoulou et al., 2013; Romeo et al., 2015).

Use as biological indicator in other projects: *X. gla*dius has not been used as a bioindicator of marine litter and microplastic ingestion in other projects.

Sea turtles

Target species

For ***OPEN WATERS*** at ***BASIN SCALE*** *and* ***MEDIUM-SCALE***

Caretta caretta Linnaeus, 1758

Source: http://www.natgeoimagecollection.com

Common name: Loggerhead turtle

Phylum: Chordata

Class: Reptilia

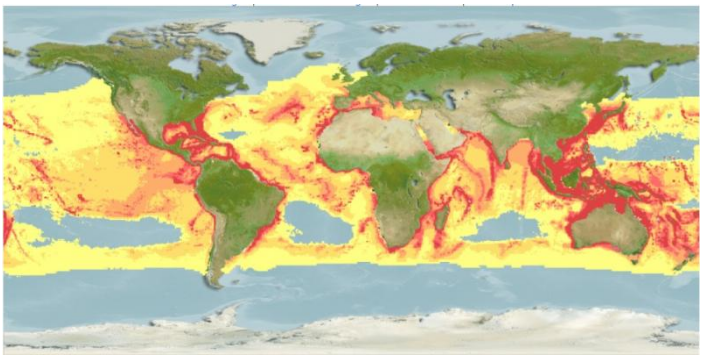
Order: Testudines

Family: CHeloniidae

Geographical distribution: Subtropical and temperate regions of the Mediterranean Sea and the Pacific, Indian, and Atlantic Oceans.

Habitat: *C. caretta* uses different habitats during its life, from oceanic to neritic areas, but in general spend most of their lives in the open ocean and in shallow coastal waters.

Size and Lifespan: The mean straight carapace length (SCL) of the mature *C. caretta* is between 80 to 100 cm, with a mean weight near to 75 kg (70 to 110 kg).



Geographical distribution of *Caretta carettas* (www.aquamaps.org)

Feeding habits: *C. caretta* is omnivorous and its feeding behaviour may change with age. It has a greater list of known prey than any other sea turtle, and its food items include sponges, corals, sea pens, polychaete worms, sea anemones, cephalopods, barnacles, brachiopods, isopods, Portuguese men o' war, insects, bryozoans, sea urchins, sand dollars, sea cucumbers, starfish, fish (eggs, juveniles, and adults), hatchling turtles (including members of its own species), algae, and vascular plants. During migration through the open sea, *C. caretta* eats jellyfish, floating molluscs, floating egg clusters, squid, and flying fish.

Commercial importance: *C. caretta* does not have commercial value, however up until the ’1970s, it was commonly captured in commercial operations and the meat, eggs, leather and fat were used.

Protection: At global level, *C. caretta* is assessed as Vulnerable in the IUCN Red List. However, for the Mediterranean, it is characterised as Least concern (Casale, P. 2015). It is also granted legislative protection under a number of treaties and laws: Annex II of the SPAW Protocol to the Cartagena Convention (a protocol concerning specially protected areas and wildlife, Appendix I of CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) and, Appendices I and II of the Convention on Migratory Species (CMS).

Knowledge on plastic ingestion: Plastic ingestion in *C. caretta* is widely reported in the Mediterranean Sea and the occurrence of marine litter in the stomachs is about 51.8% (Camedda et al. 2014; Campani et al. 2013; Casale et al. 2008; Casale et al. 2016; Gramentz 1988; Kaska et al. 2004; Lazar and Gračan 2011; Matiddi et al. 2018; Revelles et al. 2007; Russo et al. 2003; Tomás et al. 2002.

Use as biological indicator in other projects: *C. caretta* has been used as bioindicator species for macro and micro litter ingestion in the INDICIT and INDICIT II projects. Moreover, the MSFD Task Group on Marine Litter proposed “Litter ingested by sea turtles” as an impact indicator for D10. *C. caretta* is also proposed as the most representative species for the Candidate Indicator 24 concerning litter ingestion for basin-wide monitoring (UNEP/MAP SPA/RAC, 2018).

Secondary species

For ***OPEN WATERS*** at ***BASIN SCALE***

Dermochelys coriacea Vandelli, 1761

Source: http://www.sapere.it

Common name: Leatherback sea turtle

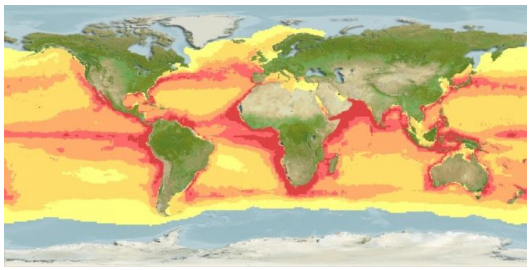
Phylum: Chordata

Class: Reptilia

Order: Testudines

Family: Dermochelydae

Geographical distribution: *D. coriacea* has a cosmopolitan global range. Of all the existing sea turtle species, *D. coriacea* has the widest distribution, reaching as far north as Alaska and Norway, and as far south as Cape Agulhas in Africa and the southernmost tip of New Zealand. *D. coriacea* is found in all tropical and subtropical oceans, and its range extends well into the Arctic Circle.



Geographical distribution of *Dermochelys coriacea* (www.aquamaps.org)

Habitat: *D. coriacea* is an oceanic, deep-diving marine turtle inhabiting tropical, subtropical, and subpolar seas. Until recently, this turtle was considered to be strictly epipelagic, but new observations have shown that it frequently descends into deep waters as it is physiologically well adapted to deep-diving.

Size and Lifespan: *D. coriacea* adults average 1–1.75 m in curved carapace length (CCL), 1.83–2.2 m in total length, and 250 to 700 kg in weight. Females usually produce several (3-10) clutches of 60-90 eggs in a reproductive season, and typically have a re-migration interval of multiple years (2+) between subsequent reproductive seasons.

Feeding habits: *D. coriacea* turtles make extensive migrations between different feeding areas at different seasons, and to and from nesting areas. It is believed that this species is carnivorous throughout its life cycle; adults feed mainly on jellyfish, tunicates and other epipelagic soft-bodied invertebrates that are abundant in the epipelagic region, with highest concentrations in upwelling areas and convergence currents.

Commercial importance: Generally speaking, there are no commercial fisheries for this species, although in some places it is used as bait in longline shark fisheries.

Protection: Globally, *D. coriacea* is assessed as Vulnerable in the IUCN Red List (Wallace et al., 2013). A partial list of international conservation instruments that provide legislative protection for Leatherbacks are: Annex II of the SPAW Protocol to the Cartagena Convention; Appendix I of CITES; Appendices I and II of the CMS; the Inter-American Convention for the Protection and Conservation of Sea Turtles (IAC); the Memorandum of Understanding on the Conservation and Management of Marine Turtles and their Habitats of the Indian Ocean and South-East Asia (IOSEA).

Knowledge on plastic ingestion: Plastic ingestion and the occurrence of marine litter in *D. coriacea* stomachs is reported in the Mediterranean Sea (Poppi et al., 2012; Russo et al. 2003) and worldwide.

Use as biological indicator in other projects: *D. coriacea* has not been used as a bioindicator of marine litter and microplastic ingestion in other projects.

Secondary species

For ***OPEN WATERS*** at ***BASIN SCALE*** *and* ***MEDIUM-SCALE***

Chelonia mydas Linnaeus, 1758

Source: fisheries.noaa.gov

Common name: Green sea turtle

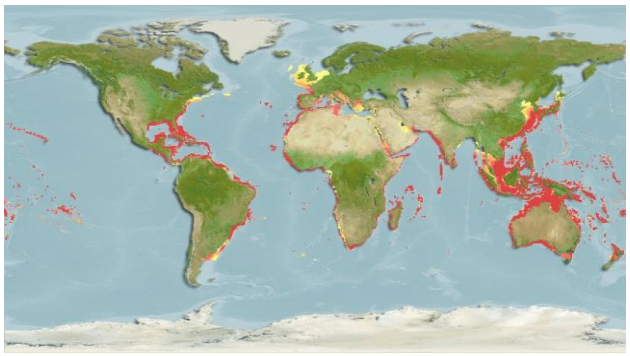
Phylum: Chordata

Class: Reptilia

Order: Testudines

Family: Cheloniidae

Geographical distribution: Widely distributed in tropical and subtropical waters, near continental coasts and around islands; rare in temperate waters. Together with the hawksbill (Eretmochelys), *C. mydas* is the most tropical of the marine turtles. Its normal latitudinal range remains within the northern and southern limits of the 20°C isotherms, and follows the seasonal latitudinal changes of these limits.



Geographical distribution of *Chelonia mydas* (www.aquamaps.org)

Habitat: Like most sea turtles, *C. mydas* is highly migratory and uses a wide range of broadly separated localities and habitats during its lifetime. After a number of years in oceanic zone, these turtles move to neritic developmental areas rich in seagrass and/or marine algae where they forage and grow until maturity.

Size and Lifespan: Adult *C. mydas* grow to 1.5 metres long. The average weight of mature individuals is 68–190 kg and the average carapace length is 78–112 cm. Females usually show nesting site fixity, and they are able to return to lay eggs near the same spot where they left the last clutch or even on the same beach from which they emerged as hatchlings.

Feeding habits: *C. mydas* is a typical solitary nektonic animal that occasionally forms feeding aggregations in shallow water areas with abundant seagrasses or algae. This species migrates from rookeries to feeding grounds, which are sometimes several thousand kilometers away.

Commercial importance: The main commercial fishing gear used to catch *C. mydas* are: entangling nets, drift-nets, harpoons, grapnels, hooks and also "turning nesting females onto their backs". *C. mydas* turtles are often taken as by-catch in shrimp trawls, set-nets, gill-nets and beach seines, and sometimes juveniles are captured with cast-nets.

Protection: Globally, *C. mydas* is assessed as Endangered by the IUCN Red List (Seminoff, 2004). *C. mydas* has been granted legislative protection under a number of treaties and laws, such as Annex II of the SPAW Protocol to the Cartagena Convention; Appendix I of CITES; and Appendices I and II of the Convention on Migratory Species (CMS).

Knowledge on plastic ingestion: Plastic ingestion and the occurrence of marine litter in the *C. mydas* stomachs is reported in the Mediterranean Sea (Russo et al. 2003) and worldwide.

Use as biological indicator in other projects: *C. mydas* has not been used as a bioindicator of microplastic ingestion in other projects.

Seabirds

Secondary species

For ***SEA SURFACE*** *and* ***COASTAL WATERS*** at ***BASIN SCALE***

Calonectris diomedea Scopoli, 1769

Source: www.ebnitalia.it

Common name: Scopoli's shearwater

Phylum: Chordata

Class: Aves

Order: Procellariiformes

Family: Procellariidae

Geographical distribution: *C. diomedea* breeds in Algeria, Croatia, France, Greece, Italy, Malta, Spain (excluding the Canary Islands), Tunisia and Turkey. The majority of the population spends the non-breeding season in the Atlantic, including areas off the west coast of Africa and east coast of Brazil.

Geographical distribution of *Calonectris diomedea* (www.iucnredlist.org)

Habitat: Pelagic movements are easily divided into frequent foraging trips around the breeding areas, rapid long-distance migrations, and smaller-scale movements within a well defined wintering ground. Breeding starts in April on barren offshore islands, where breeding pairs occupy cliffs, caves and boulder fields.

Size and Lifespan: *C. diomedea* is identifiable by its size, at 44–49 cm in length and with a 117–135 cm wingspan; weight is in the range 544–738 g. They have large brownish shearwater with mostly white underparts and rather large pale bill.

Feeding habits: Diet is mostly squid, which are obtained mainly by surface-seizing. It is regularly attracted to trawlers to feed on offal

Commercial importance: Not evaluated

Protection: *C. diomedea* is assessed as Least Concern in the IUCN Red List (BirdLife International 2015) and is listed in the EU Birds Directive Annex I and Bern Convention Appendix II. In most areas, human exploitation of *C. diomedea* has ceased or is only occasional and some breeding islands have been declared reserves.

Knowledge on plastic ingestion: There's only one paper in the Mediterranean Sea dealing with plastic ingestion by *C. diomedea*, reporting a 96% occurrence (Codina-García et al., 2013).

Use as biological indicator in other projects: *C. diomedea* has not been used as a bioindicator of marine litter and microplastic ingestion in other projects.

Secondary species

For ***SEA SURFACE*** *and* ***COASTAL WATERS*** at ***BASIN SCALE***

Puffinus yelkouan Acerbi, 1827

Source: www.pbase.com

Common name: Yelkouan shearwater

Phylum: Chordata

Class: Aves

Order: Procellariiformes

Family: Procellariidae

Geographical distribution: *P. yelkouan* is endemic to the Mediterranean basin, but its precise distribution is not well known and its numbers are disputed. The main breeding colonies are concentrated in the central and eastern basin of the Mediterranean, from Sardinia through the central Mediterranean, the Adriatic and the Aegean. During the non-breeding season some birds migrate north-eastwards towards the Black Sea, although some birds may remain close to breeding colonies or disperse around the Mediterranean Sea.

Geographical distribution of *Puffinus yelkouan* (www.iucnredlist.org)

Habitat: Pelagic movements are easily divided into frequent foraging trips around the breeding areas, rapid long-distance migrations, and smaller-scale movements within a well defined wintering ground.

Size and Lifespan: *P. yelkouan* are 30–38 cm long, with a 76–89 cm wingspan. This bird looks like a flying cross, with its wing held at right angles to the body; its colour changes from very dark brown to white as the dark upperparts and paler undersides get alternately exposed while it travels low over the sea

Feeding habits: *P.yelkouan* feeds on fish and molluscs. It follows fishing ships when offal is being thrown.

Commercial importance: Not Evaluated

Protection: *P. yelkouan* is assessed as Least concern for Europe in the IUCN Red List (BirdLife International 2015) and is also listed in the EU Birds Directive Annex I and Bern Convention Appendix II. *P. yelkouan* is under some threat from the development of holiday resorts near its breeding sites, and also from animals such as rats and cats (e.g., on the Le Levant Island, one of its major breeding location).

Knowledge on plastic ingestion: There's only one paper in the Mediterranean Sea dealing with plastic ingestion by *P. yelkouan*, which reports a71% on occurrence (Codina-García et al., 2013).

Use as biological indicator in other projects: *P. yelkouan* has not been used as a bioindicator of marine litter and microplastic ingestion in other projects.

Marine Mammals

Secondary species

For ***OPEN WATERS*** at ***BASIN SCALE***

Balaenoptera physalus Linnaeus, 1758

Source: http://marinebio.org

Common name: Fin whale

Phylum: Chordata

Class: Mammalia

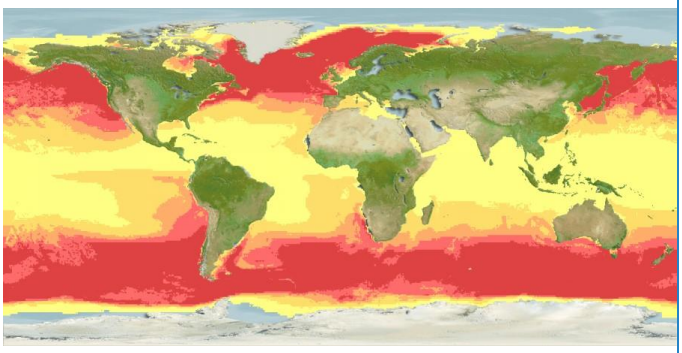
Order: Cetartiodactyla

Family: Balaenopteridae

Geographical distribution: *B. physalus* occurs worldwide, mainly, but not exclusively, in offshore waters of temperate and subpolar zones. *B. phy*salus shows some poleward migration in summer although it appears to be somehow present throughout its range during the whole year.

Habitat: *B. physalus* is a pelagic and coastal species, from shore seaward to the 1,800 m.

Size and Lifespan: Adult *B. phy*salus are about 19 m long for males and 20 m for females, with a maximum of 25 m in males and 27 m in females. It is estimated that a 25-metre whale would weigh about 70,000 kg.



Geographical distribution of *Balaenoptera physalus* (www.aquamaps.org)

Feeding habits: During autumn and winter, there is almost no feeding, at which time whales are found in lower latitudes. The diet varies between areas and seasons. Herring, capelin and other shoaling fish are eaten in both the North Atlantic and North Pacific, along with squid, and euphausiids (krill - shrimp-like crustaceans) and copepods (small crustaceans).

Commercial importance: Following depletion of Blue Whale stocks, whalers shifted their attention to Fin Whales. Populations everywhere were substantially reduced. The International Whaling Commission (IWC) set catch limits at zero for *B. phy*salus in the North Pacific and Southern Hemisphere starting in 1976. The IWC adopted a provision (popularly known as the commercial whaling moratorium) in 1982 to set all catch limits for commercial whaling to zero from 1986, although Iceland, Norway, and the Russian Federation have filed objections or reservations to the provision. Limited hunting of Fin Whales off western Greenland is permitted for "aboriginal subsistence" purposes.

Protection: *B. physalus* is assessed as Vulnerable for the Mediterranean in the IUCN Red List (Panigada & Notarbartolo di Sciara, 2012). *B. physalus* is listed on Appendix I of CITES - but this does not apply to Iceland, Norway, and Japan, who hold reservations – as well as on Appendices I and II of the CMS. Under the Agreement for Conservation of Cetaceans in the Black and Mediterranean Seas, *B. physalus* in the Mediterranean, along with other cetaceans, are protected from deliberate killing by signatories to the agreement.

Knowledge on plastic ingestion: Ingestion has been reported only in few studies; however, for the Mediterranean Fin Whale, it has been estimated that animals could consume more than 3000 microplastic particles per day, along with associated persistent, bioaccumulative, and toxic (PBT) chemicals (Baini et al., 2017; Fossi et al., 2014).

Use as biological indicator in other projects: *B. physalus* has not been used as a bioindicator of marine litter and microplastic ingestion in other projects.

Secondary species

For ***OPEN WATERS*** at ***BASIN SCALE***

Physeter macrocephalus Linnaeus, 1758

Source: http://www.acsonline.org

Common name: Sperm whale

Phylum: Chordata

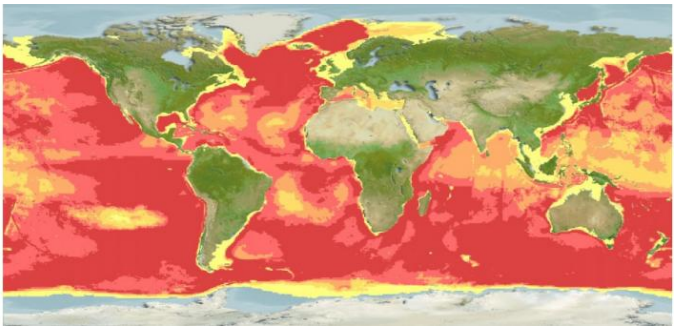
Class: Mammalia

Order: Cetartiodactyla

Family: Physeteride

Geographical distribution: It is distributed from the tropics to the pack-ice edges in both hemispheres, although generally only large males venture to the extreme northern and southern portions of the range. Females and young are usually restricted to waters at latitudes lower than about 40-50º and to areas where sea surface temperatures are higher than about 15ºC.

Habitat: *P. macrocephalus* can be found in almost all marine waters deeper than 1,000 m that are not covered by ice. In some areas, particularly in the western North Atlantic, sperm whales, especially males, can occur in shallower waters



Geographical distribution of *Physeter macrocephalus* (www.aquamaps.org)

Size and Lifespan: Newborn *P. macrocephalus* are 3.5 to 4.5 m long. Adult females are up to 12 m and adult males are up to 18 m in length. Weights of up to 57 t have been recorded.

Feeding habits: *P. macrocephalus* usually dives between 300 to 800 metres, and sometimes 1 to 2 kilometres, in search of food. Such dives can last more than an hour. They feed on several species, notably the giant squid, but also the colossal squid, octopuses, and fish such as demersal rays, although their diet consists primarily of medium-sized squid. Some prey may be taken accidentally while eating other items.

Commercial importance: The commercial value of *P. macrocephalus* (a function of its size and the quality of Sperm Whale oil) drove two massive worldwide hunts: the technologically primitive “open-boat” hunt from 1712-~1920, and modern whaling using engine-driven whaling ships and harpoon guns from ~1910-1988.

Protection: *P. macrocephalus* is globally assessed as Vulnerable but the Mediterranean subpopulation is assessed as Endangered in the IUCN Red List (Notarbartolo di Sciara et al., 2012). The species is also listed on Appendix I of CITES and Appendices I and II of CMS.

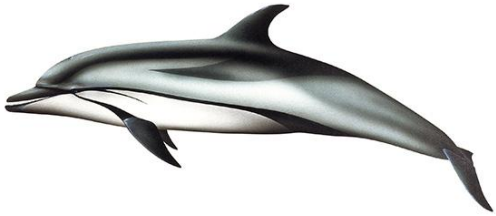
Knowledge on plastic ingestion: Plastic ingestion and the occurrence of marine litter in *P. macrocephalus* stomachs are reported in the Mediterranean Sea (de Stephanis et al. 2013; Katsanevakis et al. 2008; Mazzariol et al., 2011; Roberts et al., 2003; Viale et al., 1992) and worldwide.

Use as biological indicator in other projects: *P. macrocephalus* has not been used as a bioindicator of marine litter and microplastics ingestion in other projects.

Secondary species

For ***OPEN WATERS*** at ***MEDIUM-SCALE***

Stenella coeruleoalba Meyen, 1833



Source: sanctuaire-pelagos.org

Common name: Striped dolphin

Phylum: Chordata

Class: Mammalia

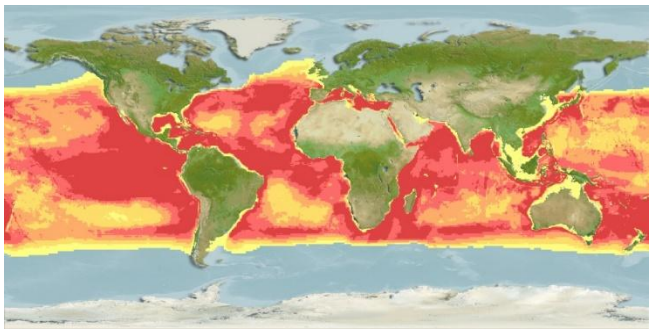
Order: Cetartiodactyla

Family: Delphinidae

Geographical distribution: *S. coeruleoalba*, are found in warm-temperate and tropical seas throughout the world including: the Mediterranean Sea, Pacific Ocean, Atlantic Ocean, Indian Ocean, Caribbean Sea, and in the northern Gulf of Mexico.

Habitat: *S. coeruleoalba* feed in pelagic to benthopelagic zones, to depths as deep as 200-700 m, in continental slope or oceanic regions.

Size and Lifespan: Adult striped dolphins are up to 2.6 m long; males are slightly larger than females. Maximum weight is about 156 kg. Newborns are about 1 m in length.



Geographical distribution of *Stenella coeruleoalba* (www.aquamaps.org)

Feeding habits: Adult *S. coeruleoalba* eats fish, squid, octopus, krill, and other crustaceans. Mediterranean striped dolphins seem to prey primarily on cephalopods and lanternfish (50-100% of stomach contents), while northeastern Atlantic striped dolphins most often prey on fish, frequently cod. They mainly feed on cephalopods, crustaceans, and bony fishes. They feed anywhere within the water column where prey is concentrated, and they can dive to depths of 700 m to hunt deeper-dwelling species.

Commercial importance: *S. coeruleoalba* are trapped in tuna purse seines fisheries in the eastern tropical Pacific, although in much smaller numbers than other dolphins. *S. coeruleoalba* is the major target of a large drive in nets fishery off Japan, where several thousand are caught each year. There appears to be some direct capture of *S. coeruleoalba* in northeast Atlantic and the Mediterranean Sea. The current ban on driftnet fishing in the Mediterranean should be implemented and enforced as a matter of priority.

Protection: *S. coeruleoalba* is globaly assessed as Least Concern but the Mediterranean subpopulation is assessed as Vulnerable in the IUCN Red List (Aguilar & Gaspari, 2012). The species is listed in Appendix II of CITES. Eastern tropical Pacific and Mediterranean populations of *S. coeruleoalba* are listed on Appendix II of the CMS, since they have an unfavorable conservation status or would benefit significantly from international co-operation organized by tailored agreements.

Knowledge on plastic ingestion: Marine litter ingestion in *S. coeruleoalb* is reported in the Mediterranean Sea and worldwide (Arbelo et al., 2013; Fernández et al., 2009; Hernandez-Milian 2014; Lusher et al., 2018; Walker and Coe, 1990).

Use as biological indicator in other projects: *S. coeruleoalba* has not been used as a bioindicator of marine litter and microplastic ingestion in other projects.

Secondary species

For ***OPEN WATERS*** at ***MEDIUM-SCALE***

Ziphius cavirostris Cuvier, 1823

Source: sanctuaire-pelagos.org

Common name: Cuvier's beaked whale

Phylum: Chordata

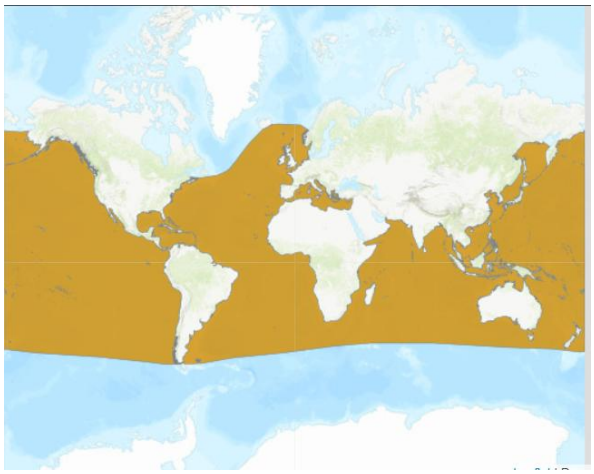
Class: Mammalia

Order: Cetartiodactyla

Family: Ziphiidae

Geographical distribution: *Z. cavirostris* is widely distributed in offshore waters of all oceans, from the tropics to the polar regions. They may have the most extensive range of any beaked whale species, and are fairly common in certain areas, such as the eastern tropical Pacific.

Habitat: Although *Z. cavirostris* can be found nearly anywhere in deep (>200 m) waters, they seem to prefer waters near the continental slope, especially those with a steep sea bottom. It is rarely found close to mainland shores, except in submarine canyons or in areas where the continental shelf is narrow and coastal waters are deep; it is mostly a pelagic species that appears to be confined by the 10°C isotherm and the 1,000 m bathymetric contour.



Geographical distribution of *Ziphius cavirostris* (www.iucnredlist.org)

Size and Lifespan: Length at birth is about 2.7 m; adults reach 7.5 m (males) and 7 m (females). Maximum recorded weight is nearly 3 000 kg.

Feeding habits: *Z. cavirostris*, like all beaked whales, appears to prefer deep waters for feeding. Dives of up to 40 minutes have been documented. Although few stomach contents have been examined, they appear to feed mostly on deep-sea squid, but sometimes also on fish and some crustaceans (MacLeod et al. 2003). They apparently feed both near the bottom and in the water column.

Commercial importance: There have been no major fisheries for this species, although small numbers have been taken deliberately in Japan, the Lesser Antilles, and incidentally elsewhere.

Protection: *Z. cavirostris* is globaly assessed as Least Concern but the Mediterranean subpopulation is assessed as Data Deficent in the IUCN Red List (Cañadas, 2012). The species is listed in Appendix II of CITES. In 2004, the Parties to the UNEP CMS Agreement on the Conservation of Cetaceans of the Black Sea, Mediterranean Sea and Contiguous Atlantic Area (ACCOBAMS) adopted a resolution recommending that human activities introducing high-intensity noise in the marine environment be avoided in the agreement area where high concentrations of *Z. cavirostris* may occur.

Knowledge on plastic ingestion: Two stranded animals in Greece had stomachs full of pieces of plastic bags, as did a stranded animal in Croatia. Poncelet in 1999 described a considerable amount of plastic debris in the stomach of a *Z. cavirostris* washed ashore on the French Atlantic coast.

Use as biological indicator in other projects: *Z. cavirostris* has not been used as a bioindicator of marine litter and microplastic ingestion in other projects.