MEDSEALITTER

Developing Mediterranean-specific protocols to protect biodiversity from litter impact at basin and local MPAs scales

Priority axis - Investment Priority-Specific Objective 3-2-1
Priority Axis 3: Protecting and promoting Mediterranean natural and cultural resources PI 6d
3.2: To maintain biodiversity and natural ecosystems through strengthening the management and networking of protected Areas

DELIVERABLE 4.6.1

Common monitoring protocol for marine litter

WP4 – TESTING

Activity 4.6: Delivering efficient, easy to apply and cost-effective protocols to monitor and manage litter impact on biodiversity

Partner in charge: University of Barcelona (SPAIN)
Contributing partners: all

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Compiled by (Authors and MEDSEALITTER Partners listed in alphabetical order):

Alex Aguilar[9]; Konstantina Andreanidou[8]; Antonella Arcangeli[6]; Fabrizio Atzori[1]; Joanne Befort[3*]; Asunción Borrell[9]; Ilaria Caliani[2]; Ilaria Campana[7]; Luis Cardona[9]; Lara Carosso[1]; Serena Carpinteri[7]; Cathy Cesarini[3**]; Roberto Crosti[6]; Gaëlle Darmon[3]; Léa David[4]; Nikoletta Digka[5]; Nathalie Di-Méglio[4]; Stefania di Vito[7]; Natalia Fraija Fernández[10]; Francesca Frau[1]; Delphine Gambaiani; Odei Garcia-Garin[9]; Patricia Gozalbes Aparicio[10]; Heleni Kaberi[5]; Agustin Lobo[9*]; Jeremy Mansui[3]; Jessica Martin[3]; Marco Matiddi[6]; Claude Miaud[3]; Marie-Aurélia Sabatte[3]; Jacques Sacchi[3**]; Jean-Baptiste Senegas[3***]; Jaime Penadés Suay[10]; Ana Pérez del Olmo[10]; Matteo Perrone[2]; Vicky Rae[8]; Juan Antonio Raga Esteve[10]; Francesco Rende[6]; Ohiana Revuelta Avin[10]; Marine Roul[4]; Jesús Tomás Aguirre[10]; Paolo Tomassetti[6]; Catherine Tsangaris[5]; Claudio Valerani[2]; Morgana Vighi[9]; Fotini Vrettou[8]; Paprapath Wongdontree[3].

[1] Capo Carbonara MPA Comune di Villasimius – ITALY.

[2] Cinque Terre National Park and Marine Protected Area – ITALY.

[3] École Pratique des Hautes Études (EPHE) – FRANCE ([3]*LDV39, [3]**RTMMF ; [3]***CESTMED).

[4] EcoOcéan Institut – FRANCE.

[5] Hellenic Centre for Marine Research (HCMR) – GREECE.

[6] Italian National Institute for Environmental Protection and Research (ISPRA) – ITALY.

[7] Legambiente ONLUS – ITALY.

[8] MEDASSET – GREECE.

[9] University of Barcelona – SPAIN ([9]*CSIC, consejo superior de investigaciones científicas)

[10] University of Valencia – SPAIN.

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1. INTRODUCTION

Reduction of marine litter is globally acknowledged as a major community challenge of our times due to its significant environmental, economic, social, political and cultural implications (Cheshire et al. 2009; Galgani et al. 2010). Marine litter is one of the main causes for sea pollution and it is dominated by plastics (Coe & Rogers 1997; Barnes et al. 2009; UNEP 2015).

First measures to tackle marine pollution were taken by the OSPAR 72/74 convention and the International Convention for the Prevention of Pollution from Ships (MARPOL 73/78), which became the main policy drivers of coastal and offshore waters monitoring. More recently, new EU directives specifically targeted the reduction of waste and asked monitoring programs to assess the progress of these measures: the Waste Directive (2008/98/EC), the Packaging Directive (94/62/EC) and the Plastic Carrier Bags Directive (2015/720/UE amending 94/62/EC) ask Member States to reduce the annual average production of waste and consumption of plastic bags. The reduction of impacts of certain plastic products on the environment was also the aim of the Single Use Plastic Directive (SUP) recently voted by the European Commission (2018/0172/EC) and of the Directive on Port reception facilities for the delivery of waste from ships. Other European directives, introducing the ecosystem-based approach, have been largely integrated in the existing measures and enforced into State legislation. These directives, such as the Water Framework Directive (WFD, EU 2000) and the UNEP/MAP Regional Plan for Marine litter Management in the Mediterranean (UNEP/MAP IG.21/9), highlight that policy drivers may change over time but similar overall purposes are maintained. In 2008, the European Commission adopted the Marine Strategy Framework Directive (2008/56/EC), whose objective is to achieve the Good Environmental Status (GES) by 2020, based on 11 qualitative Descriptors. Marine litter is the Descriptor 10 and, according to the Directive, GES is reached when the "properties and quantities of marine litter do not cause harm to the coastal and marine environment" (2008/56/EC; Galgani et al. 2010).

Notwithstanding the legislative requirement, the lack of comparable data across all seas still poses a major obstacle for a European marine assessment. Effective measures to tackle marine litter are seriously hampered by the insufficient scientific data (Ryan 2013) and the need for more accurate and coherent monitoring on marine litter is evident in order to set priorities for cost-effective marine protection actions and to monitor the effectiveness of measures (Sheavly 2007; Cheshire et al. 2009; Galgani et al. 2013a; UNEP 2015).

1.1 The Mediterranean context

The Mediterranean Sea is considered one of the seas most affected by marine litter worldwide, but information is still limited, inconsistent and fragmented (Barnes et al. 2009; Jambeck et al. 2015). The Mediterranean Sea was designated as a Special Area under MARPOL Annex V, which prohibited the disposal of garbage at sea and leaded to the establishment of adequate port reception facilities for garbage: nevertheless, the efficiency of the shoreside management of waste often remains in doubt. A pilot survey organised in 1988 by UNEP/MAP and successive assessments showed that the main sources of coastal litter in the basin are river runoff, tourist activities and coastal urban centres (MAP/UNEP, 2001; UNEP 2015). Additionally, at-sea activities such as shipping and fishing can heavily contribute to the inputs of litter in specific contexts (Coe & Rogers 1997; Carić & Mackelworth 2014).

Floating macro litter (FML) is considered a pertinent indicator of the pressure of marine litter in the marine ecosystem: it is completely included in the marine compartment, it is a "timeliness" indicator being the first portion of litter entering the sea (only successively, litter sinks to the sea bottom, is washed ashore, or breaks up into smaller particles), and can give indications on the main sources, sinks and pathways, and the effects of waste prevention measures (Thiel et al. 2003). Since marine litter is responsible for direct harm to marine species, its monitoring can also help to identify risky areas and seasons and design appropriate mitigation measures (*e.g.* Arcangeli et al. 2018; Di-Méglio & Campana 2017). At Mediterranean level,

both the up to date documents of the MSFD and the Barcelona Convention UNEP-MAP highlight the primary need for the assessment of litter pressure even in the surface layer compartment (Table 1).

Table 1 MSFD and UNEP-MAP requirements on floating litter

COMMISSION DIRECTIVE (EU) 2017/845 of 17 May 2017.	**Primary Criteria** Pressure: **D10C1** and D10C2 relate to the level of the pressure (litter and micro-litter) in the marine environment (coastline, **surface layer of the water column**, sea-floor and sea-floor sediment, as appropriate).
Integrated Monitoring and Assessment Programme of the Mediterranean Sea and Coast and Related Assessment Criteria UN Environment/MAP Athens, Greece (2017).	UN Environment/MAP will develop a specific Monitoring of floating litter protocol, on a regional basis. Common indicator (17): Floating litter (items/km^2). Min value = 0; Mx value = 195; mean value 3.9; Baseline 3-5.

The Mediterranean Sea lacked a commonly agreed species to be used as bio-indicator for the impact of biota of litter ingestion until 2011. In 2011, DG ENV asked for a further development of the indicator, and the Loggerhead turtle (*Caretta caretta* Linnaeus, 1758) was chosen as possible indicator for EU Mediterranean countries (Galgani et al. 2013a; Matiddi et al. 2017).

Further and better data are needed to develop a marine protection framework in the Mediterranean Sea that addresses marine litter effectively, thus ensuring the sustainable management and use of the marine and costal environment at a basin-scale (Cheshire et al. 2009; Galgani et al. 2013a; UNEP 2015).

1.2 Monitoring

Monitoring is intended to detect changes over time and should provide data representative of the location and time of sampling. Long-term monitoring programmes provide valuable data sets which are highly relevant to present-day policy drivers, in particular in response to MSFD requirements (Galgani et al. 2013a; Zampoukas et al. 2014). Monitoring programmes should be consistent, coherent and comparable within marine regions. The choice of the most effective methodologies (with regard to their cost-benefit, and use of the most appropriate indicator) and their implementation/adaptation to the different ongoing projects are important elements to consider in monitoring plans. The application of well-documented procedures, experienced analysts, as well as intercalibration of methodologies, will assure the production of high quality and consistent data (Zampoukas et al. 2014).

1.3 Marine Protected Areas (MPAs): Monitoring as key for a good management and governance at local scale

Marine and coastal ecosystems are highly productive and they can deliver various beneficial services that could support communities and economy. The global decline registered on the marine and terrestrial ecosystem conservation status and their productivity is mainly caused by anthropic pressures and increased environmental pollution. To mitigate the effects and build resilience to these threats, the solution is to create protected zones, such as Marine Protected Areas (MPAs), National or Regional Parks, with the implementation of effective management on local scale and, when is possible, on large scale working in a synoptic way. Protected areas maintain the full range of genetic variation, essential in securing survival of key species populations, sustaining evolutionary processes and ensuring resilience in the face of natural disturbances and human use. In this way, the ecosystem health and productivity are maintained while allowing for social and economically sustainable development. (IUCN 1999; NRC 2001; Agardy & Staub 2006; Parks et al. 2006; IUCN-WCPA 2008). Many protected areas have been established primarily to reduce the loss of biodiversity, focusing especially on vulnerable ecosystems and critical habitats, as well as on the protection of endangered species and species of economic importance.

If correctly designed and effectively managed, MPAs have an important role to protect the ecosystems (IUCN-WCPA 2008). The MPA management effectiveness is the degree to which management actions achieve the stated goals and objectives (Hockings et al. 2000, 2006). The process of evaluating management effectiveness incorporates an examination of different biological, natural, socioeconomic and governance factors that affect the management of the area. In this context, research and monitoring represent concrete actions crucial for the territory management: research contributes to understand the functioning of a system, monitoring allows the repeated observation of phenomena over time. It's important to define the state of well-being of ecosystems by key-species monitoring or through the assessment of environmental impacts such as that of marine litter pollution. In this way, the "Common monitoring protocol for ML" would allow to obtain the information about marine litter impacts useful for the management of an area. Data collection provides information on abundance, material, type of items and, therefore, on the possible sources, in addition to identify hotspots and temporal patterns. This information can be used to focus the attention on mitigating measures and to test the effectiveness of existing local and Mediterranean legislations and regulations. Starting from the specific information collected on marine litter origin and its major sources, it is possible to implement targeted practical actions creating specific programmes of environmental education and awareness-raising involving citizens, local stakeholders (i.e. fishermen), tourists, etc. Through the local stakeholders and community members involvement, in addition to obtaining the public support, it would also be possible to achieve the ultimate aim to reduce the amount of litter entering the marine environment directly targeting the source.

1.4 Scope of the document

This document intends to describe and provide practical guidelines on the application of techniques for monitoring FML and litter ingested in biota, considering in detail the parameters and covariates that can bias the results. Due to the widespread nature of marine litter within the Mediterranean, the proposed protocols describe the most effective methodologies for two spatial scales: the large offshore areas and the local coastal fringe. Moreover, as the extreme variation in shape and size of marine litter also demands a multiscale approach, protocols focus both on macro and micro litter monitoring.

Giving the similarity of techniques involved, the document is organized in two sections dedicated to methods for floating macro litter monitoring (**monitoring FML at large and local MPAs scales,** chapters 2 and 3) and for the analysis of litter ingested by indicators animal species (**monitoring macro and micro litter ingested at large and local MPAs scales,** chapter 5). Both methods are then explored considering the specific methodologies to be implemented for each platform type and/or technique (for FML) and indicator species (for ingested litter).

2. MONITORING FML AT LARGE AND LOCAL MPAs SCALES

2.1 Scope of FML monitoring (for local and large geographical scale)

Following the legislative requirements, monitoring programmes should collect information on: 1) amount, distribution and composition of litter; 2) rates at which litter enters the environment (and sources); 3) spatial and temporal variations; 4) impacts of litter.

Monitoring protocols need to adapt to the information required, *i.e*. the goal of monitoring. FML monitoring is indeed functional to:

- Evaluate trends;
- Identify accumulation areas (both seasonal and regional);
- Identify pathways and geographical sources;
- Assess changes due to mitigation measures (long-term monitoring);
- Provide information to evaluate risks and focus research and mitigation actions on specifically sensitive areas for marine biodiversity.

Effective monitoring of litter floating at sea requires a huge sample sizes to overcome the spatial heterogeneity in litter distribution (Ryan et al. 2009). For this reason, the proposed methodologies consider the cost effectiveness, efficiency and long-term sustainability of methods, also in relation to their scale of applicability.

2.2 Variables to collect and covariates influencing detectability of litter items

For an effective FML monitoring, the variables to be collected include: number of items, size class, composition/type and geographical position (Table 2). Apart from environmental parameters related with the geographical position (*i.e*. winds, currents, proximity to land), many parameters (covariates) may also influence the detectability and the identification of items and must be taken into consideration (Table 2).

Table 2. Variables and covariates influencing detectability and identification of items

Variables	**Covariates (observation parameters that could influence the sighting probability)**
Number of items	a. Sampling design and period
Size class	b. Type of platform (height and speed)
Composition/type	c. Technique (visual observation/automatic photography)
Geographical position	d. Experience of the observers
	e. Weather and visibility conditions (Beaufort, wind direction, visibility, sun glare, etc.)
	f. Strip width
	g. Size of items: lower size limit, classes
	h. Type and colour of items

a. Sampling design and period

The combination of multiple diffuse and point-source inputs and variable transportation of debris by winds and currents results in a great temporal and spatial variability in litter loads in the sea compartments. Such variability requires a well-defined sampling design with sufficiently large replication in space and time to intercept these changes. Large-scale monitoring programs, which collect information about bio-geographic regions, are usually designed to determine changes occurring at ecosystem and population level. Small-scale monitoring programs, on the contrary, provide in-depth information at specific sites and are useful for

local management. A combination of both scales would provide the information required to assess marine litter impacts in the whole Mediterranean basin, and thus the basis for management. To avoid biases in data collection, surveys must be designed considering: a) sampling stratification; b) the minimum representative sampling area, c) the minimum area to be sampled seasonally to minimize error. Pilot studies are required to identify the range of litter densities in the area and can be used to estimate variability in sample data. Power analysis would then aid to assess the most effective sample size necessary to detect a change (Ryan et al. 2009). Based on the pilot study results, the sample size needed to attain a specified level of precision can be calculated using, for example, the Burnham equation (Burnham et al. 1981).

- **Site selection**. Monitoring programmes should be consistent, coherent and comparable within marine regions and surveys. Giving the high heterogeneity of litter distribution, the criteria for the survey site selection could have crucial effect on results (UNEP/MAP 2016). Sampling should be stratified in relation to sources (urban, riverine outputs, offshore activities) to provide representative data in each location (Cheshire et al. 2009; Zampoukas et al. 2014) or it should cross areas of expected low/high litter density to cover wide range of conditions (Galgani et al. 2013a). Giving the differences in the mean amount of litter, the main drivers of litter presence and distribution and the geographical scale involved, it is suggested to stratify surveys and methodologies at least for coastal and high sea areas.
- **Temporal stratification**. Seasonality can play a key role in driving the variability of the amount and distribution of litter, which is linked to seasonal variation in oceanographic and anthropogenic factors (Arcangeli et al. 2017). Thus, stratification of surveys for the different seasons is required.
- **Frequency of sampling**. A minimum sampling frequency of one per year is required, although seasonal replication is recommended (Cheshire et al. 2009; Galgani et al. 2013a). A frequency of at least 5 surveys per season can be considered adequate to perform seasonal analysis within one year of monitoring; less surveys per season can be sufficient if more years are pooled. Within each site, at least 20 sampling units should be randomly allocated, but given the heterogeneity in the amounts of marine litter, this number might be adjusted.
- **Sample unit.** Surveys are usually based on transects, considered as sampling units to perform temporal analysis (*e.g.* trends) and including information on gradients such as distance from the coast (or from main sources of litter). The minimal length of each transect per survey must be set to avoid biases due to small sample size. To perform spatial analysis, a grid cell can be overlaid to the effort: in this case, the single cell is used as statistical unit. A minimum sampling effort per cell is also required in order to avoid outliers due to uneven effort.

b. Type of platform (height and speed)

Different platforms of observation can be used for FML monitoring: they can be categorized mainly according to their height and speed, the main factors affecting visibility and thus the detection probability of litter (especially to what regards the minimum detectable size of litter and the effective strip width):

Vessel-based surveys. Direct observations of macro-litter from vessels have been conducted worldwide since the 1980's. Small (such as dinghies), or medium size (sailing or motor) vessels can cover coastal waters, usually travelling at low speed and allowing the detection of items larger than 2.5 cm (*e.g.* Day & Shaw 1987; Thiel et al. 2003; Di-Méglio & Campana 2017). The increase of observation height and vessel speed corresponds to a loss of ability to detect small size items. Larger vessels, such as ferries, allow to survey large open sea areas, providing data limited to larger size classes (>20 cm). The use of platforms of opportunity can further enhance the survey effort, investigating high sea areas in a cost-effective way, and supporting more regular observations (Cheshire et al. 2009).

Aerial surveys. Large scale monitoring programmes have been developed through aircraft surveys to estimate the amounts of litter at sea, and locate areas of higher aggregations of litter (Lecke-Mitchell &

Mullin 1992; Pichel et al. 2007; Unger et al. 2014). Aircraft surveys allow to cover large areas but detecting only larger classes of items (*i.e.* the smallest size limit for aerial detection is *ca*. 30–40cm). Aerial surveys are considered valuable for detecting spatial differences in abundance, but the high costs of these surveys prevent from a large replication for monitoring changes over time (Galgani et al. 2013a; Ryan et al. 2009). Unmanned Aerial Vehicles such as fixed wing or multirotor drones, or other remotely controlled devices, can be used to monitor the presence of marine litter at different special scales in the sea. These devices have seen a rapid development in recent years, especially with regard to marine mammal and other marine fauna monitoring (*e.g.* Koski et al. 2009; Hodgson et al. 2013; Adame et al. 2017).

c. Technique (visual observation/automatic photography)

FML monitoring can be carried out through visual observations or remote sensing techniques:

- **Visual observation** of floating items is the most common methodology used and relies on competent, dedicated observers. Direct observations need less resource, but are fraught with other potential biases linked to differences in litter detectability due to observation conditions and platform types. The protocols here described intend to set the conditions that would guarantee consistency in the data collected
- **Automatic recording** of floating litter has been used in more recent applications and is made possible by recording systems specifically set to acquire images from ships, aircrafts or drones, travelling along defined routes (*e.g.* SeaLitterCAM, Hanke & Piha 2011; Galgani et al. 2013b). Apart from the 'traditional' RGB cameras, thermic and multi-spectral cameras are also being experimented for automated marine monitoring (Bryson & Williams 2015). The recognition analysis is performed on the video/images acquired and various algorithms for automated image analysis and object detection are being developed (*e.g.* Maire et al. 2013). Advantages of automatic recording include the reduction of human error and risk, and the permanent record of images allowing subsequent analyses (Bryson & Williams 2015). The main biases of this technique are linked to weather conditions (effect of sun glare on the images) and the post-processing recognition analyses.

d. Experience of observers

Experience of observers can influence item detection and identification, leading to incoherent results: Giving the number of items to be recorded and the vast category types, only dedicated, experienced and well dedicated observers must be used during the monitoring.

e. Weather conditions

Weather can affect the visibility and thus the detectability of litter in a number of ways. Floating litter may be less visible with increasing winds and breaking waves, thus a limit of Beaufort force equal or lower than 2 is set for all platforms. Moreover, the sun glare effect should be avoided or limited.

f. Strip width

Two methods can be applied:

- Fixed-width transect methods assume that all debris is detected within a pre-defined distance from the observer, considering a conservative strip width based on preliminary measures; these methods are applied for density estimations (*e.g.* Thiel et al. 2003; Hinojosa & Thiel 2009; Topcu et al. 2010).
- Distance sampling methods assume that the perpendicular distance to each item has to be estimated to compensate for the decreasing detection rate with the increasing distance from the observer. Separate detection curves should be estimated for different sea states. Distance sampling is applied for density estimation (Buckland et al. 1993; *e.g.* Ryan 2013; Suaria & Aliani 2014).

The main constraints of both methods are related with the accurate definition of the strip width and of the distance between the objects and the observers, measures that can be obtained with simple tools, as an inclinometer or range finder (Ryan 2013). With fixed-width transects, however, the complexity of measuring is limited only to two fixed distances (the inner and outer edge of the strip) during the whole survey. Results obtained from the concurrent application of the two methods were compared by Suaria et al. (personal communication) and, even if not completely equivalent, were very similar. Given the fact that strip transect is easy-to-use, less time consuming in terms of data analysis, and is likely to provide more realistic estimates, especially for the smallest size fractions, the protocols here described are based on the fixed-width strip transect approach.

g. Size of litter (lower size limit; classes)

Litter is broadly categorized into macro-litter ($x \geq 2.5$ cm), meso-litter (5 mm $\leq x < 2.5$ cm) and micro-litter (< 5 mm). For FML, the smallest size of items that may be recorded depends mostly on the observation platform (height, speed).

- ***Lower size limit***: the minimum size of detectable litter depends on the type of platform used and in particular on its speed and on the height of the observer. The lower size limit should be defined for each platform type.
- ***Classes***: following MSFD guidelines, during monitoring, macro-litter will be categorized into 7 classes:
  - (A: <2.5)
  - B: $2.5 \leq x < 5$ cm;
  - C: $5 \leq x < 10$ cm;
  - D: $10 \leq x < 20$ cm;
  - E: $20 \leq x < 30$ cm;
  - F: $30 \leq x < 50$ cm;
  - G: $50 \leq x < 100$ cm;
  - H: ≥ 100 cm.

h. Type and colour of objects

The MSFD technical subgroup on marine litter (TSG ML) "Guidance on Monitoring of Marine Litter in European Sea" (Galgani et al. 2013a) agreed on a masterlist of litter categories, which reviewed the original OSPAR and UNEP categories (Cheshire et al. 2009) and indicated type and colour categories for FML. This masterlist is currently under review by the EU Joint Research Center (JRC) to produce a joint common list available for monitoring marine litter across the different marine compartments (*e.g.* beach litter, FML). The use of its most recent update (available as to March 2019) is proposed for all the protocols here described (see ANNEX I for the complete list).

2.3 Basic data analysis

The ultimate goal of monitoring is the quantification of marine litter. The formula internationally used (Thiel et al. 2003) calculates the density **D** of marine litter as follows:

$$\mathbf{D = n/(w \times L)}$$

Where**: n** is the number of items observed, **w** the width of the strip (km), and **L** the length of the strip (km). Total density, and density per litter type should be calculated. Geographic Information Systems (GIS), can be used to determine the relative abundances (%) of litter on a spatial basis.

2.4 Synoptic monitoring of marine fauna

To identify risk areas and seasons for marine biodiversity, synoptic monitoring of marine fauna is recommended. Data on marine fauna can be collected by the marine litter observer within the same

monitored strip for marine litter (*e.g.* jellyfish, ocean sunfish, sea turtle sightings) or by dedicated observers monitoring macro and mega marine fauna (*e.g.* cetaceans, sharks). See chapter 4 for details and a list of potential target species.

3. SURVEY METHODS PER OBSERVATION PLATFORM/TECHNIQUE

3.1 FERRIES – LARGE VESSELS

Introduction and scope of the protocol

Large vessels, including commercial ferries, cargos and other types of large ships are especially suitable to monitor FML in offshore/large high sea areas, covering with an adequate sample size the large oceanic processes driving the distribution of floating macro litter. The height of the vessel above the sea allows monitoring a wider strip width, but the minimum size of item that can be detected is set at 20 cm.

Through the application of this protocol it is possible to determine density and characteristics of FML and its trends in large open sea areas.

Covariates

a. Sampling design and period:

A pilot study is required in order to identify the range of values of litter density in the area to be monitored. Based on the pilot study results, the sample size needed to attain a specified level of precision can be calculated (*e.g.* using the Burnham equation; Burnham et al. 1981). In general, for high sea surveys, the following indications should be considered.

Spatial stratification. It is suggested to stratify surveys and methodologies at least for the coastal and the high sea areas. In high sea areas, transects must be designed in order to be representative of the situation at least at the mesoscale level, crossing expected high/low density areas and the main stream regimes.

Temporal stratification. A seasonal stratification of surveys is also required. A frequency of at least 5 surveys per season is required in order to perform seasonal analyses within one year of monitoring.

Sampling effort required per season in high sea areas. For monitoring high sea areas with large vessels (*i.e.* ferries), 25 km^2 is the adequate sample size for almost all the subregions of the Mediterranean basins and all seasons, except for areas of very low density: in these areas, in general during Winter and Autumn, the minimum sampling area needs to be increased up to 31-40 km^2. For example, with a 50 m strip, 15 h effort at 18-26 speed knots would allow to monitor an adequate sample size for almost each season and area (see Fig. 1 and Table 3 for the minimum seasonal/survey effort required according to speed).

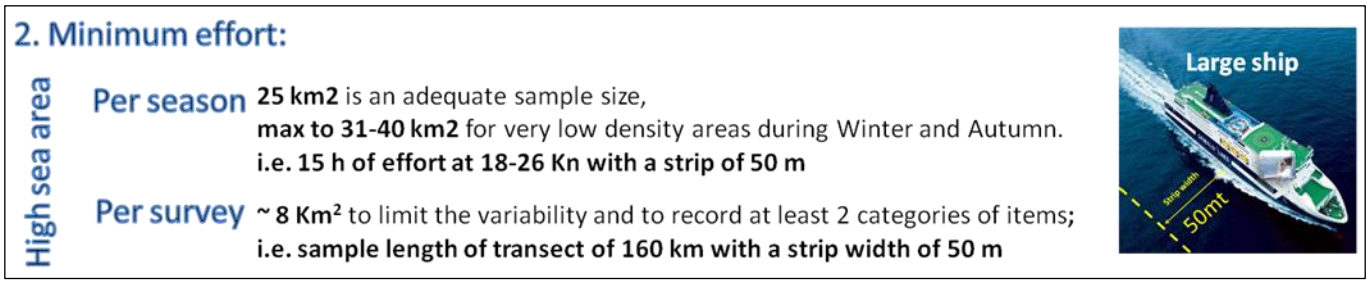


Fig. 1. Summary of indications for the optimal effort for large vessel surveys in high sea areas.

Table 3. Surface to be covered per season (lines above) and survey (lines below) according to speed

Surface to be covered per season (km²)

Type of vessel*	Speed (knots)	Strip and observer	Strip width (m)	Surface to be covered (km²)	Transect length (km)	Transect length (NM)	Nb of hours
ferry	18	1 observer, 1 strip of 50 m (side or front)	50	**25**	500	270	15

* Excel spreadsheets are available to calculate these parameters according to the specific speed and configuration of strip width, see Chapter 3.2 for examples.

ferry	26	1 observer, 1 strip of 50 m (side or front)	50	**25**	500	270	10
Surface to be covered per survey (km²)							
ferry	18	1 observer, 1 strip of 50 m (side or front)	50	**8**	160	86	5
ferry	26	1 observer, 1 strip of 50 m (side or front)	50	**8**	160	86	3

b. Type of platform (height and speed):

Large ships as ferries, cargos, oceanographic vessels, etc. are suitable to perform surveys in high sea areas. The speed of the vessel should not exceed 27 knots for an observation height about 12/25 m. It is important, however, to consider the frequency of occurrence of marine litter items within the strip: in low density areas, speed does not affect the survey if there is time to identify and record items crossed by. The speed range that would avoid items to be lost must be considered. In low density areas, an experienced observer can work up to a speed of 27 knots (so far over the maximum speed reached during the survey), while in high density areas speed should not exceed 16/18 knots. In areas with larger litter densities, the maximum speed needs to be reduced.

c. Technique (visual observation):

The observation is made mainly with the naked eyes and binoculars are used to confirm litter sightings if needed**.** A GPS is used to record the track of the monitored transect, to mark the opening and closing of transect and the waypoints that indicate the position of the sighted objects. The GPS is set for automatic detection of the track at the finest resolution. The track is automatically stored daily.

Data are collected on dedicated data collection sheets (see Fig. 2) or in the dedicated app. The characteristics of the litter items observed are noted following the classification reviewed by the MSFD TSG ML. An app for data collection is currently under development by the JRC and will be available for android and apple platforms.

d. Experience of the observers:

The experience of observers is considered one of the main potential bias in the detection probability and characterization of items, which can influence the amount of time during which the observer can keep the attention, lower detection limits, and identification capability, varying with the strip width, the type and size of object and the density of litter. Thus, data collection should be performed by experienced observers or adequately trained people.

In order to standardize the observer skills, inexperienced observer should be trained (theoretically and with practices at sea) before surveying:

- Showing them examples of the main MSFD marine litter categories observed at sea (plastic, rubber, cloth, paper, cardboard, manufactured wood, metal, glass, ceramic),

- Giving them an illustrated document with pictures of the main MSFD marine litter categories observed at sea (plastic, rubber, textile, paper, cardboard, manufactured wood, metal, glass, ceramic),

- Participating in survey to be calibrated to the size of litter.

It is also suggested to switch observers every 60 minutes to avoid fatigue and keep the attention.

MARINE MACRO FLOATING LITTER DATA COLLECTION SHEET – for FERRY and other LARGE VESSEL

Date	Observer	COD Effort	PAG
Type of platform	Platform name	Strip width	
Platform height	Item size class considered	BEG (lat-long)	Time:
Speed	Sea State	END (lat-long)	Time:
Width of strip at the window:	Distance eye-window:	Angles and name of strips for measuring litter's size:	

Cod GPS Time GPS	Sector	Buoyancy	Material							Most common Items *please specify						Other Items & Comments	Organic		SIZE (cm)	Strip of measurement	Colour	State
Specify here pause and restart (P-cod/time R- cod/time)	Fishing; Maritime FOod Sanitary Cosmetic Other Indef.	+ 0 –	ARTIF. POLYMER	GLASS	PROC. WOOD	PAPER	METAL	TEXTILE	RUBBER	Sheets	Bags*	Polystyrene*	Bottles*	Nets and lines*	Packaging*	• Other item • Changing on weather condition, speed, sea state, cloud cover, observer • FAD • PATCH – please specify 2 most important material and indicate a raw number of items observed	Seaweed/m. plant	Logs/plants parts	E 20-30 F 30-50 G 50-100 H >100 Or specify size (mm)		White Transp. Other colour* *please specify	Entire - Fragment

Notes:

Fig. 2. Data collection sheet for ferries and other large vessels.

Position of the observer. According to the type of ship, and the visibility on the deck, observers can survey both from the front and the side of the vessel (the former is preferable) (Fig. 3). In both cases, the observer is positioned on the side of the vessel in the vicinity of the bow **(**for example on the bridge**,** or the command deck**),** to have the best visibility of the strip avoiding the turbulence generated by the bow itself. Observers should stay on the side with better visibility (i.e. with less sun glare and the sun behind**).**

Different tools can help to measure and delineate the strip size, calculate the item size according to the distance, and collect data (see toolbox). To delineate the strip width from large vessels, a clinometer can be used to measure the angle of observation and the angle of the detected item: these measures, together with the height of observation, allow to estimate the width of the strip or the distance of the target. For setting the strip width, the clinometer can be used at the beginning of the survey to calculate angles, which can be subsequently marked with tape on the windows. Excel spreadsheets are provided to support through calculations. Alternatively, a simple ruler can be used along with an excel spreadsheet to calculate the corresponding measure at sea, according to personal sizes (see PRACTICAL GUIDE 1 at the end of this chapter for details).

In order to gather data on risk for alive biota, the presence within the strip of turtles and other marine organisms larger than 20 cm (or in aggregations larger than 20 cm; *e.g.* jellyfish-gelatinous plankton) should also be recorded. A synoptic monitoring of cetaceans and other macro fauna performed by other dedicated observers is strongly suggested.

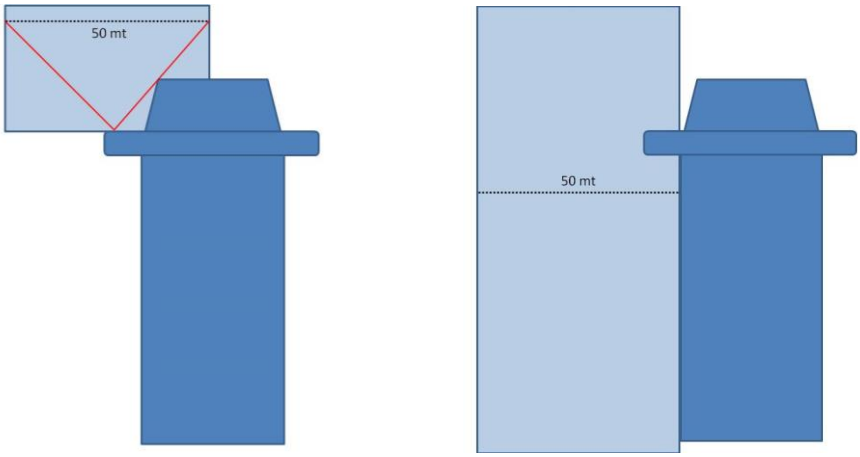


Fig. 3. Measuring the strip on the front of the vessel (left) and on the side (right).

e. Weather and visibility conditions:

For large ships there is no significant difference in the observation results below Beaufort scale 2, but there is significant difference between 2 and 3. Therefore, monitoring should be carried out with a **Beaufort sea state ≤ 2.**

f. Strip width:

Fixed strip width. For large vessels, the standard strip width is fixed at 50 m. Within this width the size of items does not affect detectability. It could be reduced to 25 m if weather conditions are not optimal.

The upper and lower limits of the fixed observational strip are calculated using a clinometer (or eventually a measuring stick or a range finder) and are continuously controlled during the survey to assure that only items spotted within the fixed strip are recorded. The strip can be measured starting from the very edge of the ship, if it is visible, or from the first point detectable by the observer**.** The distance of the inner edge and the outer edge of the strip to the route must be indicated on the data collection sheet**.** Using the clinometer or the stick range finder, the strip should be measured and the scotch tape should be placed on the window or, if outside, on a pole or a graduated stick.

g. Size of items: lower size limit, classes:

The minimum size of recorded items is 20 cm (length of one of the three sides of the object). The size classes used are those suggested by the MSFD TSG ML report "Guidance on Monitoring of Marine Litter in European Sea": E: ($20 \leq x < 30$ cm); F: ($30 \leq x < 50$ cm); G: ($50 \leq x < 100$ cm); H: ≥100 cm (Galgani et al. 2013a). Only in case of common items of known size entire and easy to recognize, *i.e.* small plastic bottles, the class D: ($10 \leq x < 20$ cm) can be recorded.

Observers are trained in advance on the size class of most common objects. A photo-catalogue with common items categorized per size class is taken as reference.

For fragments, or items of unknown size, they will be measured with a ruler: the Thalès equation is used to convert the measured size to the "real" one (see PRACTICAL GUIDE 2 at the end of this chapter for details).

h. Type and colour of items:

Items are classified following the reviewed masterlist (see ANNEX I and Fig.2). The first level of categorization of items concerns their materials: plastic (polymer artificial), glass, wood, metal, rubber, paper and textile (in line with OSPAR, UNEP and TSG_ML). For each type of material, the category (general name or second level) is then identified in more detail. Sightings that do not fall into the categories are scored as OTHER and described by the observer. For plastic, a third level classification is used for

Bags, Polystyrene and bottles. If a FAD is detected, its floating components (plastic) should be noted in the main board, while its description in the back of the data sheet. The presence of natural organic material on the surface, such as logs (from land) or seaweed (from sea), should also be noted, as it can provide information on currents and combinations of materials in the study area. All needed data are inserted in the example of datasheet shown in Fig.2**.**

TOOLBOX – what's the equipment and staff needed for this protocol?

- Staff: 1 expert/well trained observer, 1 recorder
- datasheet + joint list of items; or tablet equipped with the FMML dedicated app + charge battery pack
- GPS + charge battery pack
- Binocular
- Clinometer or measuring stick/range finder
- Measuring tape
- Tape (different colors or not),
- Transparent ruler with a strap to keep it around the neck,
- Paper data collection sheet (or app) with support
- Pen
- Optional: digital camera; computer to perform the different measurements on the excel spreadheet for marine litter from ferries
- Other: agreement with the ferry company to work on the command deck

Implementation of monitoring

1 - Prepare the material and the working position in order to be able to see and know the strip(s) width continuously (marking its edges with tape on the window or on a stick/pole).

2 - Start the GPS (or Tablet) and take note of the starting point and observation conditions (wind strength, latitude, longitude, time, speed etc.). When switching shifts, keep the same GPS track and add the name of the new observer.

2 - The observer positions him/herself comfortably to be able to see everything crossing the strip (from the hull of the ship to the external limit of the strip). If necessary, the observer can move behind the marks to assess if an item is within the strip.

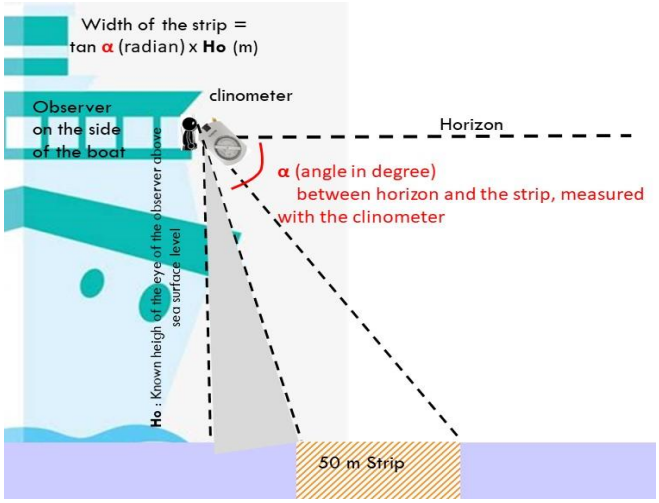
3 - For the duration of the sampling, the observer communicates to the data recorder each litter item detected within the strip and its characteristics (material, category, size, colour…). The data recorder records the time and all information on the datasheet or on the dedicated app'.

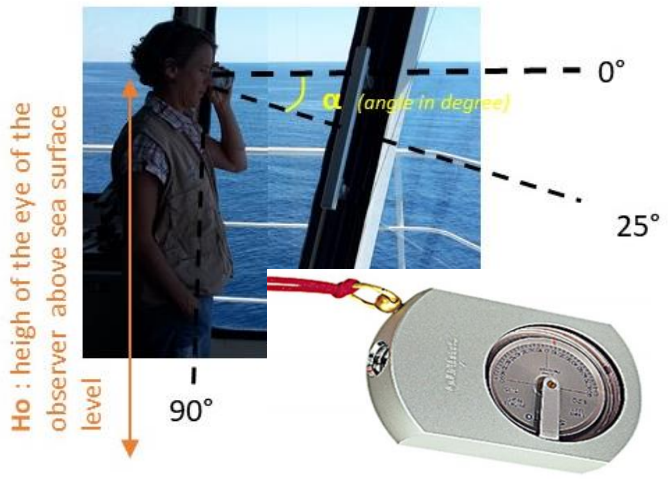
4 – When observation ends, record again the observation parameters (time, latitude, longitude, etc.).

PRACTICAL GUIDE 1. How to measure strip width from large vessels.

1. Observer on the side:

The strip will be measured with a clinometer, depending on the height of the deck where the observer is working, and marked with tape on the glass (for observations from the command deck). Everything observed below the tape limit will be considered "in the strip".





To calculate the angle that has to be measured with the clinometer to define the strip limits, the basic trigonometry theorem of Pythagore. Knowing the opposite side (strip width of 50 m) and the adjacent side (height of observation), one calculates the angle as:

$$\frac{\textbf{\textit{opposite side}}}{\textbf{adjacent side}} = \frac{\textbf{\textit{width of the strip}}}{\textbf{HO (m)}} = \textbf{\textit{tan}}\ \boldsymbol{\alpha}\ \text{(radians)}$$

Where:

HO = Known height of the eye of the observer above sea surface level (deck + observer height)

α = the angle read with the clinometer

Width of the strip = 50 m

2. Observer on the front

When the vessel characteristics prevent the observation from the side, observers can monitor from the front.

Step 1: Know the height of the deck where you will work from.

Step 2: Decide the place where the observer will stand with a good view on the sea surface. The observer should stand almost always at the same place, as the measurements will be made from there. Measure the distance eye-window (figure below).

Measurements of the distance eyes-window (at the observer position)

Step 3: To delimit the area of observation, in order to get a strip width of 50 m at the sea surface, use this equation with the following parameters in meters:

$$\frac{WS \ x \ EG}{HO} = DW$$

WS = **W**idth of the **S**trip at the sea surface (50 m required)

EG = Distance between **E**ye and **G**lass

HO = **H**eight of the Platform of **o**bservation (height deck + height eyes of the observer)

DW = Width on the window corresponding to the (50 m) observational strip for marine litter

Step 4: Measure and mark with tape the left and right edges of DW on the window (pictures below).

Measurement of the width of the strip on the window, based on calculations to get a 50 m width strip on the sea surface, from the observer's post; tapes on the window mark the right and left strip limits corresponding to the 50 m width strip on the sea surface

Metadata needed to perform the calculation:

- Side: right/left
- Angle(s) in ° for 50 m strip width

- Width of strip at the window (cm)
- Position of the observer: side / front
- Distance between the eye and the window (cm)
- Sector(s) of measurements of marine litter's size: angle in degree

An abacus has been calculated to provide needed angles for different heights and strip widths. For a strip width of 50 m, the angle to measure with the clinometer depending on height are marked in yellow.

angle lu à l'inclinomètre

Hauteur d'obs.	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60
13	74	67	61	56	52	49	45	43	40	38	36	34	32	31	29	28	27	26	24	23	23	22	21	20	19	19	18	17	17	16	15	15	14	14	13	13	13	12	12	11	11	11	10	10	9	9	9	8	8	8	8
14	79	72	66	61	56	52	49	46	43	41	38	36	35	33	31	30	29	27	26	25	24	23	22	22	21	20	19	19	18	17	17	16	16	15	14	14	14	13	13	12	12	11	11	11	10	10	9	9	9	8	8
15	85	77	71	65	60	56	52	49	46	44	41	39	37	35	34	32	31	29	28	27	26	25	24	23	22	21	21	20	19	19	18	17	17	16	16	15	14	14	14	13	13	12	12	11	11	11	10	10	9	9	9
16	91	82	75	69	64	60	56	52	49	46	44	42	40	38	36	34	33	31	30	29	28	27	26	25	24	23	22	21	20	20	19	18	18	17	17	16	15	15	14	14	13	13	13	12	12	11	11	10	10	10	9
17	96	87	80	74	68	63	59	56	52	49	47	44	42	40	38	36	35	33	32	31	29	28	27	26	25	24	23	23	22	21	20	20	19	18	18	17	16	16	15	15	14	14	13	13	12	12	11	11	11	10	10
18	102	93	85	78	72	67	63	59	55	52	49	47	45	42	40	39	37	35	34	32	31	30	29	28	27	26	25	24	23	22	21	21	20	19	19	18	17	17	16	16	15	15	14	14	13	13	12	12	11	11	10
19	108	98	89	82	76	71	66	62	58	55	52	49	47	45	43	41	39	37	36	34	33	32	30	29	28	27	26	25	24	23	23	22	21	20	20	19	18	18	17	17	16	15	15	14	14	13	13	12	12	11	11
20	113	103	94	87	80	75	70	65	62	58	55	52	50	47	45	43	41	39	38	36	35	33	32	31	30	29	28	27	26	25	24	23	22	21	21	20	19	19	18	17	17	16	16	15	15	14	13	13	12	12	12
21	119	108	99	91	84	78	73	69	65	61	58	55	52	49	47	45	43	41	39	38	36	35	34	32	31	30	29	28	27	26	25	24	23	23	22	21	20	20	19	18	18	17	16	16	15	15	14	14	13	13	12
22	125	113	104	95	88	82	77	72	68	64	60	57	54	52	49	47	45	43	41	40	38	37	35	34	33	31	30	29	28	27	26	25	24	24	23	22	21	21	20	19	18	18	17	17	16	15	15	14	14	13	13
23	130	118	108	100	92	86	80	75	71	67	63	60	57	54	52	49	47	45	43	41	40	38	37	35	34	33	32	31	29	28	27	26	26	25	24	23	22	21	21	20	19	19	18	17	17	16	16	15	14	14	13
24	136	123	113	104	96	90	84	79	74	70	66	63	59	57	54	51	49	47	45	43	42	40	38	37	36	34	33	32	31	30	29	28	27	26	25	24	23	22	22	21	20	19	19	18	17	17	16	16	15	14	14
25	142	129	118	108	100	93	87	82	77	73	69	65	62	59	56	54	51	49	47	45	43	42	40	38	37	36	34	33	32	31	30	29	28	27	26	25	24	23	23	22	21	20	20	19	18	18	17	16	16	15	14
26	147	134	122	113	104	97	91	85	80	76	71	68	64	61	58	56	53	51	49	47	45	43	42	40	39	37	36	35	33	32	31	30	29	28	27	26	25	24	23	23	22	21	20	20	19	18	18	17	16	16	15
27	153	139	127	117	108	101	94	88	83	78	74	70	67	64	61	58	55	53	51	49	47	45	43	42	40	39	37	36	35	33	32	31	30	29	28	27	26	25	24	23	23	22	21	20	20	19	18	18	17	16	16
28	159	144	132	121	112	104	98	92	86	81	77	73	69	66	63	60	57	55	53	51	48	47	45	43	42	40	39	37	36	35	33	32	31	30	29	28	27	26	25	24	23	23	22	21	20	20	19	18	17	17	16
29	164	149	136	126	116	108	101	95	89	84	80	76	72	68	65	62	59	57	55	52	50	48	46	45	43	41	40	38	37	36	35	33	32	31	30	29	28	27	26	25	24	23	23	22	21	20	20	19	18	17	17
30	170	154	141	130	120	112	105	98	92	87	82	78	74	71	67	64	62	59	56	54	52	50	48	46	44	43	41	40	38	37	36	35	33	32	31	30	29	28	27	26	25	24	23	23	22	21	20	19	19	18	17
31	176	159	146	134	124	116	108	101	95	90	85	81	77	73	70	66	64	61	58	56	54	52	50	48	46	44	43	41	40	38	37	36	34	33	32	31	30	29	28	27	26	25	24	23	23	22	21	20	19	19	18
32	181	165	151	139	128	119	112	105	98	93	88	83	79	75	72	69	66	63	60	58	55	53	51	49	47	46	44	42	41	40	38	37	36	34	33	32	31	30	29	28	27	26	25	24	23	22	22	21	20	19	18
33	187	170	155	143	132	123	115	108	102	96	91	86	82	78	74	71	68	65	62	60	57	55	53	51	49	47	45	44	42	41	39	38	37	35	34	33	32	31	30	29	28	27	26	25	24	23	22	21	21	20	19
34	193	175	160	147	136	127	119	111	105	99	93	89	84	80	76	73	70	67	64	61	59	57	54	52	50	49	47	45	44	42	41	39	38	36	35	34	33	32	31	30	29	28	27	26	25	24	23	22	21	20	20
35	198	180	165	152	140	131	122	114	108	102	96	91	87	82	79	75	72	69	66	63	61	58	56	54	52	50	48	46	45	43	42	40	39	38	36	35	34	33	32	30	29	28	27	26	25	25	24	23	22	21	20
36	204	185	169	156	144	134	126	118	111	105	99	94	89	85	81	77	74	71	68	65	62	60	58	55	53	51	50	48	46	44	43	41	40	39	37	36	35	34	32	31	30	29	28	27	26	25	24	23	22	22	21
37	210	190	174	160	148	138	129	121	114	107	102	96	92	87	83	79	76	73	70	67	64	62	59	57	55	53	51	49	47	46	44	43	41	40	38	37	36	35	33	32	31	30	29	28	27	26	25	24	23	22	21
38	216	195	179	165	152	142	133	124	117	110	104	99	94	90	85	81	78	75	71	69	66	63	61	59	56	54	52	50	49	47	45	44	42	41	39	38	37	35	34	33	32	31	30	29	28	27	26	25	24	23	22
39	221	201	183	169	156	146	136	128	120	113	107	102	97	92	88	84	80	77	73	70	68	65	62	60	58	56	54	52	50	48	46	45	43	42	40	39	38	36	35	34	33	32	30	29	28	27	26	25	24	23	23
40	227	206	188	173	160	149	139	131	123	116	110	104	99	94	90	86	82	79	75	72	69	67	64	62	59	57	55	53	51	49	48	46	44	43	41	40	39	37	36	35	34	32	31	30	29	28	27	26	25	24	23
41	233	211	193	178	164	153	143	134	126	119	113	107	101	97	92	88	84	80	77	74	71	68	66	63	61	59	56	54	52	51	49	47	46	44	42	41	40	38	37	36	34	33	32	31	30	29	28	27	26	25	24
42	238	216	198	182	168	157	146	137	129	122	115	109	104	99	94	90	86	82	79	76	73	70	67	65	62	60	58	56	54	52	50	48	47	45	43	42	41	39	38	37	35	34	33	32	31	29	28	27	26	25	24

Excel spreadsheets have also been prepared to automatically calculate the angle of observation according to the height of the observer and the desired strip width.

E.g. For observers on the side of the vessel:

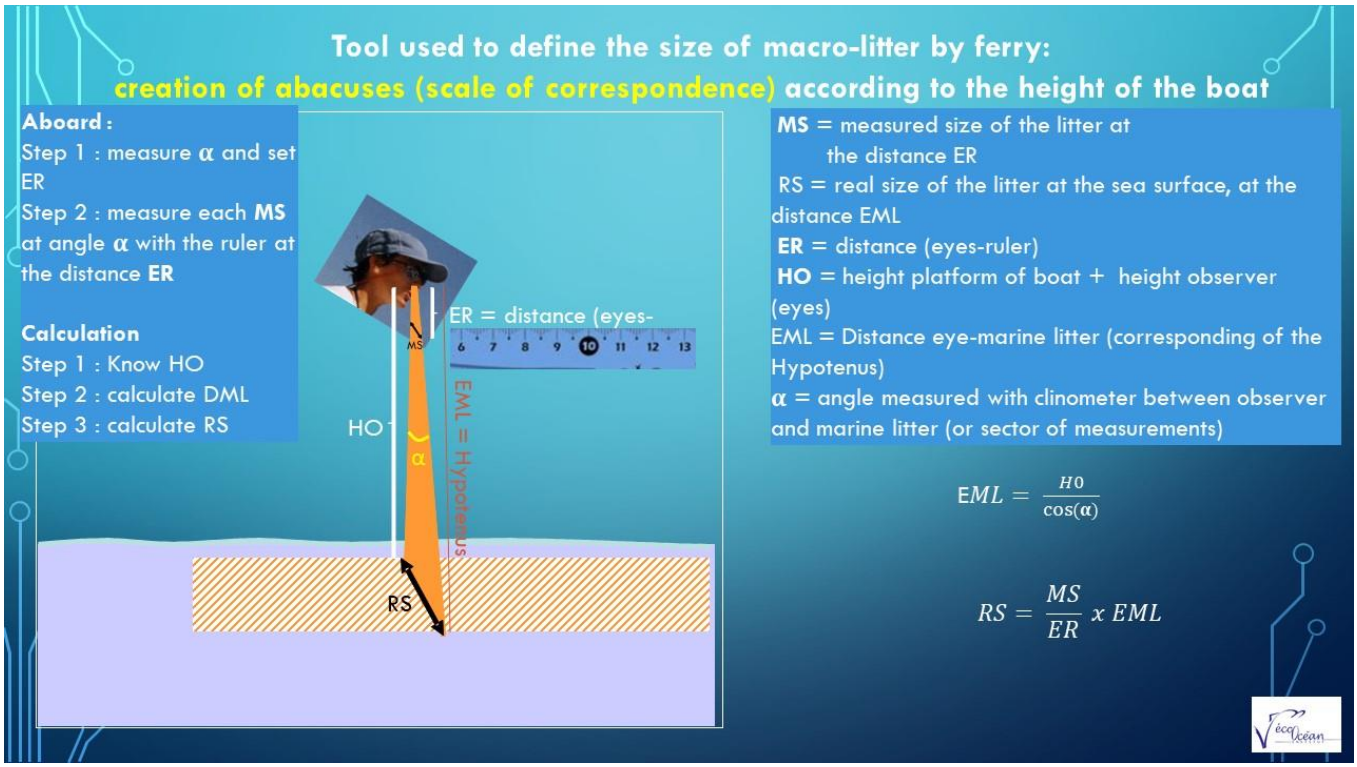
WS = Tan(alpha)*HO	tan (alpha) = WS/HO				
HO = **H**eight of the **P**latform of observation (height deck+height eyes of the observer)	26,2				
WS = **W**idth of the **S**trip at the sea surface (50 m required)	49,3				
alpha (angle to be measured with the clinometer)	28				
In case of an obstacle, and the strip is not begining at the perpendicular, at the hull, but further (case of a walkway preventing observation)					
HO = **H**eight of the **P**latform of observation (height deck+height eyes of the observer)	26,2				
Angle to delimit lower and higher limit of a 50 m strip width with clinometer		**Radians**	**TAN (a)**	**Opposite (m)**	**strip width (WS) ≈ 50 m awaited**
Angle **measured** at the nearest of the boat, where you can begin to observe (low limit of the strip)	38	0,9076	1,2799	33,5	
Angle **to determine with calculation**, farthest limit of the strip, change value until WS around 50m	17	1,2741	3,2709	85,7	52,2

And for observer on the front:

DW = (WS x EG)/HO			WS = (HP * DW) / EG	
unit = meter				
WS = **W**idth of the **S**trip at the sea surface (50 m required)	50		HO	39,2
EG = Distance between **E**ye and **G**lass	0,79		DW	1,06
HO = **H**eight of the **P**latform of observation (height deck+height eyes of the observer)	39,2		EG	0,79
DW = **D**istance between **T**apes on the **W**indow	1,01		**WS**	52,6

In yellow: the cells to be filled with observer data; in green the results of calculations.

PRACTICAL GUIDE 2. How to measure the exact size of items from large vessels.



To avoid measuring the angle for each item, a sector of measurement is defined, and all the measures of marine litter items will be made within this sector. Caution: because the clinometer measures 0° at the Horizon and 90° at the vertical, the first thing to do is to calculate the complementary angle to the one measured with the clinometer (*i.e.* measured angle - 90°).

At final, the real size (**RS**) of marine litter will be obtained with the equation:

$$RS = \frac{MS}{ER}\ x\ EML$$

Where:

ER = distance eye-ruler

EML = Distance eye-litter (corresponding to the triangle hypotenuse), and calculated with the angle of the sector of measurement (clinometer) and the height of the observation (HO) using the equation:

$$EML = \frac{H0}{\cos(\boldsymbol{\alpha})}$$

MS = measured size of the marine litter

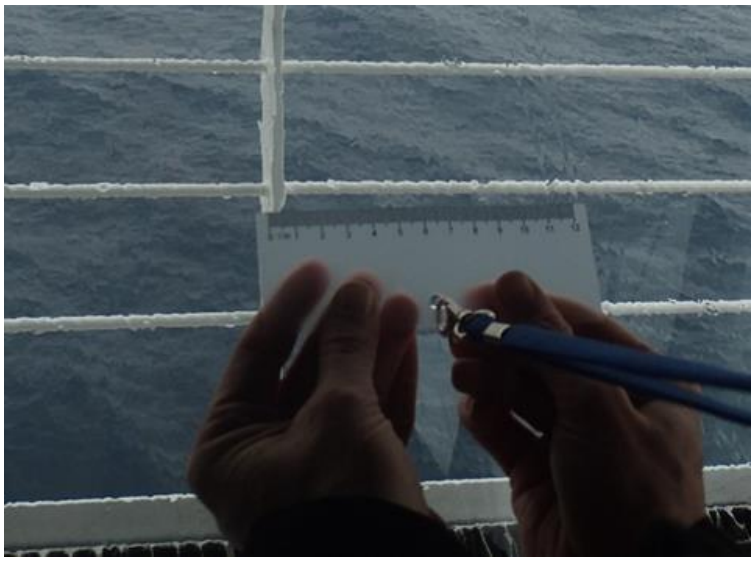
1. <u>Observer on the side</u>:

As the distance observer-litter changes from the nearest point to the further point, the measured size will differ too according to this distance. So, several sectors of measures should be delimited and the angle of the sectors known in order to calculate the real size.

The limits of the measuring sectors A, B, C are marked with tape on the window or can be visible using the balustrades as reference. Each limit is measured in degrees with the clinometer. The distance between sectors should not be larger than 10° to avoid approximation of the real size. A transparent ruler is used to measure the apparent size of the litter passing through the different sectors.

2. Observer on the front:

Each item will necessarily come towards the observer. The sector of measurement should be determined at the nearest position from the observer. The observer will see the marine litter beforehand, and will have time to prepare his ruler in hand. The ruler should be attached to his neck with a cord or a strap, to keep the distance (ER in the equation) constant (among different observers and for the same one). The ruler is transparent and can be overlapped to the litter item to check its size at a glimpse. The observer stands at his post and just records the litter observed and its size in the data recording sheet.



A transparent ruler is used to measure the apparent size of the litter passing through, at the determined sector of measurement.

3.2 MEDIUM AND SMALL SIZE VESSELS

Introduction and scope of the protocol

The protocol to be used for medium and small size vessels refers to the one used for ferry/large vessels with adaptations mainly related to the different speed and height of these vessels, and consequently to the strip width and the lower size limit of items. Medium/small vessels are suitable to survey coastal/local areas, to assess the quantity and the characteristics of floating litter.

The protocol uses the strip transect method to obtain a density value expressed as items/area (calculated as transect length x strip width). Only items within the strip are recorded.

Covariates

a. Sampling design and period:

In coastal areas, to avoid outliers and detect at least 2 different types of materials, 2 to 3 km^2 per season should be sampled and 0.14 km^2 per survey. The spreadsheets shown in Table 4 and 5 can help calculate the effort required per season, depending on the speed and strip width chosen. For example, with sailing vessel with a strip of 10 m, 15-30 h of effort at 3-5 speed knots would allow to monitor an adequate sample size for the Summer season. Or 37-56 hours with a strip of 5 m, at 4-6 knots.

Table 4. Spreadsheet to calculate the effort required per season, depending on the speed and strip width chosen.

Type of vessel	speed (knots)	strip and observer	Strip width (m)	Surface to be covered per season (km^2)	Length of transect (km)	Length of transect (NM)	Nb of hours
Small vessel	4	1 observer, 1 strip of 5 m (side)	5	2,5	500	270	67
Small vessel	4	2 observers, 2 strips of 5 m (two sides)	10	2,5	250	135	34
Small vessel	4	1 observer, 1 strip of 3 m (front)	3	2,5	833	450	112
Small vessel	4	2 observers, 2 strips of 3 m (front)	6	2,5	417	225	56
Medium-size vessel	4	1 observer, 1 strip of 5 m (side)	5	2,5	500	270	67
Medium-size vessel	4	2 observers, 2 strips of 5 m (two sides)	10	2,5	250	135	34
Medium-size vessel	6	1 observer, 1 strip of 5 m (side)	5	2,5	500	270	45
Medium-size vessel	6	2 observers, 2 strips of 5 m (two sides)	10	2,5	250	135	22

Table 5. Spreadsheet to calculate the effort required per survey, depending on the speed and strip width chosen.

Type of vessel	speed (knots)	strip and observer	Strip width (m)	Surface to be covered per survey (km^2)	Length of transect (km)	Length of transect (NM)	Nb of hours
Small vessel	4	1 observer, 1 strip of 5 m (side)	5	0,14	28	15	4
Small vessel	4	2 observers, 2 strips of 5 m (two sides)	10	0,14	14	8	2
Small vessel	4	1 observer, 1 strip of 3 m (front)	3	0,14	47	25	6
Small vessel	4	2 observers, 2 strips of 3 m (front)	6	0,14	23	13	3
Medium-size vessel	4	1 observer, 1 strip of 5 m (side)	5	0,14	28	15	4
Medium-size vessel	4	2 observers, 2 strips of 5 m (two sides)	10	0,14	14	8	2
Medium-size vessel	6	1 observer, 1 strip of 5 m (side)	5	0,14	28	15	3
Medium-size vessel	6	2 observers, 2 strips of 5 m (two sides)	10	0,14	14	8	1

Small and medium vessels are mainly used for local scale, *i.e.* MPAs. In this case, the whole area of the MPA should be covered homogeneously, including the coastal and offshore areas, and, if present, any river mouth and large current gyres.

As distribution of marine litter in coastal waters may be largely influenced by rainy or windy periods, mainly linked to seasonal patterns, data should be collected during each season. It is then suggested to repeat at least 5 surveys per season in case of 1-year surveys. For multi-year surveys, 3 surveys/season will be a good basis.

b. Type of platform (height and speed):

Small vessels include inflatable and other types of small boats (50 cm above sea surface) offering an observation height of about 1 m (Fig. 4).

Fig. 4. Small vessel (50 cm above sea surface) with an observation height of ~ 1 m.

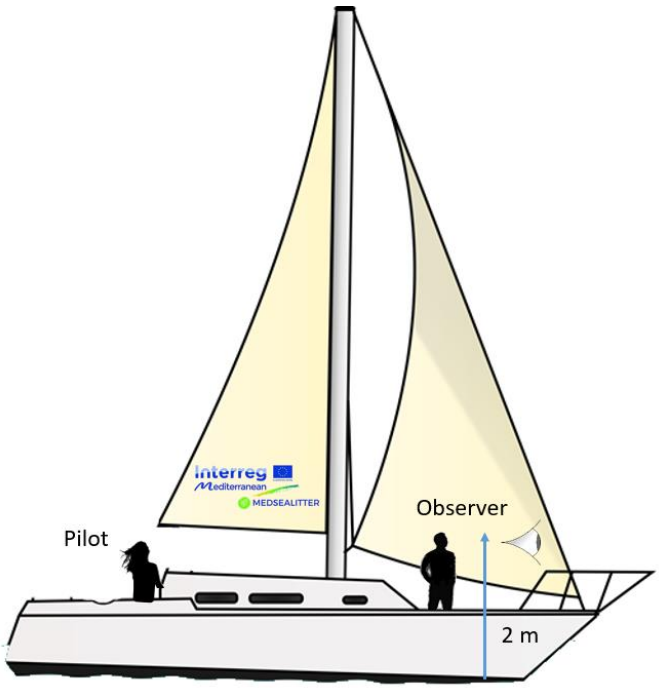


Fig. 5. Medium size vessel and position of the observer.

Medium size vessels include a wide range of motor and sailing boats. Because collection of marine litter data is made with low wind and stable navigation conditions, the sailing vessel will need to get a motor to navigate. Usually the deck is around 1 meter above sea level, and the observation height can range from a minimum of 2.5 m upwards (standing person) (fig. 5).

For a better detection of items (and to avoid foam formation around the boat), the speed of small vessels must be maximum 4 knots, and between 4 and 6 knots for medium vessels.

c. Technique (visual observation):

The strip width will be defined and delimited visually by a fishing rod attached perpendicularly to the boat, and a rope at the end of the fishing rod leaning vertically to the sea surface.

We recommend several observers positions by preferential order, allowing a large strip sampling and the avoidance of the foam that can appear on the sides:

For small vessels mainly, which can be equipped on the front (at the bow):

1) 2 observers at the bow, watching each a 3 m width (2x3 m) + 1 data recorder (option 1)
2) 1 observer at the bow (3 m) + 1 data recorder (option 2)

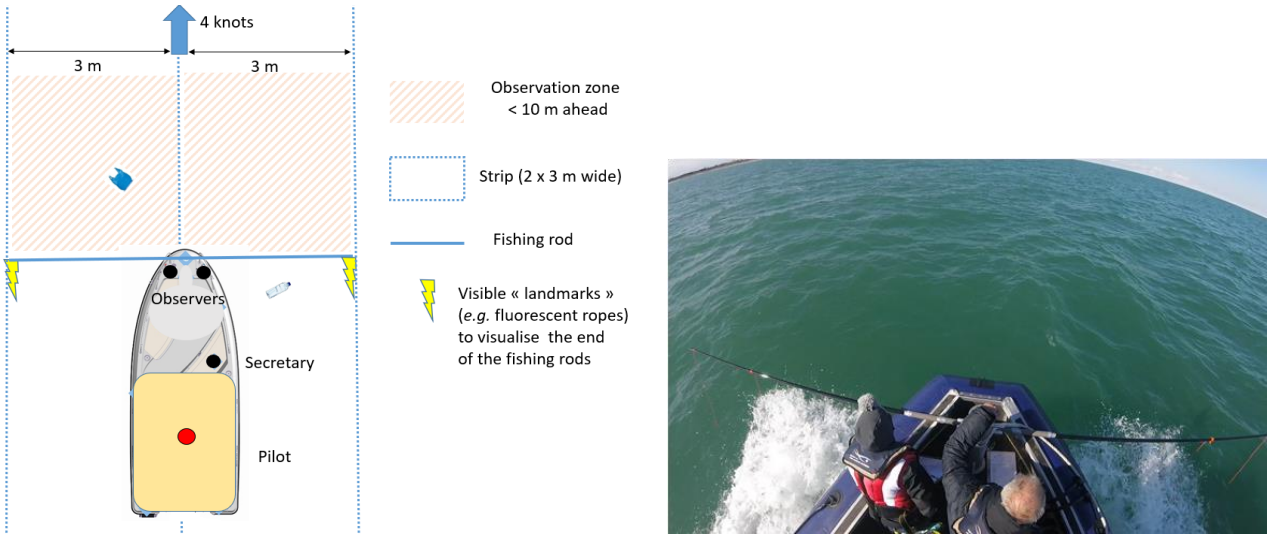


Fig. 6. Option 1 for small vessels and two observers at the bow, each watching a 3 m wide strip.

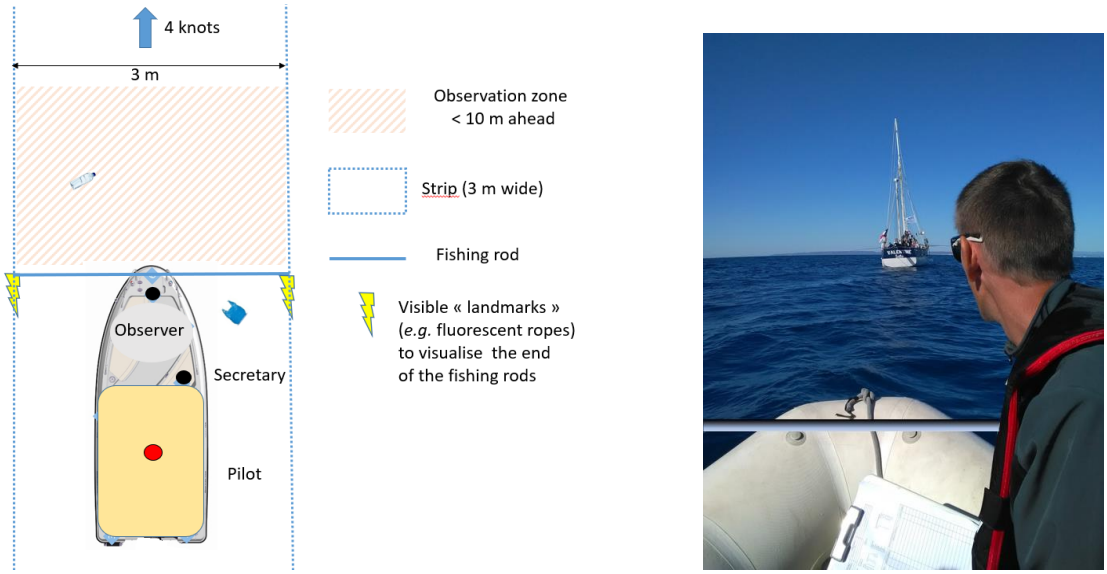


Fig. 7. Option 2 for small vessels and one observer at the bow watching a 3 m wide strip.

For small and medium vessels that can be equipped on the side:

3) 2 observers, one per side (2x5 m) + 1 data recorder (option 1)
4) 1 observer on one side (5 m) + 1 data recorder (option 2)

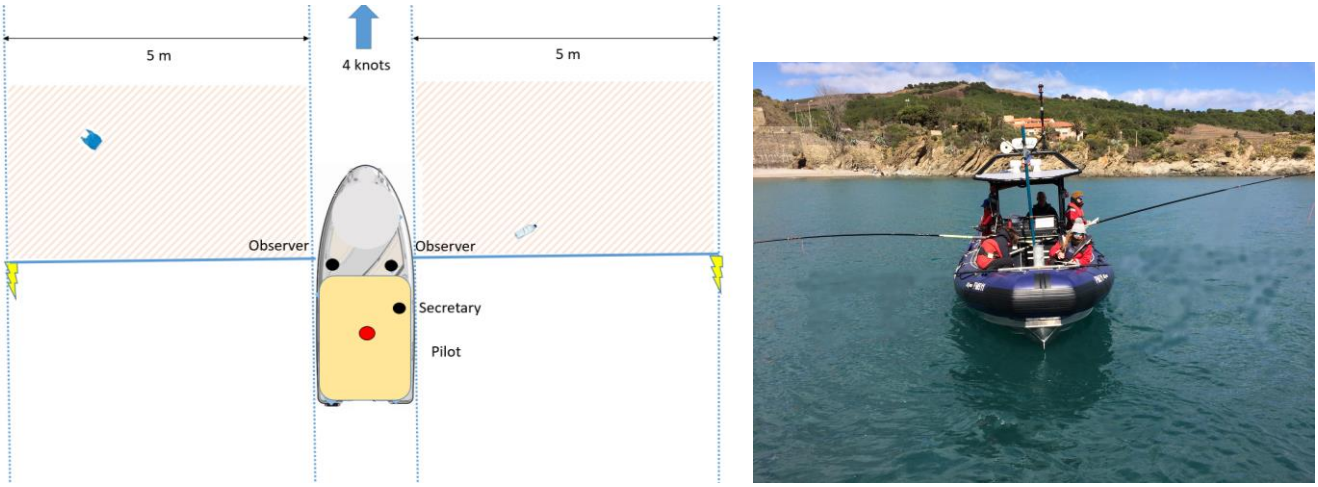


Fig. 8. Option 1 for medium vessel (and small vessel when possible)

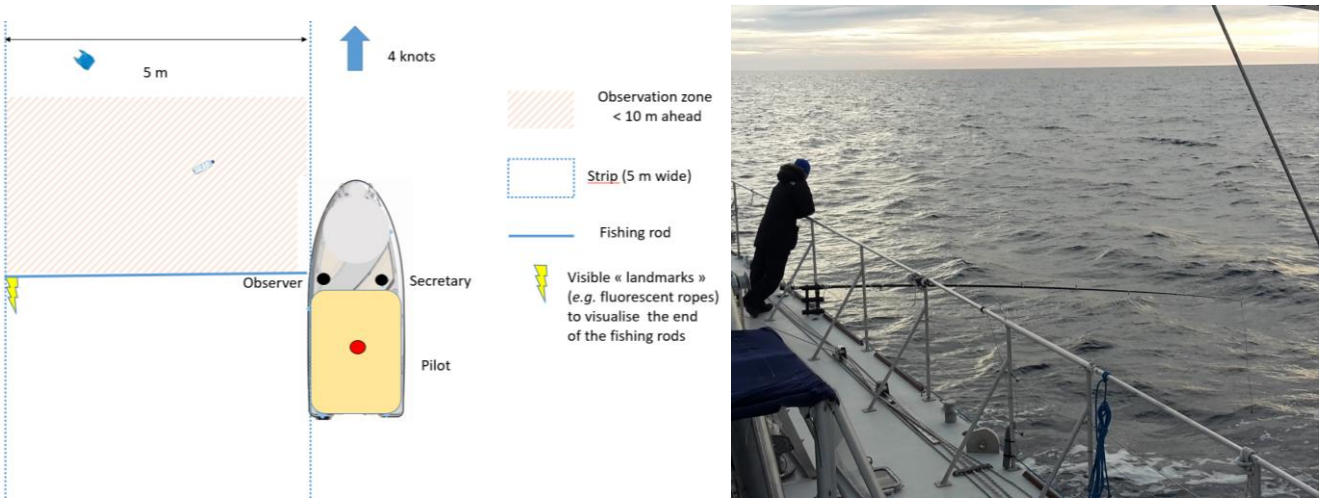


Fig. 9. Option 2 for medium vessel (and small vessel when possible)

Alternative methods to measure the strip width from small and medium size vessels are described in the PRACTICAL GUIDE 3 at the end of this chapter.

Observers can either be comfortably and securely seated or stand, but they must ensure to see the water and items near the hull. They should position in a way that the effect of sun glare on the sea is avoided. If feasible, they should switch their position every 1 hour.

Fig. 10. Position of observers at the (front) side of the vessel.

d. Experience of the observers:

Data collection should be performed by experienced observers or adequately trained people.

In order to standardize the observer skills, inexperienced observer should be trained (theoretically and with practices at sea) before surveying:

- Showing them examples of the main MSFD marine litter categories observed at sea (plastic, rubber, cloth, paper, cardboard, manufactured wood, metal, glass, ceramic),

- Giving them an illustrated document with pictures of the main MSFD marine litter categories observed at sea (plastic, rubber, textile, paper, cardboard, manufactured wood, metal, glass, ceramic),

- Participating in survey to be calibrated to the size of litter.

e. Weather and visibility conditions:

For a correct identification of items, sea state must be lower or equal to 2 on Beaufort scale. The transect orientation and the observer position have to be set in order to limit the effect of sun glare.

f. Strip width:

Different options are shown in Table 6.

Table 6. Summary of strip widths according to the vessel type and speed, number and position of observers.

Options	platform	Speed	Strip width
1 observer, at the side	Medium size vessel	4 knots	5 m
1 observer, at the side	Medium size vessel	6 knots	5 m
2 observers, at each side	Medium size vessel	4 knots	2 x 5 m (10 m)
2 observers, at each side	Medium size vessel	6 knots	2 x 5 m (10 m)
1 observer, at the side	Small vessel	4 knots	5 m
1 observer, at the bow	Small vessel	4 knots	3 m
2 observers, at the side	Small vessel	4 knots	2 x 5 m
2 observers, at the bow	Small vessel	4 knots	2 x 3 m

g. Size of items: lower size limit, classes:

The lower size limit is 2.5 cm, thus the first category to be recorded is B (See MSFD size classes).

h. Type and colour of items:

Categories are recorded according to a data collection sheet drawn from the MSFD masterlist (Fig. 11).

MARINE MACRO FLOATING LITTER DATA COLLECTION SHEET – for SMALL and MEDIUM VESSEL

Date	Observer	COD Effort	PAG
Type of platform	Platform name	Strip width	
Platform height	Item size class considered	BEG (lat-long)	Time:
Speed	Sea State	END (lat-long)	Time:

Cod GPS Time GPS	Sector	Buoyancy	Material							Most common Items *please specify									Other Items & Comments	Organic		SIZE	Colour	State
Specify here pause and restart (P-cod/time R- cod/time)	Fishing; Maritime FOod Sanitary Cosmetic Other Indef.	+ 0 –	ARTIF. POLYMER	GLASS	PROC. WOOD	PAPER	METAL	TEXTILE	RUBBER	Sheets	Bags*	Polystyrene*	Bottles*	Nets and lines*	Packaging*	Cigarette butts	Caps and lids	cotton bud	• Other item • Changing on weather condition, speed, sea state, cloud cover, observer • FAD • PATCH – please specify 2 most important material and indicate a raw number of items observed	Seaweed/m. plant	Logs/plants parts	B 2,5-5 C 5-10 D 10-20 E 20-30 F 30-50 G 50-100 H >100	White Trasp. Other colour* *please specify	Entire - Fragment

Notes:

Fig. 11. Data collection sheet for small and medium vessels.

TOOLBOX – what's the equipment and staff needed for this protocol?

- One or two dedicated observers + one data recorder
- 1 vessel
- 1 GPS (to record transect track and position each minute)
- Data sheets, 1 pencil, 1 clipboard or a mobile application on a dedicated device
- Tools to define the strip width:

  fishing rod
  - One 6-meters press-fit or telescopic carbon fishing rod (light and rigid) for each strip
  - Visible «marks» (*e.g.* fluorescent ropes) to better see the tips of the fishing rods (strip width)
  - To fix rods on the boat: fishing support or PVC pipes/duct tape/plastic and reusable cable ties/scissors

  Alternatively: clinometer (see detailed explanation on its use in the PRACTICAL GUIDE 3)
  - One clinometer to measure the angle for a strip width of 5 m and the conversion scale (or abacus) in a spreadsheet
  - Visible «marks» (*e.g.* fluorescent ropes or tape) to better see the limit of the strip (strip width)

  Alternatively: measuring stick (see detailed explanation on its use in the PRACTICAL GUIDE 3)
  - Measuring stick

Implementation of monitoring

Fishing rod:

- Attach the weighted rope to the end of the fishing rod

- Fasten the fishing rod securely so that:

  - it extends widely on the side of the boat (or front for small vessel), perpendicularly to the course
  - the rope reaches the surface of the water

- Calibrate the boat at a constant speed of 4 to 6 knots

- Start the GPS and note on the data sheet, the starting point and parameters relating to the observation conditions (wind strength, Beaufort sea state, latitude, longitude, time, etc.)

- position yourself comfortably so as to see everything that passes between the hull of the boat and the external limit of the strip

- For the duration of the sample, record for each item of litter passing within the strip the time and its characteristics (category, size, colour…), on the datasheet or on the app'.

- At the end of observation, re-record the parameters relating to the observation conditions (time, latitude, longitude, etc.)

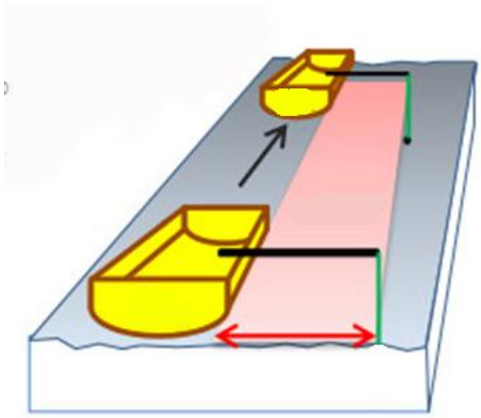


Fig. 12. Scheme showing the implementation of the fishing rod on the side of the vessel to limit the strip width.

The GPS is used to record the position each minute. The GPS time will be the link between events (begin of transect, weather changes, sightings of marine litter, end of transect) and the geographic position. The watch used by observers must be set at the same time as the GPS time.

When transect monitoring begins, time must be recorded and the GPS should already be recording the position and characteristics of the navigation. During transect monitoring, observers watch the sea surface < 10 m ahead the fishing rod looking for litter crossing the observation zone (*i.e.* "within the strip"), which is delimited by a visible landmark at the top of the fishing rod.

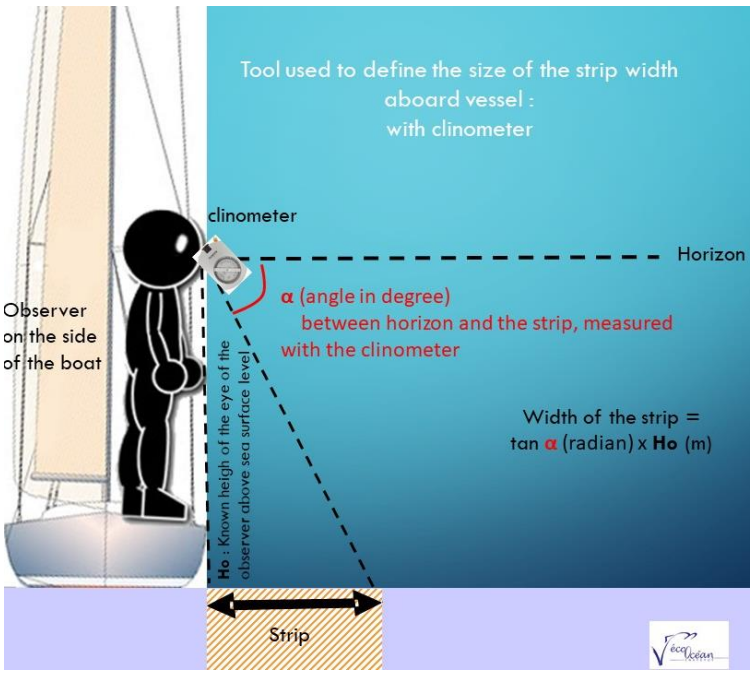
For better ergonomics and efficiency, observers should communicate their observations to the data recorder, who will take note of the time and fill in the dedicated data sheets or the dedicated app.

Observers should wear polarized sunglasses for a better detection of litters.

When transect monitoring ends, time must be recorded and the GPS stopped. The effort between begin and end of moniroring can be expressed in km or NM.

PRACTICAL GUIDE 3. How to measure strip width from small and medium vessels.

1. With clinometer:



The strip will be measured with a clinometer, depending on the height of the deck where the observer is working, and can be marked with tape on a mast stay. Everything observed below the tape limit will be considered "in the strip".

To calculate the angle that has to be measured with the clinometer to define the strip limits, the basic trigonometry theorem of Pythagore is used. Knowing the opposite side (strip width of 5 m) and the adjacent side (height of observation), one calculates the angle as:

$$\frac{\textbf{\textit{opposite side}}}{\textbf{adjacent side}} = \frac{\textbf{\textit{width of the strip}}}{\textbf{HO} \text{ (m)}} = \textbf{\textit{tan}}\ \boldsymbol{\alpha} \text{ (radians)}$$

Where:

HO = Known height of the eye of the observer above sea surface level (deck + observer height)

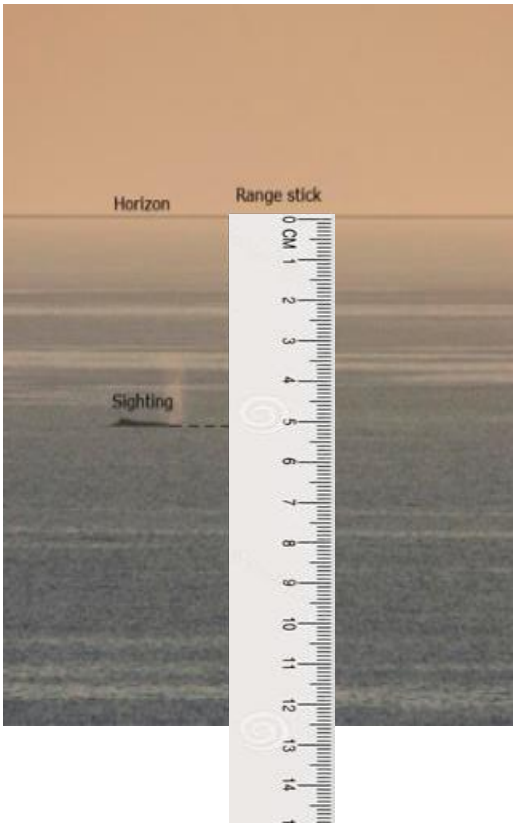
α = The angle measured with the clinometer

Width of the strip = 5 m

An abacus has been calculated to provide needed angles for different heights and strip widths. For a strip width of 5 m (in orange), the angles to measure with the clinometer depending on height (left hand column) are indicated on the top line. *E.g.: 27° for an observation height of 2.5 meters.*

Hauteur d'obs.	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
1	11,4	9,5	8,1	7,1	6,3	5,7	5,1	4,7	4,3	4,0	3,7	3,5	3,3	3,1	2,9	2,7	2,6	2,5	2,4	2,2	2,1	2,1	2,0	1,9	1,8	1,7	1,7	1,6	1,5	1,5	1,4	1,4	1,3	1,3	1,2	1,2
1,5	17,1	14,3	12,2	10,7	9,5	8,5	7,7	7,1	6,5	6,0	5,6	5,2	4,9	4,6	4,4	4,1	3,9	3,7	3,5	3,4	3,2	3,1	2,9	2,8	2,7	2,6	2,5	2,4	2,3	2,2	2,1	2,1	2,0	1,9	1,9	1,8
2	22,9	19,0	16,3	14,2	12,6	11,3	10,3	9,4	8,7	8,0	7,5	7,0	6,5	6,2	5,8	5,5	5,2	5,0	4,7	4,5	4,3	4,1	3,9	3,8	3,6	3,5	3,3	3,2	3,1	3,0	2,9	2,8	2,7	2,6	2,5	2,4
2,5	28,6	23,8	20,4	17,8	15,8	14,2	12,9	11,8	10,8	10,0	9,3	8,7	8,2	7,7	7,3	6,9	6,5	6,2	5,9	5,6	5,4	5,1	4,9	4,7	4,5	4,3	4,2	4,0	3,8	3,7	3,6	3,4	3,3	3,2	3,1	3,0
3	34,3	28,5	24,4	21,3	18,9	17,0	15,4	14,1	13,0	12,0	11,2	10,5	9,8	9,2	8,7	8,2	7,8	7,4	7,1	6,7	6,4	6,2	5,9	5,6	5,4	5,2	5,0	4,8	4,6	4,4	4,3	4,1	4,0	3,8	3,7	3,6
3,5	40,0	33,3	28,5	24,9	22,1	19,8	18,0	16,5	15,2	14,0	13,1	12,2	11,4	10,8	10,2	9,6	9,1	8,7	8,2	7,9	7,5	7,2	6,9	6,6	6,3	6,1	5,8	5,6	5,4	5,2	5,0	4,8	4,6	4,5	4,3	4,2
4	45,7	38,1	32,6	28,5	25,3	22,7	20,6	18,8	17,3	16,0	14,9	13,9	13,1	12,3	11,6	11,0	10,4	9,9	9,4	9,0	8,6	8,2	7,9	7,5	7,2	6,9	6,7	6,4	6,2	5,9	5,7	5,5	5,3	5,1	4,9	4,8

2. With measuring stick (ruler)



Formula uses Heinemann equation
see: Heinemann D. 1981. A rangefinder for pelagic bird censusing. J. Wildl. Mgmt 45: 489-493.

Arm (cm)	64
Eye height (m)	1,6
Deck Height (m)	1
Total Height (m)	2,6

Theoretical distance to Horizon (m)	6188,589048

Distance measured from horizon (cm)	Corresponding distance at sea (m)	Distance measured from horizon (cm)	Corresponding distance at sea (m)
10	16,6	42	4,0
15	11,1	43	3,9
20	8,3	44	3,8
25	6,6	45	3,7
30	5,5	46	3,6
31	5,4	47	3,5
32	5,2	48	3,5
33	5,0	49	3,4
34	4,9	50	3,3
35	4,7	51	3,3
36	4,6	52	3,2
37	4,5	53	3,1
38	4,4		
39	4,3		
40	4,2		
41	4,1		

From Patrick Lyne, Irish Whale and Dolphin Group

Any big standard ruler can be used. An Excel spreadsheet has been prepared to automatically calculate the measurements “below the horizon” corresponding to a 5 m strip. To use the spreadsheet, for each observer, first the length of the arm (from shoulder to stick hold in hand) and the observer height must be measured and entered in the spreadsheet. *E.g.: 33 cm below horizon for an observation height of 2.6 meters and arm length of 64 cm for a strip width of 5 m.*

3.3 AIRCRAFTS (PROTOCOL IMPLEMENTED FROM THE UNEP/MAP AND MSFD PROTOCOLS)

Introduction and scope of the protocol

Among the available methods for monitoring FML in the ocean, aerial surveys are useful to assess large areas, detect and identify aggregations of litter and estimate its abundance. Surveys should be designed accordingly to a line transect distance sampling technique, in which a high representation of the study area is homogenously covered. The recommended aircraft is a two-engine high-wing with flat or bubble-windows flying at constant speed and altitude. Beside of the pilot, two experienced observers and a dedicated data logger should form the crew. Environmental and weather conditions should be recorded at the start and end of all transects and any time when these changes. Considering that the lowest limit of object size for aerial detection is *ca*. 30-40cm, a limitation on the categorization of floating litter observed from aerial surveys is imposed. Applying this protocol, it will be possible to answer the following questions:

- Does this area have FML? How much?
- What is the trend on FML abundance? Is it increasing or decreasing?
- Where does the FML accumulate?
- How does the FML spread depending on the season?
- Which are the sources of FML in our study area?
- Which are the pathways of distribution for FML?
- Are the mitigation measures on FML impact having an effect?
- Which are the most sensitive areas for marine biodiversity? Which are the risks?

Covariates

a. Sampling design and period:

Line transects should be designed using the "Distance" software. The software allows creating a sampling methodology with homogeneous and highly representative coverage probability over the whole studying area, for example by using equidistant parallel lines or a systematic saw-tooth pattern. Each transect must be characterized by:

- Transect number and length.

- Date of survey and starting and ending times.

- Geographic position at the starting and ending points.

- Number of marine fauna sightings and the average distance between each two consecutive sightings (average distance = length between transects/number of sightings). This could also apply to marine litter.

- Oceanographic characteristics (*i.e*., depth, Beaufort state, cloudiness).

b. Type of platform (height and speed):

Aerial surveys can be performed on a two-engine high-wing aircraft, like a 'push-pull' Cessna 337, preferably equipped with bubble windows (Fig. 13). Aircrafts with flat windows can also be used but the reduction of the visibility of the transect strip width must be taken into account. Transects are flown at a groundspeed of *ca.*166 km/h (90 kn) and an altitude of *ca.* 230 m (750 ft), which in both cases should be maintained constant. This altitude would guarantee identification of objects bigger than 30 cm while conforming to safety aerial procedures.

c. Technique (visual observation):

A standard crew should include: pilot, recorder in the seat of the co-pilot and two experienced observers positioned behind them on each side of the plane, which will be preferably the same for all transects during

the survey. An additional observer could be dedicated to photo recording; this figure would also be greatly beneficial to switch shifts with the main observers (Fig 13).

Fig. 13. (Left) Aircraft for monitoring floating macro litter and (Right) crew made by the pilot on the left hand of the plane, data recorder in the seat of the co-pilot, two observers positioned at each side of the plane and an additional observer dedicated to photo shooting.

Sampling at the beginning of each transect:

The recorder should annotate the following items and all environmental conditions must be updated whenever any changes occur.

- Identification number and characteristics of each transect.

- Position of the sun, intensity of glare (if any as low, medium or high) and angle of glare (from the right side = 0º to 180º; from the left side = 0º to -180º).

- Geographic locations at the beginning of each transect. A GPS will continuously record the position updated every few seconds.

- Position of observers (Left, Right).

- Environmental conditions (Beaufort sea state, cloud coverage, visibility, etc.).

Sampling within effort:

1) Duties of the recorder: The recorder will take note of all data in the "Visual Survey Data Sheet" (Fig. 14). Alternatively, data can be recorded on a laptop using any specific data recording software. Otherwise, recorder can use any other suitable method for data recording. Information on the location of each sighting, which will be also recorded in the GPS, the time and angle of sighting (see below), and changes in environmental conditions will be annotated.

2) Duties for observers: Each observer will record marine litter and will communicate to the recorder the following three aspects: a) type of marine litter, b) marine litter sighting angle strip (i.e. red, yellow, blue, that will be used to estimate the distance of the observed marine litter from the transect line), and c) size of the object observed.

d. Experience of the observers:

Giving the number of items to be recorded and the vast category types, only dedicated and experienced observers must be used during the monitoring. Experience of observers can in fact influence item detection and identification, leading to incoherent results. When in need of training a new observer, this new member could be added to the crew as an additional observer as explained in the previous paragraph.

e. Weather and visibility conditions:

(Beaufort, wind direction, visibility, sun glare, etc.). Aerial surveys cover large areas and only detect very large litter items (i.e. the lowest limit for aerial detection are objects of *ca*. 30–40 cm), so they are less prone

to changes in litter detectability linked to wind strength and sea state. However, surveys must be conducted with good sea state (*i.e.*, below 3 Beaufort state), as visibility will decrease with bad weather conditions.

MARINE MACRO FLOATING LITTER DATA COLLECTION SHEET – for AIRPLANE

Date: | Flying altitude: | Sea state: | General comment:
Observers | Flying speed: | Sun glare:

Cod GPS/ TIME GPS	Observer	Transect	Sea state	Angle of sun glare	Visibility	Angle strip	Plastic, Polystyrene, Polyurethane						Proc. wood	Vegetable		Liquids		Glass	Textile	Rubber		Animal	Unidentified material		Size	Number of items	Comments
	L (Left) R (Right)		Indicate changes in Beaufort scale		Visibility values are:1-Good, 2-Moderate, 3-Poor	R (Red) Y (Yellow) B (Blue)	Bags	Boxes	Fish box	Buoys	Buckets	Fishing nets	Pallets	Seaweed/marine plant	Logs/plants parts	Oil slick	Isolated foam	Bottles	Clothing	Balloons	Tyres	Animal carcases	Ropes (plastic or textile)	Pieces (non-organic material)	Estimated size in cm: small (30-100), medium (100-200), large (>200)	Values: 1, 1-5, 5-10, 10-20, 20-50, >50	Add here any additional comment that could be relevant for the sighting

Fig. 14. Visual aerial survey data sheet

f. Strip width:

This distance will be established accordingly to the angle of sighting within three fixed-width strips (Fig. 15). These strips will be drawn on the window and the length of each strip will be estimated using a hand-held inclinometer and should be between 90º and 40º (observable area within 275 m from the transect line) (Fig. 16). The data of the angle from each detected item, together with the flying altitude, will be used to calculate the perpendicular distance of the item from the line-transect; any other object observed above 40º are outside the 275 m distance from the transect line and will not be recorded.

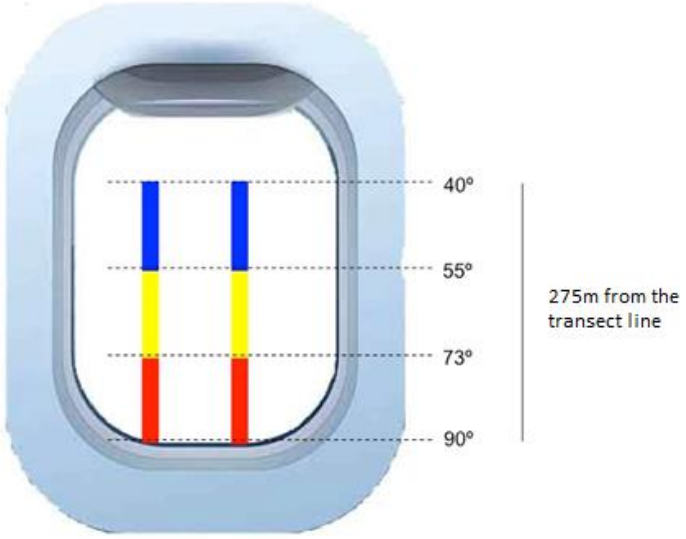


Fig. 15. Observable angles to detect marine litter within 275 m from the transect line.

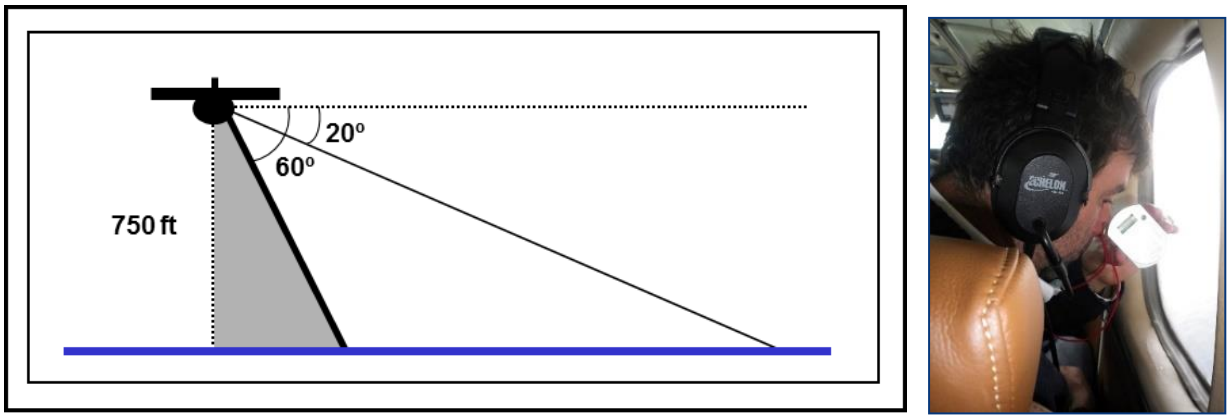


Fig. 16. Schematic drawing of the visibility from the aircraft window with angles for distance estimation. Note that with a bubble window, observers will be available to see from 0º to 90º. Marine litter will be only recorded within the 40º distance from the line transect. The maximum angle of marine fauna sighting is 20º. The grey section of the scheme represents the 90º to 60º of non-observed area from a flat-window aircraft.

g. Size of items: lower size limit, classes:

A suitable method to standardize the size of the marine litter observed is to classify the object into three main categories: Small, Medium and Large. A small object will be the one measuring ca. 30–100 cm (as an estimate, the length of a juvenile loggerhead turtle is *ca.* 30 cm); a medium-size object would measure *ca*. 100–200 cm (body length of an adult striped dolphin is *ca*. 2 m); and a large object would be > 200 cm.

h. Type and colour of items:

Different methodologies have been assessed and are currently employed for monitoring floating litter, and identifying and classifying the objects. Overall, marine litter can be classified in three different categories based on its characteristics: 1) source, 2) type of material and 3) the likely use of the item. In this protocol, we focus on the type of material. It is worth to mention the limitations posed over the accuracy of marine litter identification given the flying speed and altitude. Therefore, type and composition of marine litter objects observed will be based on a modified version of the MSFD TSG ML master list (Table 7).

Table 7. Modified master list with the list of objects observable form an aerial survey.

Plastic, Polystyrene, Polyurethane	Bags
	Boxes
	Fish box
	Buoys(*)
	Buckets
	Fishing nets
Processed wood	Pallets
Vegetable	Seaweed/marine plant
	Logs/plants parts
Liquids	Oil slick
	Isolated foam
Glass	Bottles
Textile	Clothing
Rubber	Balloons
	Tyres
Animal	Animal carcases
Unidentified material	Ropes (plastic or textile)
	Pieces (non-organic material)

(*) Only adrift buoys will be considered.

TOOLBOX – what's the equipment and staff needed for this protocol?			
Recorder (1)	**Observer (2)**	**Additional observer**	**All crew members**
- Sheets for data recording - Hard folder - GPS device -Laptop	- Pens, pencils, permanent ink pens, scissors and blank sheets - Adhesive tape of three different colours -Notebook and pen -Plasticized sheet (with protocol in it)	- Photographic camera - Notebook and pen - Watch	- Inclinometers - Binoculars - Food, drinks, dizziness pills, sun protection, sun glasses - 96º Alcohol (for cleaning windows) - Passport or ID card

3.4 AUTOMATIC PHOTOGRAPHY FROM UAVs, MANNED AIRCRAFTS AND OTHER PLATFORMS

Introduction and scope of the protocol

Methodologies for monitoring floating macroscopic litter have been mostly based on visual observation techniques applied from different platforms such as boats and airplanes (Ribic et al. 1992, Veenstra & Churnside 2011). The same platforms can be used to obtain photographs and implement automatic detection techniques for marine litter monitoring.

Automated recording of floating objects can be done through a variety of recording systems applied on Unmanned Aerial Vehicles (UAVs) or other platforms to monitor marine litter at different spatial scales in the sea. Advantages of automatic recording techniques as compared to traditional visual techniques include: reduction of human error and human risk (for pilots and/or observers); possible increase of survey effort without a subsequent increase of budget; permanent record of images, allowing subsequent statistic (re-)analyses and the answer to future questions of biological interest. Automatic photography is a reliable technique, in which the geo-referencing of observations is accurate and precise; it is constantly improving (*e.g.* through improvements in image resolution), and, when applied through UAVs, it can allow to reach inaccessible areas and repeatedly sample the same sites with minor costs than traditional aerial surveys (Bryson & Williams 2015).

The use of automated photography for marine monitoring has developed rapidly in recent years, especially with regard to marine mammal and other marine fauna monitoring (*e.g.* Koski et al. 2009; Hodgson et al. 2013), as well as surveying human activities at sea and documenting possible illegal activities, identifying litter presence and its localization in the oceans.

Independently from the platform and the instruments used for image recording, in this kind of surveys the task of recognition analysis is performed afterwards, on the video/images acquired. Various algorithms for automated image analysis and object detection are being developed and proposed, based on the characterization of pixels and the analysis of colour and/or shape of objects: these techniques are under constant improvement and their applicability on marine litter surveys is under evaluation.

The aim of this protocol is to provide a guideline for monitoring floating macro litter through the use of automatic photography techniques, applied on UAVs, small aircrafts or any kind of vessel, according to the scale and budget requirements. This protocol on field techniques and image processing is based on the results of operational experiments conducted by the University of Barcelona, CSIC and EPHE within the Studying phase (WP3) of the MEDSEALITTER project.

Covariates

a. Sampling design and period:

Spatial scale is the first thing to consider when designing a marine litter monitoring plan through automated photography. According to the monitoring scale, different types of photographic sensors can be mounted on different platforms.

For small scale monitoring, it is possible to cover photographically the whole area of interest, designing the flying/sailing routes on parallel transects, or regularly spaced concentric squares. Spacing between adjacent transects should allow approximately the 30% overlap between adjacent images. The same spacing must be considered for subsequent images, thus the shooting rate should be set according to the platform speed and the image size. Timing, height and geographic positions must be recorded

automatically from the sensor for each photo, to allow the subsequent geo-referencing. Even if building a geo-mosaic over the sea is challenging, it is possible to obtain a complete photographic map thanks to the georeferencing of images and the use of some landmarks (i.e. when flying over the area, recording pictures of the coastline). Fig. 17 shows an example of sampling design for the photographic monitoring of a small bay using a small UAV.

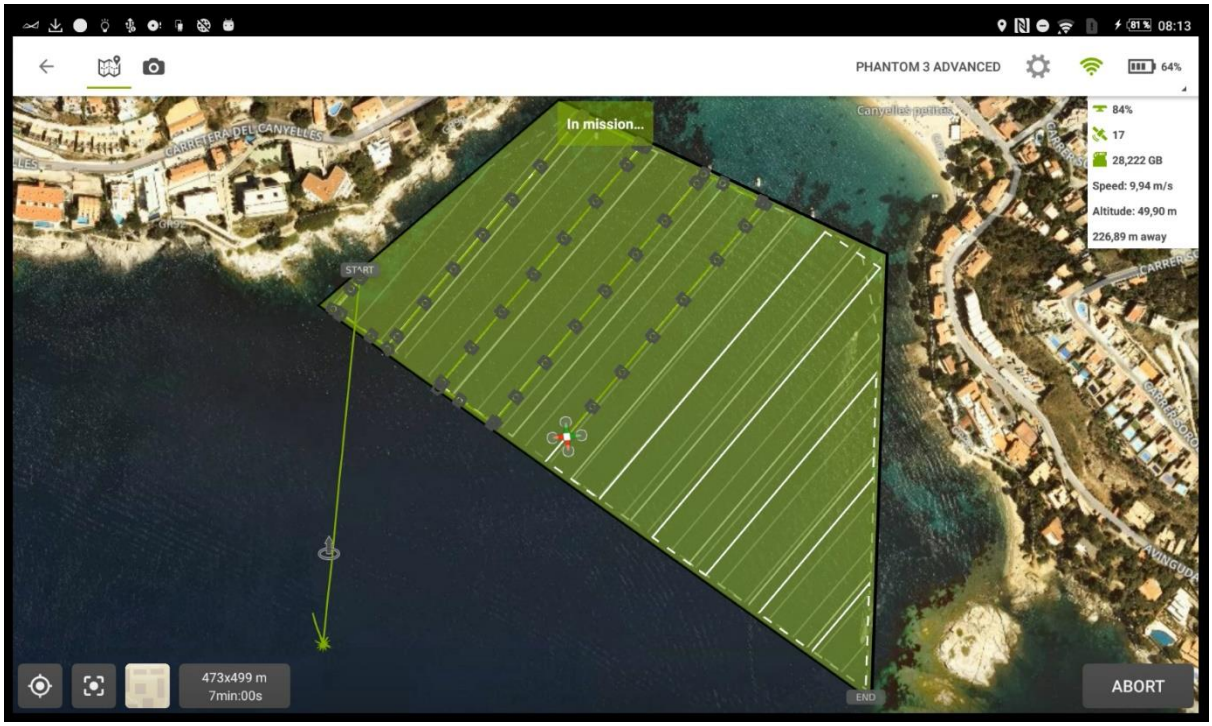


Fig. 17. Screenshot of a flight monitoring plan made through a Phantom 2 drone.

For larger areas, it is not possible to obtain a complete photographic map without a huge effort in terms of budget and time, thus the selection of smaller surface subsamples is suggested. It is recommended to select subsamples considering subareas of interest due to their ecologic, latitudinal, climatic, etc. characteristics (i.e. following a latitudinal or depth gradient). Within these smaller areas, the sampling design described above can be applied. Alternatively, the use of aerial photography from small aircrafts, could provide a more continuous image recording across the area of interest. In this case, parallel or zig-zag transects should be planned in order to cover homogeneously any possible environmental gradient.

When designing any photographic monitoring plan, it is fundamental to consider the angle of the sun (variable across seasons and with the time of the day) and plan the orientation of transects in order to limit the effect of its reflection over the water.

As for sampling period, it is suggested to reproduce the same monitoring plan at least once per season, in order to detect possible relations with currents, temperatures, and any seasonal pattern. Repeated monitoring during subsequent years provides robustness to the data obtained.

b. Type of platform:

Automated recording sensors (video and/or photographic cameras) can be mounted on a range of platforms, both flying (small aircrafts, UAVs) and sailing (ranging from a small inflatable boat with a camera attached on a pole to a large passenger ferry with a fixed sensor mounted on the top of the bow).

Each platform is characterized by a different range of speeds and heights, thus different sensors must be selected in order to maintain a minimum standard of image resolution. The selection of the most appropriate sensor should be once again done according to the monitoring scale, and the budget/time available.

When monitoring large areas (such as for basin scale surveys, or regional surveys), the use of a small aircraft is suggested, providing that sensors are selected with a resolution compatible with the height limits set by local legislation (*i.e.* increasing resolution with increasing height). Large ships, such as ferries, could also be used in case of limited budget, for opportunistic recording of images while cruising.

If monitoring is to be carried out over smaller areas, such as small MPAs or limited segments of the coastline, the use of UAVs is recommended. In this case too, it is necessary to consider local (national or even regional) safety regulations setting the maximum distance allowed from the remote controller, from the coast, from any nearby airport, and flight height limits.

Two main categories of UAV can be used for marine monitoring:

- Fixed-wing drones (Fig. 18): they have longer endurance with regard to flight distance and duration, but they present some disadvantages related to the operations of take-off and landing, especially at sea. They are less stable, sometimes limiting the quality of images recorded. Small fixed-wing drones do not transmit live recordings to the operator of the remote controller, therefore flights have to be previously programmed. Their use, due to their higher endurance, is recommended for the inspection of medium-scale marine areas and the identification of areas of high concentration of marine debris. However, considering the difficult operations of take-off and landing, the use of these UAVs is not recommended when conducting surveys from boats or from rocky coasts.

Fig. 18. Fixed-wing drone HP1, flown from the beach using a ramp-system, and recovered on the beach using a small parachute.

- Multi-copters (Fig. 19): these drones are equipped with a variable number (generally from 4 to 8) of propellers, providing a very stable structure, and allowing easy take-off and landing, and steady flights. The quality of images taken using these drones can be extremely high, allowing an accurate characterization of objects at sea. The use of multi-rotor drones, which are easier to manoeuvre, and whose recording can be transmitted directly to the control station, is recommended when operations are performed from boats or other less-stable platforms, or when high resolution photos of specific areas are required. Nonetheless, their endurance is limited, as average flight duration is 20-30 minutes. These drones are thus recommended for small-scale investigations, when a more accurate classification of sightings is needed.

Fig. 19. Multi-rotor drone.

Pilot remote-sensing surveys of marine litter can be performed using other kinds of remotely controlled systems, such as aerial balloons (Kako et al. 2012), but automated surveys can also be carried out through manned vehicles, such as small aircrafts (Fig. 20). According to local legislations, these surveys normally occur at an average height of 230 m (750 ft approximately) over the sea level. Visual observers from aircrafts could only detect large litter items (bigger than 30–40 cm), but the application of sensors on these kinds of surveys could lower substantially this limit, if cameras with adequate resolution are used.

Fig. 20. Partenavia aircraft used for aerial surveys.

c. Technique:

A series of different sensors can be applied on each platform according to the monitoring needs. The most common instruments include the 'traditional' RGB cameras (Fig. 21), which can provide very high quality (and high resolution) images and thus be used even from heights such as those reached by a small aircraft. It is important to select an adequate image resolution and photographic lens according to the planned monitoring height, considering a minimum pixel size of 2.5 cm to detect floating objects of approximately 30 cm. In good monitoring conditions, the use of these cameras allows the identification of colour, material, type and size of the items. Sun glare presence could heavily affect the quality of images obtained in the RGB visible spectrum.

Fig. 21. RGB camera Sony Alpha 7R

Other sensors can be thus coupled to RGB cameras to cope with the effect of sun glare or adverse environmental conditions: thermic cameras and multi-spectral cameras are also being experimented for automated marine monitoring (Bryson & Williams 2015).

Thermic cameras (see Fig. 22) have generally a limited resolution but could help identifying objects with a positive buoyancy that have been warmed from the sun light, such as a floating board, or even the carapax of a resting marine turtle. Moreover, these instruments are helpful to identify warmer or colder currents, like those of a water discharge, or a river mouth, that could convey a load of marine debris. Their use is suggested coupled with a visual camera, as, despite the lower resolution, they may help distinguishing items in case of sun glare presence.

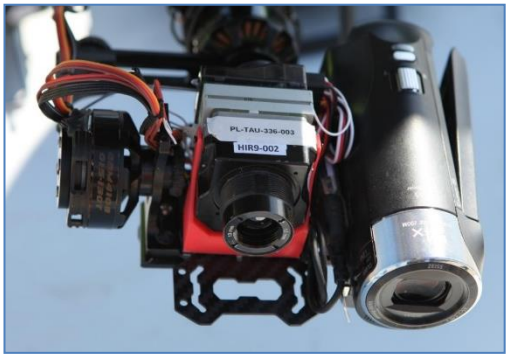


Fig. 22. Visual + thermic system, composed by a thermal imaging sensor (FLIR TAU-2 640) and a visual sensor (Sony cx240).

Multi – spectral cameras (Fig. 23) can also help the identification of floating items in case of sun glare, as their sensors are less affected by it. Also these sensors have a generally lower resolution than traditional RGBs cameras, however, they could be useful to distinguish different materials from the sea water and among them, as each material presents different spectral characteristics. Their coupled use with RGBs cameras is suggested.

Fig. 23. Multi-spectral camera Micasense Red-Edge.

d. Experience of the observers:

For this protocol no actual observers are implied, while instead two or more photo interpreters are needed if no automatic detection techniques are used. Some training of the photo interpreters is needed in order to make them familiarize with the most common categories of litter included in the master list, as well as to train them to distinguish possible effects of sun glare from actual floating items.

e. Weather and visibility conditions:

As for any other kind of survey, sea state surface (*i.e*. Beaufort scale) is a factor to consider when planning the monitoring, as the presence of white caps in the sea, like it happens with visual monitoring, could bias the probability of marine litter detection. Thus, monitoring should take place only with Beaufort < 3. When performing aerial surveys, strong winds conditions must be avoided also because they would limit the possibility to fly of both UAVs and manned aircrafts.

Visibility and the percentage of cloud covering must also be taken in consideration, as a reduced visibility (*e.g.* because of fog) or a spotted cloud covering could decrease the probability to detect floating object through automated photography.

Finally, but most importantly, the effect of sun glare reduces dramatically the probability of detecting marine litter, both when images are checked by human eye and when the detection is run automatically. It is thus important to plan monitoring when the sun glare effect is limited, preferring the early morning or the late afternoon hours, when the sun is lower on the horizon. It is also important to consider the position of the sun at each time of the day, to plan transects accordingly and avoid transects oriented against sun.

f. Strip width:

When monitoring marine areas through automatic photography, the width of transects is directly dependent on the camera resolution and lenses used, and/or the height from which the photos are taken. Therefore, according to the needs of each monitoring program, height (of flight, or of the position on a ferry or a smaller boat where the camera is mounted) can be reduced to obtain more detailed pictures but covering smaller areas, or increased to cover larger areas but with lower quality images. Conversely, sensors should be selected with a higher resolution if the position of the camera above the sea is higher.

g. Size of items: lower size limit, classes:

Size of marine litter can be easily determined knowing the resolution of each image. If the size of a pixel is known, the size of floating objects can be calculated precisely using image analysis software. The lower size limit, as explained above, is dependent on the relative curve height/resolution, that must be calculated for each platform/instrument. In an image with a pixel size of approximately 2.5 cm, it would be possible to distinguish objects of approximately 30 cm. When pixel size is reduced (due to decreasing height or increasing resolution), the probability to detect smaller objects increases.

h. Type and colour of items:

The accuracy of marine litter identification is dependent on the quality of the images taken (which in turn is dependent mainly on the type of sensor used and its altitude). Type and composition of marine litter observed must be based on the reviewed masterlist for floating objects proposed by the MSFD TSG ML (Galgani et al. 2013a, ANNEX I), despite many of the items listed in it are of difficult identification. Broader categories of floating marine litter, based at least on litter composition, could then be considered for classification.

Image processing and analysis

Once images have been recorded, and downloaded, they must be checked for marine litter presence. To this date, a fully automated detection system has not been developed yet within the MEDSEALITTER project. However, experimentation using machine learning techniques is widespread and many user-friendly applications may be available in the future.

If images are checked by photo interpreters, it is suggested that two independent persons go through each image to detect the presence of any floating item.

After this preliminary screening, a simplified automated analysis of the images should be run. To this scope, images have to undergo some processing to estimate the detectability of litter according to the parameters selected for monitoring (*e.g.* flight height, image resolution, effect of glare, minimum size of detectable litter). The processing procedure is the same for RGB and multispectral images.

Processing of images involves 3 steps:

1. Statistical analysis of detectability

On this regard, it is necessary to:

1.1. Select a sub-set of test images in which litter is present.

1.2. Interactively delineate training polygons of the different types of categories according to what the photo-interpreter can distinguish (at least "litter" and "water") (see Fig. 24).

In case polygons are drawn with QGIS (which does not allow setting an arbitrary Euclidean coordinate system), and to ensure ulterior bulk-processing, it is necessary to:

- set the photo to a coordinate reference system (CRS) with a rectangular geographic projection (*e.g.* ETRS89, UTM31N, epsg 25831);
- make sure to create the vector file in the same projection system.

Fig. 24. Training polygons for the different litter categories

1.3. Mask areas affected by sun glare. Having found sun-glare as a major cause of miss-detection, it is important to run an automatic process that detects, in a conservative way, the areas of sun-glare and creates a mask. This mask will define the area not to be used for detection (see Fig. 25 for an example, in which sun glare affects the top left corner of the image).

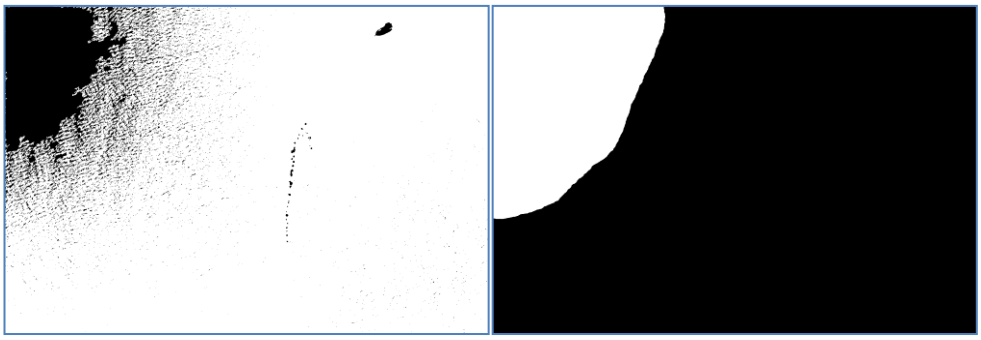


Fig. 25. Example of sun glare effect and image masking.

1.4. Extract RGB values for the polygons, run a Linear Discriminant Analysis (LDA) to visualize discrimination in LD space and classify using cross-validation to produce a confusion matrix and calculate global, user's and producer's accuracy, along with rates of True Litter (TL), True Water (TW), False Litter (FL) and False Water (FW) cases.

2. Candidate Objects extraction

Classifying every pixel of the image would be too demanding in terms of computing power, hence it is necessary an automatic process to detect patches in the image that could be objects. One possibility can be extracting the candidate objects using a threshold in software such as ImageJ and converting the obtained mask to a vector using the gdal_polygonize function (Fig. 26). After that, the vector can be used to extract target pixels from the RGB image and then classify them as water/debris using the LDA model.

Fig. 26. Example of the mask obtained from ImageJ and the respective vector of the candidate objects.

3. Classification

Using results of the LDA, all candidate objects are then classified as "Litter" (eventually, different types of litter depending on the results obtained from the previous LDA) or "water" (see Fig. 27 for an example: red dots represent TL, pink dots FL, dark blue dots TW and light blue dots FW).

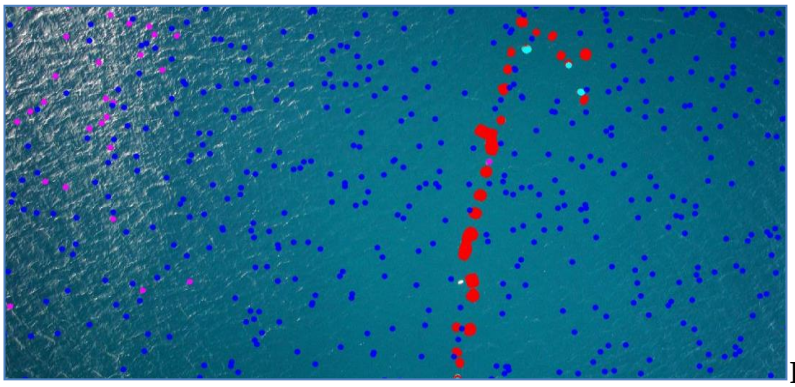


Fig. 27. Result of an image classification.

The automation of this whole process would provide a good classification of possible floating objects within each image.

Video processing and analysis

The proliferation of high-powered computers, the availability of high quality and inexpensive video cameras, and the increasing need for automated video analysis has generated a great interest in live object tracking algorithms (Sindhuja & Renuka Devi 2015; Yilmaz et al. 2016). Object tracking is the procedure for discovering moving objects beyond time using the camera in video sequences (Kothiya & Mistree 2015). Its main aim is to relate the target objects, their shape or features, and location, in successive video sequences. Object detection and classification are essential for object tracking in computer vision application (Tiwari & Singhari 2016). The basic steps for tracking an object are described below:

a) Object Detection is the process to identify objects of interest in the video sequence and to cluster their pixels. It can be done through techniques such as temporal differencing (Joshi & Thakore 2012), frame differencing (Rakibe & Patil 2013), optical flow (Sankari & Meena 2011) and background subtraction (Zhang & Ding 2012).

b) Object Representation involves various methods such as shape-based representation (Patel & Thakore, 2013), motion-based representation (Patel & Thakore 2013), colour-based representation (Zhang & Ding 2012) and texture-based representation (Lee & Yu 2011).

c) Object Tracking implies estimating the trajectory of an object in the image as it moves around a scene. Point tracking, kernel tracking and silhouette tracking are the approaches to track the object.

Detecting objects in images and videos accurately has been highly successful in the second decade of the 21st century due to the rise of machine learning and deep learning algorithms. Specialized algorithms have been developed that can detect, locate, and recognize objects in images and videos, some of which include RCNNs, SSD, RetinaNet, YOLO, and others. Google recently released a new Tensorflow Object Detection API to give computer vision everywhere a boost.

Application on UAVs, hardware & software

The **M**otion **I**magery **S**tandards **B**oard (MISB) develops standards for Motion Imagery (MI) assets. The MISB standard commonly used for Air Systems is MISB ST 0601. **STANAG** (NATO **STAN**dardization **Ag**reement) commonly refers to the specific agreement STANAG 4609 in the context of geospatial data contained in video. This agreement recommends to use MISB ST 0601 for UAS (Unmanned Air Systems). These features have applications in aerial inspections, search and rescue, law enforcement, broadcast, and may be of interest for the detection of floating items at sea. Today MISB Data is commonly found with high-end gimbals and military equipment, but is now making its way towards the rest of GIS market.

To record MISB-enabled video, hardware might need to provide a combination of a laser rangefinder, slant range, or altitude to determine target location. Some hardware may provide target location as GPS coordinates directly, others may give only the row data and the user determines location afterwards. Some instruments may give data as a KLV stream within the container, others may hide the data in the raw video bitstream. There are numerous hardware and software for encoding the video track and GPS metadata tracing and for creating and viewing a video transport stream (.ts) conforming to STANAG 4606.

The Full Motion Video technique integrated with the OBIA classification and/or the autodetection, by means of machine learning algorithms, could substantially improve the methodological protocols for the automatic monitoring of floating marine litter.

Marine biota

All the techniques above described regarding the use of sensors mounted on different monitoring platforms, can be simultaneously applied for marine macrofauna monitoring. Species that could be easily identified photographically include marine turtles, all cetacean species, and some species of large fish like the sunfish (*Mola mola*) or the Mediterranean manta ray (*Mobula mobular*). In high resolution images taken from aircrafts or drones, marine birds could also be identified (*e.g.* Fig. 28).

One of the main advantages provided by the photographic techniques is that images taken during a dedicated survey are permanently recorded and can be checked in the future when new research needs are emerged (*i.e.* the analysis of marine fauna presence and/or the identification of areas where the biota could be at risk due to the concurrent presence of litter and marine macro fauna, see next chapter 4).

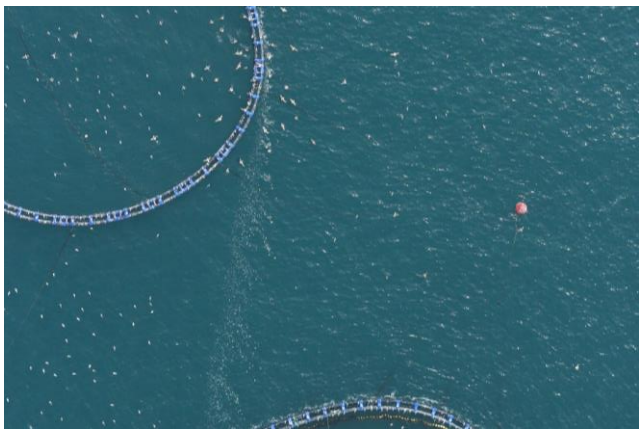


Fig. 28. Aerial photograph of fish farm nets with marine birds all around.

TOOLBOX – what's the equipment and staff needed for this protocol?

- A suitable platform (plane, UAV, etc) – and adequately trained staff to operate it
- A suitable sensor, with a technical expert to mount/dismount it if on aircrafts or large ships
- A GPS mounted on the platform or directly on the sensor (or both)
- Memory Card(s) and hard disk(s) with large memories to save images
- A computer with a good processor and possibly a good monitor to perform the photo-analyses
- Two or more photo-interpreters

4. MONITORING FML IMPACT RISK ON BIOTA THROUGH SYNOPTIC MONITORING OF KEY SPECIES OF MEGA AND MACRO-FAUNA

The objective of this activity is to identify the areas where marine fauna may be exposed to litter and quantify the associated risks of ingestion, entanglement or other impacts (*e.g.* collision, habitat loss, reduced capacity of movement, etc.). Statistical analyses may vary with available data, but rely on the combination of data on marine litter and on fauna. The first steps will be to gather the available data (observations and simulations) on FML and on mega and macro-fauna. Then, data analyses depend on the spatial scale and available data. Some examples are provided.

4.1 Step 1: Collecting data on litter distribution

Empirical data from observations at sea

They can be collected using the protocols presented in the previous chapter of this document, using different platforms ranging from small vessels to airplanes (see chapter 3).

Simulations and modeling of litter flows and accumulation areas

For now, no standard empirical data on FML is available at such large scales to allow the statistical analysis of the risky areas without a bias related to differences in protocols. Modelling can be a good way to assess marine litter accumulation areas in the entire basin. Such approach may enable considering various scenarios (*e.g.* a homogeneous initial litter density) and hypotheses (*e.g.* litter stranding zones, accumulation areas, origin and endpoint of litter items, etc.). Despite some few studies, modelling litter transport at sea is still in a relative basic state. Different tracking schemes, resolutions or model set-ups can sometimes lead to contrasted solutions. An example is described below.

Example 1: modelling floating marine litter at the entire Mediterranean Basin (from Mansui et al. work submitted for publication).

The approach here consists in investigating the spatio-temporal variability of potential FML accumulation and stranding areas at the Mediterranean scale. For this purpose, multi-annual simulations are performed using an FML distribution model developed using Lagrangian simulations, as described in Mansui et al. (2015). In such a method, virtual particles act as Lagrangian tracers and mime the marine debris transport at the sea surface. The simulation process of the particle drift consists in two stages: First, the ocean state and the velocity fields are computed by selecting an ocean general circulation model (OGCM) suitable at this scale. Then, the drift of the virtual particles is simulated thanks to an advection model using the velocity fields provided by the OGCM (the NEMO model) configured for the whole Mediterranean basin (MED12 configuration) on a 1/12° "ORCA" grid. Computing of the general transport pathways is done using the Lagrangian off-line tool ARIANE to track the virtual particles (ARIANE code available at http://www.univ-brest.fr/lpo/ariane). In the present approach, only the horizontal movements are considered, forcing the particles to remain just below the surface (first 50 cm), at the first OGCM level of velocity.

Particle input and time of advection are two key parameters in the numerical modeling of FML distribution at sea. In the present work, the same initial homogeneous particle distribution characterizes all simulations, with a spatial step of 10 km in the zonal (W to E) and meridional (N to S) directions (25,500 particles scattered in the basin). An integration time from 3 months to 1 year was considered a good compromise regarding the basin size. Finally, 1-year long runs were performed every day during 10 years and the daily particle positions were recorded in order to extract shorter integration times.

To quantify particle accumulation patterns and determine their spatio-temporal variability, a "mean binning density" index (σ) was defined according to Mansui et al. (2015). Particle accumulation or scattering can easily be distinguished thanks to the positive/negative sign of σ, respectively (initial particle density is obtained for $\sigma = 0$).

To investigate some accumulation patterns on local regions within the Mediterranean, bins of 20 km x 20 km were adopted. A sufficient number of particle trajectories in the bins was considered to ensure the robustness of the statistical analyses, and binning densities σ with advection times of 3, 6, 9 and 12 months. The origin of particles trapped in accumulation patterns was also determined to complement the information about FML potential accumulation areas. Because of the model boundary condition, particles reaching the last ocean grid cell (*i.e.* the closest to the terrestrial area) can stagnate for a long period and/or recirculate offshore after a while. For this reason, all particles that experienced long stagnation periods in a coastal strip cell were considered as stranded.

These simulations do not evidence any large or local permanent pattern of debris accumulation, in contrast to what happens in the ocean gyres. However, some seasonal patterns of FML accumulation are underlined (Fig. 29), with three largest areas in the Eastern Balearic Islands, the central Tyrrhenian Sea and off the Tunisian and Libyan coasts. Finally, according to the simulations, most modeled FML accumulation patterns occurred in the western and central Mediterranean Sea and were mainly associated to regions of high kinetic energy favoring debris concentration and scattering.

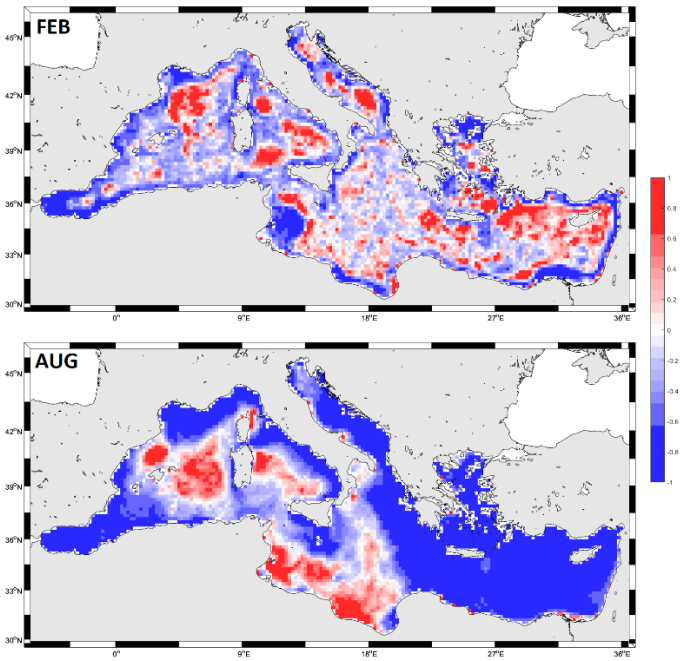


Fig. 29. Examples of simulation maps obtained from the model. Monthly mean binning densities for February (up) and August (down). Red tones are used for particle accumulation. Gray and blue tones show emptying areas.

4.2 Step 2: Collecting data on marine fauna distribution

Simultaneously to systematic monitoring of marine litter, data is collected on the marine macro-fauna species listed in Table 8. Data on these species are collected with standardised methods: line transect can be used for all groups, whereas strip transect can be used for all the groups except cetaceans (see Buckland et al. 2001). From small and medium vessels, the line transect method is recommended for all groups as the strip width used for marine litter is too narrow to detect a number of fauna large enough for analysis.

From ferry and airplane, the methodology for all groups except cetaceans can be strip transect within the marine litter strip. From any platform, the line transect method should then be used for cetaceans. A dedicated team of Marine Mammal Observers (two to three) will perform cetacean monitoring independently, in parallel with the other observers dedicated to litter monitoring.

Table 8. List of potential species of marine mega-fauna recorded in the Mediterranean.

Group	Latin name	English name
Turtle	*Caretta caretta*	Loggerhead sea turtle
Turtle	*Dermochelys coriacea*	Leatherback turtle
Turtle	*Chelonia mydas*	Green turtle
Large fish	*Mola Mola*	Ocean Sunfish
Large fish	*Xiphias glaudius*	Swordfish
Large fish	*Thunnus ssp*	Tuna
Large fish	*Fam. Istiophoridae*	Marlins
Shark and ray	Undetermined shark	Undetermined Shark
Shark and ray	*Mobula mobular*	Devil fish
Cetacean	*Delphinus delphis*	Short-beaked common dolphin
Cetacean	*Stenella coeruleoalba*	Striped dolphin
Cetacean	*Tursiops truncatus*	Common bottlenose dolphin
Cetacean	*Grampus griseus*	Risso's dolphin
Cetacean	*Globicephala melas*	Long-finned pilot whale
Cetacean	*Ziphius cavirostris*	Cuvier's beaked whale
Cetacean	*Physeter macrocephalus*	Sperm whale
Cetacean	*Balaenoptera physalus*	Fin whale

4.3 Step 3: Combining the layers in a Geographic Information System

Any GIS software can be used to overlap different layers, including also some libraries from R (R Core Team 2018). The layers can be defined as points (punctual observations), pixels (*e.g.* simulation at the pixel scale) or polygons (*e.g.* distribution ranges), representing the data of interest.

4.4 Step 4: Evaluating the overlap areas

Assuming that areas of high exposure to marine litter are related to high risks of ingestion, entanglement or collision, the objective of the risk analysis is to assess areas where high densities of fauna overlap with high densities of marine litter. The method proposed to assess the risky areas should be adjusted depending on the scale considered and the data available. Various calculations can be automatically performed in GIS software, such as the evaluation of spatial distribution from e.g. Minimum Convex Polygon or Kernel (Worton 1995) approaches.

Various analyses may be performed to predict the density probability of fauna and marine litter and determine the influence of the latter on the distribution of the former. This could be done by using *e.g.*, Kernel density estimator (Worton 1995), Species Distribution Modeling (SDM, Guisan et al. 2006), niche

analyses (*e.g.* MADIFA, (Calenge et al. 2008); K-select (Calenge et al. 2005)), Resource Selection Function (Boyce et al. 2002) or classical generalized linear models (McCullagh & Nelder 1989). Analyses can be done considering various hypotheses about the selectivity for marine litter dense areas, mono or pluri-specific approaches, and depending on the scale of what is considered available and used by fauna (Johnson 1980; Mayor et al. 2009). These modelling methods would provide predictive maps of the risks.

Examples describing the analysis of risk areas using data collected from different platforms are detailed below.

Example 2: Overlap of floating marine litter distribution with cetacean range along the Mediterranean French coast: data from medium-size vessel (from Di-Méglio & Campana 2017).

This study investigated the composition, density and distribution of floating macro-litter along the Liguro-Provençal basin with respect to cetacean presence.

Survey transects were performed in summer between 2006 and 2015 from sailing vessels with simultaneous cetacean observations. During 5,171 km travelled, 1,993 floating items were recorded, widespread in the whole study area. Sampling was not homogeneously distributed as different areas were covered each year. To overcome these differences, all records were mapped over a 1 km × 1 km grid encompassing the whole study area, for a total of 4,665 cells. Using the fTools plugins in QGIS, the total km travelled on effort, the number and type of items observed and the number of cetacean observations were associated within each cell, to calculate standardized abundance of floating litter and cetaceans. The distance from nearest coast was also extracted for each cell. To avoid biases due to poorly surveyed cells, only those with >100 m travelled on effort (4,453 cells) were selected. On this basis, Kernel density estimation was performed to show spatial clustering of floating litter and of cetacean sightings, identifying areas of higher probability of occurrence. Analysis was weighted on the abundance values and carried out using the Heatmap plugin in QGIS over a radius of 5 km, considered an adequate range for floating litter, and therefore applied also to cetaceans. The whole distribution estimates of floating litter and cetaceans were represented by the 90% density contours, used to compare ranges and to calculate the percentage of shared surface between them.

Cetacean ranges were compared with the distribution of plastic, considered the most representative category of marine litter, using the Intersect function that extracts the surface of the area of overlap between the two layers of polygons. Overlap was calculated for all cetacean species, as well as for striped dolphin and fin whale alone, that were the most sighted ones, and reported as percentage of ranges. Higher density contours (70%) were found too limiting for the purpose of this study.

Kernel analysis identified higher distribution in the eastern part of the study area for plastic objects only, for a total coverage of 5,102 km^2. Densities estimates within the 70% probability contours defined a very reduced coverage, indicating limited areas of high accumulation. The global area (included in the 90% isopleths) estimated for cetacean presence in the whole study period occupied 3,341 km^2, mostly distributed in the eastern part of the study area. The 53.3% of this range overlapped with the distribution of plastic, sharing an area of 1,781 km^2. The total range calculated for striped dolphin was 2,295 km^2 overlapping by 61.4% with plastic distribution; fin whale sightings locations described a smaller range of 678 km^2, presenting a 45.6% of correspondence with plastic presence. Main areas of co-occurrence were identified in the eastern part of the study area, where plastic density defined larger patches (Fig. 31B). Other species showed a scattered occurrence and the low number of sightings did not allow to perform a correct density estimation; however, 9 sightings of squid-eaters (sperm whale, long-finned pilot whale,

Risso's dolphin) and one sighting of bottlenose dolphin occurring within the 90% density contour of plastic were reported, accounting for more than the half of total records for these species (Fig. 30C).

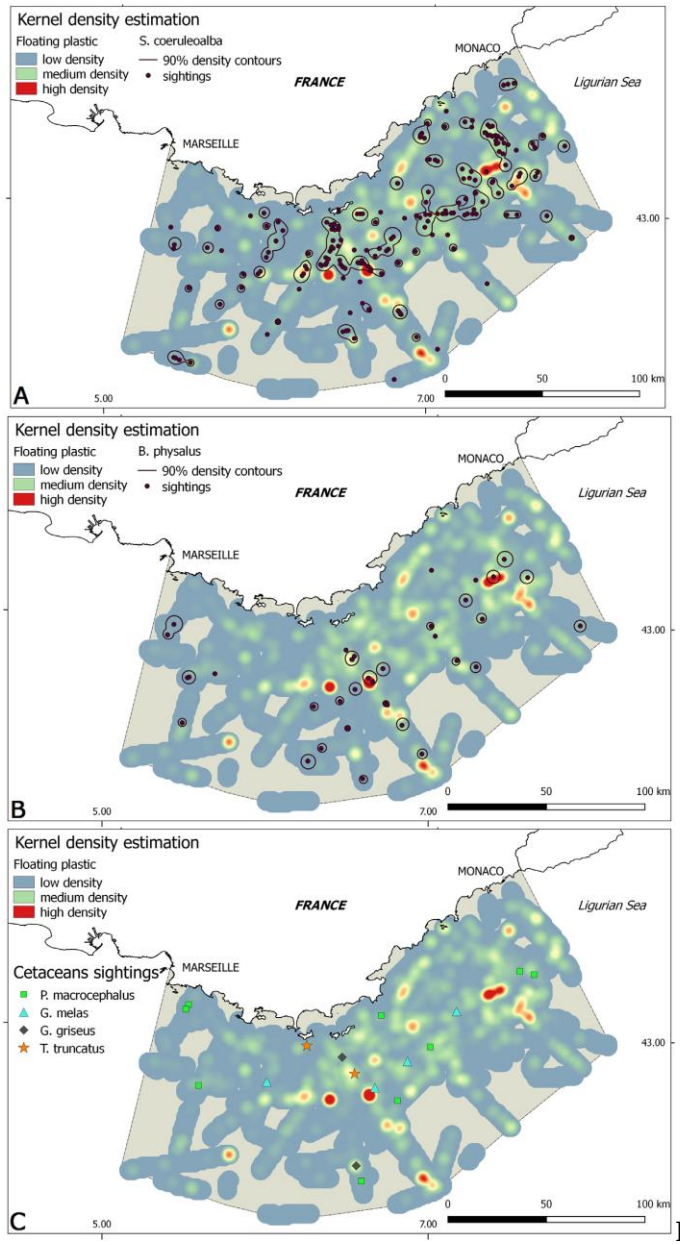


Fig. 30. Overlap between floating plastic and cetacean species. Kernel density estimation performed on 1 km × 1 km grid cells on abundance values of floating plastic and striped dolphin (A), floating plastic and fin whale (B). For other species, only the sightings locations are shown (C).

Example 3: using the MEDSEALITTER protocol to monitor litter and biota from ferries (from Campana et al. 2018)

Data on floating marine litter were collected according to the MEDSEALITTER protocol by dedicated observers along a fixed transect from Civitavecchia to Barcelona (mid-latitudes of Western Mediterranean Sea) from October 2013 to September 2016. Cetaceans observations were performed synoptically to litter monitoring by expert Marine Mammals Observers following the protocol adopted by the Fixed Line Transect (FLT) network (see Arcangeli et al. 2018 for detailed description). The study area was divided

into four sectors corresponding to the Balearic Sea, the Sardinian Sea, the most continental portion of the Bonifacio Strait, and the Tyrrhenian Sea. The amount of litter and natural debris was indicated by Density (D), estimated by applying the strip transect method, and calculated as D = N / (L*W), where (N) is the number of items recorded within the monitored area and (L) and (W) are the length and width of the strip (50 m or 100 m, depending on the weather conditions), respectively. Multiple comparisons were performed with non-parametric statistics of Kruskall-Wallis (KW) test with Mann-Whitney (MW) comparison between pairs. A preliminary analysis performed within each sector showed no significant variation of litter densities recorded within the same season among years. Therefore, the comparisons among seasons and sectors was carried out by pooling together the data collected across the three years for the same season. Spearman's Correlation was applied to the two entire datasets of litter and natural debris density, while Wilcoxon (W) test for paired samples was used to test the hypothesis of equal distribution of litter and natural debris by considering paired values for each transect. All statistical analyses were performed with the software PAST 2.17 (Hammer et al. 2001).

Spatial distribution of records was analysed over a grid of 5 km x 5 km. Using the fTools plugins in QGIS, the total km travelled on effort, the total surveyed area, and the number of artificial polymers (*i.e.* plastic) and natural items observed were associated within each cell, in order to calculate the standardised density of floating objects for each season. The distance from the nearest coast was also extracted for each cell centroid, and Spearman's Correlation was applied to investigate its possible relationships with the amount of litter.

A preliminary analysis on the gridded data showed that the mean effort in each grid cell was 2.86 km^2. To avoid biases due to outlier values in poorly surveyed cells, only those with more than 0.1 km^2 covered on effort were selected (winter: 177 cells; spring: 294 cells; summer: 304 cells; autumn: 222 cells). On this basis, sufficient data were still available to perform kernel density estimation to show spatial clustering of floating plastic and identify seasonal areas of higher probability of occurrence. Analysis was weighted on the density values, considering the large scale of the analysis, and carried out over a radius of 10 km using the Heatmap plugin in QGIS. The 70% isopleths were used to define areas of higher accumulation of floating macro-plastic, and the comparison with cetacean presence was reported for the four groups as the percentage of sightings falling within these areas.

The percentage of cetacean sightings falling within the high density areas (*i.e.* 70% isopleths; see Fig. 31) was more than 60%, during winter. As well, during spring and summer, the proportion of sightings included in the 70% isopleths of plastic was high (> 51.2%), whereas in autumn, the lowest percentage of cetacean sightings within the isopleths (11%) was recorded. The most evident overlap between the high density of plastics and cetaceans occurred in the Balearic Sea for all groups of species in all seasons (Fig.31). Fin whale and dolphin presence overlapped with high density areas of plastic in the Sardinian Sea in spring and summer; the Bonifacio Strait was an important area of overlap for fin whale in winter and for bottlenose dolphins and squid eaters (sperm whale) in spring and summer. In the central part of the Tyrrhenian Sea, a higher overlap with plastic density was observed in spring and summer for dolphins and squid eaters.

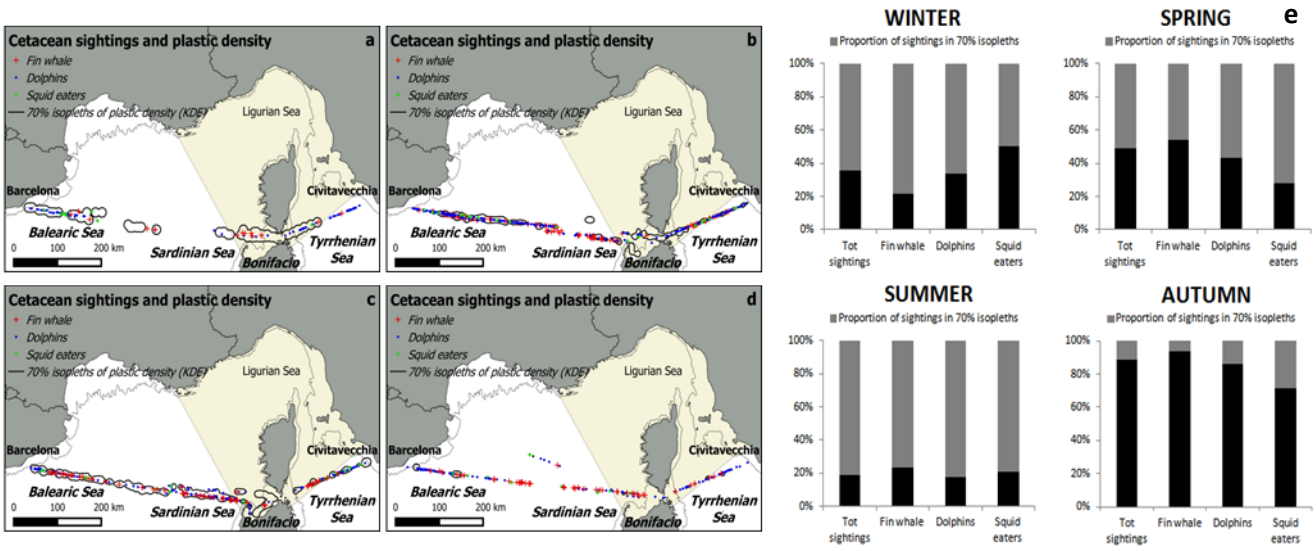


Fig. 31. (Left) Seasonal cetacean sightings and 70% isopleths of plastic density obtained from kernel density estimation along the transect from Barcelona (Spain) to Civitavecchia (Italy). a winter; b spring; c summer; d autumn. (Right) The proportions of sightings of the four cetacean groups within the isopleths are shown in grey in the histograms (e).

Example 4: Highlighting areas where sea turtles are exposed to marine litter in the Norther-Western Mediterranean area, from aerial data (from Darmon et al. 2017)

Observations of marine litter and sea turtles were made during the Marine Megafauna Aerial Survey campaign (SAMM) conducted in winter and summer 2011-2012 in the French metropolitan maritime domain (Pettex et al. 2014). For the Mediterranean façade, the campaign covered the Gulf of Lion, the North of Sardinia and the Italian waters in the Pelagos sanctuary. The surface covered was 181,377 km², with 13,762 km of transects in winter and 18,451 km in summer. The aerial overflights were performed from a Britten Norman twin plane flying at 183 m above sea surface at a constant speed of 90 knots with Beaufort sea state conditions <4. The plane was equipped with two side "bubble" windows, from which two observers noted the location and number of sea turtles and marine litter along linear 200 m wide transects. From this height, items (and individuals) larger than 20-30 cm were potentially detected in the first 2-3 m below the water surface. In total, 51 turtles were observed in winter and 332 in summer, and 8,624 and 16,481 litter items respectively during the two seasons.

The statistical analyses based on the assumption that the entire area was homogeneously sampled, with a homogeneous detection, comparable for both marine litter and sea turtles. Various libraries of the software R were used for analyses (see Darmon et al. 2017 for more details).

The marine litter and sea turtles' spatial distribution ranges were evaluated using Kernel density estimations with 95% (largest distribution range) and 10% of data (core area). Kie (2013)'s methodology was used to assess spatial distribution, selecting visually the best smoothing parameter h according to the most uniform distribution.

The 95% Kernel distribution of sea turtles extended on 316,612 km² in winter and 212,241 km² in summer, while litter distribution covered respectively 222,097 km² and 208,676 km² in the two seasons. While litter was distributed almost everywhere in the sampling areas, sea turtles were mostly occupying the North-Eastern coast of Corsica and the Balearic Islands in winter, and the area between the Balearic

Islands and Sardinia in summer. These were the main risky areas, especially in summer, when the number of observed sea turtles increased significantly.

Various methods are applicable to evaluate the overlap between two distributions, as listed in the information file associated to the function "kerneloverlap" of the library adehabitatHR in the R software. Here the overlap between marine litter and sea turtle distributions was considered as the probability of litter occurrence in the areas occupied by the turtles. This was evaluated as the volume under the litter 95% Kernel utilization distribution that was inside the turtle 95% Kernel distribution. This probability was very high in both season: 0.93 in Winter 2011 and 0.96 in Summer 2012 (Fig. 32).

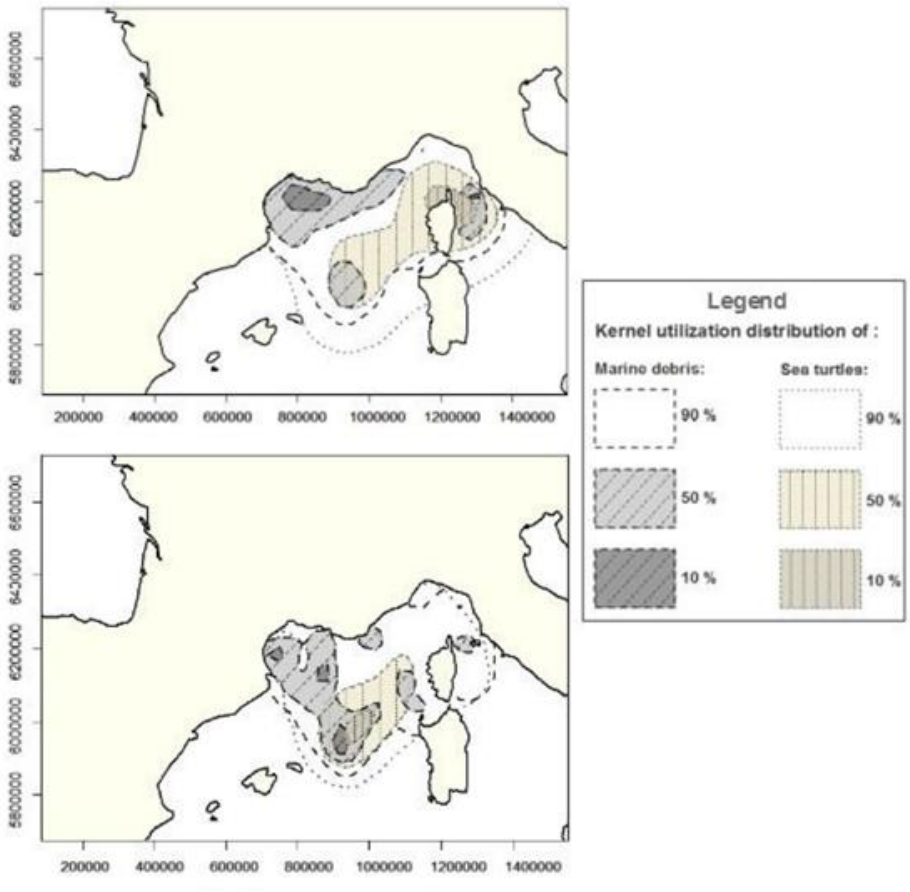


Fig. 32. Kernel home range of marine debris and sea turtle (location coordinates in Lambert 93)

The exposure of sea turtles to marine litter was evaluated using the linear distance of each turtle observation to each litter item observation. The number of locations in a radius from 50 m to 10 km every 50 m from each turtle location were calculated, and the number of items was counted at each location to assess the density of litter surrounding an individual. 99.07% of the sea turtles were surrounded by litter items in a 10 km-radius. The density of litter items was very high in summer compared to winter, with an average of 29.1 items in a 10 km-radius in winter and 88.48 items in summer.

Lastly, the authors tested whether the observed density of litter surrounding turtles could be a random process or if turtles may actively select the areas where litter accumulates (*e.g.*, preferential feeding areas where food and litter are agglomerated). For this, the observed exposure was compared to the exposure calculated from a random distribution of the same number of litter items, leaving the turtles' locations

fixed. The average observed number of surrounding items per turtle was higher than the mean number of randomized items per turtle corresponding to the probability of selectivity (*i.e*. the observed exposure was higher than the random exposure). Thus, turtles were more exposed to litter than expected by chance alone at all radius, indicating that turtles may encounter litter in the convergence current areas where both planktonic prey and litter accumulate.

4.5 Bringing the risks to light

Various protocols can be applied to evaluate the impact of litter on marine fauna. Litter ingestion in sea turtles can be evaluated using existing protocols such as the one described in this document in chapter 5. The INDICIT protocol (INDICIT consortium, 2018) also aims at evaluating the impact caused by entanglement and at describing the injuries caused by anthropogenic activities. It proposes to differentiate marine litter type, in particular distinguishing entanglement caused by active fishing from ghost nets.

A better understanding of fauna's behavioural ecology is fundamental to better evaluate the preferential feeding areas and thus to anticipate the risks. The description of diet and feeding behaviour, which can be done through various approaches (see chapter 5), can help understanding the selectivity (or avoidance) for marine litter.

Several studies focus on sea turtles because they are recognized as sentinels of their environment (indicator "Litter ingestion by sea turtles" of the Criteria D10C3 of the MSFD; New Commission Decision 2017/848/EC) and Indicator EI 18 for the Barcelona Convention covering the Mediterranean Sea). Nevertheless, these approaches tend to be developed for other taxa such as cetaceans or fish (see chapter 5).

4.6. Perspectives

The spatial and temporal stability of the risky areas should be evaluated in order to adapt management measures. This is especially important since the Mediterranean Sea, despite being highly polluted, may not allow stable litter accumulation areas due to its configuration, ecological and meteorological events as shown by simulation outputs. This may allow to evaluate the position of the Marine Protected Areas within the risky areas. This will provide arguments both to evaluate the possible role of MPAs in the monitoring of litter impacts and their capacity to efficiently participate in the implementation of restoration measures.

5. MONITORING MACRO AND MICRO LITTER INGESTED AT LARGE AND LOCAL MPAs SCALES

5.1 MACRO LITTER

5.1.1 Macro litter ingestion by sea turtles

Introduction and scope of the protocol

In the Mediterranean Sea, the loggerhead turtle (*Caretta caretta*) is considered the best indicator to monitor marine litter ingested by biota at large scale because its distribution spans the entire Mediterranean region, it is a highly migratory species and the collection of dead/stranded specimen is relatively easy. Due to the strong connection between the MEDSEALITTER project and the INDICIT project, both European co-financed projects with the common objective to harmonize protocols and adopt a single procedure among European and Mediterranean countries, it has been decided to apply the same standardized protocol on sea turtle ingestion. This protocol follows and slightly modifies the protocol proposed by the MSFD TSG ML report "Guidance on Monitoring of Marine Litter in European Sea" (Matiddi et al. 2011; Galgani et al. 2013a; Matiddi et al. 2017); it has been tested and validated during the INDICIT consortium, 2018 (https://indicit-europa.eu/protocols/) and the MEDSEALITTER programs considering basic and optional parameters proposed to stakeholders according to their logistic and time constraints. In order to have a complete harmonization of procedures between the two projects, entanglement and plastic ingestion by alive turtles are also considered in this protocol. This protocol was also submitted to the UNEP/MAP for the Joint Meeting of the Ecosystem Approach Correspondence Group on Marine Litter Monitoring.

Scope of the protocol:

Evaluate the occurrence of litter ingested by Sea turtle.

Optional: evaluate the diet of Sea turtle.

Focus species

This protocol is using data collected with the loggerhead turtle *Caretta caretta**.

**Caretta caretta* is a protected species. The operator has to verify the national laws in order to be able to handle live, dead turtles, and samples taken from them.

General design of the experiment

a. Collection of **dead** sea turtles
- Necropsy
- Separation of the digestive tract
- Collection of litter in the digestive tract
- Identification of litter items (MSFD TSG ML master list)
- Analysis (frequency of occurrence of each category, size and dry mass)
- *Optional: analysis of diet*

b. Collection of **alive** sea turtles
- Maintenance of the animals in tanks (rescue centre)
- Collection of faeces
- Collection of litter in the faeces
- Identification of litter items (MSFD TSG ML master list)

- Analysis (frequency of occurrence of each category, size and dry mass)

a. Collection of dead sea turtles

Dead turtles can be found by professional fishermen (*e.g*. by catch) or stranded on beaches (natural or induced mortality). It is the responsibility of authorized structures (NGOs, rescue centres, stranding networks, research centres, etc.) to collect and store these individuals.

The first step to implement this protocol in the area of interest (*e.g*. region and/or local MPA) is to identify the structure in charge to collect dead sea turtles in the area. A list is provided in ANNEX II to help in this identification. This list is not exhaustive, please verify local specificities if you do not find a contact.

Information on the discovery site

Species identification: Cc (loggerhead *Caretta caretta*).

Tags: If there is a tag on the flipper, specify the tag number. Indicate the presence and number of electronic chips.

Animal Identification Code: the INDICIT consortium (https://indicit-europa.eu/protocols/) proposes the following code: 2 letters for the country_2 letters for the location (*e.g*. region or institution)_the species initials_year_month_day_the number of turtle per order of collection during the year (*i.e*. FR_GR_CC_2018_05_05_4, corresponds to the 4th loggerhead found by the rescue centre of Le Grau du Roi in France the 5th of May 2018). The type of sample can be specified afterward.

Contact: Name, contact (phone, mail) and institution of the observer(s) (data collector).

Date of discovery (dd/mm/yyyy), **location of discovery** and coordinates (X, Y: in decimal degrees, or specify the coordinate system).

Description of the animal's body condition:

Conservation status or decomposition level (5 levels):

- Level 1: ALIVE,
- Level 2: FRESH (Dead recently, turtle in good conditions),
- Level 3: PARTIALLY (Internal organs still in good condition; autolysis (swollen); bad smell; colour changes in skin),
- Level 4: ADVANCED (Skin scales raised or lost; still possible to record CCL and presence of ingested plastic (only FO%) & entanglement),
- Level 5: MUMMIFIED (Part of the skeleton and the body are missing; GI tract lost).

Discovery circumstances (4 categories):

- Stranding: Animal found stranded on the beach or on the shoreline,
- By-catch/Fisheries: Animal captured actively by fishermen (*e.g*. ingestion of a hook, trapped in a net, brought back by fishermen, etc.),
- Found at sea: Animal discovered on sea surface,
- Dead at the recovery centre: The animal arrived alive, but died during its recovery.

Probable cause of death/stranding:

- Bycatch/Fisheries related: Presence of an ingested hook, decompression sickness, individual trapped in gear net (in this case, fill in the column "Entanglement type" and "Litter causing entanglement"), individual asphyxiated in a fishing gear…,
- Entanglement in litter: Entanglement in litter items other than related to fishing activity. Please fill the column "Entanglement type" and "Litter causing entanglement",
- Ingestion of litter: digestive obstruction, perforation or other symptoms,
- Anthropogenic trauma: Collision with a boat or a propeller, individual wounded with a knife, stick or harpoon…,
- Natural trauma: shark attack, etc.,
- Natural disease: Related to malnutrition, buoyancy trouble, cachexia, dermatitis, conjunctivitis, rhinitis…,
- Oils: Ingestion or external impregnation with oils,
- Healthy: No remarkable damages, injury or disease,
- Unidentified: Impossible to know the cause of death/stranding,
- Other.

Health status (level of body condition):

- Poor condition (concave plastron),
- Fair condition (flat plastron),
- Good condition (convex plastron).

By-catch engine cause:

- Longline
- Trawl
- Fishing net (drifting, gillnet, trammel)
- Fishing rod
- Non-identified
- Other

Main injuries:

- FRACTURE (On carapace, head, jaws, plastron or bones, usually caused by boat collisions),
- AMPUTATION (Partial: one or more flippers need to be amputated, or total: one or more flippers missing),
- SECTIONING (Cuts or shearing produced by different kinds of litter usually on flippers or neck),
- ABRASION (Lost or wear of scales produced by the friction of material adhering to the animal or causing entanglement).

Affected body part:

- RFF for the right front flipper,
- LFF for the left front flipper,
- RRF for the right rear flipper,
- LRF for the left rear flipper,

- neck,
- carapace,
- plastron,
- head,
- several (if several parts of the body are impacted),
- other.

Entanglement type (according to 3 categories):

- Active (bycatch): Related to active fishing gear, *e.g*. the caught individual has been released by a fisherman (no fishing gear remaining on the animal), or a part of the active entangling net has been cut (by someone or the animal itself) to release the entangled individual (part of the fishing gear remaining attached to the animal). The presence of a hook is considered as active entanglement,
- Passive: The individual entangled in a litter which is either not related to fishing activity or related to fishing activity but was abandoned at sea for a long time (signs of old age; please specify in the column "Notes"),
- Undetermined: wounds/lacerations traces without fishing gear/marine litter remaining.

Litter causing entanglement:

- Pieces of net (N),
- Monofilament line (nylon) (L),
- Rope or pile of ropes (R),
- Plastic bag (Pb),
- Raffia (Rf),
- Other plastics (Ot),
- Multiple materials (Mu),
- Unknown (Unk).

Other descriptive parameters (e.g. sex, fat reserves, etc.).

Biometric measurements (Standard Curved Carapace Length (CCL), notch to tip)

Turtle necropsy[1]

Collection of the gastrointestinal system (GI):

Remove and separate the plastron from the carapace through an incision on the outside edge.

Expose the GI by removing the pectoral muscles and the heart of the animal.

Clamp the oesophagus proximal to the mouth and clamp the cloaca, the closest to the anal orifice. Remove the entire GI and place it on the examination surface.

Isolate the different parts of GI (oesophagus, stomach, intestines) by strangling and cutting between 2 clamps, the gastro-oesophageal sphincter and the pyloric sphincter.

[1]This protocol has to be applied in authorized facility, and follows the standardized protocol (INDICIT consortium, 2018). The list of these facilities is provided in ANNEX II.

Extraction of the gut content:

Separate the 3 parts of the GI (oesophagus, stomach, intestines) by adding a second strangling at the cut edge to prevent spillage of the contents.

Open each GI section lengthways using a scissor and slide the material directly out of the section on a 1 mm mesh sieve.[1]

Clean out the content with abundant tap water.

Inspect the content for the presence of any tar, oil, or particularly fragile material that must be removed and treated separately.

Rinse all the material collected on the 1 mm sieve and store it in jars with 70% alcohol or in zipped bags at -20ºC, reporting on the label the sample code (individual code and respective GI section).

Note the presence of any digestive occlusion or perforation caused by litter.

Litter classification

Sort by visual observation the collected material on a petri dish and dry all items, (marine litter, food remain and natural no food remain) at room temperature or in a stove at 35°C maximum. For each GI section of the necropsied individual, classify the litter items according to the categories provided by the protocol INDICIT (https://indicit-europa.eu/protocols/), as in Table 9.

Table 9. Standard categories of litter to be used for identification of litter ingested by sea turtles.

Ind Plastic	Industrial plastic granules (usually cylindrical but also oval spherical or cubical shapes) or suspected industrial items, used for the tiny spheres (glassy, milky, …)
Use she	Remains of sheet, *e.g.* from bags, cling-foils, agricultural sheets, rubbish bags etc.
Use thr	Threadlike materials, *e.g.* pieces of nylon wire, net-fragments, woven clothing
Use foa	All foamed plastics *e.g.* polystyrene foam, foamed soft rubber (as in mattress filling), PUR used in construction etc.
Use frag	Fragments, broken pieces of thicker type plastics, can be a bit flexible, but not like sheet-like materials
Other (Use Poth)	Any other plastic items, including elastics, dense rubber, balloon pieces, soft airgun bullets.
Other Litter (non plastic)	All the non-plastic litter, cigarette butts, wood, metal, paper items
Natural Food (Foo)	Natural food remains
Natural No Food (Nfo)	Anything natural, but which cannot be considered as food (stone, wood, pumice, etc.)

Collection of data

[1]If you want to apply the protocol for diet, please refer the paragraph on diet analysis at the end of Chapter 5.1.1.

Record the dry mass of food remains (undigested material from the animal diet) and of natural no food remains (any natural item not derived from the animal diet), and for each litter category record the following parameters:

- Dry mass (grams, precision 0.01 g),
- Number of ALL items: record all counted items,
- Total number of plastic items: record only plastic items,
- Occurrence: record the presence or absence of ingested litter.

b. Collection of alive sea turtles

Alive sea turtles can be found by professional fishermen (*e.g.* by catch) or stranded on beaches. It is the responsibility of authorized structures (NGOs, rescue centres, stranding networks, research centres, etc.) to collect these individuals, which are treated in rescue centres.

The first step to implement this protocol in the area of interest (*e.g.* region and/or local MPA) is to identify the rescue centre in charge of sea turtles in the area. A list is provided in ANNEX II to help in this identification. This list is not exhaustive, please verify local specificities if you do not find a contact.

If the sea turtle dies at the rescue centre after excreting plastic items, this sample should be added to the necropsy file including the excreted items from the intestine column.

Information on the individual

Species identification: Cc (loggerhead *Caretta caretta*).

Tags: if existing tag on the flipper, specify the tag number. Indicate the presence and number of electronic chips.

Animal Identification Code: the INDICIT consortium (https://indicit-europa.eu/protocols/) proposes the following code: 2 letters for the country_2 letters for the location (*e.g.* region or institution)_the species initials_year_month_day_the number of turtle per order of collection during the year (i.e. FR_GR_CC_2018_05_05_4, corresponds to the 4th loggerhead found by the rescue centre of Le Grau du Roi in France the 5th of May 2018). The type of sample can be specified afterward.

Contact: Name, contact (phone, mail) and institution of the observer(s) (data collector).

Date of discovery (dd/mm/yyyy), **location of discovery** and coordinates (X, Y: in decimal degrees, or specify the coordinate system).

Biometric measurements (Standard Curved Carapace Length (CCL), notch to tip).

Collection of faeces

The collected faeces will be analysed only for the individuals remaining at least 1 month in the rescue centre. The faeces are collected during 2 months after the individual's arrival.

Tanks:

Carefully rinse the turtle with water to avoid contamination and place the animal in an individual tank.

Put a 1 mm filter in all the discharge tubes of the tank.

Control the water tank daily by filtering through the 1 mm mesh sieve according to the following methods:

- Collect the faeces manually with a 1 mm mesh dip net,

- Put a 1 mm mesh flexible collector in the drain tube,
- Place a 1 mm mesh rigid sieve under the drain.

Samples that cannot be analysed directly can be conditioned in a tube or a zipped bag, identified with a permanent marker (animal identification code and date of collection) and stored at -20 °C or in 70º alcohol at room temperature, pending the laboratory analyses.

Collection of litter from faeces

Wash the sieves and collectors with abundant water above a rigid sieve (1 mm mesh).

Sort by visual observation the collected material on a petri dish and dry all items (marine litter, food remains and no food remains) at room temperature or in a stove at 35 ºC maximum.

Litter classification and collection of data

The protocol is the same as that for dead sea turtles.

Optional: Diet analysis

The aim of this protocol is to identify the diet with the classical method (biological fragment determination by visual observation) and the eDNA method (analysis of remaining DNA in the gut content).

This protocol is applied during the necropsy, thanks to the extraction and washing of the gut content.

Equipment

For sample collection (eDNA) and conditioning:

- Bucket of 8 litres minimum
- Needleless syringe or disposable pipette
- 50 ml Falcon tube
- Graduated 100 ml cylinder
- Beaker
- Spatula
- Precision balance
- Absolute ethanol, demineralized water, Sodium acetate

For visual identification and conditioning:

- Identification guide
- Stereoscope
- Camera
- Plastic bag (storing of hard parts)
- Tube of several size filled with 95° alcohol (storing of fresh material)

Extraction of digestive content and storage

All the equipment must be dipped into a 0,5% domestic bleach solution (calcium hypochlorite) during at least 2 hours before use.

Slide the digestive content directly on a 1 mm mesh sieve as described above, ensuring to place a bucket underneath the sieve.

Rinse thoroughly the content on the sieve with tap water, while collecting the rinsing water in the bucket (Fig. 33).

If the bucket is full before the complete washing of the digestive tract, homogenize the liquid with a spatula, take a sub-sample of 1 L and store it in a bottle (previously cleaned with bleach solution). Repeat sub-sampling several times if necessary.

Once the litter is separated for macro-plastics analysis (see above), collect all the remaining material on the sieve and store it in tubes or zipped bags, reporting the sample code (individual code and respective GI section).

If the samples cannot be analysed directly, the tubes/zipped bags must be stored at -20 °C, until further analysis.

Fig. 33.

eDNA sampling and storage

Once the entire digestive content is rinsed through the sieve, mix the content of the bucket with the sub-samples (if any) with a spatula (Fig. 34).

Fig. 34.

Collect 45 ml of this solution directly from the bucket using the needleless syringe or a disposable Pasteur pipette and store it in the 50 ml Falcon tube (Fig. 35).

The Falcon tube is filled with 33 ml of absolute ethanol and 1.5 ml buffer of molar mass sodium acetate

The Falcon tube containing eDNA should then be tagged with the code of the individual and stored at 4 °C.

Fig. 35.

Visual identification of digestive content

Prior to visual identification, dry the digestive content from each GI section at room temperature during 24 h minimum. Then, sort the dry materials by the main prey groups encountered (*e.g.* crustaceous, gastropods, bivalves, echinoderms, algae, unidentified, etc.) (Fig. 36). Identify each prey group item to the lowest possible taxonomical resolution, using the stereomicroscope with identification guides, if needed, and, when feasible, with the support of identification experts.

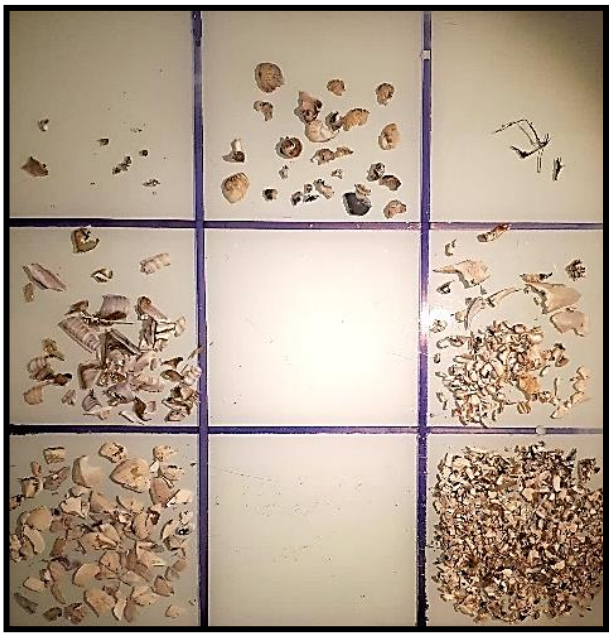


Fig. 36. Dry digestive content sorted into 7 groups.

5.2 MICRO LITTER

5.2.1 Micro litter ingestion by fish

Introduction and scope of the protocol

Fish are recommended bioindicators for monitoring microlitter ingestion in the Mediterranean Sea (Galgani et al. 2013a, Fossi et al. 2018). This protocol aims to evaluate occurrence of microlitter ingestion in fish species in Mediterranean MPAs. It follows the MSFD TG 10 Guidelines (Galgani et al. 2013a) and it is based on the DeFishGear protocol for monitoring microplastic litter in biota (Tsangaris et al. 2015) with modifications for improvement, both in terms of target species selection and sample processing for the detection of microplastics.

Selection of species

Criteria for the selection of target species for monitoring microplastic ingestion in the Mediterranean Sea include: species distribution throughout the Mediterranean basin, gut length, home range and vagility, commercial value and the documented occurrence of marine litter in gut content (Bray et al. 2019). At local scale (*e.g*. inside MPAs) the target species should reflect the environmental conditions in which they have been collected. For this reason, animals with a long transit time should be avoided.

Based on the above criteria, the most suitable target species were identified for different habitats: *Engraulis encrasicolu*s (pelagic); *Hygophum benoiti, Myctophum punctatum* and *Electrona risso* (mesopelagic), *Boops boops* (benthopelagic), *Mullus barbatus* (demersal), and *Chelidonichthys lucerna* (benthic) (Bray et al 2019).

However, *Boops boops* is the recommended target fish species because of:

- high frequency of occurrence of microplastic ingestion (Deudero & Alomar 2015);
- high spatial variability of microplastic ingestion (Nadal et al. 2016).

In addition, this species is among the target species considered for monitoring microplastics by UNEP/MAP (UNEP/MAP WG.439/Inf.12.2017). The report on UNEP/MAP IMAP indicator 24 addressing litter impacts on biota (UNEP/MAP SPA/RAC 2018) also proposes *Boop*s sp among the fish species to be used for monitoring microplastic ingestion together with *Mullus* sp. Furthermore, *B. boops* is used as bioindicator for chemical contaminants monitoring in the UNEP/MAP MED POL programme.

Although *B. boops* is the target species proposed in this protocol, due to fishing limitations of this species, it is not always available within MPAs (depending of the fishing techniques applied within MPAs).

Alternative target species can be used if *B. boops* is not available (Bray et al. 2019).

Selection of extraction method for the detection of microplastics

Sample processing for the detection of microplastics in the gastrointestinal tract (GI) of the fish includes the digestion of the GI with a chemical agent in order to degrade organic matter and facilitate detection of microplastics. The digested material is subsequently filtered and microplastic particles are retained on the filter. Currently, various digestion methods are being used for the extraction of microplastics in marine organisms.

The selection of microplastic extraction method in this protocol was based on testing of different methods in terms of digestion efficiency, microplastic recovery as well as the time required for digestion (see the "Report of testing activities and results" document) during the studying phase of the MEDSEALITTER

project. Based on these tests, digestion with 15% H_2O_2 at 60 °C is the recommended extraction method for the detection of microplastics in fish in the current protocol. 10% KOH at 40 °C was also found effective in terms of digestion efficiency and microplastic recovery although required more time than H_2O_2 for the digestion process.

Collection of fish

Fish sampling can be carried out in collaboration with fishermen or by MPA staff. The location of the fish catch must be known and recorded. Recommended sampling frequency is twice per year and a minimum number of 50 samples per species per location should be used. The following information should be recorded: fishing location, sampling gear used, species, date and time of capture, depth. Fish may eject stomach contents during sampling so care must be taken to discard such specimens. Immediately after sampling, the fish are rinsed, frozen and stored at -20 ºC until analysis. Fish samples should be transported frozen at the reference laboratory (see list in ANNEX II) for sample processing for the detection of microplastics.

Sample processing for the detection of microplastics

Fish sample processing for the detection of microplastics in the current protocol is as follows.

1) Fish preparation

Fish are thawed in the laboratory at room temperature.

2) Biometric measurements of the fish

• Weigh the whole fish (mandatory).

• Measure total length of the fish (from the tip of the snout to the end of the caudal fin) (mandatory).

• Measure its circumference with a tape (the most convex part of the fish at the end of the extended pectoral fins).

• Record visible deformations.

• Record gender.

• Record maturity stage.

3) Dissection of the fish

• Extract the entire GI.

• Weigh and rinse the GI with purified water (*e.g.* milli Q).

• Do not include the liver for microplastic analysis. Isolate the liver and store it for parasitology analysis (see Ana Perez-del-Olmo of the University of Valencia for the relative protocol) (optional).

• Place a filter paper in a petri dish (blank sample) in the working area during fish dissection to test for airborne contamination.

4) Digestion of the GI (Fig. 37)

• Place the entire GI in a suitable Pyrex beaker (150 ml for GI ≤2 g or 250 ml for GI ≥2 g). To avoid losing content, digest the entire GI and not just its content. The GI can be divided in two subsamples for faster digestion since time required for digestion depends on the amount of tissue to be digested.

• Add 20 ml 15% H_2O_2 per gram of tissue to the beaker (1:20 w/v). Prepare the required volume of 15% H_2O_2 daily (by mixing equal volumes of H_2O_2 30% and distilled water) in a graduated cylinder. H_2O_2 containers must be kept away from light. The required volume of 15% H_2O_2 in each sample is added gradually (in 2 aliquots if GI ≤2 g or more aliquots for GI ≥2 g).

• Cover samples with aluminum foil throughout the digestion process (2-4 days, depending on the sample weight).

• Place the beaker on a hot plate (several beakers can be placed on the same plate) or in a water bath at 60 °C throughout the digestion process until the organic matter is digested (translucent solution that may be of various colors). If organic matter is not fully removed by the time H_2O_2 is close to evaporation, add more 15% H_2O_2 until nearly all of the organic matter is digested.

• Stir the solution in the beaker every 20 minutes (shake the beaker by hand).

• To prevent the organic material from sticking to the walls of the beaker, do not leave the hot plate on at night or set it at a very low temperature.

• Change the foil if it gets damaged by H_2O_2 not to contaminate the samples.

• Use a blank sample to test for possible ambient contamination (add similar volume of 15% H_2O_2 as that used in the samples in a beaker without samples, and follow the protocol described in the steps 4-9).

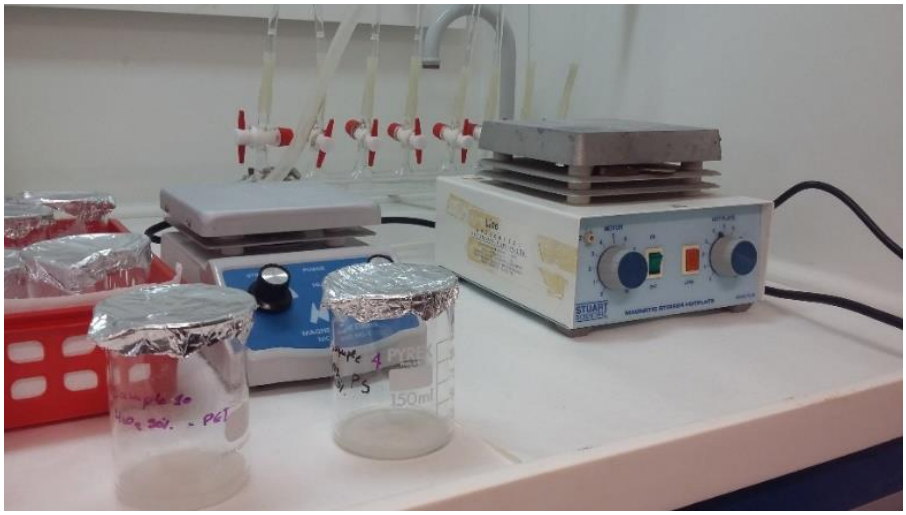


Fig. 37. Digestion process.

5) Dilution and homogenization of the digested sample (Fig. 38)

• Add 100 ml of distilled water (d H_2O) to the beaker, add a magnet and place on a magnetic stirrer (high speed for 1-2 minutes).

• Let stand 1 to 2 minutes.

Fig. 38. Dilution and homogenization process over magnetic stirrer.

6) Vacuum filtration of the digested sample (Fig. 39)

• Carefully place a GFC filter on the Buchner funnel (porcelain or glass fit). It is recommended to use a 500 ml vacuum flask for a more ergonomic handling.

• The filters used are as follows (Fig. 40): GFC 1.2 µm 47 mm in diameter.

• Empty the contents of the beaker into the funnel (rinse the magnet in the beaker with d H_2O and the walls of the beaker and funnel) and filter under vacuum.

• To avoid contamination, carry out filtration in a glove box (Captair Pyramid style laptop - Erlab, see Fig. 39).

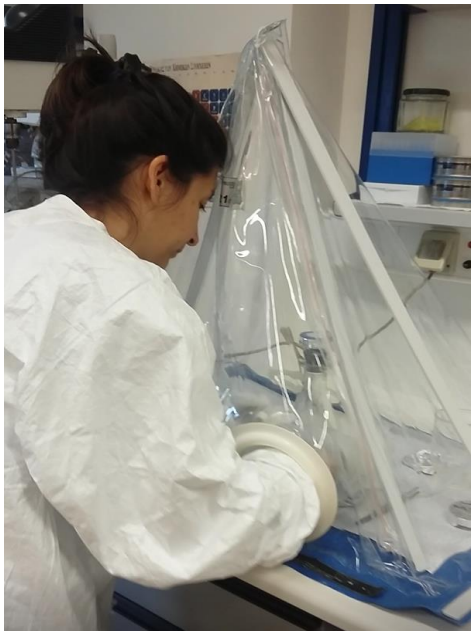


Fig. 39. Filtration process

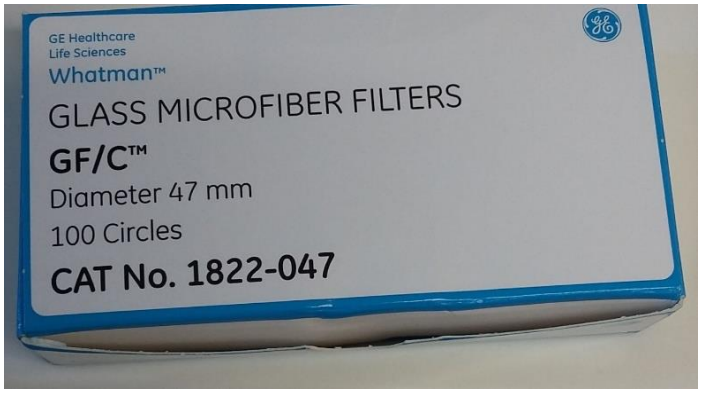


Fig. 40. Filters to be used for the filtration process (0.2 µm and 1.6 µm are also suitable knowing that the micro plastics sought are much larger)

7) Drying of samples

• Remove the filter from the Buchner funnel by sliding it directly into a glass Petri dish (plastic Petri dish can be used if completely covered inside and outside with aluminum foil).

• Place the Petri dishes in a clean cupboard for drying filters at room temperature.

8) Observation of samples under stereo-microscope - Identification of microplastics (Fig. 41 & 42)

• 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. Note position of the particles that should be checked. The Petri dish can be marked in 9 zones to note in which zones the different particles are found.

• Check the particles with a tweezer: when a particle easily disintegrates in pieces in contact with the clamps it is usually tissue. Suggestions to identify microplastics include the following: no cell structure, uneven, sharp and crooked edges, uniform thickness and distinctive colors (blue, green, yellow, etc.).

• Photograph, count and record type, color and maximum length of microplastic particles using image analysis software. Categorize microplastic particles according to the MSFD TSG ML Guidelines (see table 11 and ANNEX I for an updated list of marine litter categories).

• If performing Fourier Transformation Infrared (FTIR) analysis for polymer identification, particles can be moved on the outside of the filter (Fig. 41) before the FTIR analysis (to have easy access to the latter). If performing MICRO-FTIR a different membrane should be used (*e.g.* aluminium, gold, silver).

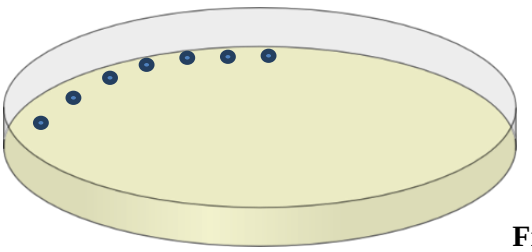


Fig. 41. Plastic particles positioned on the contours of the filter.

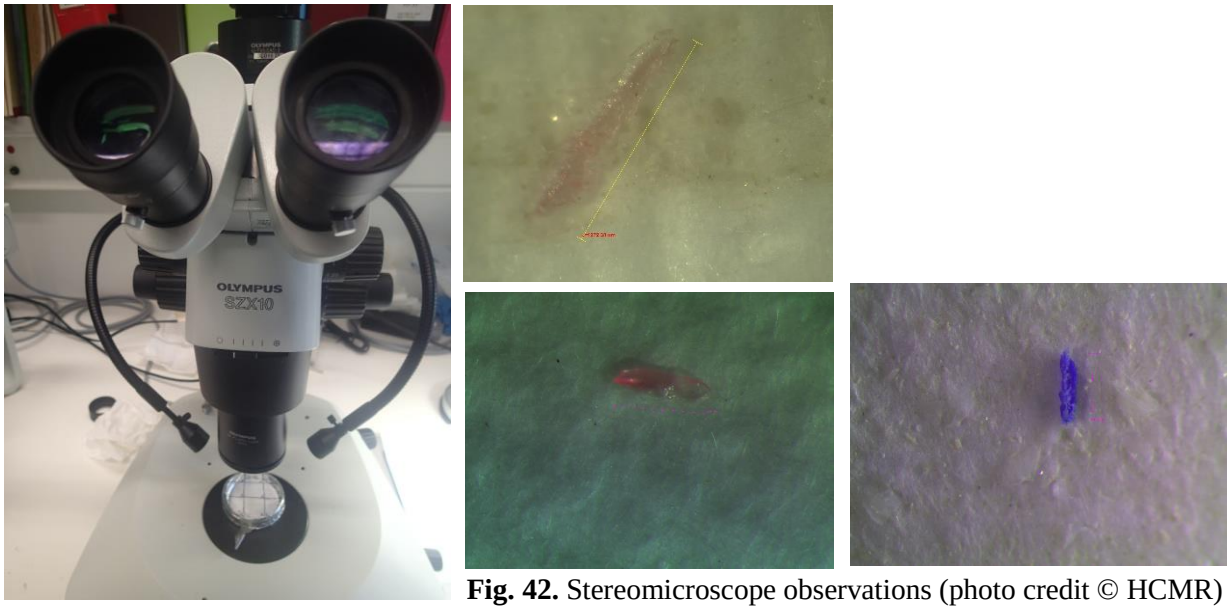


Fig. 42. Stereomicroscope observations (photo credit © HCMR)

9) OPTIONAL: Plastic polymer identification (Fig. 43)

FTIR spectroscopy is used to determine the polymer composition and confirm the polymer origin of the detected particles. Alternatively, Raman spectroscopy can be used for polymer analysis. FTIR spectroscopy can be used for analysis of particles > 200 μ, while μFTIR and FPA-FTIR coupled with microscopy or Raman spectroscopy can be used for analysis of smaller size particles. It is recommended to analyze at least 10% of the detected microplastics as suggested by the MSFD Guidelines (Galgani et al. 2013a). However, FTIR/Raman spectroscopy is often not available (not all MEDSEALITTER partners are equipped) and thus this analysis is considered optional for the project. MEDSEALITTER partners Reference laboratories competent to perform plastic polymer identification are listed in ANNEX II.

Fig. 43. Spectroscope FTIR Analyses.

Summary of necessary material

- Distilled water and wash bottles
- Alcohol 70°
- 150 and 250 ml beakers
- 100 ml test tube
- 15% H_2O_2

- Disposable scalpels, fine forceps, fine scissors and small clamps (for dissection)
- Magnetic stirrer and hot plate (or magnetic stirrer heater)
- Glove box to prevent environmental contamination during filtration
- Clamp (for magnetic magnet and filter handling)
- Vacuum filtration system with Buchner funnel (porcelain or glass fitter)
- Precision tweezers (fine and pointed) for micro-plastic handling on filters and FTIR
- Glass Petri dishes (x 400)
- GFC filters 0.2, 1.2 or 1.6 µm 47 mm diameter for filtration (x400)
- Aluminum foil
- Precision scale
- Stereo microscope with associated analysis software
- Optional: FTIR spectrometer and computer with associated analysis software

Contamination precautions

Synthetic clothing (*e.g.* fleece) should be avoided during fish sampling. To ensure there is no contamination of the fish samples from the nets, it is recommended to apply FTIR analysis on the net used for fish sampling.

Glass material should be used where possible and all glassware and tools (*e.g.* tweezers, scissors etc.) should be rinsed thoroughly with purified water (*e.g.* Milli Q). Staff should wear natural fiber laboratory clothes or Tyvec suites. Sample processing should be done in closed areas with little ventilation and air circulation (*e.g.* from air conditioning). Samples should be covered by foil paper during digestion and when not in use. It is recommended to use covers during sample rinsing and filtration (*e.g.* glove bag, laminar flow cabinet or other closed cover) and to cover filters with glass lids during observation under the stereomicroscope. Procedural blank samples should be used in all steps of sample processing and the results provided by blanks should be less than 10% of the other samples, otherwise the whole process should be repeated.

Recovery of microplastics by the applied extraction procedure must be tested on samples of fish gastrointestinal tissue enriched with specific number (*e.g.* 10 particles/sample) of different types of virgin plastic particles. The number of particles detected after sample processing is used to calculate % recovery of microplastics.

Reporting units

Frequency of occurrence (%) of ingested microplastics for each species is calculated as the percentage of the individuals examined with ingested microplastics.

Abundance (N) of microplastics ingested per individual (average number of items/individual) for each species is calculated as a total and per category of microplastics. Since currently there are inconsistencies in the literature in reporting abundance of ingested microplastics, it is recommended to report average number of items per individual both considering all individuals examined and only individuals found with ingested microplastics.

The number, length and weight of the individuals examined for each species should be reported.

Recovery rate of microplastics is reported.

5.2.2 Micro litter ingestion by polychaeta

Introduction and scope of the protocol

This protocol aims to evaluate occurrence of microplastic ingestion in polychaeta species in Mediterranean MPAs. Guidelines for the selection of target polychaeta species are presented and polychaeta family/species are proposed as targets for assessing microplastic ingestion. The protocol follows the methodology for microplastic detection described in the previous section for fish species, with adaptations for polychaeta. For example, the whole body of worms instead of their gastrointestinal tract is used for microplastic extraction following the approach used for the detection of microplastics in small invertebrates, such as lugworms (Van Cauwenberghe et al. 2015).

Selection of species

Although microplastic ingestion and related effects have been shown in polychaeta under laboratory trials, information on microplastic ingestion in polychaeta species under field conditions is very scarce (Wright et al. 2013, Van Cauwenberghe et al. 2015, Gusmão et al. 2016). For example, in the Mediterranean Sea only one study reports microplastic ingestion in *Saccocirrus papillocercus* from Sardinia, Italy (Gusmão et al. 2016).

To study the interaction of polychaeta with microplastics, some ecological and pragmatical aspects have to be considered, such as feeding guild, habitats and sampling availability. Based on these considerations, guidelines referring to the selection process of best polychaeta family/species to be used as target can be outlined. Under an ecological point of view, families/species with feeding guild and ways of life that maximize interactions with microplastics should be selected and studied preferentially. Pragmatic issues, such as availability of the family/species at the right scale, sampling feasibility, size of organisms, should also be considered. Taking in account also the availability of previous studies on certain species, a selection of some polychaeta families that could be used to assess ingestion of microplastics is proposed.

The selected families are: Arenicolidae, Maldanidae, Orbinidae, Flabelligeridae, Sternaspidae, Ampharetidae, Pectinariidae, Terebellidae, Oweniidae, Sabellariidae, Chaetopteridae, Amphinomidae, Euphrosinidae, Eunicidae, Onuphidae, Aphroditidae, Chrysopethidae, Glyceridae, Nephtydae, Polynoidae, Polynoidae, Sigalionidae, Sphinteridae, Saccocirridae. The selected species are: *Arenicola marina, Dasybranchus caducus, Aphrodita aculeate, Laetmonice hystrix, Harmothoe* spp., *Sternaspis scutata, Sabella pavonina, Sabella spallanzanii, Sabellaria alveolata, Saccocirrus papillocercus*.

In the framework of theMEDSEALITTER project, a second level of selection was applied: A species was selected based on its feeding guild, its wide geographical distribution, its availability within seasons, and its presence in different habitats along the marine coastal areas.

The species selected as indicator of microplastic ingestion was *Sabella spallanzanii* (Sabellidae family, fig. 44), due to its ecological features (Table 10) as feeding strategy (it can filter large quantities of sea water) or habitat distribution (it can live both in polluted areas and in clean ones). It is a very common species that can be sampled all year round along the Mediterranean coasts; it is very frequent in ports and harbors, on artificial substrata and natural hard bottoms. The most studied polychaeta species, the lugworm *Arenicola marina*, was not selected due to its scarceness in the natural habitats along the Mediterranean coast. Moreover, due to a decrease in the abundance of this species during the last decades, it is currently not easy to find and sample.

Fig. 44. *Sabella spallanzanii* specimen next to an annular seabream.

Table 10. *Sabella spallanzanii* ecological and biological features.

Species Name	*Sabella spallanzanii* (Viviani, 1805)
Common name	Mediterranean fanworm, peacock feather duster, European fanworm
Distribution range	Subtropical
Depth range	From shallow waters to 30 m
Lifestyle	Sessile
Geaographic distribution	Indo-West Pacific Ocean, Northeast Atlantic Ocean and Mediterranean Sea
Max lenght	70 cm
Feeding strategy	Suspension filter feeder that feeds on bacteria, zooplankton and phytoplankton and suspended particles of organic matter
Colour	The colour of the tentacles is variable but they are usually banded in orange, purple and white or they may be a uniform pale grey.
Biology	The flexible tube can reach up to 50 cm in length and the tentacles up to 20 cm. Mating: Females produce a pheromone attracting and signalling the males to shed sperm which in turn stimulates females to shed eggs, a behaviour is known as swarming. Gametes are spawned through the metanephridia or body wall rupturing (i.e. "epitoky", wherein a pelagic, reproductive individual, "epitoke", is formed from a benthic, nonreproductive individual, "atoke"). After fertilization, most eggs become planktonic; although some are retained in the worm tubes or burrowed in jelly masses attached to the tubes (egg brooders). Life Cycle: Eggs develop into trocophore larvae, which metamorphose into juvenile stage (body lengthened), and later develop into adults.

Selection of extraction method for the detection of microplastics

Four different chemical digestion protocols were tested to select the best method for the detection of microplastic ingestion by Polychaeta. The tests led to the results briefly summarized below and available in the “Report of testing activities and results” document.

Protocol 1 (Foekema et al. 2013) and 2 (Rochman et al. 2015): organic material underwent an incomplete digestion.

Protocol 3 (Li et al. 2015): organic material was totally digested.

Protocol 4 (Avio et al. 2015): organic material underwent an incomplete digestion and big fragments remained undigested.

Based on these results, protocol 3 was selected as the best method to digest Polychaeta tissues. The protocol is described in detail below.

Collection of samples

Polychaeta sampling can be carried out by hand by the MPA staff. At least 10/20 samples per species per location should be used, and recommended sampling frequency is twice per year. The following information should be recorded: sampling location, species, date and time of sampling, depth. Immediately after sampling, the worms must be frozen and stored at -20 ºC until analysis. The samples should be removed from the tube, rinsed with filtered distilled water, put in an adequate labeled jar and transported frozen at the reference laboratory (a list is provided in ANNEX II) for sample processing and microplastics detection.

Sample processing for the detection of microplastics

1) Polychaeta preparation

Worms are thawed at room temperature in the laboratory.

2) Measurements of the polychaeta

• Weigh the whole Polychaeta with a precision scale.

• Measure total length (fan or tentacles included).

3) Digestion of the polychaeta (Fig. 45)

• Place the entire animal in a suitable Pyrex beaker.

• Add 20 ml of H_2O_2 (15% or 30%) per gram of tissue to the Pyrex beaker containing the entire animal. The amount of H_2O_2 (30%) required must be prepared in a graduated cylinder soon after the digestion process (do not store it for better efficiency) and H_2O_2 containers must be kept away from light.

• Cover samples with aluminum foil throughout the digestion process.

• Place the beaker on a hot plate or a waterbath (several beakers can be placed on the same plate) at 65 °C and incubated it for 24 hours and then at room temperature for 48 hours (Fig. 45).

• Stir the solution every 20 minutes.

• Prepare a control sample to test possible ambient contamination (with only H_2O_2 in a beaker and following the entire protocol described above and below).

• Change the foil when it gets damaged by H_2O_2 to avoid sample contamination.

• If organic matter is not fully removed by the time H_2O_2 is close to evaporation, add more H_2O_2 until nearly all of the organic matter is digested.

Fig. 45. Digestion process: waterbath incubation (left); room temperature incubation (right).

4) Dilution and homogenization of the digested sample

• Add 100 ml of distilled water (d H_2O) to the beaker and place it on the magnetic stirrer (high speed for 1-2 minutes).

• Let stand 1 to 2 minutes.

5) Vacuum filtration of the digested sample (Fig. 46)

• Carefully place the filter (previously weighed) on the Buchner funnel (porcelain or glass fit). It is recommended to use a vacuum flask of 500 ml for a more ergonomic handling.

• The filters used are as follows: GFD 2.5 µm 47 mm in diameter.

• Empty the contents of the beaker into the funnel (rinse the magnet in the beaker with dH_2O and the walls of the beaker and funnel).

• To avoid contamination, carry out filtration in a glove box (Captair Pyramid style laptop - Erlab, Fig. 46) and place aluminum foil on a Buchner funnel during filtration. The base of the pyramid should be covered with paper towel to avoid contamination (dark blue plastic particles have been observed).

Fig. 46. Filtration system.

6) Drying of samples (fig. 47)

• Remove the filter from the Buchner funnel by sliding it directly into a glass Petri dish (or plastic, by completely covering it inside and outside with aluminum foil to avoid contamination).

• Leave the aluminum-covered boxes slightly open and place them in a clean cupboard.

Fig. 47. Drying process: glass Petri with filter in an air incubator (left) and in a glass desiccators (right).

7) Observation of samples under stereo-microscope – identification of microplastics (Fig. 48)

For quantification and characterization of microplastics, filters are examined under a stereomicroscope.

• To avoid opening the Petri dish (and not contaminate the sample), first observe with the lid and note what is observed and what should be checked with the tweezer.

• Observe the whole filter in the Petri dish under a stereomicroscope, with a magnification up to 150x.

• Petri dishes can be crisscrossed with a marker in 9 zones (to note in which zones the particles are).

• When the particles easily disintegrate in pieces in contact with the clamps it is generally tissues (in 2 pieces it is generally plastic).

• No need to cover the stereo microscope (cover only the Petri dish).

• With 200 µm magnification, it's difficult to pick the particles and determine if it is plastic without NIRS.

• Analysis software associated with the stereo-microscope could help to measure and identify colors.

• Particles can be moved outside the filter before FTIR analysis (to have easy access to the latter).

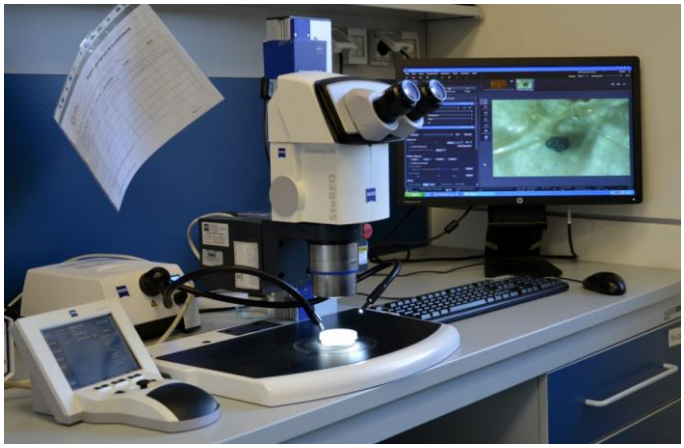


Fig. 48. Filter stereomicroscope analysis and image acquisition with a specific software.

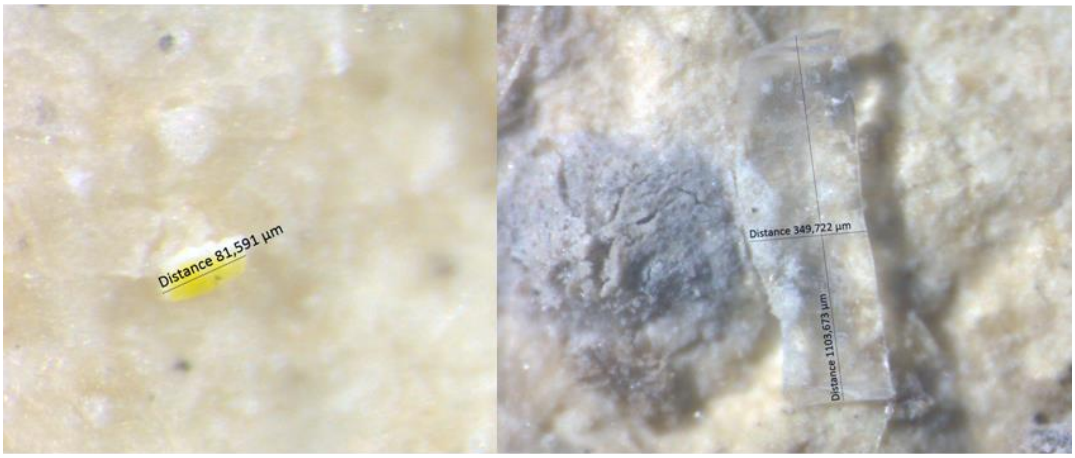


Fig. 49. Detected microplastic images measured by mean of image analysis software.

The microplastic particles are photographed, counted and categorized according to maximum length, color, and type, following the MSFD Guidelines (Galgani et al. 2013a) (See Table 11 and ANNEX I for an updated list of marine litter categories).

Table 11. Categories of microplastics from TSG ML masterlist of litter categories (Galgani et al. 2013a).

Microplastics	General name	TSG ML General Code
Fragments	Plastic fragments rounded <5mm	G103
	Plastic fragments subrounded <5mm	G104
	Plastic fragments subangular <5mm	G105
	Plastic fragments angular <5mm	G106
Pellets	Cylindrical pellets <5mm	G107
	Disks pellets <5mm	G108
	Flat pellets <5mm	G109
	Ovoid pellets <5mm	G110
	Spheruloids pellets <5mm	G111
Filaments	Filament <5mm	G113

Films	Films <5mm	G114
Foamed plastic	Foamed plastic <5mm	G115
Granules	Granules <5mm	G116
Styrofoam	Styrofoam <5mm	G117

8) OPTIONAL: Plastic polymer identification (Fig. 50)

Spectroscopic analyses are optional and can vary according to the type of the spectrometer.

• Connect FTIR to MicroLab Software.

• Don't let the samples dry out to avoid that the particles get stuck to the filter when you move them to the spectrometer crystal.

• Clean the FTIR glass with acetone.

• Carefully place the plastic particles to be analyzed (>200 µm) on the FTIR crystal using fine tweezers.

• There may be irregularities in the curves obtained when fabrics or when water remains on the particle.

• A complete library of the spectra of plastics that can be observed is needed

• A good match is at least 85%.

Fig. 50. FTIR spectroscope.

Summary of necessary material

• Distilled water and wash bottles
• 150 and 250 ml beakers
•100 ml test tube
• 30 % H_2O_2 at 30%
• Disposable scalpels, fine forceps, fine scissors and small clamps (for dissection)
• Magnetic stirrer and hot plate (or magnetic stirrer heater)
• Glove box to prevent environmental contamination during filtration

• Clamp (for magnetic magnet and filter handling)
• Vacuum filtration system with Buchner funnel (porcelain or glass fitter)
• Precision tweezers (fine and pointed) for micro-plastic handling on filters and FTIR
• Glass Petri dishes
• GFD filters 2.5 µm 47 mm diameter for filtration
• Aluminum foil
• Precision scale (Mettler Toledo)
• Stereo microscope with associated analysis software
• Optional: FTIR and computer with associated analysis software

Contamination precautions

Glass material should be used where possible and all glassware and tools (*e.g.* tweezers, scissors, etc.) should be rinsed thoroughly with purified water (*e.g.* Milli Q). Staff should wear natural fiber laboratory clothes. Sample processing should be done in closed areas with little ventilation and air circulation for example from air conditioners. Samples should be covered by foil paper during digestion and when not in use. It is recommended to use covers during sample rinsing and filtration (*e.g.* glove bag, laminar flow cabinet or other closed cover) and to cover filters with glass lids during observation under the stereomicroscope. Procedural blank samples should be used during all steps of sample processing.

Reporting units

Frequency of occurrence (%) of ingested microplastics for each species is calculated as the percentage of the individuals examined with ingested microplastics.

Abundance (N) of microplastics ingested per individual (average number of items/individual) for each species is calculated as a total and per category of microplastics. Since currently there are inconsistencies in the literature in reporting abundance of ingested microplastics, it is recommended to report average number of items/individual, both considering all individuals examined and only individuals found with ingested microplastics. When using pooled samples, abundance (N) of microplastics is reported per weight of the animals (average number of items/g wet weight) for each species as a total and per category of microplastics.

The number, length and weight of the individuals examined for each species should be reported.

6. HOW TO SELECT THE MOST APPROPRIATE PROTOCOL? COST-BENEFIT ANALYSIS OF MARINE LITTER MONITORING TECHNIQUES

Each of the sub protocols proposed (with reference to the main platform and method used for monitoring) has been associated with an approximate estimation of its cost, level of expertise required and potential performers, main limitations and benefits, based on the MSFD TSG ML "Guidance on monitoring of marine litter in European Seas" (Galgani et al. 2013a), and updated/adapted with the results obtained from the testing activities performed during the pre-testing and testing phases of the MEDSEALITTER project.

According to the MSFD Guidance, cost estimates include: cost of labour in different phases of monitoring, cost of equipment and other running costs (ship time, etc.). These are very rough estimates, as the staff-costs vary considerably across countries.

The criteria that can support the decision of which protocols to adopt for monitoring include (as from the Guidance):

Level of maturity - The extension to which the protocol has been tested and applied;

Technical/Equipment - Requirements for technical equipment in terms of: *LOW – €1.000-10.000; MEDIUM - €10.000 – 50.000; HIGH - >€50.000;*

Expertise - Level of expertise required for sampling, analysis and data interpretation:

LOW - trained personnel without specific professional formation; *MEDIUM – trained personnel with specific professional formation; HIGH - high expertise and special skills required.*

Cost - Total costs incurred. *LOW: €1.000-10.000; MEDIUM: €10.000 – 50.000; HIGH: >€50.000.* Please note that these are only approximate estimations, as they depend greatly on staff costs, existing equipment and whether or not the protocol makes use of existing monitoring programmes and/or maritime operations;

Level of detail generated - Potential of the protocol to generate details and information in terms of material, nature and purpose of the items sampled, which can be attributed to specific and distinct sources.

Geographic applicability - Potential of the protocol to be applied in any geographic area/region

Limitations - Key aspects inherent to the protocol and/or factors that can limit its applicability and/or generation of reliable & comparable data.

Benefits and opportunities to reduce costs - Main advantages of each techniques and opportunities that can improve cost-effectiveness, *e.g.* by making use of other monitoring programmes, and/or maritime operations, in which the protocol can be integrated.

Table 12. Estimated costs, level of expertise, limitations and benefits of FML monitoring techniques.

Method/Protocol	Large vessels (visual)	Small and medium vessels (visual)	Aerial (Visual)	Aerial (Photo)	Drone (Photo)
Level of maturity	H	H	H	M	M
Technical/equipment	L	M	H	H	H
Required expertise					
Sampling	L/M	L/M	M	H	H
Analysis of samples	H	H	H	H	H
Statistical analysis	H	H	H	H	H
Possible performers (Vt: VOLUNTEERS; C/A: CONSULTANTS & AGENCIES; S: SCIENTISTS)	VT; C/A; S	C/A; S	C/A; S	C/A; S	C/A; S
OVERALL	L/M	M	M	H	H
Cost					
Collection of samples	L	M	H	H	M/H
Analysis of samples	M	M	M	H	H
Statistical analysis	M	M	M	M	M
Equipment	L	M	H	VH	M/H
OVERALL	L/M	M	M/H	H	M/H
Level of detail generated	L (size > 20 Cm)	M (size > 2.5 Cm)	L (size > 30 Cm)	L/M	H
Geographic applicability	H	M	H	H	M
Limitations	Observations affected by weather/sea conditions; the minimum detectable size of litter is 20 cm.	Can be expensive according to the platform used; observations affected by weather/sea condition.	Expensive; observations affected by weather/sea conditions; can detect only large floating items (>30 cm); scarce discrimination of litter types.	Expensive; observations affected by weather/sea conditions. Unless automated, the process of analysis can be expensive and time consuming.	Observations affected by weather/sea conditions. Unless automated, the process of analysis can be expensive and time consuming.
Benefits and opportunities to reduce costs	Costs reduced thanks to the integration in ongoing vessels operations and/or coupling with marine fauna monitoring programmes; wide coverage. Possibility to replicate surveys across seasons and years, allowing robust statistical analyses.	Can be coupled to marine fauna monitoring to reduce costs. Higher detail of the observations generated; monitoring can be adapted to necessities of sampling (specific areas/seasons).	Can be coupled with marine fauna monitoring to reduce costs. Very large area coverage .	Very large area coverage and high detail of observation generated. Images available for future analyses. Automation of analyses can reduce the overall cost and time dedicated to analyses.	Very high detail of observation. According to the technology used, can be easily adopted to routine low-cost monitoring of small coastal areas. Automation of analyses could further reduce costs.

Table 13. Estimated costs, level of expertise, limitations and benefits of ingested marine (micro and macro) litter monitoring techniques.

Method/Protocol	Macro litter (sea turtle)	Micro litter (fish)	Micro litter (polychaeta)
Level of maturity	H	H	M
Technical/equipment	L	M/H	H
Required expertise			
Sampling	L/M	H	H
Analysis of samples	M	H	H
Statistical analysis	M	M	M/H
Possible performers (Vt: VOLUNTEERS; C/A: CONSULTANTS & AGENCIES; S: SCIENTISTS)	C,S,Vt	S	S
OVERALL	M	M/H	M/H
Cost			
Collection of samples	M	M	M/H
Analysis of samples	M	H	H
Statistical analysis	M	M	M
Equipment	L	H	H
OVERALL	M	M/H	H
Level of detail generated	M (size > 1 mm)	M/L (SIZE < 5 mm)	M/L (SIZE < 5mm)
Geographic applicability	M	M	L
Limitations	Depends on the availability of animals.	Depends on the geographic coverage of species and the availability of animals. Costs and expertise needed for micro-litter analyses are still high.	Depends on the geographic coverage of species and the availability of animals. Costs and expertise needed for micro-litter analyses are still high.
Benefits and opportunities to reduce costs	Potential to collaborate with rescue centres for collecting dead turtles. Wide coverage across the mediterranean thanks to the wide distribution of Caretta caretta.	Potential to collaborate with rescue centres for collecting dead turtles; fish monitoring programs and/or the fish market to collect fish. Species are selected to garantee wide coverage across the Mediterranean.	The indicator species is still to be selected; sampling relatively easy. Depending on the distribution of the species could provide information on large areas.

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ANNEX I. JOINT COMMON LIST FOR MARINE LITTER MONITORING (MSFD TSG-ML modified masterlist updated as at March 31st 2019)

TSG_ML General-Code	Level 1 - Materials	TSG_ML General-Code	level 2 - use	TSG_ML General-Code	level 3 - general type	TSG_ML General-Code	level 4 - type	description/examples	TSG_ML General-Code	level 5 - specific type	TSG_ML General-Code	size classes	TSG_ML General-Code	size classes 2nd level	level 6 - Single Use Items (EU-Directive; in brackets: items not surely included in the Directive)	OSPAR- Code	UNEP- Code	MEDITS	ICES
	Artificial polymer materials		packaging	G1			4/6-pack yokes, 6-pack rings, other packaging for tin cans									1	PL05	L1j	A14
				G2	Bags	G3	Shopping/carrier Bags	incl. identifiable pieces of such bags							SUP	2	PL07	L1a	A3
				G2	Bags	G4	Small plastic bags	freezer bags, tissue packets, etc. incl. identifiable pieces of such bags								3	PL07	L1a	A3
				G2	Bags	G5	Parts remaining from rip-off plastic bags								112	PL07	L1a	A3
				G6	Bottles & containers		Drink bottles				G7	Drink bottles <= 0.5l			SUP	4	PL02	L1b	A1
				G6	Bottles & containers		Drink bottles				G8	Drink bottles > 0.5l			SUP	4	PL02	L1b	A1

	Artificial polymer materials		packaging	G6	Bottles & containers	G9	Cleaner bottles & containers	detergent, toilet cleaner, glass cleaner etc.								5	PL02	L1b	A1
				G6	Bottles & containers	G10	Food containers incl. fast food containers								SUP	6	PL06	L1c	A11
				G6	Bottles & containers		body care and cosmetics bottles & containers	suncream, aftersun lotion, shower gel, toothpaste	G11	Beach use related body care/cosmetic bottles & containers						7	PL02	L1b	A1
				G6	Bottles & containers		body care and cosmetics bottles & containers	suncream, aftersun lotion, shower gel, toothpaste	G12	Non-beach use related body care/cosmetic bottles & containers; unidentified cosmetic bottles & containers						7	PL02	L1b	A1
				G6	Bottles & containers	G13	Other bottles & containers (drums)									12	PL02	L1b	A11
				G6	Bottles & containers		Engine oil bottles & containers				G14	Engine oil bottles-containers <50cm				8	PI03	L1j	A11
				G6	Bottles & containers		Engine oil bottles & containers				G15	Engine oil bottles-containers >50cm				9	PI03	L1j	A11
				G6	Bottles & containers	G16	Jerry cans (square plastic containers with handle)									10	PI03	L1j	A11
				G6	Bottles & containers	G17	Injection gun containers	e.g. for silicone, grease								11	PL24	L1j	A11

	Artificial polymer materials		packaging	G18	crates, boxes, baskets			not fish boxes								13	PL13	L1j	A11
			vehicle related	G19	vehicle parts			artificial polymer materials/fibre glass parts of cars & other transport vehicles								14	PL24	L1j	A14
			packaging	G20	Plastic caps and lids	G21	Plastic caps/lids drinks								SUP	15	PL01	L1j	A4
				G20	Plastic caps and lids	G22	Plastic caps/lids of chemicals, detergents (non-food)									15	PL01	L1j	A4
				G20	Plastic caps and lids	G23	Plastic caps/lids unidentified									15	PL01	L1j	A4
				G20	Plastic caps and lids	G24	Plastic rings from bottle caps/lids	rings breaking off from a bottle cap when twisted off								15	PL01	L1j	A4
			smoking related	G25	Tobacco pouches / plastic cigarette box packaging											48	PL24	L1j	A14
				G26	Cigarette lighters											16	PL10	L1j	A14
				G27	Cigarette filters										SUP	64	PL11	L1j	A14
			utility items	G28	Pens and pen lids			writing utensils mainly made of artificial polymers								17	PL24	L1j	A14
				G29	Combs/hair brushes/sunglasses											18	PL24	L1j	A14

	Artificial polymer materials		packaging		food packets and wrappers		Crisps packets /sweets wrappers /lolly sticks		G30	Crisps packets/sweets wrappers					SUP	19	PL24	L1j	A14
			packaging		food packets and wrappers		Crisps packets /sweets wrappers /lolly sticks		G31	Lolly sticks					(SUP)	19	PL24	L1j	A14
			recreation related	G32	Toys and party poppers											20	PL08	L1j	A14
			Non-packaging food consumption related	G33	Cups and cup lids										SUP	21	PL24	L1j	A14
					Cutlery/plates/trays/straws /stirrers	G34	Cutlery, plates and trays			cutlery					SUP	22	PL04	L1j	A14
					Cutlery/plates/trays/straws /stirrers	G34	Cutlery, plates and trays			plates and trays					SUP	22	PL04	L1j	A14
					Cutlery/plates/trays/straws /stirrers	G35	Straws and stirrers			straws					SUP	22	PL04	L1j	A14
					Cutlery/plates/trays/straws /stirrers	G35	Straws and stirrers			stirrers					SUP	22	PL04	L1j	A14
			packaging	G2	Bags	G36	heavy-duty sacks	e.g. fertiliser or animal feed sacks								23	PL07	L1a	A3
				G3	Bags	G37	Mesh bags			Mesh bags for vegetables, fruits & other products						24	PL15	L1a	A14
			Clothing (clothes, shoes)	G39	Gloves	G40	Gloves (washing up)									25	PL09	L1j	C5
				G39	Gloves	G41	Gloves (industrial/professional rubber gloves)									113	RB03	L1j	C5

	Artificial polymer materials		fisheries related	G42	Crab/lobster pots and tops										SUP Fishing Gear	26	PL17	L1h	A14
			utility items	G43	Tags (fishing and industry)											114	PL24	L1j	A14
			fisheries related	G44	Octopus pots										SUP Fishing Gear	27	PL17	L1h	A14
			aquaculture		Bags	G45	Mesh bags			Mussels nets/ net sacks/ oyster nets & nets pieces						28	PL15	L1j	A14
				G46	Oyster trays (round from oyster cultures)											29	PL24	L1j	A14
				G47	Plastic sheeting from mussel culture (Tahitians)											30	PL24	L1j	A14
			undefined		Rope, string, cord	G49	Rope (diameter > 1cm)									31	PL19	L1i	A7
					Rope, string, cord	G50	String and cord (diameter < 1cm)			String/cord (diameter < 1cm) not from dolly ropes or unidentified						32		L1i	A7
					Rope, string, cord	G50	String and cord (diameter < 1cm)			String and filaments exclusively from dolly ropes					SUP Fishing Gear	32		L1i	A7
			fisheries related	G52	Nets and pieces of net						G53	Nets and pieces of net < 50 cm			SUP Fishing Gear	115	PL20	L1f	A8
				G52	Nets and pieces of net						G54	Nets and pieces of net > 50 cm			SUP Fishing Gear	116	PL20	L1f	A8

	Artificial polymer materials		fisheries related	G52	Nets and pieces of net	G56	Tangled nets/cord			Tangled dolly rope					SUP Fishing Gear	33	PL20	L1f	A8
				G52	Nets and pieces of net	G56	Tangled nets/cord			Tangled nets and rope without dolly rope/mixed with dolly rope					SUP Fishing Gear	33	PL20	L1f	A8
					Fishing line	G59	Fishing line (tangled & not)								SUP Fishing Gear	35		L1g	A5
					Fish boxes	G57	Fish boxes - plastic								(SUP Fishing Gear)	34	PL17	L1h	A11
					Fish boxes	G58	Fish boxes - expanded polystyrene								(SUP Fishing Gear)	34	PL17	L1h	A11
				G60	Light sticks (tubes with fluid) incl. packaging										SUP Fishing Gear	36	PL17	L1h	A14
				G61	Other fishing related items (e.g. other than fishing line monofilaments, metal hooks, rubber bobbins)			delete from list? Clarify what is included in addition to all other fishing items							SUP Fishing Gear	48	PL24	L1h	A14
					Floats/Buoys	G62	Floats for fishing nets								SUP Fishing Gear	37	PL14	L1h	A14
					Floats/Buoys	G63	Buoys	diverse use e.g. for marking fishing gears, shipping routes, mooring etc.								37	PL14	L1j	A14
			shipping related	G64	Fenders											48	PL24	L1j	A14

	Artificial polymer materials		utility items	G65	Buckets											38	PL03	L1j	A14
			packaging	G66	Strapping bands											39	PL21	L1i	A10
				G38	Cover packaging	G67	Plastic sheets, industrial packaging									40	PL16	L1j	A14
			undefined	G68	Fibre glass items and fragments											41	PL22	L1j	A14
			Clothing (clothes, shoes)		Headware	G69	Hard hats/Helmets									42	PL24	L1j	A14
			hunting related	G70	Shotgun cartridges											43	PL24	L1j	A14
			Clothing (clothes, shoes)		Footwear		Shoes/sandals/flip flops		G71	artificial polymer footwear						44	CL01	L1j	F2
			utility items	G72	Traffic cones											48	PL24	L1j	A14
			undefined	G73	Foam sponge		Other foam sponge items or pieces	e.g. mattresses								45	FP01	L1j	A14
			packaging	G73	Foam sponge	G74	Foam packaging /insulation									45	FP01	L1j	A14
			undefined		fragments		Plastic/polystyrene pieces				G75	Plastic/polystyrene pieces < 2.5 cm	G78	Plastic pieces < 2.5 cm		117		L1j	A14
			undefined		fragments		Plastic/polystyrene pieces				G75	Plastic/polystyrene pieces < 2.5 cm	G81	Polystyrene pieces < 2.5 cm		117		L1j	A14
			undefined		fragments		Plastic/polystyrene pieces				G76	Plastic/polystyrene pieces 2.5 cm-50cm	G79	Plastic pieces 2.5-50cm		46		L1j	A14

	Artificial polymer materials		undefined		fragments		Plastic/polystyrene pieces				G76	Plastic/polystyrene pieces 2.5 cm-50cm	G82	Polystyrene pieces 2.5-50cm		46		L1j	A14
			undefined		fragments		Plastic/polystyrene pieces				G77	Plastic/polystyrene pieces > 50 cm	G80	Plastic pieces > 50 cm		47		L1j	A14
			undefined		fragments		Plastic/polystyrene pieces				G77	Plastic/polystyrene pieces > 50 cm	G83	Polystyrene pieces > 50 cm		47		L1j	A14
			packaging	G84	CD, CD-box											48	PL24	L1j	A14
			packaging			G85	commercial salt packaging	incl. heavy-duty sacks and other commercial salt containers e.g. for conserving products								48	PL24	L1j	A14
			recreation related	G86	Fin trees (from fins for scuba diving)											48	PL24	L1j	A14
			utility items	G87	Masking tape			incl. any tape duct tape, packaging tape, etc.								48	PL24	L1j	A14
			utility items	G88	Telephone (incl. parts)			mobile and any other type of telephone								48	PL24	L1j	A14
			construction related	G89	Plastic construction waste e.g. pipes and tubes e.g. for cables			e.g. drainage & waste pipes, plastic tubes for cables, insulation, construction foam								48	PL24	L1j	A14

	Artificial polymer materials		agriculture	G90	Plastic flower pots											48	PL24	L1j	A14
			sewage/aquaculture	G91	Biomass holder from sewage treatment plants and aquaculture											48	PL24	L1j	A14
			fisheries related	G92	Bait containers/packaging										(SUP Fishing Gear)	48	PL24	L1h	A14
			utility items	G93	Cable ties											48	PL24	L1j	A14
			personal hygiene&care	G95	Cotton-bud-sticks		Plastic cotton-bud-sticks								SUP	98	OT02	L5d	A14
				G96	Sanitary towels/panty liners/backing strips										SUP Sanitary	99	PL24	L5d	A13
				G97	Toilet fresheners											101	PL24	L5d	A14
				G98	Diapers/nappies										SUP Sanitary	102	PL24	L5d	A12
			medical related	G99	Syringes/needles											104	PL12	L1j	A14
				G100	Medical/Pharmaceuticals containers/tubes											103	PL24	L1j	A14
			packaging	G2	Bags	G101	Dog faeces bag									121	PL07	L1a	A14
			Clothing (clothes, shoes)	-	Footwear		Shoes/sandals/flip flops		G102	Flip-flops						44	RB02	L1j	F2
			undefined	G124	Other plastic/polystyrene items (identifiable)			identifiable items not fitting in any other category								48	PL24	L1j	A14

	Rubber		recreation related	G125	Balloons, balloon ribbons, strings, plastic valves and balloon sticks									SUP	49	RB01	Lb2	C2
	Rubber		recreation related	G126	Balls										53	RB01	Lb2	C6
			Clothing (clothes, shoes)		Footwear	G127	Rubber boots								50		Lb2	C1
			vehicle related		Tyres, belts, inner tubes, wheels	G128	Tyres and belts								52	RB04	Lb2	C4
					Tyres, belts, inner tubes, wheels	G129	Inner-tubes and rubber sheet								53	RB05	Lb2	C6
	Mixed		vehicle related		Tyres, belts, inner tubes, wheels	G130	Wheels								52		Lb2	C4
	Rubber		utility items	G131	Rubber bands (small, for kitchen/household/post use)										53	RB06	Lb2	C6
			personal hygiene&care	G133	Condoms (incl. packaging)			packaging not rubber						SUP Sanitary	97	RB07	Lb2	C6
			undefined	G134	Other rubber pieces			identifiable items not fitting in any other category							53	RB08	Lb2	C6
	Cloth/textile	G135	Clothing (clothes, shoes)	G137	Clothing		-	-							54	CL01	L5a	F1
		G135			Footwear		Shoes/sandals/flip flops		G138	leather and/or cloth footwear					57	CL01	L5a	F2
			recreation related	G139	Backpacks & bags										59	CL02	L8-L9	F3
			packaging	G140	Sacking (hessian)										56	CL03	L8-L9	F3
			utility items	G141	Carpet & Furnishing										55	CL05	L5b	F3

	Cloth/textile		utility items	G143	Sails, canvas										59	CL03	L8-L9	F3
	Mixed		personal hygiene&care	G144	Tampons and tampon applicators									SUP Sanitary	100		L5d	F3
	Cloth/textile		undefined	G145	Other textiles including pieces of cloth, rags etc.										59	CL06	L8-L9	F3
G146	Paper/ Cardboard		packaging	G149	Paper packaging	G147	Paper bags								60	PC03	L7	E3
G146				G149	Paper packaging	G148	Cardboard (boxes & fragments)								61	PC02	L7	E3
G146			undefined	G156	fragments		Paper fragments								67		L7	E3
G146			packaging	G149	Paper packaging		Carton/Tetrapack		G150	Carton/Tetra pack Milk					118	PC03	L7	E3
G146				G149	Paper packaging		Carton/Tetrapack		G151	Carton/Tetra pack (non-milk)					62	PC03	L7	E3
G146				G149	Paper packaging	G152	Cigarette packets	incl. plastic covering of cigarette packets							63	PC03	L7	E3
G146			non-packaging food consumption related	G153	Cups, food trays, food wrappers, drink containers		Cups								65	PC05	L7	E3
G146				G153	Cups, food trays, food wrappers, drink containers		food trays, food wrappers, drink containers								67	PC03	L7	E3
G146			utility items	G154	Newspapers & magazines										66	PC01	L7	E3
G146			recreation related	G155	Tubes & other pieces of fireworks										67	PC04	L7	E3

G146	Paper/ Cardboard		undefined	G158	Other paper items			identifiable items not fitting in any other category							67	PC05	L7	E3
G146			personal hygiene&care	G95	Cotton-bud-sticks		Paper/card cotton-bud-sticks								67	OT02	L5d	E3
G170	Processed/ worked wood		packaging	G159	Corks			including plastic corks							68	WD01	L6	E1
G170				G160	Pallets										69	WD04	L6	E4
G170				G162	Crates, boxes, baskets										70		L6	E1
G170			fisheries related	G163	Crab/lobster pots									SUP Fishing Gear	71	WD02	L6	E1
G170				G164	Fish boxes									(SUP Fishing Gear)	119		L6	E1
G170			non-packaging food consumption related	G165	Ice-cream sticks, chip forks, chopsticks, toothpicks										72	WD03	L6	E1
	Mixed		utility items	G166	Paint brushes										73		L8-L9	E1
G170	Processed/ worked wood		recreation related	G167	Matches & fireworks										74		L6	E1
G170			undefined	G173	Other wood			wooden items not fitting in any other category e.g. planks, boards, beams			G171	Other wood < 50 cm			74		L6	E1

G170	Processed/worked wood		undefined	G173	Other wood			wooden items not fitting in any other category e.g. planks, boards, beams			G172	Other wood > 50 cm				75	WD06	L6	E1
	Metal		packaging		containers		Cans	(< 4 L)?	G174	Aerosol/ Spray cans industry						76		L8-L9	B8
					containers		Cans	(< 4 L)?	G175	Cans (beverage)						78	ME03	L3a	B1
					containers		Cans	(< 4 L)?	G176	Cans (food)						82	ME04	L3b	B2
					containers		Cans	(< 4 L)?	G190	Paint tins						86		L3c	B8
					containers		Cans	(< 4 L)?	G188	Other cans						89		L8	B8
				G177	Foil wrappers, aluminium foil											81	ME06	L3b	B8
					containers	G178	Bottle caps, lids & pull tabs									77	ME02	L3b	B8
			recreation related	G179	Disposable BBQ's											120		L8-L9	B8
			utility items	G180	Appliances (refrigerators, washers, etc.)											79	ME10	L3d	B5
			non-packaging food consumption related	G181	Tableware (e.g. plates, cups & cutlery)											89	ME01	L8-L9	B8
			fisheries related	G182	Fishing related weights, sinkers, lures										SUP Fishing Gear	80	ME07	L3f	B3

	Metal		fisheries related	G184	Lobster/crab pots										SUP Fishing Gear	87	ME07	L3f	B3
		G186	industry related		Industrial scrap											83		L3d	B8
			packaging		containers	G187	Drums & barrels	e.g. oil, chemicals								84	ME05	L3d	B4
		G191	undefined		Wire, wire mesh, barbed wire											88	ME09	L8-L9	B8
			vehicle related	G193	vehicle parts / car batteries			Cars/other transport vehicles parts made mainly of metal, incl. non-household batteries								89		L8-L9	B6
		G194	construction related		Cables											89+90		L3e	B7
			utility items	G195	Household Batteries											89	OT04	L8-L9	B8
			undefined	G197	Other metal objects			identifiable items not fitting in any other category			G198	Other metal pieces < 50 cm				89		L8-L9	B8
				G197	Other metal objects			identifiable items not fitting in any other category			G199	Other metal pieces > 50 cm				90		L3d	B8
	Glass/ ceramics		packaging	G200	Bottles incl. Pieces of bottles											91	GC02	L4a	D2
				G201	Jars incl. Pieces of jars											93	GC02	L8-L9	D1
			utility items		Light bulbs and flourecent light tubes	G202	Light bulbs									92	GC04	L8-L9	D4

	Glass/ ceramics		utility items		Light bulbs and flourecent light tubes	G205	fluorescent light tube									92	GC05	L8-L9	D4
			Non-packaging food consumption related	G203	Tableware (e.g. plates & cups)											102	GC03	L8-L9	D4
			construction related	G204	Construction material (brick, cement, pipes)											94	GC01	L8-L9	D4
			fisheries related	G207	Octopus pots										SUP Fishing Gear	95	GC08	L8-L9	D4
			undefined		other ceramic/pottery items			identifiable items not fitting in any other category								96	GC08	L4c-L4d	D4
					Other glass items			identifiable items not fitting in any other category								93	GC08	L8-L9	D4
					pieces of glass			not counted on OSPAR unless identifiable as bottle or jar									GC07	L4b	D3
	Mixed		medical related		other medical items (swabs, bandaging, adhesive plasters etc.)			identifiable items not fitting in any other category								105	OT05	L8-L9	F3
			personal hygiene & care		other personal hygiene and care items			identifiable items not fitting in any other category							SUP Sanitary	102		L5d	F3
	Artificial polymer materials		recreation related		plastic remains of fireworks			Rocket caps, fuse covers, exploding parts of battery fireworks								48	PL24	L1j	A14
			personal hygiene&care		wet wipes										SUP Sanitary	102	PL24	L5d	A14

ANNEX II. LIST OF RESCUE CENTERS AND REFERENCE LABORATORIES FOR MACRO AND MICRO LITTER INGESTION ANALYSES.

COUNTRY	INSTITUTION	TYPE	ACTIVITIES*	WEBSITE	LOCATION	AREA OF WORK
CROATIA/ ADRIATIC	Croatian Institute for Biodiversity and Biota	Research centre	1	http://www.hibr.hr/	Croatia	Eastern Adriatic
FRANCE	CESTMed	Rescue centre	1.2	www.cestmed.org	Le Grau-du-Roi, France	French continental Med
FRANCE	RTMMF	Stranding network	1.2	http://lashf.org/rtmmf/	Sète, France	French continental Med
FRANCE	RTMMF-CARI Corsica	Stranding network	1.2	http://lashf.org/rtmmf/	Corte, France	Corsica
FRANCE	CRFS	Rescue centre	1.2	https://centre-de-rehabilitation-de-la-faune.business.site/	Antibes, France	French continental Med
FRANCE	LDA 34	Veterinarian laboratory	2	http://www.herault.fr/service/laboratoire-veterinaire	Montpellier, France	French continental Med
FRANCE	LDA 30	Veterinarian laboratory	2	http://lda.gard.fr/accueil.html	Nïmes, France	French continental Med
FRANCE	UMR 5175 CEFE	Research centre	1, 2, 3, 5	https://www.cefe.cnrs.fr/	Montpellier, France	French continental Med
GREECE	ARCHELON, the Sea Turtle Protection Society of Greece	Rescue center	1, 2	https://www.archelon.gr	57 Solomou Street, GR-104 32, ATHENS, Greece	Entire Greece
GREECE	School of Veterinary Medicine, Aristotle University of Thessaloniki	Research Center (University)	1, 2	http://www.vet.auth.gr	Aristotle University of Thessaloniki, Faculty of Veterinary Medicine, University	Entire Greece

					Campus, GR-54124, Thessaloniki	
GREECE	Amvrakikos Wetlands National Park (MPA)	Stranding network	1	http://www.amvrakikos.eu/	1 Katsimitrou & Kommenou - Arta, 47100, GREECE	Amvrakikos Gulf, Ionian Sea
GREECE	National Marine Park of Zakynthos (MPA)	Stranding network	1	http://www.nmp-zak.org	1, Eleftheriou Venizelou str., GR-29100, Zakynthos	ZAKYNTHOS, Ionian Sea
GREECE	Hellenic Centre for Marine Research	Research Center	1,2, 3, 4	http://hcmr.gr	46,7km Athinon-Souniou Ave., GR-19313, Anavyssos, Greece	Entire Greece
ITALY	CRES Centro di Recupero del Sinis delle tartarughe e dei mammiferi marini	Rescue center	1, 2, 3	http://www.areamarinasinis.it/it/attivita/cres-centro-di-recupero-del-sinis/index.aspx?m=53&did=1665	P.zza Eleonora, 1, Càbras, ORISTANO	Sardinia Island, Western Med sub-region
ITALY	IAS-CNR Istituto per lo studio degli impatti Antropici e Sostenibilità in ambiente marino del Consiglio Nazionale delle Ricerche	Research Center	3	http://oristano2.iamc.cnr.it/	Loc. Sa Mardini, 09170 Torregrande, ORISTANO	Sardinia Island, Western Med sub-region
ITALY	CRAMA Centro Recupero Animali Marini Asinara	Rescue center	1,2	https://crama.org/	via Principe di Piemonte 2, 07046 Porto Torress, SASSARI	Sardinia Island, Western Med sub-region

ITALY	LAGUNA di NORA Centro Recupero Cetacei e Tartarughe marine	Rescue center	1, 2	http://www.lagunadinora.it/sezione.php?idsez=5	Laguna di Nora Loc. Nora, 09010 Pula - CAGLIARI	Sardinia Island, Western Med sub-region
ITALY	University of Cagliari, UNICA (DISVA, Dipartimento di Scienze della Vita e dell'Ambiente – Sezione di Biologia Animale ed Ecologia)	Research Center (University)	3	http://corsi.unica.it/bioecologiamarina/	Via Tommaso Fiorelli, n° 1 09126 – CAGLIARI	Sardinia Island, Western Med sub-region
ITALY	University of Sassari, UNISS	Research Center (University)	3	https://www.uniss.it/	Piazza Università 21, SASSARI	Sardinia Island, Western Med sub-region
ITALY	IMC Istituto Marino Costiero	Research Foundation	3	https://www.fondazioneimc.it/en/	Località Sa Mardini, 09170, Torregrande, ORISTANO	Sardinia Island, Western Med sub-region
ITALY	Istituto Zooprofilattico di Oristano	zooprophylactic institute	1, 2, 3	http://www.izs-sardegna.it/cs_sedi_oristano.cfm	via Atene, 2, 09170 ORISTANO	Sardinia Island, Western Med sub-region
ITALY	Istituto Zooprofilattico di Tortolì	zooprophylactic institute	1, 2, 3	http://www.izs-sardegna.it/cs_sedi_tortoli.cfm	Via Aresu, 2 – Tortolì	Sardinia Island, Western Med sub-region
ITALY	Acquario di Calagonone	Aquarium	1	https://www.acquariocalagonone.it	Via La Favorita, 08022 Cala Gonone NU	Sardinia Island, Western Med sub-region
ITALY	Centro Ricerche Tartarughe Marine - Osservatorio del Golfo di Napoli - Stazione Zoologica Anton Dohrn	Research Institute	1	http://www.szn.it/index.php/it/divulgazione/centro-ricerche-tartarughe-marine	Porto del Granatello, 80055 Portici NA	Campania Region Western Med sub-Region

ITALY	Istituto Zooprofilattico Sperimentale Lazio e Toscana	zooprophylactic institute	1	http://www.izslt.it/	Via Appia Nuova, 1411 – 00178 Roma	Lazio Region Western Med sub-region
ITALY	Istituto Zooprofilattico Sperimentale della Sicilia	zooprophylactic institute	1	http://www.izssicilia.it/	via Gino Marinuzzi, 3 90129 PALERMO	Sicily Island Central Mediterranean sub-region
ITALY	Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale"	zooprophylactic institute	1	http://www.izs.it/IZS/	Campo Boario | 64100 TERAMO	Abruzzo/ Molise Adriatic sub-Region
SPAIN	Universitat de Valencia	Research Center (University)	2, 3, 4	https://www.uv.es/uvweb/cavanilles-institute-biodiversity-biology/en/cavanilles-institute-biodiversity-evolutionary-biology-1285893448913.html	Parque Científico - Carrer del Catedrátic José Beltrán Martinez, 2, 46980 Paterna, Valencia	Valencian region
SPAIN	Xarxa de rescat d'animals marins de Catalunya	Regional rescue network	1	http://mediambient.gencat.cat/ca/05_ambits_dactuacio/patrimoni_natural/fauna-autoctona-protegida/xarxa-rescat-fauna-marina/	faunamarina.daam@gencat.cat; arcasur@gencat.cat; rgutierrez@gencat.cat	Catalan region
SPAIN	Universitat Autonoma de Barcelona	Research Center (University)	1, 2, 3	www.uab.cat	Plaça Cívica 08193 Bellaterra (Cerdanyola del Vallès)	Catalan region
SPAIN	Universitat de Barcelona	Research Center (University)	2, 3	www.ub.edu	Av. Diagonal 643, 08028 Barcelona, SPAIN	Catalan region

SPAIN	Fundación Oceanogràfic	Research Veterinary Centre / Rescue center	1	https://www.oceanografic.org/	Carrer d'Eduardo Primo Yúfera, 1 46013 Valencia - España	Valencian region
SPAIN	CREMA	Rescue center	1	http://www.auladelmar.info/crema	Calle Pacifico 80, 29004 Málaga	Southern Spanish Mediterranean
SPAIN	Asociación EQUINAC	Rescue center	1	https://asociacionequinac.org/	El Ejido (Almería)	Southern Spanish Mediterranean
SPAIN	Fundación Palma Aquarium	Rescue center	1	https://palmaaquarium.com/es/acuario/fundacion-palma-aquarium/fundacion-palma-aquarium	C/ Manuela de los Herreros i Sorà, 21 07610 Palma de Mallorca	Balearic Islands
SPAIN	CREM-Aquàrium	Rescue center	1	http://aquariumcapblanc.com/CREM	Carretera Cala Gració. 07820.Sant Antoni de Portmany. Ibiza.	Balearic Islands
SPAIN	MAPAMA (Ministerio de Agricultura, Pesca, Alimentación y Medio Amb.)	National authority	1	https://www.mapama.gob.es/	Marta Martínez-Gil, mmgil@mapama.es	Mediterranean Spain
SPAIN	Consejería de Agua, Agricultura y Medio Ambiente (Oficina de Impulso Socioeconómico del Medio Ambiente)	Stranding Network Coordinator	1	http://www.carm.es	mariaj.gens@carm.es; fescribanocanovas@gmail.com	Murcia region
SPAIN	Conselleria de Agricultura, Medio Ambiente, Cambio Climático y Desarrollo Rural	Stranding Network Coordinator	1	http://www.agroambient.gva.es/es	gomez_jualop@gva.es	Valencian region

SPAIN	Agencia de medio ambiente y agua	Stranding Network Coordinator	1	https://www.agenciamedioambienteyagua.es/	carogue38@hotmail.com; msvivas@agenciamedioambienteyagua.es	Andalucia region
SPAIN	CIRCE	Stranding Network Collaborator	1		renaud@stephanis.org	Andalucia region
SPAIN	EBD	Stranding Network Collaborator	1		joan.gimenez@csic.es	Andalucía region
SPAIN	PROMAR	Stranding Network Collaborator	1		rosahval@hotmail.com	Almería region
SPAIN	CREMA	Stranding Network Coordinator	1		crema@auladelmar.info	Málaga region
SPAIN	CECAM	Stranding Network Coordinator	1		ziphio@hotmail.com	Ceuta-Melilla region
SPAIN	CRAM	Stranding Network Collaborator	1		elsa@cram.org / vet@cram.org	Catalan region
SPAIN	SUBMON	Stranding Network Collaborator	1		manelgazo@submon.org	Catalan region

***ACTIVITIES LEGEND**

1 Sea Turtle rescue and handling
2 Litter ingestion by sea turtles
3 Micro litter ingestion by fish
4 Micro litter ingestion by invertebrates
5 Diet of sea turtle