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Guidance on the Monitoring of Marine Litter in European Seas

An update to improve the harmonised monitoring of marine litter under the Marine Strategy Framework Directive

MSFD Technical Group on Marine Litter

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Abstract

The Marine Strategy Framework Directive (MSFD) Technical Group on Marine Litter developed the 'Guidance on monitoring of marine litter in European seas' in 2013 to enable EU Member States to launch monitoring programmes for MSFD Descriptor 10: 'no harm caused by marine litter'. The maturity of methodological protocols for marine litter monitoring has increased over the last 10 years, based on research advances and Member States' efforts.

This document updates the previous guidance to facilitate the harmonisation of the monitoring framework for the MSFD, including protocols, recommendations, and information required to increase the comparability of data and assessments among Member States. The document comprises chapters dedicated to the protocols for monitoring marine litter across different marine environmental compartments (i.e. the coastline/beach, the surface layer of the water column, the seafloor/seabed) and types of litter (i.e. macro litter, mesolitter, microlitter, ingested litter and microlitter by biota, and entanglement with litter).

Foreword

The Marine Directors of the EU and all EU Member States have developed a common strategy to support the implementation of Directive 2008/56/EC, the Marine Strategy Framework Directive (MSFD). The main aim of this strategy is to ensure coherent and harmonious implementation of the Directive among EU Member States. The focus is on methodological questions related to a common understanding of the technical and scientific implications of the MSFD. In particular, one of the objectives of the strategy is the development of non-legally binding and practical documents, such as this guidance document, on various technical issues pertaining to the Directive.

To support and advise on the policy development and implementation process, the MSFD Technical Group on Marine Litter (TG ML) was set up as part of the MSFD Implementation Strategy. The TG ML acts through the mandate of the Marine Directors of the EU. It is led by the Directorate-General for Environment and chaired by the Spanish Centre for Public Works and Experimentation and the European Commission's Joint Research Centre. Members include EU Member State delegates, representatives of the parties to the Regional Sea Conventions, other stakeholders and invited technical experts. The TG ML reviews scientific developments and prepares technical guidance and information documents to support EU Member States in implementing the MSFD. Further information can be found on the TG ML page of the Joint Research Centre's MSFD Competence Centre website (http://mcc.jrc.ec.europa.eu/dev.py?N=41&O=434&titre_chap=TG %20Marine %20Litter).

The present document updates the previous *Guidance on monitoring of marine litter in European seas*, published in 2013, to facilitate the harmonisation of the monitoring framework for the MSFD, including protocols, recommendations and information required to increase the comparability of data and assessments among Member States.

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1 Introduction

The Marine Strategy Framework Directive ([1]) (MSFD) is the EU policy for the protection of the marine environment. The MSFD requires European Member States to develop strategies and establish monitoring programmes to assess the state of marine waters and to achieve or maintain the Good Environmental Status (GES) in European seas.

'Marine litter' is defined by the United Nations Environment Programme (UNEP) as 'any persistent, manufactured or processed solid material discarded, disposed of or abandoned in the marine and coastal environment' (UNEP, 1995) ([2]). Marine litter consists of anthropogenic items that have been discarded in the sea or rivers or on beaches; transported to the sea by rivers, sewage, stormwater or winds; accidentally lost, including lost at sea in bad weather; or deliberately left on beaches and shores by people. Increasing levels of marine litter have led to growing concern worldwide regarding its environmental impact (e.g. UNEP, 2016). Marine litter is covered by Descriptor 10 (D10) of the MSFD, which requires that 'properties and quantities of marine litter do not cause harm to the coastal and marine environment'.

Marine litter research has expanded its borders into different areas of knowledge, developing new monitoring methodologies for different environmental compartments and types of marine litter, since the 2013 publication of the previous guidance on marine litter monitoring in European seas (Galgani et al., 2013). Ongoing discussions have highlighted the variability of methods, data formats and data accessibility, which hinders comparability, as well as differences in the provisions for GES assessment.

Harmonised methods and comparable data and formats are needed to establish trends and determine threshold values, which are set in relation to a reference condition. The Commission Decision (EU) 2017/848 ([3]) defines 'threshold value' as 'a value or range of values that allows for an assessment of the quality level achieved for a particular Criterion, thereby contributing to the assessment of the extent to which good environmental status is being achieved' and specifies that 'threshold values are intended to contribute to Member States' determination of a set of characteristics for Good Environmental Status and inform their assessment of the extent to which Good Environmental Status is being achieved'.

The MSFD Technical Group on Marine Litter (TG ML) has carried out its work since 2011, investigating outstanding issues, such as the assessment of harm caused by marine litter (Werner et al., 2016); approaches to the identification of land- and marine-based sources of marine litter (Veiga et al., 2016); monitoring of riverine litter (González et al., 2016); the identification of the most abundant marine beach litter items (Addamo et al., 2017); the definition of beach litter baselines (Hanke et al., 2019) and threshold values (van Loon et al., 2020); and the development of the *Joint List of Litter Categories for Marine Macrolitter Monitoring* (Fleet et al., 2021). Furthermore, the MSFD TG ML has explored the Directive needs and started to develop approaches to determining threshold values for the various marine litter criteria (van Loon et al., 2020; Werner et al., 2020; Vasilakopoulos et al., 2022).

This document aims to update the guidance presented in 2013 (Galgani et al., 2013), including with regard to the methods and protocols that have been developed, which have reached a degree of maturity that allows for harmonised monitoring. This harmonisation will reduce differences in the collection, classification and reporting procedures among EU Member States, increasing the comparability of the data and allowing environmental assessment at the regional and European levels. Simultaneously with the preparation of this document, information on the current scientific background has been compiled to update the existing guidance, resulting in the production of an additional technical report supplementing the chapter on floating marine macro litter (FMML) (Vighi et al., 2022).

([1]) Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008 establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive) (OJ L 164, 25.6.2008, p. 19).

([2]) Marine litter definition put forward by UNEP and adopted during the intergovernmental conference on the Global Programme of Action for the Protection of the Marine Environment from Land-based Activities, Washington, DC, 1995 (UNEP(OCA)/LBA/IG.2/7, p. 54).

([3]) European Commission, Commission Decision (EU) 2017/848 of 17 May 2017 laying down criteria and methodological standards on good environmental status of marine waters and specifications and standardised methods for monitoring and assessment, and repealing Decision 2010/477/EU (OJ L 125, 18.5.2017, p. 43).

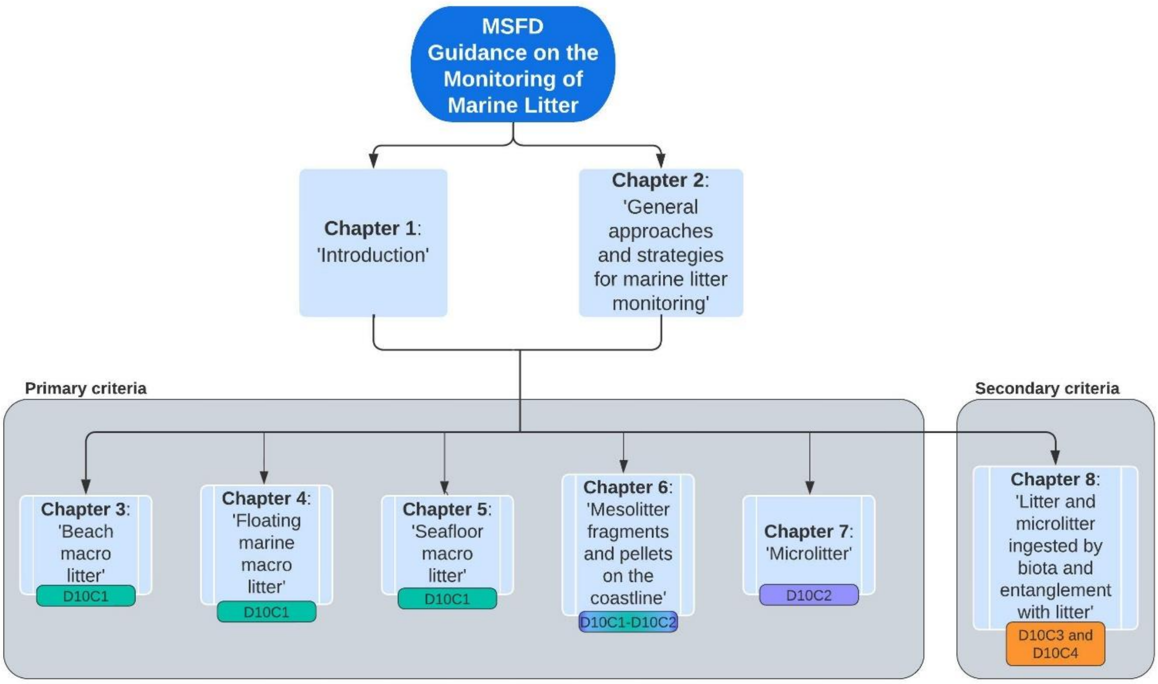
1.1 Use and structure of the guidance on monitoring marine litter

This guidance document on marine litter monitoring is primarily intended to provide the EU Member States and other national authorities, regional bodies and intergovernmental and international organisations responsible for marine litter management with the information necessary to facilitate the strategic design and effective implementation of harmonised marine litter monitoring programmes.

The document is divided into eight main chapters (Figure 1.1). Chapter 2 provides information on the marine litter monitoring requirements for successfully implementing MSFD D10, including the conceptual approaches and aims of the harmonised monitoring framework. Chapters 3–7 describe the specific monitoring strategies, methods, and assessments for each marine environmental matrix, including quality assurance / quality control (QA/QC) measures and data processing and reporting approaches. These chapters are structured according to the criteria of D10, with Chapters 3–5 corresponding to the primary criterion (D10C1) for monitoring marine litter, excluding microlitter. Chapter 6 considers methods for monitoring mesolitter fragments and pellets on beaches. Chapter 7 includes recommendations for monitoring microlitter (D10C2) on the water surface and in sediments, and subsequent sample processing and analytical methods.

Methods for criteria D10C3 and D10C4 are compiled in Chapter 8, including protocols for monitoring litter and microlitter ingested by organisms (D10C3), and methods for assessing entanglement with litter (D10C4).

Figure 1.1. General structure of the MSFD Guidance on monitoring of marine litter.



2 General approaches to and strategies for marine litter monitoring

This chapter discusses general topics associated with the monitoring of marine litter and the requirements under the MSFD, including advice on the harmonisation of the monitoring framework, the data requirements, and the costs and efforts of monitoring, and a general overview of existing monitoring protocols.

Monitoring is crucial to assess the state of the environment by providing reliable and objective information to address specific policy questions and concerns. Therefore, monitoring programmes should be designed as long-term processes and in accordance with the purposes of monitoring, which may include the assessment of environmental status, including temporal and spatial trends; the identification of sources and hotspots; the assessment of the degree of achievement of targets; or the evaluation of the effectiveness of measures. However, monitoring requirements also depend on measuring techniques and reliable, comparable, and fit-for-purpose data at affordable costs, to implement MSFD D10 successfully.

The revision of monitoring frameworks and programmes to follow harmonised approaches that take into account the degree of maturity and the research development of methodologies favours the consistency and robustness of data. It also facilitates the exchange and comparability of data among institutions to address policy concerns.

2.1 Monitoring requirements under the Marine Strategy Framework Directive

The EU MSFD covers diverse aspects of marine GES by monitoring numerous environmental parameters for the holistic assessment of the marine environment, ranging from marine biodiversity to multiple anthropogenic pressures (e.g. marine contaminants, marine litter or energy, including underwater noise).

Article 11 of the MSFD provides legally binding requirements for establishing and implementing coordinated monitoring programmes to assess EU waters' environmental status. Article 11(1) specifies that:

> *On the basis of the initial assessment made pursuant to Article 8(1), Member States shall establish and implement coordinated monitoring programmes for the ongoing assessment of the environmental status of their marine waters on the basis of the indicative lists of elements set out in Annex III and the list set out in Annex V [of the MSFD], and by reference to the environmental targets established pursuant to Article 10.*
>
> *Monitoring programmes shall be compatible within marine regions or subregions and shall build upon, and be compatible with, relevant provisions for assessment and monitoring laid down by Community legislation, including the Habitats and Birds Directives, or under international agreements.*

In addition, Article 11(2) indicates that:

> *Member States sharing a marine region or subregion shall draw up monitoring programmes in accordance with paragraph 1 and shall, in the interest of coherence and coordination, endeavour to ensure that:*
>
> *(a) monitoring methods are consistent across the marine region or subregion so as to facilitate comparability of monitoring results;*
>
> *(b) relevant transboundary impacts and transboundary features are taken into account.*

EU Member States reported on their monitoring programmes under Article 11 in 2014 and had updated these programmes by 2020, in line with Article 17(2) of the MSFD. After the first cycle of implementation, to facilitate future updates of the initial assessment and ensure comparability between Member States' assessments, in 2017 the European Commission released a new Commission Decision (Decision (EU) 2017/848). It clarified, revised and introduced criteria, methodological standards, specifications and standardised methods to be used by Member States for marine litter monitoring instead of those previously laid down in Commission Decision 2010/477/EU. Commission Decision (EU) 2017/848 establishes four criteria for determining GES under D10 (Box 2.1).

Regular assessment of the state of the environment is crucial for adaptive management processes within the MSFD and the European Regional Seas Conventions (RSCs). Moreover, the Zero Pollution Action Plan ([4])

([4]) See European Commission (undated), 'Zero Pollution Action Plan' (https:/ec.europa.eu/environment/strategy/zero-pollution-action-plan_en) provides an approach to addressing environmental pollution and targets for reducing litter at sea. Marine litter has been identified as one of the main global challenges by the international scientific community and it is specifically addressed by UN Sustainable Development Goal 14 ('Life below water'). It is covered by the ministerial declaration of the UN Environment Assembly on strengthening actions for nature to achieve the sustainable development Goals ([5]) and launched negotiations on the Global Plastic Pollution Treaty ([6]) during its fifth session (UNEA-5). Marine litter and microplastics are also covered by the global monitoring processes supported by the G20 ([7]), the Blue Planet initiative of the Group on Earth Observations ([8]) and the UNEP / Global Partnership on Marine Litter ([9]). Therefore, actions and best practices should be linked to these external global monitoring schemes and other activities to provide comparable quantitative assessments on a large scale.

Box 2.1. Marine litter criteria and methodological standards under the MSFD, as specified in Commission Decision (EU) 2017/848

Primary criteria

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

Specifications: 'Litter shall be monitored on the coastline and may additionally be monitored in the surface layer of the water column and on the seabed. Information on the source and pathway of the litter shall be collected, where feasible.'

D10C2 – 'The composition, amount and spatial distribution of microlitter on the coastline, in the surface layer of the water column, and in seabed sediment are at levels that do not cause harm to the coastal and marine environment.'

Specifications: 'Microlitter shall be monitored in the surface layer of the water column and in the seabed sediment and may additionally be monitored on the coastline. Microlitter shall be monitored in a manner that can be related to point-sources for inputs (e.g. harbours, marinas, waste-water treatment plants, storm-water effluents), where feasible.'

Secondary criteria

D10C3 – 'The amount of litter and microlitter ingested by marine animals is at a level that does not adversely affect the health of the species concerned. Member States shall establish threshold values for these levels through regional or subregional cooperation.'

D10C4 – 'The number of individuals of each species which are adversely affected due to litter, such as by entanglement, other types of injury or mortality, or health effects. Member States shall establish threshold values for the adverse effects of litter, through regional or subregional cooperation.'

Specifications: 'For D10C3 and D10C4: the monitoring may be based on incidental occurrences (e.g. strandings of dead animals, entangled animals in breeding colonies, affected individuals per survey).'

([5]) UN Environment Assembly (2022), *Ministerial declaration of the United Nations Environment Assembly at its fifth session – Strengthening actions for nature to achieve the sustainable development goals*, UNEP/EA.5/HLS.1, Nairobi.

([6]) UN Environment Assembly (2022), *Resolution adopted by the United Nations Environment Assembly on 2 March 2022 – End plastic pollution: Towards an international legally binding instrument*, UNEP/EA.5/Res.14, Nairobi.

([7]) See Japanese Ministry of the Environment (2021), G20 Report on Actions against Marine Plastic Litter – Third information sharing based on the G20 implementation framework: 2021, 2nd edition, Tokyo (https://www.env.go.jp/content/900505935.pdf).

([8]) See Group on Earth Observations, Blue Planet initiative (undated), 'Marine Litter' (https://geoblueplanet.org/marine-litter/).

([9]) See UNEP (2021). 'Global Partnership on Marine Litter (GPML) digital platform', presentation (https://wedocs.unep.org/handle/20.500.11822/37070)

2.2 Harmonising the monitoring framework under the Marine Strategy Framework Directive

Harmonisation of monitoring approaches, in particular for sampling design, marine litter categories, analysis techniques and data reporting, is vital to combine and compare datasets. Numerous international and national frameworks are working on developing harmonised approaches to marine litter and microlitter (e.g. the UN, the RSCs, the National Oceanic and Atmospheric Administration (NOAA)); at the European level, a major effort is represented by the TG ML.

The aims of marine litter monitoring in the MSFD framework are to:

1. generate compatible and comparable data for each marine environmental compartment to allow spatial and temporal trend analysis;
2. identify the main marine litter sources to support the development of mitigation measures;
3. assess whether GES has been achieved or maintained, and if environmental status is improving, remaining stable, or deteriorating;
4. assess the progress towards achievement of environmental targets and the effectiveness of mitigation strategies.

Monitoring may have different aims and purposes at different stages of the management cycle; however, the harmonisation of monitoring frameworks is fundamental to inform policy and support the implementation of the MSFD towards provisions such as reduction targets, the definition of threshold values, and the determination of GES, based on compatible and comparable quantitative data and assessments.

This updated guidance on monitoring marine litter is intended as a tool to aid EU Member States in complying with the new decision requests by presenting the state of the art in methodologies for marine litter monitoring. EU Member States' efforts, research developments and the involvement of non-governmental organisations (NGOs) in the last few decades, have indeed increased the level of maturity of the diverse litter and microlitter methodologies used during the monitoring processes (see Section 2.5), creating a base for harmonisation and comparability of results, through regional cooperation, as requested by Article 6 of the MSFD. Furthermore, the MSFD requires an approach that generates information and data of sufficient quality and comprehensibility through incorporating QA/QC approaches into the different stages of the monitoring framework (i.e. from the definition of monitoring programmes to the reporting stage). The respective chapters elaborate on the QA/QC aspects relevant to each marine compartment. The EU Member States' adoption of the *Joint List of Litter Categories for Marine Macrolitter Monitoring* (Fleet et al., 2021) is considered in the monitoring approach presented, and this will pave the way for the consistency, compatibility and comparability of assessments of D10C1 under the MSFD.

2.2.1 Spatial distribution of survey sites – site selection strategies

The strategy for selecting survey sites is related to the purpose and design of the monitoring. It has implications for the monitoring analysis, affecting the comparability of monitoring programmes even if the same sampling methods are used.

Site selection may depend on many characteristics, such as the potential land and sea sources of marine litter, pathways, potential accumulation areas and the presence of protected areas or areas of particular environmental or social value. Another strategy is the random selection of sites that may meet certain requirements based on the purpose of the monitoring. In this sense, Commission Decision (EU) 2017/848 specifies the environmental compartments to be selected and, where possible, prioritises areas that provide information on potential sources of marine litter (see Box 2.1).

It should be ensured that survey sites are representative of the state of litter in the study area, regardless of the other characteristics considered in their selection. Replication is necessary to assess the intrinsic variability of litter abundance and distribution in the study area. The determination of the locations and the minimum sample size are still under discussion for some of the methods and environmental compartments. In these cases, priority should be given to monitoring programmes that focus on measuring the state of the environment and the spatial and temporal trends that allow for their statistical evaluation.

The selection of marine litter monitoring sites can be compatible with their integration into other studies or monitoring programmes or can be made through opportunistic sampling as a cost-effective approach (e.g. visual observations from ferry lines, seafloor litter monitoring as part of scientific trawl surveys for stock assessment), seeking a compromise between representativeness for marine litter monitoring and the opportunity to reduce costs. However, it is essential to analyse their suitability for marine litter monitoring, together with the trade-off between the representativeness of monitoring and the opportunity to reduce costs.

2.2.2 Quality assurance / quality control approaches

Implementing QA/QC processes provides measures to improve the rigour and the quality of data and results (Erickson et al., 1991). Data and information generated from the assessments should be of sufficient quality to answer policy questions and facilitate decision-making. Incorporating harmonised QA/QC approaches should ensure the quality and integrity of marine litter monitoring data at all stages of the monitoring programme (i.e. from monitoring programme design to storage and reporting of data and information).

The approaches and measures used in QA/QC may vary depending on the stage of the monitoring programme, the method used or the marine environmental compartment addressed. Elements such as harmonising terminology to reduce inconsistencies have been developed for macro litter items in the *Joint List of Litter Categories for Marine Macrolitter Monitoring* (Fleet et al., 2021). Reference materials and inter-laboratory comparisons (e.g. the quality assurance of information on marine environmental monitoring in Europe project (QUASIMEME) (van Mourik et al., 2021), the Joint Research Centre (JRC), the Bundesanstalt für Materialforschung und -prüfung (BAM)) are essential for ensuring accuracy and reliability and identifying and addressing biases to improve the quality of microlitter measurements. Currently, inter-laboratory comparisons targeting microlitter are in the early stages, but are expected to be further developed in the coming years. Specific recommendations for sampling and data processing are detailed for each marine environmental compartment in the subsequent chapters of this guidance document.

Four general aspects should be considered when adopting QA/QC measures at different phases of the design of monitoring programmes.

- Harmonised operational procedures. This should include the design of the monitoring, the selection of methods to ensure that data are comparable, and the specification of procedures and responsibilities through protocols or field manuals.
- Harmonised terminology. The use of common terminology (e.g. litter item categories, litter characteristics) will reduce inconsistencies in data reporting and allow comparison of results between regions and marine environmental compartments.
- Management and traceability. Management and traceability systems should be implemented in the different phases of the monitoring programme to control, plan and manage the tasks of the monitoring programme.
- Control and evaluation of critical phases. Systematic quality checks and random assessment evaluations should be in place at each stage of the monitoring programme to detect potential failures or errors (e.g. checking data in report against raw data).

However, a complete discussion of specific QA/QC procedures at all phases of a monitoring programme is beyond the scope of this report.

2.3 Data requirements for monitoring

Monitoring programmes should follow the practices set out in their data management policy/strategy for handling collected or generated data that can be used for reporting and decision-making. In addition to ensuring data management and security, these practices should support comprehensive data analysis and quality and control requirements to ensure that the data meet the requirements of the MSFD.

This guidance document provides recommendations on data collection and units (i.e. standard categories of items), but it is beyond the scope of this guidance to provide specific recommendations on data handling and reporting.

2.3.1 Data units

The data's recording, format and units may vary depending on the questions to be answered and the sampling methods used in each marine environmental compartment. Commission Decision (EU) 2017/848 sets out the units of measurement to be reported for each D10 criterion under the MSFD in the decision's 'Specifications and standardised methods for monitoring and assessment' sections (Box 2.2).

The data should also be accompanied by auxiliary data that describe a dataset and the monitoring process (i.e. metadata). Metadata used in monitoring activities typically include three types of metadata: administrative metadata (e.g. unique identification, date, location, surveyor/scientist responsible, keywords), technical metadata (e.g. equipment used, opening, exposure, file format) and descriptive metadata (e.g. environmental variables, short description). Information on the specific metadata requirements for each method is provided in Chapters 3–8.

Box 2.2. Units of measurements for the MSFD D10 criteria, as specified in Commission Decision (EU) 2017/848

Primary criteria

D10C1 – 'Amount of litter per category in number of items:

— per 100 metres (m) on the coastline,
— per square kilometre (km^2) for surface layer of the water column and for seabed'.

D10C2 – 'Amount of micro-litter per category in number of items and weight in grams (g):

— per square metre (m^2) for surface layer of the water column,
— per kilogram (dry weight) (kg) of sediment for the coastline and for seabed'.

Secondary criteria

D10C3 – 'Amount of litter/micro-litter in grams (g) and number of items per individual for each species in relation to size (weight or length, as appropriate) of the individual sampled'.

D10C4 – 'Number of individuals affected (lethal; sub-lethal) per species'.

2.3.2 Data handling and reporting

Data handling and reporting of MSFD marine litter data refer to raw and interpreted data (information): data on the occurrence and composition of litter (Article 8), on progress towards GES (Article 9) and targets (Article 10), and on the impact of measures and actions (Article 13). The separation between raw data and interpreted information offers a basis for interpreting the Directive's phrase 'data and information' in Article 19(3).

The content and formats used for the reporting are agreed upon by the European Commission and Member States in the context of the MSFD Common Implementation Strategy (MSFD CIS). The discussions are dealt by the Working Group on Data, Information and Knowledge Exchange (WG DIKE) and steered by the EC Directorate-General for Environment (DG ENV) and the European Environment Agency (EEA).

Data are often made available through data infrastructures, such as the European Marine Observation and Data Network (EMODnet), following the principles and legal requirements of the Infrastructure for Spatial Information in the European Community (INSPIRE). Other platforms or infrastructures are also involved in collecting marine litter data relevant to the MSFD at different levels (e.g. at the national or regional level), establishing coordinated linkages and collaboration between the different data infrastructures.

2.4 Cost and efforts needed for marine litter monitoring

Marine litter monitoring requires efforts and resources that depend on the priorities/scope set during the design of the programmes. An important factor determining the costs of marine litter monitoring is the marine space that is planned to be monitored and its influence on the management measures. For example, monitoring beach litter in remote and inaccessible beaches is more costly and requires more effort than monitoring easily accessible ones. Similarly, monitoring seafloor litter in deep waters (e.g. > 800 m depth) requires substantial infrastructure, equipment and know-how, which increase the costs. Decision-making tools may help design effective and efficient monitoring programmes (e.g. to determine the spatial and temporal resolution needed or possibilities for the integration of techniques). Cooperation between Member States in the execution of monitoring programmes, the use of EU services/data products (e.g. Copernicus products) and joint monitoring with other ongoing programmes (e.g. the International Bottom Trawl Survey (IBTS)) are some examples of optimally using resources to reduce monitoring costs.

The main points that need to be taken into consideration for a cost-effective monitoring programme are summarised below.

Technological improvements for marine litter monitoring are expected to improve data quality and accuracy, increase the spatial coverage of monitoring and lead to faster data acquisition. Nevertheless, to date, the development of such methods has still not reached the desired level of integration in the monitoring programmes, as, in many cases, they are considered high cost. This is the case for aerial imaging of floating macro litter and the use of autonomous underwater vehicles (AUVs) for deep seafloor litter monitoring. For microlitter monitoring, laboratory equipment is another factor affecting the overall costs, especially if targeting the identification of microlitter of < 300 μm.

Although many D10 criteria require scientific expertise, sophisticated equipment, and the use of ships, there are certain cases in which volunteers and citizens can be trained in marine litter monitoring. This is the case for beach litter monitoring. Through citizen science and community engagement programmes a high level of spatio-temporal monitoring coverage can be achieved in a cost-effective way. The engagement of citizens is expanding to groups of divers for monitoring the shallow seafloor. As with any citizen-science-based programme, thoughtful design and ongoing quality control are essential elements of success, but Member States should support and validate the data generated for the requirements under the MSFD.

The integration of marine litter monitoring into other monitoring programmes is encouraged. For seafloor litter in trawlable areas (soft bottoms), the integration with trawling for monitoring fish stocks (e.g. IBTS; see Kammann et al., 2017) is already a common approach in most Member States. Unfortunately, this is not the case for the Mediterranean region, resulting in a severe data gap for this region ([10]). For the shallow seafloor and/or rocky bottoms, integration should be sought with other monitoring programmes related to the sublittoral zone (e.g. Nature 2000, introduced by the Habitats Directive 92/43/EEC) and to the monitoring for the needs of Descriptors 1 and 6 (see, for example, Angiolillo et al., 2023).

Monitoring of FMML and floating marine microlitter can be integrated into the monitoring programmes of other Descriptors that make use of ships, such as those for Descriptors 1, 4, 5 and 8. For floating macro litter, visual observations from ferries have been used by many Member States; however, these require a large workforce. For macro litter and microlitter in biota, the same approach can be followed (e.g. integration with other monitoring programmes collecting fauna). For microlitter ingestion by fish, monitoring can be done through integration with the IBTS trawling programme and subsequent sharing of fish samples. For other kinds of animals (beached turtles or birds), integration should be sought with Descriptor 1 and/or with specific networks and NGOs that record and handle beached animals, including protected species. Therefore, only authorised people should handle live and dead animals or parts of them.

Re-designing the monitoring programme on a 6-year cycle is envisaged, although improvements may be made if possible. Based on the experience and knowledge obtained from previous cycles, a more cost-effective programme can be designed by updating the selection of monitoring sites and spatio-temporal coverage, by improving integration with other programmes and by fine-tuning monitoring practices.

Details on monitoring costs of or the effort required for specific protocols are provided in the subsequent chapters. A general summary of the estimated cost per year, the effort per survey and the level of expertise for each of the protocols proposed in this guidance is given below (Table 2.1). Please note that these are very rough estimates, as the staff and equipment costs vary considerably across countries.

([10]) See EMODnet (2021), *'European seafloor litter standardized, harmonized and validated datasets 2006/2021 v2021'* (https://sextant.ifremer.fr/record/72155279-1315-48ce-99a1-48a957ed599b/)

Table 2.1. Summary table of estimated costs of and effort needed in applying different protocols on a three-step scale.

Estimated cost-effort and level of expertise																				
Marine compartment		Beach	FMML			Seafloor			Mesolitter and pellets	Microlitter			Biota							
MSFD criteria		D10C1	D10C1			D10C1			D10C1 and D10C2	D10C2			D10C3					D10C4		
Protocol		Visual and collection	Visual	Imagery	Unmanned aerial vehicles	Visual (shallow)	Trawl	Imagery	Beach – collection	Surface trawls	Grab and core-based	Beach – collection	Seabirds	Turtles	Marine mammals	Fish	Mussels	Turtles and marine mammals	Seabirds – nesting	Benthic organisms
Estimated cost	Collection of samples	L/M	L (b)/H	H	M/H	M/H	L/M	H	L/M (a)	L/M	M	L/M (a)	M/H	M/H	H	L	L	M	M	M
	Analysis of samples	L/M (a)	M/H	M/H	M/H	M/H	L	M	L	M/H	M/H	M	M	M	M	H	H	L	L	L
	Equipment required	L	L	H	H	M	M	H	L	M/H	M/H	M	L	L	L	M/H	M/H	L	L	M
Estimated effort	Working – hours required for sample collection and analysis	L/M (a)	M	M/H	M/H	L/M (a)	L/M (a)	M/H	M	M/H (c)	M/H (c)	M/H (c)	M	M	M	M/H	M/H	L	L	L
Required expertise	Sampling	L/M	L/M	H	H	H/M	L/M	H	L/M	M/H	M/H	M	M	M	M	H	H	L	L	L/M
	Analysis	M	H	M	M	L	L	M	M	M/H	M/H	M/H	M	M	M	M/H	M/H	L	L	L

	Statistical analysis	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	L/M	L/M	L/M
Potential performers		VT, P	P	P	P	P	P	P	VT, P	P	P	P	VT, P	VT, P	VT, P	P	P	VT, P	VT, P	P

([a]) Depending on litter quantities.
([b]) If ships of opportunity are used.
([c]) Depending on microlitter quantities and the method of analysis used.
NB: For cost per year, L = low (EUR 1000–10000), M = medium (EUR 10000–50000) and H = high (EUR 50000–100000). For effort per survey, L = low (< 8 working hours), M = medium (8–40 working hours) and H = high (> 41 working hours). For required expertise, L = low (trained personnel without specific professional formation), M = medium (trained personnel with specific professional formation) and H = high (high expertise and special skills required). For potential performers, VT = volunteers and P = professionals.

Source: Adapted and updated from Galgani et al. (2013).

2.5 General overview of protocols

The methods/protocols presented in this update of the guidance are the results of the last years of development of methodologies for monitoring the environmental status and measuring progress toward GES. These protocols must be effective in assessing progress towards targets and measuring the impact of measures. Moreover, protocols need to be flexible enough to be integrated into combined monitoring programmes and to continue to address changes triggered by the implementation of specific measures.

Monitoring methods that provide high-level detail may be more suitable for addressing measures related to specific litter types, for instance monitoring beach litter by using a detailed categorisation of the litter items. However, other proposed protocols may be applied or combined where limitations or incompatibilities exist, thus offering approaches that can enable the assessment of targets linked to trends or measures.

Table 2.2. provides an updated overview of the available protocols, from the previous guidance (Galgani et al., 2013) to support the appropriate selection of protocols based on the following characteristics:

- The level of maturity refers to the extent to which the protocol/method has been tested and systematically applied in different regions for a period. The following scale has been used to categorise the maturity of protocols: high, when the protocol has been applied systematically for more than a decade and extensively in one or more regions; medium, when the protocol/method has been applied systematically in a few countries/regions for less than a decade; and low, when the protocol/method is under development/has been only tested in a couple of pilots.
- The level of detail generated is the potential of the protocol/method to generate details and information in terms of the material, nature and purpose of the items sampled, which can be attributed to specific and distinct sources.
- Geographical applicability refers to the potential of the protocol to be applied in any geographical area/region.
- The main limitations are the key aspects inherent to the protocol and/or factors that can limit its applicability and/or generation of reliable and comparable data.

Table 2.2. Updated overview of monitoring protocols.

D10 criterion	Marine environment matrices	Method/protocol	Level of maturity (development compared with previous guidance)	Level of detail generated	Geographical applicability	Limitations
D10C1	Beach	Visual/ collection	High (maintained)	High (size ≥ 2.5 cm)	High	Distribution and accumulation of litter may be affected by weather and sea conditions Variability among sites
	Floating	Visual	High (maintained – improved with the use of dedicated apps)	Medium (size ≥ 2.5 cm)	High	Observations affected by weather and sea conditions
	Floating	Automated camera survey (imagery)	Medium (increased – in development)	Low/medium/high (depending on the dispositive/camera and platform used (height and resolution))	High	Still needs to be adapted for routine use Depends on good weather and sea conditions Analysis can be time-consuming
	Floating	Aerial (aircraft) survey (visual)	Medium (increased)	Low (size ≥ 30 cm)	High	Depends on good weather and sea conditions Focused on large floating macro items
	Floating	Aerial (unmanned aerial vehicle) survey	Low (in development)	Medium/high (depending on the dispositive/camera used (height and resolution))	High	Depends on good weather and sea conditions Analysis can be time-consuming
	Seafloor (shallow)	Visual	Medium (maintained)	Medium	High	Restricted to accessible areas
	Seafloor	Bottom-trawl surveys	High (increased – systematically applied for more than a decade)	Medium	Medium	Restricted to flat and smooth bottoms, in continental shelves Affects the seafloor structure

D10 criterion	Marine environment matrices	Method/protocol	Level of maturity (development compared with previous guidance)	Level of detail generated	Geographical applicability	Limitations
	Seafloor	Video imagery	Medium (maintained)	Medium	Medium	Affected by turbidity conditions
D10C1-D10C2	Mesolitter and pellets	Beach sediment	Low (in development)	Medium	Medium (limited to sandy beaches)	Restricted to sandy beaches Distribution and accumulation of litter may be affected by weather and sea conditions Variability among sites
D10C2	Floating microlitter	Water manta trawl	Medium/High (increased – systematically applied for more than a decade)	Medium/ High (depending on the mesh size: usually 300 µm)	High	Affected by weather and sea conditions Clogging problems Potential contamination from the vessel and tow rope fibres
	Seafloor microlitter	Sediment collection	Medium (increased)	High (size ≥ 20 µm)	Medium (only in soft sediments)	Limited representativeness Restricted to soft sediments
	Beach microlitter	Beach – collection	Low	Medium/ High	Medium (limited to sandy beaches)	Restricted to sandy beaches Distribution and accumulation of litter may be affected by weather and sea conditions High variability among sites
D10C3	Biota	Seabirds (ingestion)	High (maintained)	Medium (size ≥ 1 mm)	Medium	Depends on availability of dead seabirds Affected by feeding selectivity and behaviour Depends on geographical coverage of species

D10 criterion	Marine environment matrices	Method/protocol	Level of maturity (development compared with previous guidance)	Level of detail generated	Geographical applicability	Limitations
		Turtles (ingestion)	Medium/high (increased)	Medium (size ≥ 1 mm)	Medium	Depends on availability of animals Affected by feeding selectivity and behaviour Depends on geographical coverage of species
		Marine mammals (ingestion)	Low (maintained)	Medium (size ≥ 1 mm)	Medium	Depends on availability of animals Affected by feeding selectivity and behaviour Low rates of ingested litter Depends on geographical coverage of species
		Fish (ingestion)	Low (maintained)	Medium	High	Depends on geographical coverage of species. Level of maturity (low)
		Mussels (ingestion)	Low (maintained)	Medium	High	Level of maturity (low)
D10C4	Biota	Turtles and marine mammals (entanglement)	Low (maintained)	Low/medium	Medium	Depends on availability of entangled animals
		Seabirds (nesting materials)	Medium/low (increased)	Low/medium	Medium	Depends on geographical coverage of species.
		Benthic organisms	Low/medium	Low/medium	Medium	Depends on geographical coverage of selected species.

Source: Adapted and updated from Galgani et al. (2013).

3 Beach macro litter

3.1 Introduction

Within the MSFD (Directive 2008/56/EC) and Commission Decision (EU) 2017/848, criterion D10C1 has been defined as 'The composition, amount and spatial distribution of litter on the coastline, in the surface layer of the water column, and on the seabed, are at levels that do not cause harm to the coastal and marine environment'. This criterion refers to items larger than 5 mm (macro litter and mesolitter). Moreover, the MSFD requires, when feasible, the collection of information on the sources and pathways of marine litter to pinpoint the prioritisation and elaboration of targeted prevention, reduction and mitigation measures. In addition to identifying tailor-made management approaches, the MSFD requires assessment of their effectiveness to feed into the decision-making process of the subsequent MSFD implementation cycles.

In September 2020, the Commission published a threshold value for marine macro litter (> 2.5 cm) on coastline, paving the way for reducing harm to European regional seas from beach litter to a sufficiently precautionary level. EU Member States' experts have agreed that the median value of beach litter within a country subregion has to be less than 20 items per 100 m of coastline to stay under the threshold as part of GES for marine litter (van Loon et al., 2020). The reduction of beach litter in Europe in order to move towards achieving GES requires a combination of efforts at different levels. These include legislative measures at the EU level, such as the European Plastics Strategy (European Commission, 2018), the Single-Use Plastics Directive ([11]), the Water and Waste policies, the Green Deal and the Circular Economy Action Plans; measures in the context of the European Regional Seas Conventions and Action Plans against marine litter; national initiatives ranging from the country level to the municipality levels, including awareness-raising targeting different societal actors.

3.1.2 Background and state of the art

Beach surveys for macro litter assessment are the most common marine litter monitoring (Ryan et al., 2009). Litter monitoring on the European Regional seas' coasts has developed from several community-based campaigns, mostly of NGOs (Galgani et al., 2013). Originally designed to heighten public awareness or make a simple assessment of the magnitude of the problem, they have developed over the past 40 years into a tool for monitoring litter washed ashore and/or deposited on beaches (e.g. Schulz et al., 2015). In 2013, the MSFD TG ML published operational guidelines on how to monitor beach macro litter on the European coastline to address the need for obtaining harmonised beach macro litter data and support Member States in setting up their beach macro litter monitoring programmes (Galgani et al., 2013). Indeed, the comparison of beach litter data among assessment programmes and Member State is one of the aims of the MSFD. While some Member State already have beach litter monitoring programmes in place (e.g. countries in the Helsinki Commission (HELCOM) region and those in the Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR) region; Schulz et al., 2013; HELCOM 2020), other countries only recently started to set up their marine litter monitoring programmes within the context of the MSFD (e.g. Italy; see Fortibuoni et al., 2021).

Most beach litter protocols that were used on European coasts during the first MSFD cycle focused on the collection and visual identification and classification of litter items found at shoreline sites. However, the protocols applied in some countries differed in terms of sampling units (type, size and positioning criteria), frequency and timing of the surveys, size limits for and classes of litter items to be surveyed, classification lists and quantification units (number, weight or volume of items per stretch of coastline or per surface area of coastline) (Hanke et al., 2019). The application of several protocols between and within European Regional seas made it difficult to compare data.

In 2017, within the TG ML and with the support of EMODnet, the first pan-European beach litter dataset for 2012-2016 was compiled and analysed to derive baselines for the MSFD (Hanke et al., 2019). The analysis involved data from 22 European countries and four marine regions. Data from 3063 surveys performed on 389 European beaches were considered. The biggest challenge faced during the data compilation phase was dealing with the heterogeneity of data formats, data quality and protocols used during the beach surveys. The outcomes of this analysis were considered in the revision of the beach macro litter monitoring guidance. One

([11]) Directive (EU) 2019/904 of the European Parliament and of the Council of 5 June 2019 on the reduction of the impact of certain plastic products on the environment (OJ L 155, 12.6.2019, p. 1).

of the key findings was that five litter item classification lists were used, each featuring different levels of litter item category aggregations and total category numbers (Hanke et al., 2019). In response, the *Joint List of Litter Categories for Marine Macrolitter Monitoring* was prepared by the TG ML in close collaboration with Member States and the RSCs (Fleet et al., 2021). This list is based on a hierarchical system that facilitates the recording of litter items at different levels of detail. This enables the compatibility and comparability of results obtained through different marine litter recording schemes used for beach litter or those performed in other compartments of the marine environment (Fleet et al., 2021). The benefits of comparable data (also linked to the implementation of large-scale policy measures against litter) are evident. Indeed, in recent years, cooperation among the RSCs and the EU Member States led to a better harmonisation of data collected under different policy and legislative frameworks; a comparison of the latest versions of the different protocols (Table 3.1) shows an overall alignment of the most critical aspects, even if there is still room for improvement and additional efforts are needed. This chapter represents a further step towards harmonising the monitoring of litter on the coastline among Member States and the RSCs.

3.2 Scope

The TG ML has evaluated existing methods for monitoring litter on the coastline regarding their capacity to meet the MSFD requirements. The TG ML recommends a harmonised method that can be applied to assess beach litter in all European Regional seas, thus ensuring the consistency, compatibility, and comparability of monitoring data from coastal assessments of litter within and among regions. In this chapter, the methodology for conducting beach macro litter surveys within the MSFD is thoroughly described, and QA/QC aspects are addressed. In addition, an overview of other beach macro litter survey methods is presented.

3.3 Definitions and terminology

- Macro litter. Litter items larger than 25 mm in the longest dimension, with no set upper limit.
- Mesolitter. Litter items from 5 mm to 25 mm in the longest dimension.
- Microlitter. Litter items smaller than 5 mm in the longest dimension, with no set lower limit
- Monitoring campaign. The long-term process of carrying out one or more surveys in one or more survey sites with a certain frequency and within a given time period.
- Monitoring programme. A national or regional scheme for monitoring and assessing marine litter pollution.
- Monitoring protocol. A detailed description of the procedural method for monitoring marine litter pollution, including a reference list of litter types.
- Monitoring strategy. It outlines the survey sites and the associated survey sites selection criteria, the timing and frequency of the surveys, and the survey method.
- Sampling unit. A fixed section of coast covering the whole area from the water edge to the back of the beach (base of dunes, cliff, vegetation line or human artefacts), where the survey is carried out.
- Survey (or sampling). The process of recording data related to a sampling unit at a given time.
- Survey site (or sampling site). A beach or section of a large beach chosen for placing one or more sampling units.

Table 3.1. Comparison of the main aspects of the different beach macro litter monitoring protocols adopted by the MSFD TG ML (this guidance) and the RSCs (i.e. OSPAR, HELCOM, the Barcelona Convention and the BSC)

	Litter size	Materials / main categories	List and item categories	Reporting unit	Survey frequency and timing	Sampling unit definition	Sampling unit length	Litter removal	Beach typologies	Selection of beaches (partial)
EU MSFD (1)	> 2.5 cm Plus 15 categories of litter that are always recorded, even if < 2.5 cm	AP, R, C/T, P, WW, M, G/CE, CH, O, F	Joint list (Fleet 2021) 183 categories	Items/100 m	Four times a year	A fixed section of beach from the water's edge to the back of the beach (obstacles)	100 m	Yes	Urban, semi-urban, remote/ natural	The beaches should be spatially stratified to reflect: - different pressures and different levels of exposure; – different development and urbanisation levels.
OSPAR (2)	> 5 mm ([a])	AP, R, C, P, WW, M, G, CE, SW, MW	OSPAR list 126 categories	Items/100 m	Four times a year	A fixed section of beach from the water's edge to the back of the beach (obstacles)	100 m	Yes		The survey sites should be representative of the litter sources. The beaches should not be subject to any other litter collection activities.
HELCOM (3)	> 5 mm	AP, R, C/T, P, WW, M, G/CE, U	Different coding lists (Joint list, OSPAR (2010) or MARLIN (2013))	Items/100 m	Three times a year	A fixed section of beach from the water's edge to the back of the beach (obstacles)	100 m	Yes	Urban, semi-urban, remote/ natural	At least one beach is not included in the regular cleaning process and is frequented by few visitors. Beaches are preferably in rural areas.
Barcelona Convention (4)	> 5 mm	AP, R, C, P, WW, M, G, C, SW, MW, F, PW	IMAP list 131 categories:	Items/100 m and items/m^2	At least two times a year	A fixed section of beach from the water's edge to the back of the beach (obstacles)	100 m	Yes	Urban, peri-urban, rural	The beaches should not be subject to any other litter collection activities.
BSC	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

([a]) Only items > 2.5 cm are used for assessment.
NB: AP, artificial polymer materials; C, cloth; CE, ceramic; CH, chemicals; F, faeces; G, glass; IMAP, integrated monitoring and assessment programme of the Mediterranean Sea and coast and related assessment criteria; M, metal; MW, medical waste; U, undefined; n/a, not available; P, paper/cardboard; PW, paraffin/wax; O, organic food waste; R, rubber; SW, sanitary waste; T, textile; WW,

processed/worked wood. In dark green are the aspects that are fully harmonised; in light green are the aspects that are partially harmonised but still not fully in line; in orange are the aspects that have been addressed but would require additional effort to become harmonised.

Sources: (1) This guidance; (2) OSPAR Commission (2020); (3) HELCOM (2021); (4) UNEP/MAP (2019).

3.4 Marine Strategy Framework Directive methods for beach macro litter surveys

Capitalising upon the lessons learned from implementing the first MSFD cycle, the following protocol for carrying out beach macro litter monitoring has evolved.

3.4.1 Monitoring strategy

3.4.1.1 Survey site selection

Survey sites should, whenever possible, have the following characteristics:

- a minimum length of 100 m along the water edge (i.e. sufficient to have at least one sampling unit);
- composed of sand or gravel;
- low to moderate slope;
- clear access to the sea (not blocked by breakwaters or jetties);
- accessible to survey teams year-round.

Within the above constraints, the location of survey sites should be spatially stratified to reflect:

- different pressures and different levels of exposure to litter (e.g. close to river mouths, close to harbours/marinas, presence of touristic facilities nearby);
- different development and urbanisation levels, including a balanced mix of urban, semi-urban, and remote/natural beaches (Table 3.2 and Figure 3.1).

Table 3.2. Main characteristics of different beach typologies representing different levels of urbanisation.

	Environment	Accessibility	Habitation and accommodation	Services and facilities
Urban	Located in front of urban areas, with a wide range of well-established public services (shopping areas, business districts, etc.)	Accessible by both public and private transport	Large population and large-scale residential and tourist accommodation	Extensively developed range of services and facilities provided for beach users
Semi-urban	Located in the surroundings of the urban areas, adjacent to or within a small coastal town with small-scale community services	Accessible by both public and private transport	Small residential populations and/or many beach users during the bathing season; presence of accommodation facilities (hotels, bed and breakfast, campsites)	A reduced range of services and facilities provided for beach users
Remote/natural	Remote and natural environment, located away from small towns or villages, a predominance of natural elements and absence of community services	Accessible by private transport, boat or walking; includes those beaches that are closed to the public	Absence of residential population, housing and tourist accommodations	Absence of services and facilities for beach users

NB: The table is indicative, and some deviations may occur.

Figure 3.1. Examples of survey sites in Italy characterised by different level of development and urbanisation: (a) urban, (b) semi-urban and (c) remote/natural.

(a) (b) (c)

Source: Maps data – Google, ©2019.

The survey sites should be the same as those monitored in the first MSFD cycle to compare results over time. It is possible to replace one or more of the survey sites among those monitored until 2020, but only in the case of profound changes that make it impossible to monitor a site indefinitely (destruction of the coast, new positioning of breakwaters, inaccessibility, etc.). Changes should be kept to a minimum, and, where possible, the new monitored survey sites should have the same characteristics as the original ones. New survey sites can be established, by applying the selection criteria above, if this increases the representativeness of beaches at the country-region level. It is of utmost importance that the characteristics of their survey sites and any changes in the characteristics are recorded and saved for future reference (see below and Annex I – 'Survey site (beach) Identity Form (AI)').

There is no agreed statistical method for recommending a minimum number of survey sites represent a certain length of coast, a specific region or a country. It depends on the purpose of the monitoring, the geomorphology of the coast, the number of sites available that meet the criteria presented above, and trade-offs between available resources and monitoring needs. The sampling effort necessary to assess litter concentrations within a given region is, for instance, dependent on the desired level of detection (i.e. to detect small-scale spatial differences in litter quantity and composition, more sampling sites are required) and the heterogeneity of pressures.

It is proposed that beach litter surveys should be performed in at least four survey sites within a country-subregion (e.g. France – western Mediterranean Sea). This approach (four surveys per year in four survey sites) is in compliance with the method for assessing the threshold value for beach litter and would provide a sufficient number of surveys (over 40) within a 3-year period (van Loon et al., 2020).

It should be highlighted that all necessary precautions should be taken to ensure that surveys will not pose any threat to endangered or protected species such as sea turtles, shorebirds, marine mammals, or sensitive beach vegetation/habitats. In many cases, this could exclude protected areas from survey areas; however, this will depend on local management arrangements.

3.4.1.2 Survey site metadata

For each survey site, metadata on the characteristics of the site should be recorded and saved to facilitate the analysis and interpretation of results. Using the form provided in Annex I – 'Survey site (beach) identity form (AI)' is suggested. This form needs to be filled out once for each survey site and then updated if significant changes to the characteristics of the site occur (e.g. creation of a new residential area nearby).

The information that should be recorded for each survey site includes the following (see Annex I – 'Survey site (beach) identity form (AI)' for metrics and units):

- the total length of the coast/beach;
- the latitude and longitude of the central point of the beach (to identify the position of the beach);
- degree of urbanisation (urban, semi-urban, remote/natural);
- features related to the back of the beach (i.e. cliffs, dunes, rocks, forest, bush, crops, fields, built-up area, road, other);
- features related to the development behind the beach (e.g. camping, road, hotels);

- the main orientation of the coast/beach (i.e. the direction the coast is facing when looking from the coast to the sea);
- coastline curvature (i.e. linear, concave, convex or sinusoidal);
- beach substrate (i.e. percentage of sand, pebbles, rocky coast);
- objects in the sea that influence the currents (e.g. reefs);
- beach slope (i.e. level, gentle slope, moderate slope, steep slope);
- beach access (i.e. pedestrian, vehicle, only boat);
- beach usage (e.g. tourism and recreation, fishing) indicating for each usage if it is primary or secondary and whether it is seasonal or not;
- estimated number of people using the beach (seasonal average);
- any other relevant information (e.g. an incidental large-scale touristic event such as a surfing competition which may create a litter peak).

Some of this information can be obtained from maps and similar sources (e.g. Google Earth™ images), although this information should be checked by direct observation at the site. The collection of metadata would ideally be a task for a national or local coordinator of the beach litter surveys, who can access the required information and collect the information in a uniform way for all beaches.

3.4.1.3 Survey frequency and timing

Preferably four surveys per year should be carried out for each survey site. The proposed periods in which the surveys are to be performed are the following:

- winter – January
- spring – April
- summer – July
- autumn – October

These periods are more or less evenly distributed throughout the year. However, regional or even local conditions might prevent the performance of surveys in the periods proposed. Weather conditions (e.g. snow) in particular could prevent surveys in winter or spring. In addition, a high volume of tourists and extremely hot weather might hinder surveys in July. Surveys should not be undertaken during periods when there is a risk of affecting endangered or protected species, such as sea turtles and birds (i.e. nesting period).

While using harmonised monitoring periods among the countries is highly recommended, it is up to the national coordinators of beach litter surveys to choose the survey periods best suited for their regions.

3.4.1.4 Sampling unit

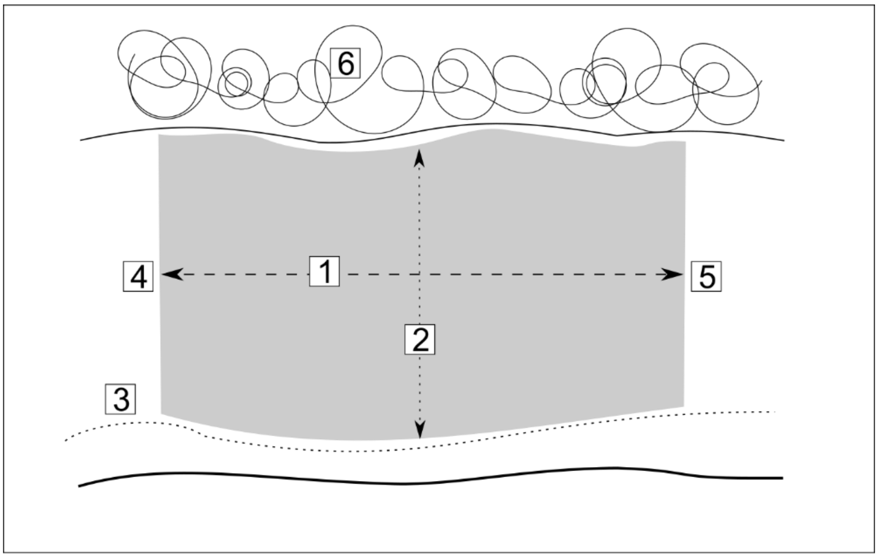
According to Commission Decision (EU) 2017/848, the unit of measurement for beach macro litter is the amount of litter per category in terms of number of items per 100 m of the coastline.

The sampling unit is a stretch of coast of 100 m in length covering the area from the water's edge to the back of the beach measured at half the actual width as a curved line on curved beaches or a straight line on straight beaches. Examples of how to measure the length of the sampling units are provided in Figure 3.2(b). Please note that, if the monitored stretch deviates slightly from the suggested 100 m length, the results must be normalised to 100 m when reported.

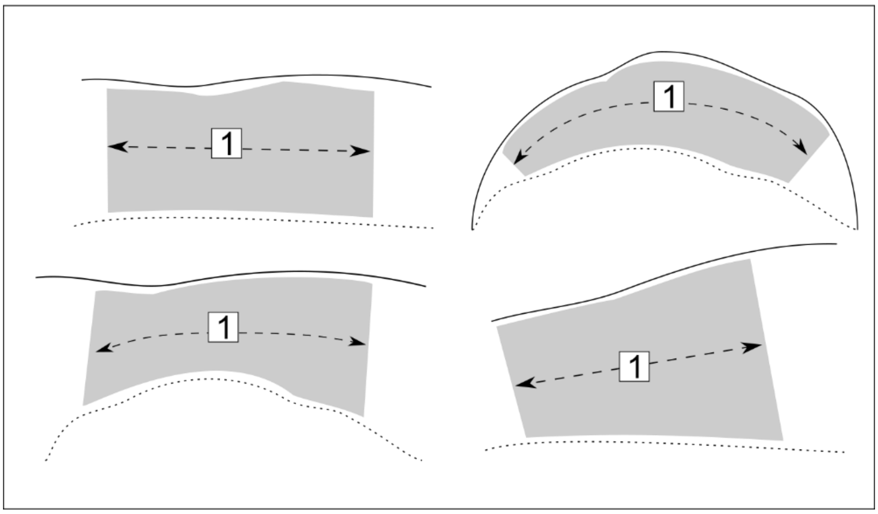
Sampling units should represent the general characteristics of the survey site and the overall state of litter within it. The sampling units should not be placed on the edges of a beach or on parts of the beach that have a higher likelihood of accumulating litter. In addition, the sampling unit should not be placed in potential litter hotspots, such as areas near the entrance of the beach, near coastal parking lots or directly in front of hotels. Based on these considerations, a set of potential sampling units should be identified and a random selection of survey units should then be made from this set (e.g. dividing the coast into 100 m sections and randomly choosing a number of these sections as sampling units) (Figure 3.3). However, existing sampling units from long-running monitoring programmes (e.g. those used for the first MSFD monitoring cycle) should continue to be surveyed.

Figure 3.2. (a) Sampling unit characteristics and (b) suggested method to measure the length of the sampling unit in differently shaped beaches

(a)



(b)



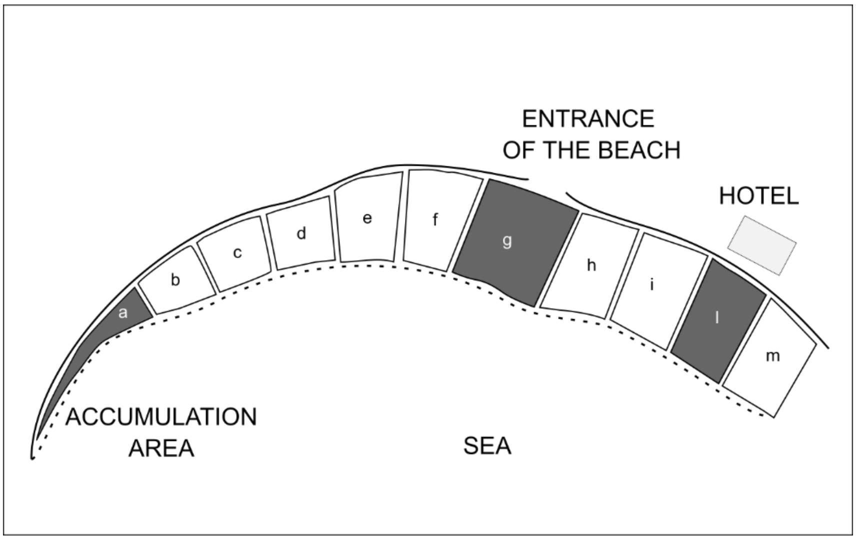
NB: Numbers refer to the following: 1, sampling unit length; 2, sampling unit width; 3, edge of the water; 4 and 5, GPS coordinates of the sampling unit; and 6, back of the beach.

Source: Created by the authors.

In heavily littered survey sites (i.e. where a 100 m stretch requires more than 1 day of work to be surveyed) (Figure 3.4), a smaller sampling unit (at least a 50 m stretch of coast covering the area from the water's edge to the back of the beach), representative of the situation, can be monitored. Note that the results must be normalised to 100 m when reported to obtain comparable results.

Monitoring more than one sampling unit at the same survey site allows an estimation of the sample variability (e.g. the sample mean and standard error).

Figure 3.3. Example of how to select the sampling unit(s): once potential hotspots (shaded sections: a, g and l) are excluded, the sampling unit(s) should be chosen randomly from the remaining 100 m sections of the beach (unshaded sections b, c, d, e, f, h, i and m)



Source: Created by the authors.

Figure 3.4. Examples of heavily littered sites.

(a) (b)


Sources: Photo credits – (a) Vlachogianni, T., and (b) Fortibuoni, T.

3.4.1.5 Sampling unit metadata

The same sampling units should be monitored for all surveys planned in the monitoring programme. The sampling units' coordinates should be documented (sampling unit start latitude/longitude and sampling unit end latitude/longitude) (see Figure 3.2(a)). If absolutely necessary (e.g. because of the construction of a tourist facility), the sampling units can be replaced with new units within the same stretch of coast. In such cases, new metadata must be recorded. As beach litter distribution is usually heterogenic, even on a small scale, the replacement sampling unit must be very close to the original one. Coordinates obtained by GPS are useful for identifying the sampling units; easily identifiable landmarks can be used, provided that their presence and position are consistently maintained over time.

The following information can be collated once for each sampling unit (using the form provided in Annex II – 'Sampling unit identity form (A2)' is suggested) and, once recorded in a database, can be used for all future surveys:

- the sampling unit length, measured along the beach curve at the mid-point between the water's edge and the back of the beach (see Figures 3.2 and 3.5);
- the sampling unit width (perpendicular to the shoreline line), defined as the distance between the water edge and the back of the beach (base of dunes, cliff, vegetation line or human artefacts) and measured at half its length – beach width should be measured at the mean water level in areas with small tidal amplitudes and at the mean high tide level for areas with high tidal amplitude (see Figures 3.2(a) and 3.5);
- start/end GPS coordinates;
- direction of the prevailing winds;
- direction of the prevailing water currents;
- name, distance to and position of the nearest town, and the size its residential population;
- distance to and position of the nearest food/drink outlet and the months in which the food/drink outlets are present;
- name, distance to and position of the nearest harbour and the type of shipping using the harbour (e.g. passenger, merchant, fishing, military, recreational);
- name, distance to and position of the nearest river mouth;
- distance to and position of the nearest wastewater or stormwater discharge point;
- distance to and position of the nearest shipping lane and the type and intensity of marine traffic.

Much of this information can be obtained from maps and similar sources (e.g. Google Earth™ images), although this information should be checked by direct observation at the site.

Figure 3.5. Examples of sampling units starting from the water's edge and extending to the back of the beach

(a)

(b)

NB: In part (a), the back of the beach is defined by the presence of trees and vegetation; in part (b), it is defined by the dunes.

Sources: Photo credits – (a) T. Vlachogianni and (b) T. Fortibuoni.

3.4.2 Survey protocol

3.4.2.1 Survey metadata

For each survey performed on a sampling unit, the following data should be recorded (using the form provided in Annex III – 'Marine litter monitoring survey form (A3)' is suggested):

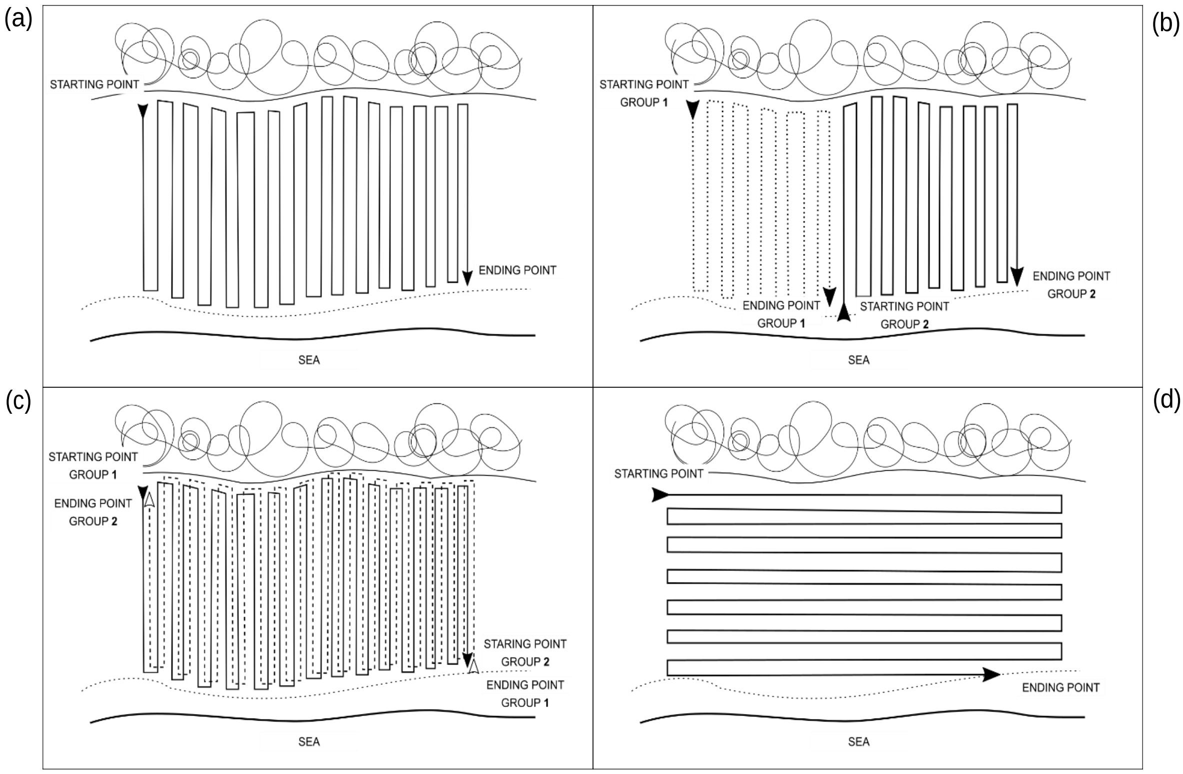
- sampling unit code/name;
- survey date;

- surveyors' names and contact details;
- length of the surveyed sampling unit, which may differ slightly from the suggested 100 m, measured along the beach curve at the midpoint between the water's edge and the back of the beach (see Figures 3.2(b) and 3.5);
- date of the last known cleaning action (e.g. municipality beach cleaning, clean-up days);
- weather conditions during the dates of the surveys;
- any deviation from the sampling protocol (e.g. transect length reduction or displacement of the transect, sampling outside the expected period, subsampling) and motivation (e.g. extreme weather events, flooding, new infrastructures in place);
- special circumstances and events that could have caused unusual litter in terms of abundance and/or type (e.g. clean-up actions, mechanical cleaning, beach party or competition, cargo losses nearby, extreme weather conditions);
- information on any entangled fauna encountered during the survey (details of the organism, nature of entanglement, live or dead).

3.4.2.2 Litter sampling

To ensure that all macro litter items are recorded in the sampling unit, a systematic sampling approach should be deployed. Some examples are shown in Figure 3.6. All items found on the surface of the sampling unit must be recorded (litter items should not be dug up). Items entangled in seaweed or other natural materials should also be considered.

Figure 3.6. Examples of litter sampling approaches. (a) recommended path, transverse to the water's edge, (b) different groups of surveyors can monitor different sections of the transect at the same time, (c) different groups of surveyors monitoring the whole section but in opposite directions and (d) path parallel to the water's edge



Source: Created by the authors.

3.4.2.3 Litter size ranges and classification

A lower limit of 2.5 cm in the longest dimension is set for macro litter items monitored during beach surveys. However, the specific objects listed in Table 3.3 should be recorded in all cases, even if they measure less than 2.5 cm. An upper size limit has not been established.

Table 3.3. Items from the Joint List that should be recorded during the macro litter surveys even if smaller than 2.5 cm in the longest dimension

Code	Name
J182	Metal fisheries-related weights/sinkers, and lures
J178	Metal bottle caps, lids and pull tabs from cans
J195	Metal household batteries
J21	Plastic caps/lids drinks
J100	Plastic medical/pharmaceuticals containers/tubes/packaging
J22	Plastic caps/lids chemicals, detergents (non-food)
J23	Plastic caps/lids unidentified
J24	Plastic rings from bottle caps/lids
J91	Plastic biomass holder from sewage treatment plants and aquaculture
J32	Plastic toys and party poppers
J60	Plastic fishing light sticks / fishing glow sticks including packaging
J257	Foamed plastic packaging
J27	Tobacco products with filters (cigarette butts with filters)
J131	Rubber band (small, for kitchen/household/post use)
J125	Rubber balloons

Source: Adapted from Fleet et al. (2021).

The MSFD TG ML *Joint List of Litter Categories for Marine Macrolitter Monitoring* (Fleet et al., 2021) should be used for classifying the litter items sampled. Using the most detailed level of the joint list is highly recommended. The manual for applying the Joint List classification system provides detailed information on how to classify litter items (Fleet et al., 2021).

Litter items can be classified and recorded either on-site or in a sheltered place (e.g. a lab) after the sampling has been completed (e.g. in the case of bad weather conditions and/or heavily littered beaches); however, the latter approach should be avoided for weathered or fragile items, which can easily disintegrate, potentially leading to an overestimation of these litter items.

For specific purposes, it may be worth recording additional data regarding litter items (Cau et al., 2019), for example:

- the expiry date and/or the production date reported on food packaging or other containers;
- the geographical origin of the item if the label or the barcode is readable;
- whether it is deposited *in situ* or whether it was washed out from the sea;
- weight per material category (i.e. chemicals, clothes/textile, food waste (organic), glass/ceramics, artificial polymers/plastic, paper/cardboard, rubber, processed/worked wood);
- the size of litter items, since this can provide a link to litter quantities (the manual for the application of the *Joint List of Litter Categories for Marine Macrolitter Monitoring* (Fleet et al., 2021) provides recommended approaches to recording the size of objects).

3.4.2.4 Litter removal

All items sampled during the survey must be removed from the sampling unit. The litter collected should be disposed of properly. Local, regional or national regulations and arrangements for waste disposal should be followed. Larger items that cannot be removed (safely) by the surveyors should be marked, for example, with paint spray (that meets environmentally friendly standards) so that they are not counted again in the next surveys.

3.4.2.5 Survey equipment and consumables

The following equipment and consumables are recommended when carrying out the surveys:

- a hand-held GPS unit (with extra batteries);
- a measuring wheel or a 100 m decametre;
- flag markers/stakes;
- a sturdy 30 cm ruler;
- a clipboard and field sheets (one per team) and/or a mobile phone or a tablet for recording litter items;
- pencils and rubbers;
- printed list of items (the Joint List codes and names are recommended);
- high-resolution cameras (e.g. digital single-lens reflex cameras (DSLR), mirrorless, smartphones);
- protective gloves;
- bags to collect the litter (mesh bags can be used for bigger items);
- a rigid container and sealable lid to collect sharp items such as needles;
- a first aid kit.

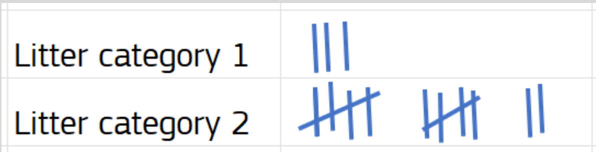
Box 3.1 provides practical tips on conducting surveys and Box 3.2 lists safety considerations.

Box 3.1. Practical tips on monitoring beach macro litter

Print the field form from Annex III – 'Marine litter monitoring survey form (A3)'. This will ensure harmonisation.

Items that easily break or get entangled and are weathered must be sorted and classified on-site to avoid errors.

To record the items one by one, a quick method is to use slashes marks on the litter items recording sheet: in the example, this approach makes it convenient to count items in groups of five at the conclusion of the survey.



To speed up the survey, the items can first be grouped into categories according to the Joint List and then counted together

Arranging the litter types on the field list according to the most frequent items found can facilitate the recording of the litter items found.

To ensure that the sampling unit's entire area is sampled and no parts are left out, small flags moved along the beach during the survey can be used to mark subsections.

Field forms should be entered into a database or digital storage medium (e.g. a spreadsheet) within 3-days of the field surveys. This will ensure a good recollection of the litter observations and field conditions.

A tablet or smartphone with access to the European Commission's European Commission's 'Online photo catalogue of the joint list of litter categories' web could help the categorisation of the items. Unusual or not recognisable litter items can be photographed for further evaluation.

Box 3.2. Safety considerations

Safety should be the number one priority during any survey activity. Since this work is carried out in the field, there are a few inherent hazards. Caution should be used, and the general safety guidelines presented below should be followed.

Start the monitoring about 1 hour after high tide to prevent surveyors from being cut off by the incoming tide.

Check and avoid circumstances that may lead to unsafe situations for surveyors: heavy winds, slippery rocks and hazards such as rain, snow or ice.

Wear appropriate clothing (sturdy shoes and gloves) when handling litter, as they may have sharp edges. If you come across a potentially hazardous material (e.g. oil or chemical drums, gas cans, propane tanks), contact competent authorities to report the item. Do not touch or sniff the material or attempt to move it.

Large, heavy objects should be left in place. Do not attempt to lift heavy litter items; instead, report them to the appropriate authorities for removal.

When in doubt, do not pick it up! If the item is potentially hazardous (e.g. ammunition), report it to the appropriate authorities.

Be aware of your surroundings and be mindful of trip and fall hazards.

Always carry a first aid kit. The kit should include an emergency water supply, sunscreen and bug spray.

Understand the symptoms of heat or cold stress and the actions to treat them.

Make sure to carry enough water.

Carry a means of communication for emergencies, for example, a mobile phone.

Let someone know where you are and when you expect to return.

3.5 Quality assurance / quality control

Implementation of consistent QA/QC practices should be considered early and throughout the beach macro litter monitoring process, including the monitoring strategy design, the sampling and classification, the data processing and reporting. Although there are many facets to QA/QC, the most important elements when surveying macro litter are related to the survey sites' locations and their respective number, the timing of surveys, the positioning of the sampling unit on the survey site, the collection and classification of litter items, the data control and reporting and the metadata documentation.

3.5.1 General quality assurance / quality control measures

Establishment of a beach macro litter monitoring organogram. It is recommended that the Member States establish an organisational chart with clear-cut and distinct roles for each type of staff (including third parties, NGOs) involved in the design and implementation of the beach macro litter monitoring strategy. Some proposed roles include national coordinators, local coordinators, fieldworkers/surveyors, and data managers with one or more of the following QA/QC related roles. These roles can include:

- establishment of a national macro litter monitoring strategy;
- selection of survey sites in compliance with this guidance;

- training, coordination, and supervision of field workers;
- collection and recording of data and metadata related to the survey sites, the sampling unit and the survey;
- establishment of contact with local municipalities and local NGOs to better plan the surveys and obtain the latest information on beach-cleaning activities;
- performance of quality control for the monitoring data (checking the correctness of the data directly before and after their entry into a database and undertaking an annual quality control examination of national beach macro litter data);
- management of the national macro litter monitoring dataset, including all related data and metadata.

Establishment of an advanced training programme. High-quality training is essential to ensure data quality, and it needs to include the development of operational (field) skills. It is recommended that the surveyors are engaged on a long-term basis to maintain experience and knowledge of how the monitoring should be performed. Member States should provide adequate training for the fieldworkers who participate in the surveys. Staff and/or volunteer training programmes should also incorporate information on the results and outcomes of the beach macro litter surveys so that field workers can understand the context of the macro litter assessment programme. In addition, the training programme should include hands-on calibration exercises to ensure, among other aspects, that macro litter items are attributed correctly to the litter types included in the field protocols. Inter-calibration exercises with neighbouring countries and at the regional sea level could be carried out if necessary.

3.5.2 Quality assurance / quality control measures related to sampling

When performing the beach macro litter sampling, the following best practice measures are recommended to reduce biases and/or errors.

Sampling design. Identifying appropriate survey sites (sampling site) types and numbers is paramount for establishing a comprehensive macro litter monitoring programme. Survey sites should be identified by using a stratified randomised approach. An initial pool of survey sites should be identified with locations that reflect different human-induced pressures leading to different litter densities and compositions. From this pool of locations, the actual sites should be selected randomly, considering the characteristics mentioned in Section 3.5.1. A sufficient number of survey sites should be chosen considering the coastline length of a country and the diversification of pressures (in terms of intensity or typology).

Sampling unit. To ensure that the sampling unit reflects the overall beach status, it should be positioned in the most representative section of the beach. This means that the parts of the beach where litter might tend to accumulate should be avoided and the sampling units should be located at least 50 m from points of access to the beach. In addition, special attention should be given to surveying exactly the same sampling unit in each seasonal survey and keeping to precisely the same 100 m stretches of the beach during each survey. If a sampling unit has to be moved for some reason (e.g. erosion of the coastline), it must be defined as a new sampling unit.

Representative sampling. Beach macro litter sampling is influenced by many factors, such as extreme weather phenomena and clean-up operations. It is recommended that the beach macro litter survey be postponed to at least 14-days after a weather-related or clean-up-related event that may have affected the abundance of macro litter on the beach. In addition, sampling units could be explicitly marked as national monitoring areas (e.g. by putting signs on the coastline) to discourage people from removing litter from that site. Moreover, a national database could be set up to register all coastal clean-up activities.

Replicate surveys. To increase the accuracy and precision of beach macro litter data from beach surveys, replicate surveys in close proximity to the sampling unit can be conducted. Average values can then be used for assessments. For research purposes, the individual replicate survey data can be stored separately.

3.5.3 Quality assurance / quality control measures related to sample processing

The sample processing elements of a beach macro litter survey address the litter items’ removal, sorting and classification. The following best practice measures are recommended.

Litter item collection. All the litter items found (without digging) on the beach within the boundaries of the sampling unit must be collected. Litter items that easily break or get entangled and/or are weathered must be classified on-site to avoid the introduction of errors in their numbers due to fragmentation or entanglement during transport and processing.

Litter items sorting and classification. All macro litter items collected should be classified according to the categories of the Joint List of macro litter items. The photo guide can help the surveyors identify and categorise the litter items ([12]). Pieces of litter that are recognisable (e.g. a piece of a drinking bottle) should be registered as such (see also Fleet et al., 2021). Unusual or unknown litter items or recognisable litter items, that are not attributable to litter types from the field protocol, should be recorded along with a description and a photograph. In this way, emerging litter types can be identified and considered for inclusion updates/revisions of the protocol. It is recommended that the most experienced members of the surveying team supervise the final classification of the litter items.

3.5.4 Quality assurance / quality control measures related to data processing and reporting

All data and metadata should be reported using a 'standardised' data reporting sheet. The local coordinator should undertake the data collation and data quality assurance for each survey. Once submitted to the national database, the data should undergo additional control by the national data manager. The national coordinator will undertake responsibility for the review and final approval of uploaded data and will clarify any issues with local coordinators. Annual checklist documentation will provide an incentive for national coordinators, at the end of the monitoring year, to check that all surveys have been carried out and that all relevant information has been collected and entered into the appropriate beach litter database. This would ensure a high level of consistency within each region and create a hierarchy of quality assurance on data acquisition. The use of such a system will also support a comprehensive analysis of the data providing the opportunity to undertake statistically robust comparisons over time and between survey locations (Cheshire et al., 2009). Relevant databases that serve the needs of the Member States are made available by EMODnet (https://www.emodnet-chemistry.eu/marinelitter) for European Regional Seas, and by the OSPAR Commission for the north-east Atlantic Ocean (http://beachlitter.ospar.org).

3.6 Costs and efforts needed for beach monitoring

Compared with the methods described in this guidance for other compartments, beach litter monitoring is far less burdensome in terms of efforts and costs. In most cases, the survey sites can be easily reached by car and on foot. The operators need a low/medium level of expertise to collect and categorise the items, as long as expert supervisors train the participants and perform accurate quality control during and after the collection, especially regarding the marine litter item subcategories. The equipment required to perform the beach surveys is mainly related to the safety of the operators, while, for the actual collection and classification of the items, the expenses are low (see Section 3.4.2.5). The time required for collecting the items may vary greatly depending on the state of the beach and, thus, the quantity of litter accumulated. In general terms, 1-day may be sufficient to monitor one survey site (100 m length), including the collection, characterisation and disposal of the items. The relatively simple and easy-to-apply protocol for coastlines allows several Member States to involve NGOs and citizen science projects, which may drastically increase the cost-effectiveness of beach monitoring programmes under the MSFD. Beach litter data can be analysed with basic statistical methods and software, at least for a general overview and reporting purposes.

3.7 Other beach macro litter monitoring methodologies – an overview

In addition to the beach macro litter monitoring method described in this guidance, which provides data for the MSFD reporting requirements, several other methods are used to perform beach macro litter surveys, with different aims. The data from these surveys could potentially be compared with the MSFD monitoring data by using the *Joint List of Litter Categories for Marine Macrolitter Monitoring*. While it is not possible to specify a best method in general, it should be kept in mind that it is important to adopt the most appropriate monitoring methodology taking into account the aims of the study, the characteristics of the sites monitored,

([12]) See European Commission (undated), 'Online photo catalogue of the Joint List of Litter Categories': (https://mcc.jrc.ec.europa.eu/main/photocatalogue.py?N=41&O=457&cat=all).

the staff involved and other specific aspects of the survey. It is also important to acknowledge that each methodology has its pros and cons and strengths and limitations that need to be considered when analysing and interpreting the data (Velander and Mocogni, 1999).

Beach macro litter studies are commonly categorised into two main types: accumulation and standing stock studies (Ryan et al., 2009). Accumulation studies require the initial removal of all litter from the site, followed by regular surveys to record and remove all litter. The data collected over time may provide an estimate of the litter's flux on the shoreline (e.g. Prevenios et al., 2018). To have a realistic estimate of loading rates (fluxes), a high frequency of sampling is needed, which may pose substantial challenges in terms of high time costs (Smith and Markic, 2013). Conversely, carrying out surveys four times a year, as suggested in this guidance, allows the assessment of long-term balance between input and output (standing stock). In contrast, surveys that are run more frequently provide information on what is arriving over a shorter time frame (GESAMP, 2019).

3.7.1 Rapid surveys

Rapid surveys for beach litter (i.e. surveys that can be completed in a short time and are not based on a detailed assessment of litter types) may be used to provide an initial 'snapshot' of the distribution and abundance of marine litter. They can be useful in the case of a major natural disaster (e.g. a tsunami or typhoon); to collect a qualitative or semi-quantitative estimate of litter abundance and composition that is sufficient to direct further recovery operations or monitoring design; to provide a baseline to inform the development of a routine monitoring programme; and to identify accumulation hot spots for possible intervention. This kind of survey is not intended for application where detailed information about litter amounts, composition and fluxes is required.

For instance, in the frameworks of the Interreg Mediterranean Actions for Marine Protected Areas project (AMARE) (https://amare.interreg-med.eu) and the Interreg Mediterranean Plastic Busters Marine Protected Areas (https://plasticbustersmpas.interreg-med.eu) project, rapid surveys for beach litter were performed with small boats (5-6 m) operating at low speed (1-12 knots) from 20 m to 100 m from the shore along the coast of Corsica (France). The presence of litter was recorded for low accumulation zones (2-10 litter items per site, which were usually a 5-30 m apart) and high accumulation zones (> 10 litter items per site). The position of accumulation areas was recorded using GPS. A detailed assessment of litter types was not performed.

The development of image capture using aerial photography has proved to be useful for rapid assessments of litter, allowing large-scale coverage (GESAMP, 2019). These methods and their limitations are described in the subsequent sections.

3.7.2 Imaging techniques

Imaging techniques are particularly useful for detecting litter in dense vegetation (e.g. reed beds), and for non-destructive observations in sensitive habitats (e.g. salt marshes) and remote or inaccessible coastlines. Indeed, distant and rugged coastline segments are usually challenging to monitor conventionally. A variety of remote, aerial monitoring methods have been implemented, using fixed-wing aircraft (Kataoka et al., 2018), bush planes (Moy et al., 2018), balloons (Nakashima et al., 2011), aerial vehicles (Papakonstantinou et al., 2016; Deidun et al., 2018; Martin et al., 2018) and webcams (Kako et al., 2018; Kataoka et al., 2018).

Unmanned aerial vehicles (UAVs) / unmanned aircraft systems (UASs) (e.g. drones) may be good technological options for beach macro litter monitoring (e.g. Martin et al., 2018; Papakonstantinou et al., 2021). Their use has the advantages of high image acquisition frequency, high spatial resolution, the ability to fly at low altitudes below clouds, and high mobility (Bao et al., 2018). UAVs/UASs can be used to acquire georeferenced red, green and blue (RGB) images along the coastline cost-effectively and rapidly (Deidun et al., 2018). A post-processing system based on visual interpretation of the images allows the localisation and identification of the marine litter within the scanned area and the estimation of its spatial and temporal distribution (Merlino et al., 2020). Deep learning algorithms can automatically identify and quantify marine litter (Fallati et al., 2019). However, it has been shown that monitoring with UAVs/UASs may lead to an underestimation of beach litter compared with human inspection since, for instance, hidden and transparent items cannot be detected (Merlino et al., 2020). Another limitation of using UAVs/UASs is adverse weather conditions because surveys cannot be carried out on windy or rainy days.

Another method that could be used for inaccessible beaches is based on the acquisition of high-resolution images through vessel-based photography surveys (Papachristopoulou et al., 2020) or by applying image processing techniques to archived shoreline aerial photographs (Kataoka et al., 2018). Vessel-based photography provides a good trade-off between high-quality photographic documentation, spatial coverage, processing time, and operational cost. At the same time, unlike other remote methods, it could easily be performed by non-experts (Papachristopoulou et al., 2020). However, it is worth noting that the quantification of beach litter abundance through remote photography may result in an underestimation of litter densities when the quality and resolution of the images are poor, for instance, as a consequence of bad illumination due to bad weather or it being impossible to navigate close to the coast.

3.7.3 Participatory science and community-based initiatives

Even though there is no internationally accepted definition of citizen science, the term mainly refers to the involvement of non-professional volunteers, typically in data collection, but also in other phases of the scientific process, such as data interpretation, problem definition or the dissemination of results (Bonney et al., 2009; Haklay, 2015).

Participatory science is a more inclusive term that refers to 'research conducted in partnership between trained experts and members of a "community" or CSOs [civil society organisations]', including non-governmental organisations (NGOs) (Gall et al., 2009, p. 12). Throughout the years, NGOs have significantly contributed to providing data and information on the temporal and spatial distribution of marine litter found stranded on beaches, and participatory science campaigns have proved to be an essential tool to fill in the marine litter knowledge gaps (Hidalgo-Ruz and Thiel, 2015). In many cases, environmental NGOs can produce fit-for-purpose and accurate beach litter monitoring data for institutional purposes (Vlachogianni et al., 2020).

3.7.3.1 Clean-up and removal

Community-based beach litter initiatives mainly focus on clean-up and removal actions (e.g. Ocean Conservancy's International Coastal Cleanup, NOAA Marine Debris Program, Clean Up the Med campaign, SeaCleaner) rather than research/monitoring actions. These actions may generate estimates of litter amounts at a particular site. Community-based projects that focus on research and monitoring (citizen science) can produce good-quality data on litter (e.g. van Der Velde et al., 2017; Vincent et al., 2017; Chen et al., 2020; Haarr et al., 2020; Kideys and Aydin, 2020; Vlachogianni and Scoullos, 2023), provided that volunteers are trained, and professionals/scientists supervise and guide them. A rigorous citizen science programme requires intensive coordination and communication with the volunteer participants. The resulting data must be controlled, reviewed and validated by experts to remove mistakes and spot unlikely results that are from errors or misunderstandings in data acquisition (GESAMP, 2019). The general public's involvement in research can generate added value in addition to producing new data; for example, it can raise awareness, strengthen custodianship for the local environment and increase pressure on policymakers to act (Merlino et al., 2015; GESAMP, 2019).

3.7.3.2 Hotspot surveys

While the MSFD coastline litter surveys are based on the repeated monitoring of a fixed set of beaches, litter quantification on other beaches may provide important complementary information and help to identify litter hotspots that might require special attention, potentially reducing litter input to the marine environment, for example, through specific local measures. Such surveys would ideally use the same protocols as the MSFD surveys and thus enable a comparison of data.

3.7.3.3 Rare events monitoring and early warning

Opportunistic beach litter surveys involving citizens can provide a cost-effective approach to documenting relatively rare events such as animals' entanglement in litter or to following the spread of massive quantities of litter items along the coastline due to unexpected events, such as a cargo loss or an accident along the coastline. This type of survey is based on massive amounts of citizens' engagement, resulting in the broader distribution of the observations in space and time and an early warning system. Dedicated websites and/or apps can facilitate the collection of this data (e.g. https://seawatcher.info-rac.org).

3.7.3.4 *The EEA Marine Litter Watch*

The EEA has developed the Marine Litter Watch to strengthen Europe's knowledge base and thus provide support to European policy implementation. MLW offers tools – an app ([13]) and a public database ([14]) - to collect and share data on marine litter on beaches. A web portal ([15]) is also available for the communities to manage their events and data. Communities organise events on beaches and make surveys with the Marine Litter Watch app to report on litter items found.

3.8 Resources

When recording and analysing litter on the coastline, Member States and other communities can benefit from the set of tools and resources developed in the last decade to collect, store, visualise and analyse data.

EMODnet. EMODnet is a European initiative funded by the Directorate-General for Maritime Affairs and Fisheries. The initiative is divided into seven thematic areas, each focused on a specific topic. EMODnet Chemistry (https://emodnet.ec.europa.eu/en/chemistry) started in 2009, intending to support the MSFD implementation with a data management plan (Molina-Jack et al., 2019). Marine litter was included among the target parameters in 2017. In recent years, a joint task has been performed to develop a standard data structure for marine litter at the European level. It was modelled to host MSFD monitoring data, following the OSPAR beach litter database (OSPAR-MCS) approach and taking into account the MSFD TG ML and UNEP / Mediterranean Action Plan requirements (Molina-Jack et al., 2019). The collaborative action between the JRC, the TG ML, and EMODnet Chemistry for gathering official MSFD monitoring data for calculating European baselines and thresholds gave the database an initial boost, including a large number of datasets in 2018 (Partescano et al. 2021).

LitteR. LitteR (Schulz et al., 2019) is an open-source statistic tool for analysing litter data developed as an R package (R Core Team, 2021), to support OSPAR and EU scientists and policymakers. This package offers a simple user interface for analysing litter data in a consistent and reproducible way. It contains routines for data quality control, outlier analysis, descriptive statistics, trend analysis and regional aggregation of states and trends. The tool produces a detailed analysis report in HTML format, from which tables and figures can be copied (https://cran.r-project.org/web/packages/litteR/). Schulz et al. (2017, 2019) provide more background information on the statistical data analysis of beach litter.

([13]) See EEA (undated-a), 'Marine Litter Watch', https://marinelitterwatch.discomap.eea.europa.eu/Index.html
([14]) See EEA (undated-b), 'Marine Litter Watch data viewer', https://www.eea.europa.eu/themes/water/europes-seas-and-coasts/assessments/marine-litterwatch/data-and-results/marine-litterwatch-data-viewer/marine-litterwatch-data-viewer
([15]) See EEA (undated-a), 'Marine Litter Watch', https://marinelitterwatch.discomap.eea.europa.eu/Index.html

4 Floating marine macro litter

4.1 Introduction

The occurrence of anthropogenic objects, mainly made of plastic, floating at sea has been described for decades (Venrick et al., 1973; Morris, 1980). MSFD criterion D10C1 includes the amount of floating litter ('The composition, amount and spatial distribution of litter on the coastline, in the surface layer of the water column, and on the seabed, are at levels that do not cause harm to the coastal and marine environment'), and the evaluation of its spatial distribution, composition and sources, according to Commission Decision (EU) 2017/848.

Floating Marine Macro Litter (FMML) referring to any floating object larger than 2.5 cm in size, poses a direct threat to marine organisms, which may be adversely affected by ingesting whole items or pieces of larger litter items, or by becoming entangled in litter items such as bags, nets and other fishing gear (e.g. Boerger et al., 2010; van Franeker et al., 2011; Domènech et al., 2019). Moreover, FMML is a precursor of marine microlitter, whose effects on living organisms are still under investigation and which could be a vector for transportation of invasive species, or for the adsorption/release of harmful chemical compounds that might be mobilised during FMML degradation processes, or after its ingestion by marine organisms (e.g. Aliani and Molcard, 2003; Teuten et al., 2009; Hahladakis et al., 2018).

4.2 Scope and key questions

According to the requirements of the MSFD and to comply with the necessity of producing reliable and comparable data within and among regions, monitoring should assess the environmental status, the temporal and spatial trends, and the main sources and pathways of litter items in the marine environment in order to determine the level of achievement of environmental targets and/or the effectiveness of measures. Thus, monitoring programmes should collect information on: (i) amount, distribution, and composition of litter; (ii) rates at which litter enters the environment (and sources); (iii) spatial and temporal variations of (i) and (ii); and (iv) impacts of litter.

Monitoring protocols must supply quantitative data in response to the assessment needs. For this reason, and to allow the assessment of trends, they need to be coordinated, coherent, consistent, and comparable across the European seas. Furthermore, data should be produced in a comparable format to allow their integration across monitoring programmes. However, the most appropriate protocols should be selected according to their suitability for achieving the relative aims and objectives of monitoring, which determine the temporal and spatial scales of application, the frequency of sampling, replication needs, etc. The goal of FMML monitoring can functionally be to evaluate trends; identify pathways, geographical sources, and potential accumulation areas or seasons; assess changes due to mitigation measures (long-term monitoring); provide information to evaluate risks for and focus mitigation actions on sensitive areas for marine biodiversity.

This chapter compiles the recommended protocols for FMML monitoring, investigate their differences and applicability, and identifies their potential limitations to fulfil the requirements of the MSFD. The protocols aim to create harmonised monitoring approaches to ensure data comparability between programmes and across regions. They address FMML monitoring at the local scale and in the open sea, and consider the different approaches needed with regard to the observation conditions provided by the monitoring platforms and, consequently, the range of sizes of litter that can be detected from them. This chapter also addresses the issue of data QA/QC for trend analysis, considering monitoring data derived from the use of platforms of opportunity and from new monitoring methods such as aerial photography.

4.3 Strategy for floating marine macro litter monitoring

FMML monitoring protocols should define the spatial and temporal scales of application, sampling units and replicates.

4.3.1 Spatial distribution of monitoring

Monitoring programmes should be consistent, coherent, and comparable within marine regions and surveys. Given the high heterogeneity of litter distribution, the criteria used to select the survey site could have a crucial effect on results (UNEP/MAP, 2016). The selection of the monitoring site is highly dependent on the purpose and the monitoring method and may be made based on certain characteristics of interest to reduce variability, or through a random selection to allow extrapolation to other sites or areas.

Given the differences in the main drivers, distribution, amount of litter, and the geographical scale involved, stratifying the sampling in relation to sources (urban, riverine outputs, offshore activities) is suggested in order to provide representative data in each location (Cheshire et al., 2009; Zampoukas et al., 2014). Alternatively, sampling could be designed to cross-areas of expected low (e.g. open sea) and high (e.g. close to harbours/marinas) litter density, identified based on the results of preliminary exploratory surveys that should be performed to assess the variability of litter distribution. Based on this initial phase, a routine programme should be established that includes areas of interest that cover the widest range of conditions and is stratified for at least coastal and high sea areas. Furthermore, the selection of other specific areas should be considered (e.g. in estuaries, areas of touristic or commercial traffic) (Vighi et al., 2022).

The monitoring areas from the previous MSFD cycle should be maintained to allow the comparison of results over time; new locations may be added based on the above considerations. Changes or modifications to areas should be limited, and newly designated areas should maintain the same characteristics as the original locations.

4.3.2 Timing of monitoring

Seasonality may play a key role in driving the variability of the amount and distribution of litter, which is linked to seasonal variations in oceanographic and anthropogenic factors (Arcangeli et al., 2017). As the observation of FMML is dependent on weather conditions such as the sea state and the wind speed, temporal (i.e. seasonal) stratification of surveys is highly recommended. However, the organisation of monitoring must be flexible enough to take the variability of environmental conditions into account and to allow rescheduling observations to meet appropriate conditions. Ideally, monitoring should be performed after a minimum duration of calm sea to prevent any bias related to recent storms or heavy seas.

Preliminary monitoring should be performed with a higher frequency to assess the variability of litter quantities over time. Subsequently, a minimum sampling frequency of one per year is required, although seasonal replication is recommended (Cheshire et al., 2009).

In some cases, the timing of surveys can depend on the schedule of the observation platforms. Regular patrols of coast-guard ships, ferry lines or touristic trips may offer frequent opportunities for observations, especially during the calm weather conditions that are required. Sharing information and experiences regarding the preliminary monitoring among local and regional authorities and at the EU level will be important for the organisation of harmonised and cost-effective monitoring of the European seas.

4.3.3 Sampling units and replicates

To perform temporal analysis (e.g. to assess trends), surveys are usually based on transects, considered as sampling units, and include information on gradients such as distance from the coast (or from main sources of litter). A minimum transect length for the surveys must be set to avoid biases due to small sample sizes.

A grid cell may be overlaid on the monitoring effort to perform spatial analysis: in this case, the single cells are considered statistical units. A minimum sampling effort per cell is also required in order to avoid outliers due to uneven effort. Sampling units should be randomly allocated within each monitoring site, provided that the heterogeneity in the amounts of marine litter is taken into account.

Replicates are a combination of monitoring sites and monitoring occasions. Due to the variability of monitoring needs and specificities, it is not possible to give general advice on how many replicates is an adequate number; this must be defined according to the variation across sites and seasons, determined within pilot studies.

4.4 Litter categories for floating marine macro litter

For consistent monitoring results, litter items must be grouped into categories according to their size, material and type. The approach used to categorise FMML is linked to the *Joint List of Litter Categories for Marine Macrolitter Monitoring* used for the other environmental compartments to allow cross-comparisons across compartments (Fleet et al., 2021).

4.4.1 Size categories

Since items are usually observed but not collected, the size of FMML items is the main indicative parameter of their volume or weight (which vary according to the material). The size of an object is defined here as its largest dimension, width or length, as visible during the observation. Any piece of litter that can be identified as an item should be counted as one item.

Lower size limit. The minimum size of the observed litter depends on the observation conditions, specifically the platform's speed and height. Although a lower size limit of 2.5 cm is recommended, any alternative limit should be specified for each platform type.

Classes. As visual observation will not permit the exact measurement of object sizes, size range classes must be introduced for reporting purposes. It is important that a common approach is used, as the data will be combined in common databases. The TG ML has recommended reporting the size of macro litter according to agreed size ranges in order to allow quantitative reporting and link the reporting to the MSFD assessments (Galgani et al., 2013; Fleet et al., 2021; Vighi et al., 2022).

The size determination/reporting scheme should cover the following classes:

- A. $2.5 \leq x < 5$ cm
- B. $5 \leq x < 10$ cm
- C. $10 \leq x < 20$ cm
- D. $20 \leq x < 30$ cm
- E. $30 \leq x < 50$ cm
- F. ≥ 50 cm

4.4.2 Litter item categories

The categories of FMML items should be consistent with the categories selected for beach litter, seafloor litter and others to allow comparisons between environmental compartments, particularly between beach and surface floating litter. Thus, the quality of data collected within FMML monitoring relies on the unambiguous identification of litter-type categories through a commonly agreed list. The *Joint List of Litter Categories for Marine Macrolitter Monitoring* (Fleet et al., 2021) has been adopted by EU Member States for MSFD litter monitoring.

The Join List of Litter Categories is organised into six levels with increasing classification details: level 1 (classification by material), level 2 (classification by use), level 3 (classification by general type), level 4 (classification by type), level 5 (classification by specific type) and level 6 (classification by size class). The highest level of detail includes 183 categories. An online photo catalogue supports the identification of items to facilitate their categorisation during monitoring ([16]).

4.4.3 Source attribution of floating marine litter

The spatial distribution of FMML, in combination with information on local currents, tides and river discharges, gives indications of physical sources (i.e. the litter input zone) and pathways, which is very valuable information that may help to design appropriate measures and check the efficiency of existing ones.

4.5 Observation parameters to be considered within monitoring protocols

Essential data to be collected during FMML monitoring should include the geographical coordinates, number, size class, composition, and type of items. However, the observation of floating marine litter is subject to numerous variables related to the observation conditions that may influence the detectability and identification of items, which must be taken into account in any protocol. These may be divided into operational parameters related to the technique used, the sampling design and the characteristics of the observation platform, environmental parameters related to the geographical position or weather conditions, and parameters related to the properties of observed marine litter items.

([16]) See European Commission (undated), 'Online photo catalogue of the Joint List of Litter Categories' (https://mcc.jrc.ec.europa.eu/main/photocatalogue.py?N=41&O=457&cat=all).

4.5.1 Operational parameters

- Transect strip-width. Width of the defined observation transect. For image-based methods the observation area is defined by the sensors, lens and the observation height.
- Observation height. Vertical distance between the observer's eyes and the water surface.
- Observation distance (maximum). Maximum distance between the observer's eyes and the far end of the transect width.
- Observation angle. Radial angle between the transect direction and the observed item.

4.5.2 Environmental parameters

- Wind speed and direction (Beaufort wind force scale – see Annex IV – 'Beaufort wind force scale')
- Sea state (Douglas scale – see Annex V – 'Douglas scale – state of the sea')
- Light conditions (sun direction and intensity)
- Visibility (quality of vision, in terms of the maximum detectable item distance, potential impairment by fog, etc.)

4.5.3 Marine litter object properties

- Location (Infrastructure for spatial information in the European Community (INSPIRE) compatible geographical coordinates).
- Lower/upper size ranges (detection limit/detection probability). These should be set for each platform/technique according to the height of observation.
- Size classes.
- Type of items: Items should be categorised according to the *Joint List of Litter Categories for Marine Macrolitter Monitoring* (Fleet et al., 2021; see Section 4.4.2).
- Further levels of classification could include a description of the:
    - item state (general description of the item, including the status of degradation);
    - buoyancy (under surface, on surface, above surface);
    - item colour
    - item shape

4.6 Practicalities of monitoring protocols

To produce comparable results, protocols must include a description of how to implement FMML monitoring and how to process the collected information, from the necessary equipment and staff to data compilation, elaboration and further use. The compilation of data in the same format across observing institutes and areas/regions should make it possible to plot the FMML distribution over time and consequently couple this information with current oceanographic models.

4.6.1 General equipment

- Datasheet (app/laptop/paper forms).
- Observers with binoculars / photographic equipment (optional).
- GPS.
- Notebook and pen (optional).

4.6.2 Data analysis

Geographic information systems (GISs) may be used to determine the relative abundances (%) of litter on a spatial basis. Steps for basic data analysis and presentation include:

- exploratory statistics;
- geostatistical analyses;
- modelling (based on current oceanographic models);
- map plotting;
- graphs;
- density mapping.

The goal of monitoring is the quantification of FMML. The formula used internationally to calculate the density, D, of marine litter is:

$$D = {}^{n}\!/_{(w \times L)}$$

where *n* is the number of items observed, *w* is the width of the strip (km), and *L* is the length of the strip (km) (Thiel et al., 2003). The total density and density per litter type should be calculated.

4.6.3 Optional synoptic monitoring of marine fauna

To identify areas and seasons that may pose a risk to marine biodiversity due to the high occurrence of FMML, the synoptic monitoring of marine fauna is recommended (e.g. Arcangeli et al., 2019). Data on marine fauna (e.g. jellyfish, ocean sunfish, sea turtle) can be collected by FMML observers within the same fixed-width monitored strip, or dedicated observers can use distance sampling techniques to monitor/record the presence of marine macrofauna and megafauna (e.g. cetaceans, sea turtles, sharks).

4.7 Protocols for visual monitoring of floating marine macro litter

This section includes a subset of protocols that provide harmonised approaches to quantifying FMML using observer-based (visual) methods, performed from different types of platforms. The protocols are intended to be used for monitoring from large ships, small or medium sized vessels, and aircraft (see Table 4.1).

The most commonly applied method for the visual monitoring of FMML is based on the designation of transects with a fixed width, measured from either the side or the front of the vessel during navigation (Vighi et al., 2022). The linear distance-to-object technique has been used in several studies (e.g. Bergmann et al., 2016; Sá et al., 2016; Currie et al., 2017), but the results obtained with the two methods are not completely comparable.

As a general rule, a minimum of one dedicated and experienced observer must perform the observation, quantification and identification of floating litter items. As intensive surveying of the sea surface leads to fatigue and potential observation errors, the transect length or the observation shifts should be reduced. It is also suggested that observers should be switched at least every 60 minutes to avoid fatigue and to maintain their attention. However, it is recommended that observers perform shorter transect observations of 20 – 30 minutes to avoid underestimating litter density.

Table 4.1. Recommended classification of platforms for visual monitoring of FMML based on the observation height.

Platform	Size of the ship/vessels	Types of ships/vessels	Observation height	Recommended maximum speed (for observations)	Maximum fixed width of strip	Suitable use
Vessels						
Vessel	Large	Ferries, cargo ships, oceanographic vessels, etc.	10–25 m	25 knots 16/18 knots in high-density areas	50 m	Offshore – large scale

Platform	Size of the ship/vessels	Types of ships/vessels	Observation height	Recommended maximum speed (for observations)	Maximum fixed width of strip	Suitable use
Vessel	Medium	Medium-sized ships: sailboats, motorboats, fishing boats, oceanographic vessels, etc.	> 2 m and < 10 m	6–15 knots	20 m	Offshore / coastal waters
Vessel	Small	Inflatable boats, rigid-hulled inflatable boats, etc.	≤ 2 m	4 knots	3 m	Coastal waters – local scale
Aircraft						
Aircraft	-	-	ca. 750 ft	n/a	ca. 275 m	Large scale monitoring programmes

NB: n/a, not applicable.

4.7.1 Protocol for visual monitoring survey from vessels

Although some studies (e.g. Vighi et al. 2022) use the distance sampling method to record FMML synoptically with top predators monitoring, strip transect (fixed width) is the commonly used method of estimating FMML density in offshore and large-scale areas. Therefore, conducting visual surveys based on fixed-width strip transects to monitor FMML is recommended.

Large (e.g. commercial ferries, cargo ships) or medium sized (e.g. oceanographic vessels, medium sized fishing boats) vessels are suitable for monitoring FMML in offshore/large areas, covering the large oceanic processes that drive FMML distribution with and adequate sample size. Considering the logistical characteristics and potential constraints of these types of vessels, opportunistic monitoring might be a cost-effective approach. For coastal and local scales, the monitoring of FMML is often related to potential land-based sources and the coastal distribution of litter; however, some adaptations related to the speed of the vessel and the observation height should also be considered during the definition of the strip width.

4.7.1.1 Survey frequency

Although sampling in offshore and large-scale areas is usually carried out using opportunistic platforms, a preliminary monitoring at a higher frequency is recommended to assess the variability of litter quantities over time. A minimum sampling frequency of once per year is required, and seasonal sampling is recommended (Cheshire et al., 2009). Ideally, it is also recommended that the number of surveys per season be increased to perform seasonal analysis for a single monitoring year (Arcangeli et al., 2020).

Since the distribution of marine litter in coastal waters may be influenced by local/regional environmental factors, such as rainy or windy periods, knowledge of local temporal variations should be used to define sampling frequency. Each seasonal collection of data should be performed at a similar time point each year, considering seasonal surveys to assess the variability of environmental factors at each sampling site.

4.7.1.2 Survey coverage

There is no agreed statistical method to determine the minimum representative coverage per survey that can be extrapolated to all regions and/or density ranges of litter for offshore and coastal waters. However, the Burnham approach may facilitate the initial assessment of the minimum transect area required for a given precision when a pilot study cannot be conducted in the study area (Burnham et al., 1980; Arcangeli et al., 2020).

4.7.1.3 Transects

The usual fixed width of the transects for FMML visual surveys is set in the range of 10–50 m for large and medium-sized vessels in offshore waters, with a maximum speed of 25 knots and an observation height of 25 m for large vessels such as cargo ships or ferries. Nevertheless, to define a strip width that will allow the detection of objects as small as 2.5 cm (sizes classes A–F, see Section 4.4.1), the following three factors must be considered: observer height, speed, and observation time/fatigue. If it is not possible to define a fixed width sufficient to detect items in the smaller size classes (i.e. A size category), a higher range of detectable sizes should be considered.

The strip width can be defined using an inclinometer in front of the bow or at the side of the vessel. When monitoring from the side, it is strongly recommended to perform monitoring from the side less affected by the sun glare effect and with the best visibility. The strip width measure should be continuously controlled, or marked on the vessel (e.g. using a marking system to delimitate the strip width, as in Figure 4.1) during the survey to ensure that only items spotted within the strip are monitored.

Figure 4.1. Examples of a marking system to control the strip width of the transect: (a) marking system on large or medium-sized vessels and (b) marking system on small vessels

(a) (b)

Source: Interreg Mediterranean MEDSEALITTER project.

Medium-sized or small vessels (see Table 4.1) are suitable for assessing the quantity and the characteristics of FMML larger than 2.5 cm (size classes A–F, see Section 4.4.1) in coastal and small-range areas. Transect widths are normally set in the range of 3–20 m, at a speed of 2–10 knots and with an observation height of 1.5 m to 9 m, depending on the type of vessel used (e.g. Arcangeli et al., 2018; Suaria et al., 2020; Vighi et al., 2022). However, for coastal and local-scale monitoring, the strip width and observation height should be adjusted to allow for the detection and observation of items as small as 2.5 cm across the entire width of the transect. Additional transects may be added during the same survey if additional observers participate in the monitoring (e.g. 3 m fixed width strip at each side of the bow). The fixed-width strip could be defined and delimited visually using an inclinometer or physically using a rod (or similar) of known length attached perpendicularly to the boat.

For small vessels, with an observation height of ≤ 3 m, it is recommended that parallel transects be used to cover a determined area of interest due to the visibility limitations (i.e. limited angle of observation, sun glare effect).

4.7.1.4 Litter size range

Since macro litter includes any item larger than 2.5 cm in its longest dimension, efforts should be made to record any item down to this lower limit. However, when conducting visual observations in offshore waters with medium/large vessels, the observation height and the strip width may limit the detectability of the smaller litter items over the entire transect width. In these cases, a size range class with a larger minimum size may be selected that allows all objects within the size range and above to be detected across the strip width. The acquired data will then not be comparable to data considering the lower size limit of 2.5 cm.

The relevance of items larger than 50 cm in the statistical analysis of data obtained from short and narrow coastal transect-based surveys may be questionable due to the limited representativeness of this method for larger items.

4.7.1.5 Detectability

Depending on the type of vessel and visibility on the deck, observers may survey from the side of the vessel. The observer should be positioned on the bridge or the command deck of the vessel, on the side, in the vicinity of the bow, to have the best visibility of the strip, but avoiding the turbulence generated by the bow itself. Observers should stay on the side with better visibility (i.e. less affected by sun glare and with the sun behind them). Binoculars can be used to confirm litter sightings if needed. The observation height from small vessels may hinder visibility depending on the angle of observation obtained when defining the width strip; it is recommended that narrow strips be defined to facilitate the observation and detectability of objects within them. Photographs can be taken to facilitate the identification of some litter items, but additional observers should be assigned to this task.

Weather conditions might also hinder the detectability of litter items, particularly the smaller ones. To avoid this bias, it is recommended that monitoring is performed when the wind force is ≤ 3 on the Beaufort scale to avoid reduced visibility of items due to sea conditions and wave mixing.

4.7.1.6 Survey and sampling metadata

The following data should be recorded within each survey transect through the dedicated app:

- name of the vessel;
- start/end geographical coordinates;
- course over the ground / ground track;
- region;
- country and country identifier code;
- sea state (Douglas scale);
- weather description;
- visibility range;
- average wind direction (Beaufort scale);
- transect width;
- transect length;
- number of observers;
- observation height;
- minimum size range observed and surveyed;
- speed (maximum / minimum and average);
- start/end time of observation;
- total time of observation

4.7.1.7 Survey equipment and consumables

Other than the observation platform, the equipment used for FMML visual monitoring is very limited. The instruments that may facilitate the task include the following:

- a system for training and calibrating size classification (e.g. inclinometer, ruler, marker stickers);
- a system for visually marking the observation area (e.g. inclinometer, ruler, marker stickers);
- binoculars (optional);

- a GPS for determining ship speed and geographical coordinates, and extra batteries;
- a tablet computer for documenting the results (through a dedicated app/programme; e.g. the JRC Floating Litter Monitoring app);
- high-resolution cameras (e.g. digital single-lens reflex camera or mirrorless cameras) (optional);
- protective equipment (e.g. sunglasses, sunscreen, cap).

Box 4.1 provides some tips on visual monitoring from vessels.

Box 4.1. Tips on conducting visual monitoring surveys from vessels

Determine the observation area with a visual marking system, if possible. Check often that the markings correspond to the defined transect.

Use a tablet computer with a dedicated app/programme.

Grouping the most frequent categories of litter items can facilitate the recording of items observed during the survey.

An additional observer can photograph the litter items and patches in areas of medium/low density and associate the photographs with the records taken by the main observer. The subsequent analysis of the images may provide more detailed information on the observed items.

An additional tablet or smartphone with access to the Online Photo Catalogue ([17]) of the Joint List of Litter Categories may facilitate the swift identification of unknown litter items.

4.7.2 Protocol for visual monitoring on aircrafts

Aerial surveys are useful for assessing FMML in large-scale monitoring programmes, and for detecting and identifying aggregations of litter. Aerial surveys are usually performed on small, high-wing, two-engine airplanes, equipped with bubble windows for improved visibility. Observers sit at the two sides of the aircraft and the co-pilot acts as the data recorder. Considering that the lower limit of object size for aerial detection is ca. 30–40 cm, a limitation on the categorisation of FMML observed from aerial surveys is imposed. Concurrent visual-based and image-based observations were performed from this type of platform, and results highlighted that the densities of FMML detected by aerial photography were higher than those detected through the observer-based method (Garcia-Garin et al., 2019).

4.7.2.1 Survey frequency

The frequency of aerial monitoring should be set using an analogous approach to that used for visual observations from vessels. At least one survey per year is required, and seasonal replications are recommended to evaluate intra-annual variability; however, the cost-effectiveness of this methodology is high and its use for FMML monitoring in conjunction with other purposes (e.g. cetacean monitoring) is recommended.

4.7.2.2 Survey coverage

There is no agreed statistical method to determine the minimum representative coverage per survey that can be extrapolated to all regions and/or density ranges of litter for offshore and coastal waters.

([17]) See European Commission (undated), 'Online photo catalogue of the Joint List of Litter Categories' (https://mcc.jrc.ec.europa.eu/main/photocatalogue.py?N=41&O=457&cat=all).

4.7.2.3 *Transects*

Surveys should be designed according to the fixed width transect technique in which a high representation of the study area is homogenously covered. Since the observable area of the transect corresponds to an angle of observation between 90° and 40°, for flights performed at around 750 ft, the observation strip width ranges from 200 m to 275 m (e.g. Lambert et al. 2020). The distance from the detected items to the transect line can also be established according to the angle of sighting estimated using a hand-held inclinometer. The angle from each item detected, together with the flying altitude, should be used to calculate the perpendicular distance of the item from the transect line.

4.7.2.4 *Litter size range*

The size ranges proposed by the TG ML (see Section 4.4.1) should be used whenever item identification is feasible. However, as a suitable method of standardising the size of the observed marine litter, items can be classified into three main categories: small (measuring ca. 30–100 cm), medium (measuring ca. 100–200 cm) and large (measuring larger than 200 cm).

4.7.2.5 *Detectability*

The size ranges determined in Section 4.4.1 may be difficult to assign to each item observed, mainly due to the observation height and speed of the aircraft. Since atmospheric and sea conditions also influence the detectability of litter objects from aircraft, monitoring should be performed when the wind force is ≤ 3 on the Beaufort wind force scale.

4.7.2.6 *Survey sampling metadata*

Data on litter should take into consideration the characterisation of each transect (geographical coordinates at the starting and ending points, oceanographic characteristics, etc.), in addition to the number of sightings and the average distance between consecutive sightings (average distance = length between transects / number of sightings). The following data should be recorded within each survey transect, through the dedicated app:

- name of the aeroplane;
- start/end geographical coordinates;
- course over the ground / ground track;
- region;
- country and country identifier code;
- sea state (Douglas scale);
- weather description;
- visibility range;
- average wind direction (Beaufort scale);
- transect width;
- transect length;
- number of observers;
- observation height;
- minimum size range observed and surveyed;
- speed (maximum / minimum and average);
- start/end time of observation;
- total time of observation.

4.7.2.7 *Survey equipment and consumables*

Other than the different observation platform, the equipment used for FMML visual monitoring from aircraft is analogue to that used from vessels. The instruments that may facilitate the task include the following:

- a system for training and calibrating size classification (e.g. inclinometer, ruler, marker stickers);
- a system for visually marking the observation area (e.g. inclinometer, ruler, marker stickers);
- binoculars;
- a GPS for determining aeroplane speed and geographical coordinates, and extra batteries;
- a tablet computer for documenting the results (through a dedicated app/programme; e.g. the JRC Floating Litter Monitoring app);
- protective equipment (e.g. sunglasses).

Box 4.2 provides some tips on visual monitoring from aircraft.

> Box 4.2. Tips on conducting visual monitoring surveys from aircraft
>
> Determine the observation area with a visual marking system on the window of the aircraft. Check often that the markings correspond to the defined transect.
>
> Grouping the most frequent categories of litter items together can facilitate the recording of items observed during the survey.
>
> An additional tablet or smartphone with access to the online photo catalogue ([18]) of the Joint List of Litter Categories may facilitate the swift identification of unknown litter items.

4.8 Protocols for image-based monitoring of floating marine macro litter

According to the scale and budget requirements, image-based monitoring may be performed using different platforms, such as small aircraft, any kind of vessel and UAVs (e.g. Bryson and Williams, 2015; Garaba et al., 2018; Garcia-Garin et al, 2020; Vighi et al., 2022). 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: these techniques are under constant improvement and their applicability to marine litter surveys is under evaluation.

4.8.1 Types of platforms and sensors

4.8.1.1 *Platforms*

Automated recording sensors (video and/or photographic cameras) may be mounted on a range of platforms, both flying (small aircraft, UAVs – e.g. Garcia-Garin et al., 2020; Garaba et al., 2018) 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 – de Vries et al., 2021). Each platform is characterised 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 combination of platform and sensor should once again be made according to the required monitoring scale and the available budget/time.

([18]) See European Commission (undated), 'Online Photo catalogue of the Joint List of Litter Categories' (https://mcc.jrc.ec.europa.eu/main/photocatalogue.py?N=41&O=457&cat=all)

4.8.1.2 Sensors

A series of different sensors may be applied on each platform according to the monitoring needs. The most common instruments include RGB cameras, which can provide high-resolution images and thus be used even from the flight height of a small aircraft. Under good monitoring conditions, the use of these cameras allows the identification of the colour, material, type and size of items, but the sun glare effect could heavily affect the quality of images obtained in the visible RGB spectrum. It is important to select an adequate image resolution and photographic lenses according to the planned monitoring height, considering a minimum pixel size of 2 cm and a frequency of four images per second. Lower observation heights, longer focal lengths and higher-resolution sensors can increase image resolution to allow the detection of the smaller sizes classes of FMML.

Other sensors may be coupled to RGB cameras to cope with adverse environmental conditions: thermic cameras and multi-spectral cameras are also being experimented with for automated marine monitoring (Bryson and Williams, 2015). Thermic cameras generally have a limited resolution, but could help identify objects with a positive buoyancy that have been warmed from the sunlight, such as a floating board. It is suggested that their use be coupled with an RGB camera, as they may help distinguish items when sun glare. Multi–spectral cameras can also help identify floating items in cases of sun glare, as their sensors are less affected by its effects. Despite these sensors generally having a lower resolution than traditional RGB cameras, they could be useful for distinguishing between different materials and between materials and the seawater, as each material has different spectral characteristics.

4.8.1.3 Survey frequency

The frequency of image-based monitoring, analogous to that of visual observations, should be based on knowledge of the temporal variation of marine litter. Reproducing the monitoring plan several times per season is suggested in order to detect possible trends related to main currents, temperature changes and any seasonal pattern; this can be adjusted to the temporal variation characteristics of the study area. Continuous monitoring programmes at the same site in subsequent years should ensure robustness of the data obtained.

4.8.1.4 Survey coverage

There is no agreed statistical method to determine the minimum representative coverage per survey that can be extrapolated to all regions and/or density ranges of litter. Coverage should be determined based on the purpose of the monitoring programme.

4.8.1.5 Transects

The width of transects is directly dependent on the camera resolution and lenses used, and/or the height of the operating sensor. Depending on the needs of each monitoring programme, the height (of flight, or of the camera position on a ferry or on a smaller boat) can be reduced to obtain more detailed pictures but cover smaller areas, or increased to cover larger areas but with lower-quality images. Sensors with higher resolution should be selected if the position of the camera above the sea is higher.

4.8.1.5.1 Small monitoring scale

For small-scale monitoring, it is possible to photographically cover the whole area of interest using a camera mounted on a pole attached to a small/medium sized vessel or a simple drone, designing the flying/sailing routes on parallel transects, or regularly spaced concentric squares. The spacing between adjacent transects should allow an approximately 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 geographical positions for each photo must be recorded automatically by a sensor to allow their subsequent geo-referencing.

4.8.1.5.2 Large monitoring scale

For larger areas, it is not possible to obtain a complete photographic map without considerable efforts in terms of budget and time. The use of aerial photography from small aircraft could provide a more continuous image recording across the area of interest. Parallel or zigzag transects should be planned to homogeneously cover any possible environmental gradient. To photographically monitor large areas over fixed surveys, mounting a recording camera on the bow of cruise ships, cargo ships or ferries (e.g. the JRC *Sealittercam*; Hanke and Piha, 2011; González-Fernández et al., 2022) is also an option.

4.8.1.6 Litter size range

The monitoring height, the lenses used, and the sensor definition should be considered in the selection of the optimal resolution to detect the largest possible size range of litter. A minimum resolution of 2 cm per pixel is considered adequate for monitoring litter items of more than 25–30 cm in length from aerial platforms (i.e. aircraft or UAVs). Detection of smaller sizes can be improved by reducing the observation height, and using cameras with high-resolution sensors (e.g. 100 megapixels) and/or cameras equipped with longer focal length lenses.

For sensors and cameras installed on vessels, the height of observation – or the height at which the camera is installed in relation to the sea surface – the type of lens and the definition of the sensor must also be considered. This approach allows for the detection of items in the lower size range of litter; however, the upper size range may be underestimated, depending on the size of the path covered in each image.

4.8.1.7 Detectability

Monitoring should be performed from a calm sea to prevent reduced visibility of items due to sea conditions and wave mixing. As in the previous protocols, monitoring should be carried out when wind force is ≤ 3 on the Beaufort wind force scale.

4.8.1.8 Image processing and analysis

Automated detection systems (e.g. user-friendly apps, machine learning systems) could be used to help detect marine litter according to the parameters selected for monitoring (e.g. flight height, image resolution, the effect of glare, minimum size of detectable litter). The processing procedure would involve three major steps: (i) statistical analysis of detectability, (ii) candidate object extraction and (iii) classification. Nevertheless, a human operator should perform the final validation.

4.8.1.9 Survey sampling metadata

Data on litter should take into consideration the characterisation of each transect (geographical coordinates at the starting and ending points, oceanographic characteristics, etc.), in addition to the number of sightings and the average distance between consecutive sightings (average distance = length between transects / number of sightings). The following data should be recorded within each survey transect, through the dedicated app:

- type of platform (i.e. UAV, vessels);
- types and models of cameras and lenses used;
- types and models of sensors used;
- start/end geographical coordinates;
- course over the ground / ground track;
- region;
- country and country identifier code;
- sea state (Douglas scale);
- weather description;
- visibility range;
- average wind direction (Beaufort scale);
- transect width;
- transect length;
- observation height;
- observation angle of each camera and sensor;
- percentage of overlap between images;

- minimum size range observed and surveyed;
- speed (maximum / minimum and average);
- start/end time of observation;
- total time of observation.

4.8.1.10 Survey equipment and consumables

The following equipment is the minimum recommended for conducting the survey:

- cameras and sensors;
- lenses (if needed);
- batteries and extra batteries for cameras and sensors (for unwired systems);
- batteries and extra batteries for UAVs and control systems (only for monitoring with UAVs);
- autonomous power supply system (e.g. uninterruptible power supply)
- data storage system and extra data storage (e.g. hdds, ssd, etc.)
- UAV control system (only for monitoring with UAVs);
- control system for cameras and sensors (except in continuous recording);
- attachment system for mounting cameras and sensors on vessels;
- tools;
- protective equipment for operators (e.g. sunglasses, sunscreen, cap).

4.9 Data and metadata reporting

The data analysis of litter needs to be performed at different spatial and temporal scales, and should be harmonised when reporting monitoring results, as this is crucial for comparing data.

The data obtained from the application of the protocols described above will be a list of georeferenced items classified according to the *Joint List of Litter Categories for Marine Macrolitter Monitoring* (Fleet et al., 2021) and size classes. The use of a laptop or other portable devices with a dedicated app to collect FMML data has a clear advantage over paper documents.

4.9.1 Floating Litter Monitoring app

The JRC has developed a system based on a mobile computer app, the Floating Litter Monitoring (FLM) app, which has been field tested within the Policy-oriented marine environmental research for the European regional seas (PERSEUS), the Improving environmental monitoring in the Black Sea II (EMBLAS II) and the Riverine and marine floating macro litter monitoring and modelling of environmental loading (RIMMEL) projects (e.g. Gónzalez-Fernández and Hanke, 2017; González-Fernández et al., 2022). The FLM app aims to enable the large-scale acquisition of comparable data based on observations and/or image-based systems in offshore and inshore waters and rivers, using the harmonised litter categories of the *Joint List of Litter Categories for Marine Macrolitter Monitoring* developed by the MSFD TG ML in collaboration with the RSCs (Fleet et al., 2021).

The FLM app has been designed for data acquisition by monitoring authorities, scientific projects, NGOs and the general public, where data can be managed by users, depending on the assigned role, and data managers. The app also enables the setting up of coordinated actions based on group codes for the subsequent management of datasets. The FLM app can contribute to harmonising the monitoring of FMML. For further information and access to the web platform, see the Commission’s floating litter monitoring web page (https://floating-litter-monitoring.jrc.ec.europa.eu).

4.10 Quality assurance / quality control

A high level of consistency within and among regions would support a comprehensive analysis of the data providing the opportunity to undertake statistically robust comparisons over time and across survey locations.

The implementation of consistent QA/QC processes should be considered and implemented from the initial development of FMML monitoring programmes, following best practice measures to reduce potential biases and errors.

4.10.1 Quality assurance / quality control related to monitoring and sampling

The following measures are recommended for FMML monitoring and sampling processes.

- Appropriate techniques and platforms should be selected according to monitoring needs.
- Monitoring of FMML should be stratified into selected coastal/open sea transects. Initial pilot studies are recommended to select locations and define a sampling frequency that reflects the impact of human activities and pressures, and the temporal variation of local/regional environmental factors, particularly for coastal monitoring.
- Seasonal surveys are recommended to provide greater precision and accuracy and robust data for trend analysis.
- Advanced training programmes or hands-/eyes–on training courses with comparisons of observations and use of artificial targets should be held to guarantee minimum standards of observations from each platform. Similar events should be organised periodically at the EU level with further implementation at the national level within the EU Member States.
- Monitored sizes range categories might vary according to the platform and monitoring scale used but should include a range covering relevant small items and be based on the MSFD *Joint List of Litter Categories for Marine Macrolitter Monitoring* (Fleet et al., 2021).
- Calibration approaches should be implemented to guarantee the consistency and comparability of the monitoring data between areas and over time.

4.10.2 Data processing and reporting measures

Data acquisition should be organised effectively between Member State authorities and scientific research projects to ensure a consistent data reporting.

All data should preferably be reported through laptop or tablet computer devices with a dedicated app for collecting FMML data. If there are technical issues, data can be collected manually and then transferred to the dedicated apps to provide a consistent digital FMML database that allows a comprehensive analysis of the data within each region.

4.11 Cost and efforts needed for monitoring floating marine macro litter

Several factors influence the cost of FMML monitoring, including staff, equipment and field implementation requirements. Costs for monitoring when using a dedicated platform/activity could be high due to the involvement of vessels or aircraft; however, these costs may vary widely between regions. Integrated multidisciplinary monitoring programmes, targeted monitoring, the use of volunteers and the development of electronic tools to simplify data collection (apps, automated detection systems), would contribute to reducing costs and maximising the use of existing resources.

4.11.1 Using opportunities for observation

Costs and efforts could be reduced by connecting FMML monitoring to other activities (e.g. by using ferries or regular cruises as observation platforms). Placing a dedicated person on board a ferry for a selected short coastal transect repeated at appropriate intervals, appears to be a very cost-effective methodology, which may provide a quantification of FMML in a short time. However, although this would drastically reduce operational costs, visual monitoring still requires the employment of dedicated personnel. The use of volunteers could further reduce staff costs, provided that volunteers are properly trained and protocols are rigorously applied. Finally, staff costs would be further reduced if photographic methods and automated detection algorithms were used, as then only one staff member in charge of the maintenance of technical equipment, would be needed.

Other opportunities for performing FMML observation could be provided by scheduled coastal oceanographic cruises, coast-guard patrols, touristic cruises, etc. However, any monitoring programme would need to be adapted to the available opportunities, and some compromises for the ideal observation transect might be needed.

4.11.2 Estimating costs and efforts

The selection of a given protocol should be made according to the monitoring needs in terms of the spatial resolution and level of detail needed, the budget and the available staff/equipment. To facilitate this selection process, an estimation of the cost and effort (including the cost of labour, equipment and other running costs), the level of technical equipment and expertise needed for data collection and analysis, and the applicability has been provided for each protocol proposed (Table 4.2). Given the high variability of labour costs among countries, these are only rough estimates provided to support the decision of which protocols to adopt for monitoring. However, the quantification of the costs is highly dependent on the technique (visual/photographic) and platform used and should consider any available equipment or opportunity that could reduce costs.

Table 4.2. Overview of the approaches recommended for FMML monitoring with relative indications of the technical equipment and expertise required, possible performers, costs associated with the different phases of implementation, detail generated, scale of applicability and a summary of the main pros and cons

	Large vessels (visual)	Small/medium vessels (visual)	Ship-based imagery	Aerial surveys (visual)	Aerial (aircraft) photography	Aerial (UAV) photography
Technical equipment	L	M	H	H	H	M
Expertise	L/M	L/M	H	M/H	H	H
Possible performers	V; P	V; P	P	P	P	P
Costs (sampling; analysis; equipment)	L/H; M; L/H Overall: L/M	M; M; M Overall: M	M; M; M Overall: M	H; M; H Overall: M/H	H; M; VH Overall: H	M/H; M; M/H Overall: M/H
Detail generated	M (size > 20 cm)	H (size > 2.5 cm)	M/H (depending on height and resolution)	L (size > 30 cm)	L/M (depending on height and resolution)	M/H (depending on height and resolution)
Spatial scale of applicability	H	M	H	H	H	L/M
Benefits opportunities to reduce the costs	Wide coverage. Can be integrated with ongoing vessels' operations and/or coupled with marine fauna monitoring programmes to allow replicated surveys across seasons and years. Trained volunteers can be employed to reduce staff costs.	High detail of observations. Can be adapted to necessities of sampling (specific areas/seasons). Allows precise assessments on a local scale. Can be coupled with marine fauna monitoring. Trained volunteers can be employed to reduce costs.	Can produce extremely highly detailed observations and allows FMML assessment over large areas. Automation of analyses can further reduce costs.	Allows FMML assessment over large areas and correlations with potential sources (shipping/fisheries). Can be coupled with marine fauna monitoring or other ongoing monitoring activities to reduce costs.	Large area coverage and highly detailed observations. Recorded images can be used for several subsequent analyses. Automation of analyses can reduce the overall cost and time dedicated to analyses.	Can produce extremely highly detailed observations. Basic platforms and sensors can be easily adopted for routine low-cost monitoring of small coastal areas. Automation of analyses can further reduce costs.

NB: VH, vey high; H, high; L, low; M, medium; P, performers, V, volunteers.

Source: Adapted from Vighi et al. (2022).

4.12 Other methodologies

Riverine litter monitoring

Visual and image-based techniques are also applicable for monitoring floating litter on rivers by performing observations from bridges or similar places (González et al., 2016). Depending on the height of the observation station and the river flow rate, either the large ferry or the medium-sized boat protocols can be used for determining the observation strip width, the sample size (time effort) and the categorisation of the litter. The height of the observation station would also determine the resolution needed for photographic methods. The comparability of data between riverine and marine monitoring is important for quantifying FMML and identifying its main sources.

Net tow surveys for macro litter and mesolitter

A physical sampling of floating macro litter requires large net openings to be operated on the sea surface. Given the occurrence of macro litter items, this would require significant dedicated ship time and specific equipment (e.g. Lebreton et al., 2018; Vighi et al., 2022). This method may be applicable for floating mesolitter, size range relevant to ingestion by marine biota, and in line with the monitoring of floating microlitter, but further research is needed.

Satellite imagery

Satellite imagery has also been investigated in the last decade to detect and track FMML and aggregations, such as windrows, as proxies for marine litter monitoring (e.g. Arias et al., 2021; Cózar et al., 2021), however, it is not ready yet for standardised and systematic FMML monitoring programmes (Vighi et al., 2022). Analysing some sources and modelling dispersal by applying current data from remote sensing via satellite has the potential to become an efficient and reliable tool to support the design of monitoring programmes in large marine areas.

5 Seafloor macro litter

5.1 Introduction

Criterion D10C1 ('The composition, amount and spatial distribution of litter on the coastline, in the surface layer of the water column, and on the seabed, are at levels that do not cause harm to the coastal and marine environment') of D10 includes the amount of litter deposited on the seafloor, with analysis of its composition, spatial distribution and, where possible, source, according to Commission Decision (EU) 2017/848 (replacing Decision 2010/477/EU).

Comparable data and baselines are needed to establish trends and compare current data against threshold values. The existing methods for monitoring litter on the seafloor reflect the difficulties associated with applying compatible and harmonised methods and their limitations. Any location is characterised by different depths, and the nature of the bottom may be sandy, muddy or rocky. As a consequence, different methods are applied to monitor litter on the seafloor (e.g. trawling, diving, imagery) (GESAMP, 2019). Moreover, monitoring litter on the seafloor may be challenging for some Member States and coastal areas because of limited resources; therefore, there is a need to set up a list with priority areas to monitor.

Coordinated national or regional monitoring programmes for litter on the seafloor within Europe started in 2013 through experimental monitoring. The most common approaches to evaluating seafloor litter distribution use opportunistic sampling during trawling surveys. This type of sampling is usually coupled with regular fisheries surveys (marine reserve, offshore platforms, etc.) and programmes on biodiversity monitoring, since methods for determining seafloor litter distribution (e.g. trawling, diving, video) are similar to those used for benthic and biodiversity assessments.

Monitoring programmes for demersal fish stocks, undertaken as part of the Data Collection Framework (DCF), provide data using harmonised protocols, which may support the monitoring of litter at the European scale. Data are collected regularly through existing International Bottom Trawl Surveys in the North Sea (NS-IBTS), the Atlantic Ocean (IBTS, North-East Atlantic Surveys), the Baltic Sea (Baltic International Trawl Survey (BITS)), the Mediterranean Sea (Mediterranean International Trawl Survey (MEDITS)), and the Adriatic Sea (Solea monitoring project (SOLEMON)), according to MSFD requirements.

5.2 Background and the state of the art

The seafloor, from inter-tidal to abyssal depths, has been identified as an important sink for marine litter (Ramirez-Llodra et al., 2011). Data have been obtained from varying locations and depths using different methodologies (divers, video footage, or sampling by bottom trawls).

Both abundance and spatial distribution of seafloor litter show considerable variability. The distribution of litter on the seafloor is strongly influenced by hydrodynamics, geomorphology and human factors. In general, the abundance of marine litter is much greater in shallow coastal areas than on the deeper parts of the continental shelf. For instance, near metropolitan areas, densities may exceed 100000 items per km^2 (Galgani et al., 2015). In these coastal areas, activities related to fishing and tourism significantly contribute to the littering of the seafloor with notable temporal, particularly seasonal variations, while dumping activities that pre-date the introduction of international regulations (e.g. the London Convention) influence the offshore litter distribution. Considering existing data, the Mediterranean Sea may be the most affected European sea (Galgani et al., 2015; Canals et al., 2021).

Long-term monitoring of litter on the seafloor has been performed regularly in some EU countries such as Germany, Spain, France and Italy, and non-EU countries such as the United Kingdom. In the United Kingdom, the results from the plastic caught in nets have not changed since 1999 (Maes et al., 2018). Consistent results were also obtained at several sites in the Spanish Mediterranean Sea in a study carried out between 2007 and 2017, with 1 323 hauls on shelves, except for the Alboran Sea, where a decrease was measured (García-Rivera et al., 2018).

Other studies indicate an increase in litter amounts. For example, at the margins of the Gulf of Lion (France; Gerigny et al., 2019), trend studies (70 stations, depth 40-800 m) have determined a slight but statistically significant increase since 2013.

In the Baltic Sea, a survey performed by seven countries conducting 2 377 hauls (53 cruises between 2012 and 2017) also showed an increase in the occurrence of plastic in the last 2-years; however, no trend for fishing-related litter was detected (Zablotski and Kraak, 2019). A weak but statistically significant increase in seafloor litter representing non-natural materials in the Baltic Sea was also seen between 2012 and 2016 (HELCOM, 2018).

In contrast, a significant decrease in the total litter quantity (kg/km^2) between 2011 and 2016 was found in the north-central Adriatic Sea (Strafella et al., 2019).

However, the evaluation of trends may be challenging when the aim is to detect slight changes. A power analysis of IBTS-related sampling by the Centre for Environment, Fisheries and Aquaculture Science (Cefas) indicated that detecting a 10 % change over 5 or 10 years is unlikely without massive sample sizes (Maes et al., 2015). However, a 50 % change over 5 or 10 years looks readily detectable with current designs based on fish stock surveys such as the IBTS. Annual variations in litter transport, such as seasonal changes in the flow rate of rivers and related turbidity currents, further complicate the interpretation of temporal trends. Other seasonal factors include the intensity of currents, swell and downwelling/upwelling.

Due to the persistence of many litter materials, monitoring litter on the seafloor must consider accumulation processes over past decades. Timescales for observations should therefore be adapted, for example by requiring pluriannual deep seafloor surveys. Finally, seafloor litter assessments need to be planned with defined protocols, including the definition and specification of the survey location; the choice of sampling units; the methodology for collection, classification and quantification of litter; and the process for data integration, analysis and reporting of results.

Research activities focusing on evaluating litter on the seafloor have suggested some priority topics (Canals et al., 2021). These include (i) the evaluation of the catching and detecting potentials of different possible approaches and gear, (ii) the localisation of accumulation areas and supporting tools, such as modelling or seabed maps of sedimentation, to identify areas to be targeted by reduction measures, and to enable the backtracking of transportation schemes and sources, (iii) an analysis of existing data to characterise the most important sources, and (iv) the improvement of imaging tools (automated analysis, image resolution, etc.) for video protocols. A combined approach using both trawl surveys and visual/imaging surveys may be the best set-up for future monitoring of seafloor macro litter.

5.3 Scope and key questions to be addressed

This chapter evaluates existing methods for monitoring litter on the seafloor with respect to their capacity to fulfil the requirements of the MSFD. It proposes harmonised methods that can be applied to assess litter in regional seas, ensuring the comparability of seafloor assessments of litter within and between regions and at the European scale. A strategy is proposed, listing criteria, sites of interest and constraints. Complementary methodologies are also proposed for specific questions. Finally, it addresses data QA/QC requirements. An outlook for the needs of developments and research is provided.

Because of limited resources, the monitoring of litter on the seafloor is determined by each Member State at the national level, depending on the priority areas to be monitored. The strategy to be employed may consist of regularly monitoring selected areas, comparable to the approach used in beach litter surveys to identify and report litter trends over time (Hanke et al., 2019).

Opportunistic approaches may be used to minimise monitoring costs. Valuable information can be obtained from ongoing monitoring of benthic species in marine protected areas, during pipeline camera surveys, the cleaning of harbours and diving activities. Additional monitoring might have to be put in place to cover all areas and create a consistent monitoring network. The sampling strategy should enable the generation of detailed data in order to allow the assessment of the most likely sources of litter, the evaluation of trends and pressure/impact (ingestion, entanglement, and contaminants) relationships and the possibility of evaluating the effectiveness of measures.

The TG ML proposes using protocols based on existing trawl surveys and two protocols based on diving and imagery, which fit the MSFD requirements and will support harmonisation at the European level if applied transnationally. The monitoring strategy for the seafloor can partly be based on ongoing monitoring already developed at the European level. Indeed, existing fishery stock assessment programmes cover most European seas annually, facilitating harmonisation across Member States and data management. Key information on seafloor litter typology, sources, localisation and trends can be obtained. Trawling (otter or beam trawl) is an efficient method for large-scale evaluation and monitoring of seafloor litter, but a much better understanding of how different fishing trawls catch litter from the seafloor is needed. However, when the same gear is used, seafloor data from trawling represent a resource that can be used as a base for marine litter assessments at the transboundary level.

Nevertheless, it must be noted that trawling is a destructive monitoring method, and some sampling locations in rocky areas are incompatible with trawling. Indeed, potential litter accumulation areas (e.g. trenches, seamounts and canyons) cannot be covered by trawling approaches. Designing and developing an adequate monitoring programme will have to consider these limits and consider non-destructive imagery approaches. In a combined approach, protocols based on imaging techniques are efficient approaches to monitoring, particularly in deep-sea areas. These protocols are based on the use of submersibles, remotely operated vehicles (ROVs), AUVs and towed underwater cameras (TUCs). Only some countries will have to consider deep-sea areas in terms of monitoring seafloor litter. The strategy is to be determined by each Member State at the national level, depending on affected areas, but previous scientific results indicate that priority should be given to coastal canyons (e.g. Pierdomenico et al., 2019; Canals et al., 2021).

In this chapter, guidelines are provided for the following:

- visual surveys,
- trawl surveys,
- image-based surveys.

5.4 Visual surveys

Underwater visual surveys are the most common method of estimating marine litter density in shallow areas (GESAMP, 2019). The shallow seafloor is considered separately from other compartments, as it requires a dedicated monitoring strategy, and the approach can differ from those applied to other seafloor depths. The depth limit here is defined as 30 m, which is within the limits of recreational diving and provides enough bottom time to perform safe surveys. Underwater visual surveys are well adapted to monitoring marine protected areas and may address the lack of data where other methods, such as trawls, cannot be employed. As the shallow seafloor is the more accessible seafloor area, there are additional opportunities for data gathering through participatory science with non-scientific communities (e.g. Consoli et al., 2020); these opportunities are not available for the deep seafloor. The abundance of marine litter in shallow coastal waters is generally high in bays, where litter disposed of locally is more likely to accumulate on the bottom because of weaker currents (Katsanevakis, 2008; Stagličić et al., 2021). Furthermore, wave or upwelling-induced cleaning of the seafloor is less important in small bays, where there is usually much less transport. As the logistics related to scientific diving are demanding, opportunistic monitoring – that is, the add-on of litter monitoring to surveys performed for other purposes, such as biodiversity assessments – might provide a cost-effective approach.

Although the most commonly used method to estimate marine litter density in shallow coastal areas is to conduct underwater visual surveys by scuba diving, snorkelling has also been used in very shallow waters (usually < 10 m depth) and for larger items of marine litter (nets/fishing gear). The most common methodology is to perform strip transects, where the observer travels along the centre line searching for litter and counting all items within the strip (e.g. Fortibuoni et al., 2019, Pasternak et al., 2019).

5.4.1 Technical requirements

Knowledge of temporal variation is used to choose the sampling frequency. The minimum sampling frequency for any site should be annual, and at a similar time of the year. Ideally, it is recommended that locations are surveyed every three months (allowing an interpretation in terms of seasonal changes). Sites should be selected that have flat and uniform substrates without risks of wrecks, munitions and/or endangered or protected species.

The easiest methodology for underwater visual surveys with scuba diving is to perform 'strip transects'. The observer travels along a line searching for litter and counting all items within a predefined strip. The transect length (L) must be measured. All litter items within 2 m or 4 m (w) on both sides of a nylon line are recorded and removed, if possible. The strip area (A) is defined as $A = 2 * w * L$. It has to be considered that underestimations of the abundance might occur, especially when counting small items or in the case of high turbidity.

Surveys should be conducted through a minimum of two transects for each site (GESAMP, 2019). Unbiased design-based inference requires allocating transects randomly in the study area or on a grid of systematically spaced lines randomly crossing each other. However, with a model-based approach such as density surface modelling (DSM), it is not required that the transects are located according to a formal and restrictive survey sampling scheme, although good spatial coverage of the study area is desirable. Transects may be defined with a nylon line, marked every 5 meters with resistant paints, and deployed using a diving reel while scuba diving. This way, the transect is well-defined, and its length is easily and accurately measured. Another option might be to not physically define the line but move along an imaginary line using a compass. However, when no real line is deployed, there are difficulties in accurately estimating transect length. A vessel with a GPS may help to assess the transect length in this case by measuring the distance between the start and endpoint of the dive or by summing the lengths for a sequence of positions along the line (in which case, the divers could display their position, for example by towing a buoy) (Katsavenakis, 2009).

The nature of the bottom/habitat is also recorded. The length of the transects may vary between 20 m and 200 m, depending on the depth, the depth gradient, the turbidity, the habitat complexity and the litter density (Katsanevakis, 2009). Results are expressed as litter density (e.g. items/km^2 or items/100 m^2).

5.4.2 Use of volunteers in shallow water surveys

Recreational scuba divers can provide valuable information on seafloor litter in shallow waters. They can access and have the skills and equipment needed to collect, record, and share information about the litter they encounter underwater. Many dive clubs and shops organise underwater clean-ups, often in partnerships with NGOs or local governments. For some Member States, the involvement of volunteer divers might be a good opportunity for litter monitoring in shallow waters, but standardisation and conformity with the common methodologies, protocols and tools proposed here should be achieved.

For example, the Dive Against Debris (DAD) project run by the Professional Association of Diving Instructors (PADI) AWARE Foundation provides a harmonised methodology, field protocol and data reporting process for scuba divers to remove and report marine litter found on the seafloor. As such, data are directly comparable between survey sites globally. Data acquisition can be further harmonised by considering the effort of the survey (e.g. Consoli et al., 2020; Scotti et al., 2021). As the DAD project encourages divers to also report debris-free sites, it yields also presence-absence data. Divers are encouraged, but currently not required, to conduct surveys at the same dive site on a regular (monthly) basis to build data identifying temporal trends in seafloor litter. All resources are freely available to download from the DAD project website (https://www.diveagainstdebris.org/) and the PADI AWARE Foundation has numerous data sharing agreements with various entities to provide bespoke datasets at the local, national and regional levels. Volunteers submit their data to the global data set via the free mobile app or the online submission form. Every DAD survey submitted undergoes and internal quality review process to ensure data integrity. The DAD project and similar projects provide a cost-effective monitoring tool that Member States can implement nationally to build quantitative baselines regarding the types and quantities of seafloor debris and facilitate ongoing assessment.

5.5 Trawl surveys

Trawling (otter and beam) has been employed for large-scale seafloor litter evaluation and monitoring (e.g. Goldberg, 1995; Galgani et al., 2000; Maes et al., 2018; Spedicato et al., 2019). There are some restrictions in rocky areas and soft sediment, as the method may not be suitable and/or may underestimate the litter quantities present.

General strategies to investigate seabed litter are similar to the methodology for benthic ecology. The occurrence of international surveys of bottom trawls such as the IBTS (Atlantic Ocean), the BITS (Baltic Sea), MEDITS (Mediterranean Sea) and SOLEMON (Adriatic Sea) provides useful and valuable means for monitoring marine litter. These use standard gears, depending on the region (GOV and BACA nets in the Atlantic, TVL and TVS nets in the Baltic Sea, GOC73 nets in the Mediterranean and a modified beam trawl in the Adriatic Sea) and provide some harmonised and common conditions for sampling (mesh size, duration of tows, large sampling surface covered) and hydrographical and environmental information (surface & bottom temperature, surface & bottom salinity, surface & bottom current direction & speed, wind direction & speed, swell direction and height). Moreover, specific equipment is used to calculate the swept area of the net. In some cases, when the horizontal opening of the trawl is not evaluated for each tow, surfaces can be calculated by estimating the opening of the trawl (e.g. Fortibuoni et al., 2019). The TG ML recommends using these ongoing and continuous programmes to collect data on marine litter on the seafloor in combination with other visual and imaging methods. However, bottom trawling has a significant impact on benthic ecosystems. Thus, creating a new monitoring programme to monitor seafloor litter may not be justified from an environmental perspective when other methods are available (GESAMP, 2019).

Data on seafloor litter should be reported as items/km^2 before further processing and reporting according to Commission Decision (EU) 2017/848.

5.5.1 Technical requirements

5.5.1.1 Atlantic Ocean, North Sea and Baltic Sea

Litter data collection using trawl surveys started in the 1990s in the north-east Atlantic Ocean (within the IBTS programme; Maes et al., 2018). The International Council for the Exploration of the Sea (ICES) Working Group on Marine Litter (WGML) has recently developed a unique protocol for marine litter assessment using trawling programmes; its application is mandatory for ICES surveys (ICES, 2022). This protocol harmonises the procedures for collecting and reporting marine litter data during existing fish stock surveys. It has been discussed within the TG ML and modified to provide an accurate methodology applicable to MSFD monitoring (facilitating the evaluation of sources, trends, data analysis, etc.).

In the North Sea, the sampling grids are based on statistical rectangles of 1° longitude × 0.5° latitude (30 × 30 nautical miles). Each rectangle is usually fished by ships of two different countries (two hauls per rectangle) or a single country fishing more than once in every rectangle (e.g. in the Skagerrak and the Kattegat, Sweden). In the Baltic Sea, the station allocation is different and stratified by depth intervals, and only one country covers each area. All countries have a standard haul duration of 30 minutes (defined as starting at the moment when the vertical net opening and door spread are stable), using the same 36/47 GOV demersal trawl in the North Sea (ICES/IBTS, 2017), BACA nets in the Bay of Biscay and on the Iberian coast, and TV-3 (TVS and TVL) bottom trawl in the Baltic sea (ICES/BITS, 2017), sampling at 3.5-4 knots (2.3-2.73 knots in the BITS) between 20 m and 200 m depth with 20 mm mesh nets (3 knots in the IBTS between 15 m and 800 m).

5.5.1.2 Mediterranean Sea

Litter data collection using trawl surveys started in the 2010s in the Mediterranean Sea (within the MEDITS programme; Spedicato et al., 2019). The protocol is derived from the MEDITS survey (see the protocol manual (MEDITS working group, 2017)). It is also a reference for associated countries, including Bulgaria and Romania in the Black Sea. The hauls are positioned following a depth-stratified sampling scheme with a random drawing of the positions within each stratum. The number of hauls in each stratum is proportional to the surface of these strata, and the hauls should be made in the same position from year to year. The depth strata (10–50 m, 50–100 m, 100–200 m, 200–500 m and 500–800 m) are fixed in all areas. The total number of hauls for the Mediterranean Sea is approximately 1300 every year, covering the shelves and slopes from 10 countries in the Mediterranean (MEDITS working group, 2017). The haul duration (defined as starting at the moment when the vertical net opening and door spread are stable) is fixed at 30 minutes at depths of less than 200 m and 60 minutes at depths of over 200 m, using the GOC73 trawl with a mesh size of the cod end of 10 mm of mesh side, which corresponds to about 20 mm of mesh opening and sampling between May and July, at 3 knots (MEDITS working group, 2017). The length of the mesh side is defined by the International Organization for Standardization as ‘the distance between two sequential knots or joints, measured from centre to centre when the yarn between those points is fully extended’ (ISO 1107:2017 (2022)).

5.6 Image-based surveys

Seafloor image-based surveys are increasingly used to study the abundance and distribution of litter on the seafloor and its interactions with marine organisms (Canals et al., 2021). The most commonly used platforms for image acquisition of marine litter are submersibles, ROVs, AUVs and TUCs. Visual surveys can be performed at all depths and on all sea bottoms, including those not accessible to bottom trawls, such as steep slopes, rocky bottoms, and ultra-deep areas, down to the oceanic trenches (e.g. Galgani and Lecornu, 2004; Bergmann and Klages, 2012; Miyake et al., 2011; Ioakeimidis et al., 2015; Tekman et al., 2017; Chiba et al. 2018), and allow the precise geo-referencing of each litter item. In addition, these methods enable small-scale observations, which are essential for identifying litter–biota interactions and accumulation areas. Furthermore, image-based systems are harmless to organisms. Two disadvantages of the image-based approaches to seafloor litter quantification are that the minimum size of litter that can be identified depends on the resolution achievable by the cameras and that items covered by sediment or entrapped within seagrass, coral reefs or fine-scale rocky structures cannot be detected. The ability to physically sample and bring litter items onboard for examination is also severely limited. As a result, seafloor-imaging surveys may easily underestimate litter items, and some objects (e.g. fragments) could barely be identified by conventional imagery.

There are some available protocols where litter is counted on routes and expressed as items/km², especially when using TUCs, ROVs, and submersibles at variable depths above the deep seafloor (Galgani et al., 1996). These technologies enable the evaluation of the densities through standardised approaches (transect lengths and widths, sampling units, etc.). Considering the improvement in visual sampling, there is a need to develop a data management approach for data acquired through direct observations, such as on the shallow seafloor, or through imagery techniques. This requires identifying essential metadata sets and agreeing on common reporting formats. This area is underdeveloped and will be considered within the EMODnet data management system for the next MSFD cycle.

5.6.1 Shallow seafloor

In some circumstances, diving may be unsuitable, difficult or impossible because of inadequate conditions, such as heavy boat traffic and cold water temperatures, because the legal requirements for diving are very strict, because the costs are high or because there is a lack of diving personnel with the proper scientific/technical requirements. Imaging-based approaches to seafloor monitoring (e.g. ROVs or TUCs) may also be employed for shallow surveys. The shallow depth can allow the use of simple equipment and thus reduce monitoring costs. Recording videos during the monitoring enables data analysis using appropriate software, thus improving data collection.

Towed low-cost camera set-ups (Fakiris et al., 2022) or sledges (Lundqvist, 2013) can be also employed for shallow seafloor macro litter monitoring.

The types of litter should then be recorded using the categories defined by the MSFD Joint List (Fleet et al., 2021).

5.6.2 Deep seafloor

The deep sea includes waters and sediment below depths of approximately 200 m (Danovaro et al., 2010). Only some areas/countries (e.g. France, Greece, Ireland, Italy, Norway, Spain and Portugal) are concerned with the deep seafloor along the European coasts, including submarine canyons, seamounts, cold seeps, open slopes and deep basins. Sampling difficulties and costs largely restrict monitoring in those deep-sea areas. Litter that reaches the seabed may already have been transported for considerable distances, only sinking when weighed down, for example, by fouling. The consequence is an accumulation in canyons that tunnel litter, often around large cities, rather than in the open sea (Chiba et al., 2018). These high litter densities result from residual ocean circulation patterns and, more locally, from the morphology of the seabed (around rocks and/or in depressions or channels) and the deep submarine extensions of coastal rivers (Pierdomenico et al., 2019). Specific equipment is necessary for slopes and rocky bottoms, including ROVs, AUVs and/or submersibles, which may be very expensive to operate, especially in deep-sea areas. Data collection is usually performed irregularly, using protocols based on existing seafloor monitoring and research activities to study seafloor litter and benthic biodiversity through opportunistic approaches (e.g. Enrichetti et al., 2020, Canals et al., 2021). ROVs, which are less complicated than submersibles and generally cheaper, are recommended for litter surveys of the deep seafloor.

For the monitoring, video transects should be linear and at least 200 m long. If there is a deviation from the initial track, it is essential not to count the same items several times. Three video transects for each area surveyed are recommended. The start of the dive is defined as the moment at which the ROV (or other cameras/vehicles) dives into the seawater. The end of the dive is defined as when ROV is at the surface / on the deck. The start of the transect is defined as the moment at which the ROV is at the bottom, and the end of the transect is when the ROV leaves the bottom (i.e. is off the bottom). The area surveyed is calculated by multiplying the transect length by the visual field (width) of the video. When possible, two laser beams can be used to measure the size of objects and distances on the seafloor. Altimeters are necessary to evaluate the altitude, and then, depending on the focus of the camera, the surface of the area sampled during surveys can be estimated (GESAMP, 2019). ROVs (or other cameras/vehicles) should be in continuous recording mode at a constant slow speed (e.g. 0.5-2 knots) and a constant height from the bottom (e.g. < 1.5 m). Each object observed along the transect (within the constant field of view of the camera) has to be recorded and counted to obtain information about litter abundance and occurrence. Each item has to be classified based on the type of object, according to the list of main categories provided in the MSFD Joint list (Fleet et al., 2021). Data must be annotated in a data sheet, which should be completed for each dive. Results are usually expressed as items/km^2 when the explored surface measurement is possible, and items/km (or items/100 m) when the surface cannot be measured. Site information should be recorded, such as location, date, time, geographical coordinates, depth, substrate types, speed, distance from the coast and other relevant observations.

Adopting a common protocol will lead to a significant standardisation level among the countries that apply it as their sampling strategy. Usually, transect routes are strategically distributed to delineate surveys along canyon heads, floors and flanks at various depths to obtain a visual picture of the overall distribution. Images are also referenced on navigation logs, providing the time of observation, water depth and geographical position along a given transect route. Single frames may be extracted from video records for further analysis and identification (GESAMP, 2019).

Given that surveys might be performed by ROV classes with different equipment, or other more sophisticated instruments, it is very important to record any extra equipment and the characteristics of the instrumentation to harmonise these among the teams performing surveys. Technological instruments should provide controlled sampling, precise data on geographical position and depth, high-definition video or photos, and reference points (e.g. laser beams) to use as a metric scale for measuring the width of the visual field. The video survey should allow the recording of the precise position of litter items.

5.7 Data recording and management

It is necessary to combine different methodologies according to the requirements of the various seafloor types and depths, and approaches designed to ensure comparable assessments. For trawling surveys, templates for data recording sheets based on this system have been integrated into the ICES ([19]), MEDITS ([20]) and SOLEMON ([21]) manuals. Data on litter should be collected using these templates and the items categories listed for the seafloor. Site information and trawl sampling characteristics such as the date, the position and type of trawl, the speed, the distance, the sampled area, the depth and the hydrographical and meteorological conditions, should be recorded. Data sheets should be filled out for each trawl and compiled by survey. If multiple counts (transects/observers) are run at any given site, then a new sheet should be used for each trawl shot. After each survey, data must be processed for analysis and reporting. Furthermore, for litter items, the weight, a picture and a note of attached organisms may further complement the classification of objects.

Monitoring litter on continental margins must be co-organised and coordinated within two groups: (i) the ICES / the IBTS (north-east Atlantic Ocean and Baltic Sea) and (ii) MEDITS (Mediterranean Sea). The inclusion of litter monitoring through the IBTS/MEDITS programmes has been organised within the EU through the Scientific, Technical and Economic Committee for Fisheries (STECF) and its Subgroup on Research Needs (SGRN), with the support of the Data Collection Framework (DCF) from the Directorate-General for Maritime Affairs and Fisheries. Litter data management has been organised at the regional institution level through the OSPAR Commission (ICES Database of Trawl Surveys (DATRAS)), HELCOM (ICES-DATRAS) and MEDITS data management systems.

EMODnet Chemistry, a pan-European network of organisations supported by the EU, developed a central database for seafloor litter in 2017. A common infrastructure for managing all the data is well adapted to address protocols and reporting heterogeneity. It was modelled following the ICES-DATRAS approach and considering the European TG ML and MEDITS requirements. Using a single data model and common data formats based on consolidated monitoring initiatives has allowed the collation of multiple data streams in a uniform and standardised way. It will also the creation of a basic dataset from which to compute seafloor baselines.

Furthermore, the EMODnet visualisation products will provide an easy tool to display available litter data and allow partial comparison of homogenised European data. They are a straightforward way to promote access to interesting data for a wide variety of stakeholders. For example, data from the north-east Atlantic Ocean and the Baltic Sea are directly collected by EMODnet from the OSPAR/ICES-DATRAS database. The temporal coverage of the seafloor litter collection is from 2006 to 2018, with a total of 3 600 seafloor surveys. Up to now, this can be considered the most comprehensive collection of homogenised data available at the pan-

([19]) https://ices-library.figshare.com/articles/report/ICES_Manual_for_Seafloor_Litter_Data_Collection_and_Reporting_from_Demersal_Trawl_Samples/21435771
([20]) https://archimer.ifremer.fr/doc/00832/94436/
([21]) https://podaci.ribarstvo.hr/files/SOLEMON-Handbook_2019_Ver_4.pdf

European level (Molina Jack et al., 2019). EMODnet's visualisation products provide an accessible way to display the available data and allow their partial comparison. The products provide information such as total abundances, litter composition or abundances of relevant litter types. Marine litter formats and instructions for data gathering, raw litter data, and aggregated collections and visualisation products are accessible through the EMODnet Chemistry web portal (http://www.emodnet-chemistry.eu/) (Galgani et al., 2017, 2022). Aggregated marine data collections and the visualisation products are in the public domain and freely available for all users with acknowledgement of the source (https://www.emodnet-chemistry.eu/marinelitter). The methodology used in the generation of visualisation products is described by Le Moigne et al. (2019).

5.8 Seafloor litter categories

Because marine litter degradation is increased by light, oxygen and wave action, the persistence of marine litter is increased on the seafloor and deep seafloor, generating variable outcomes for the nature of litter found. Moreover, the gear types and their ground contact used in different areas may affect the types of litter caught, making a comparison between areas and surveys more complex. Another important factor influencing the composition of benthic litter is related to the source of litter. Typically, the analysis of sources must indicate the importance of and differences between sea- and land-based litter, but also the differences between different activities or sectors. Although marine litter is strongly affected by processes affecting transport, fishing has been shown as a main source of litter in some fishing or aquaculture grounds (e.g. Fortibuoni et al., 2019). Similarly, specific types of marine litter were also found in areas affected by tourism, for example around beaches, such as those in the Mediterranean Sea (Stagličić et al., 2021). This may affect the selection of monitoring sites, such as shallow waters.

A standardised litter classification system has been defined and adopted by Member States for use in MSFD implementation (i.e. the MSFD Joint List; Fleet et al., 2021) in accordance with the types of litter found at the regional level, providing common main categories for all regions. This list allows for assessments with comparable categories across marine environmental compartments. The list main categories provide the basis for a hierarchical system, including subcategories. It considers nine main categories of material (artificial polymer materials, chemicals, cloth/textiles, metals, rubber, glass/ceramics, processed/worked wood, paper/cardboard and organic food waste) and 183 subcategories.

5.9 Interactions with criterion C4 of Descriptor 10

As litter is widely distributed on the seafloor and interacts with / affects marine biota in different ways, through entanglement, providing new substrates, or covering/smothering marine fauna (e.g. Anastasopoulou and Fortibuoni, 2019), some of these interactions can be easily measured through seafloor visual surveys (e.g. by diving or using ROVs). The imagery technology provides a well-adapted platform for documenting the entanglement of marine fauna, especially on seafloor areas dominated by sessile suspension feeders, structuring coralligenous assemblages, mesophotic and deep-sea sponge and coral aggregations, termed 'animal forests' (Galgani et al., 2018). Because of their branching and massive morphology, these habitat-forming species have strong potential for monitoring temporal and spatial trends of entanglement events caused by marine litter, especially by abandoned, lost or otherwise discarded fishing gear (ALDFG) (Galgani et al., 2018; Angiolillo and Fortibuoni, 2020).

Considering the importance of this type of data on litter for a better understanding of interactions with marine organisms, it is necessary to perform litter surveys using videos (deep waters) and by diving (shallow waters) that simultaneously allow the assessment of MSFD primary (D10C1) and secondary (D10C4) criteria.

No specific database exists for this approach, but future work, within the next MSFD cycle, will need to consider merging databases on seafloor images/pictures and interactions to facilitate the evaluations of interactions between litter and organisms, especially in fishing grounds where ALDFG are most often present and abundant.

5.10 Quality assurance / quality control

While assuring the quality of data employed for assessments in a policy context is important, the implementation of practical QA/QC measures for seafloor litter monitoring is challenging. The use of agreed and harmonised monitoring protocols and the provision of relevant agreed metadata sets are crucial.

Several contracting parties from OSPAR and the programme for the assessment and control of marine pollution in the Mediterranean (MEDPOL) use their fish stock surveys for seafloor litter monitoring, an approach that is already adopted as a common indicator in the OSPAR, Barcelona Convention and HELCOM regional plans. Adopting a common protocol will lead to significant standardisation among the countries that apply it as their sampling strategy. This is considered an adequate approach, although litter quantities are probably underestimated when using GOV fishing nets due to their limited bottom contact and given the restrictions in rocky or hard bottom areas. How to compare the data collected during trawl surveys with data gathered using different methodologies remains an open question. A conversion factor between beam trawling and GOV trawling would help compare data from different surveys.

Data recording and management should be undertaken through an online, relational database system under the quality control and direction of skilled managers. Regional/country coordinators should review and approve the uploaded data. This would ensure a high level of consistency within each region and create a hierarchy of quality assurance on data acquisition. For the IBTS, the BITS and MEDITS, sampling data are collected in the DATRAS and MEDITS databases together with data on hydrographical and environmental conditions. This process may also support quality insurance for data on litter. The ICES is considering data for OSPAR and HELCOM areas, while MEDITS has included litter data to be analysed within a specific subgroup of experts. The occurrence of WISE/EMODnet with modules dedicated to MSFD indicators may also be considered to develop a specific module for criteria from Descriptor 10, including litter on the seafloor.

6 Mesolitter fragments and pellets on the coastline

6.1 Introduction

Within the MSFD and Commission Decision (EU) 2017/848, the following two criteria target marine litter on the coastline.

- Criterion D10C1. 'The composition, amount and spatial distribution of litter on the coastline, in the surface layer of the water column, and on the seabed, are at levels that do not cause harm to the coastal and marine environment'. The size distribution of marine litter in the marine environments is continuous, where mesolitter items are between 5 mm and 25 mm and should be included under criterion D10C1. Note that identifiable mesolitter, such as bottle caps and cigarette butts, are considered in the beach macro litter monitoring. However, a specific protocol allowing fit-for-purpose monitoring of mesolitter fragments is required because these are known to be monitored unreliably, resulting in poorly comparable data (Hanke et al., 2019).
- Criterion D10C2. 'The composition, amount and spatial distribution of microlitter on the coastline, in the surface layer of the water column, and in seabed sediment, are at levels that do not cause harm to the coastal and marine environment. Member States shall establish threshold values for these levels through cooperation at Union level, taking into account regional or subregional specificities'. This criterion refers to items smaller than 5 mm in their longest dimension, including plastic items called microplastics. To date, there are no guidelines for monitoring and no threshold value set for the assessment of microlitter, including plastic pellets, on EU beaches. The objective of this guidance is to propose a harmonised EU method of monitoring mesolitter fragments and plastic pellets on the European coastline.

6.1.1 Scope

The aim of this guidance is to present a harmonised EU methodology for monitoring mesolitter fragments and plastic pellets along the European coastline.

6.1.1.1 Mesoplastic fragments (5 – 25 mm)

Mesoplastic fragments, considered a predominant fraction of mesolitter, are secondary products, that is, they originate from plastic objects that experienced fragmentation during their use or their journey as litter (e.g. abrasion, shocks, photodegradation). They can be divided into several categories according to their morphologies observed in the environment and based on the classification proposed by the EMODnet vocabulary for microlitter (Vinci et al., 2021): (A) fragments, (B) filaments, (C) films, (D and E) foams including foamed polystyrenes, and (F) Others (Figure 6.1; Vinci et al., 2021).

6.1.1.2 Plastic pellets

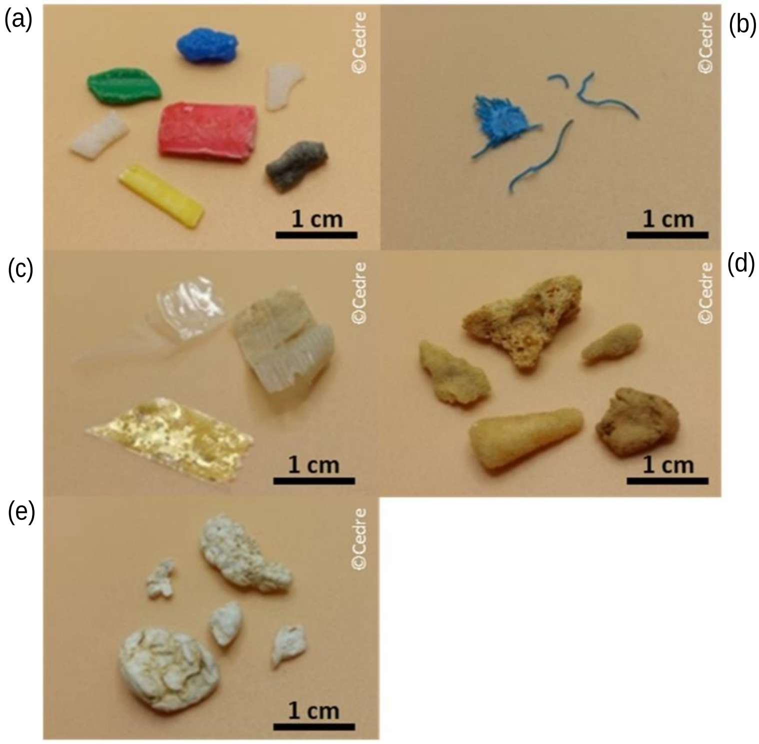
Among the diversity of microplastics found in European waters and on the coastline, plastic pellets (also called plastic nurdles) appear to be of importance. Plastic pellets are the raw material used by the industry to produce a large majority of plastic products. A pellet is defined as a 'small mass of preformed moulding material, having relatively uniform dimensions in a given lot, often used as feedstock in moulding and extrusion operations' (International Organization for Standardization (ISO) 472:2013) ([22]). Plastic pellets constitute one major category of microplastics found in the environment. In the EU, estimations of annual operational and incidental losses in the environment are between 16 000 and 167 000 tonnes (OSPAR Commission, 2018). Plastic pellets are ubiquitous in aquatic environments. They are found on beaches, at the sea surface, in seabirds and in seafloor sediments (Turner and Holmes, 2011; Lechner et al., 2014; Mani et al., 2016; Moreira et al., 2016; Karlsson et al., 2018; Corcoran et al., 2020; van Franeker et al., 2021; Hunter et al., 2022; Cedre, 2023).

Most plastic pellets are between 2 mm and 5 mm, and they come in a range of colours (Figure 6.2). These size classes and morphological properties are advantageous in the context of monitoring, as it is relatively easy to collect samples and analyse them in order to identify plastic pellets and provide an assessment of

([22]) https://www.iso.org/obp/ui/#iso:std:iso:472:ed-4:v1:en this pollution. Some specific forms of pellets are bio-beads and flakes. Bio-beads are usually rough black pellets, which are used in waste-water treatment plants (Turner et al., 2019). Flakes are shredded plastics, which are used as a basic material for plastic production (OSPAR Commission, 2018). They are not considered in the current protocol as it is not possible to distinguish them from other plastic fragments issued from litter fragmentation.

Plastic pellets are targeted by several EU measures and regional action plans (e.g. action C.1.1 of the second OSPAR regional action plan, which covers 2022-2030, and action RS5 of the HELCOM regional action plan on marine litter (HELCOM, 2021)). Currently, plastic pellets are monitored in several EU countries (e.g. Germany, France, the Netherlands) and by various global citizen science programmes (e.g. nurdle patrol, international pellet watch) using different protocols, making the comparison of collected data difficult and highlighting the need for a harmonised protocol to monitor the presence of plastic pellets along the European coastline.

Figure 6.1. Different morphologies of mesoplastic fragments: (a) fragments, (b) filaments, (c) films, (d) foams and (e) foam polystyrene fragments



Source: Centre of Documentation, Research and Experimentation on Accidental Water Pollution (Cedre).

Figure 6.2. Examples of plastic pellets found on the European coastline.

Source: Centre of Documentation, Research and Experimentation on Accidental Water Pollution (Cedre).

6.2 Strategy for mesoplastic fragments and pellet monitoring on the coastline

6.2.1 Locations

The proposed protocol is adapted for sandy beaches.

Overall, the surveys can be performed on the sandy sites used for the macro litter monitoring programme, including urban, semi-urban and natural beaches, and beaches exposed to different pressures and sources of litter (e.g. harbour, touristic activities, river outlets, pellet transporters or other pellet handler companies). The use of the same site allows comparisons between macro litter and pellets/mesoplastics results and logistical and cost mutualisation for operators.

To perform the surveys, the selected coastal sites should meet the following criteria:

- sandy beach with fine sand (a grain size below 1 mm);
- a minimum length of 100 m;
- low to moderate slope;
- accessible to survey teams year-round (with some exceptions, see Section 6.2.2);
- clear access to the sea (not blocked by natural or artificial structures such as breakwaters or jetties);
- not an accumulation site (also called a hotspot).

6.2.2 Frequency, cycle and timing

The goal of the beach pellet / mesoplastic fragment monitoring is to assess abundance, distribution and trends. Therefore, surveys should be performed several times a year and always within the same periods every year. It is proposed that the beach pellet / mesoplastic surveys should be aligned with the frequency used for the beach macro litter programme, with four equidistant surveys per year. Therefore, it is recommended to conduct four surveys per year with one survey per season, as follows:

- winter – January–March,
- spring – April–June,
- summer – July–September,

- autumn – October–December.

In specific cases, the number of surveys can be reduced similarly to the reduction in the beach macro litter monitoring. In northern Europe and Arctic sites, the autumn and winter samplings may not be possible due to weather conditions (presence of snow and ice on the sites). In some touristic areas, the summer sampling may not be possible due to site trampling and clean-up activities. Sampling may also not be possible in cases of substantial strandings of algae or seagrass. On macrotidal beaches with relatively low concentrations of pellets and mesoplastic fragments, it is recommended that sampling be undertaken 1 or 2 days after springtides (e.g. in the Atlantic region – Spain, southern France and Portugal – spring tides are 0 or 1 day after the full/new moon, and in the North Sea region spring tides are 2 days after the full/new moon), as springtides give the most recent and concentrated accumulation of pellets and mesoplastics (Wenneker et al., 2022).

It is also recommended to conduct the sampling during dry weather and avoid days with heavy rain, as rain may disturb the sand surface, the pollution distribution and the ability to visually detect the pellets and mesolitter fragments.

6.3 Sampling protocol

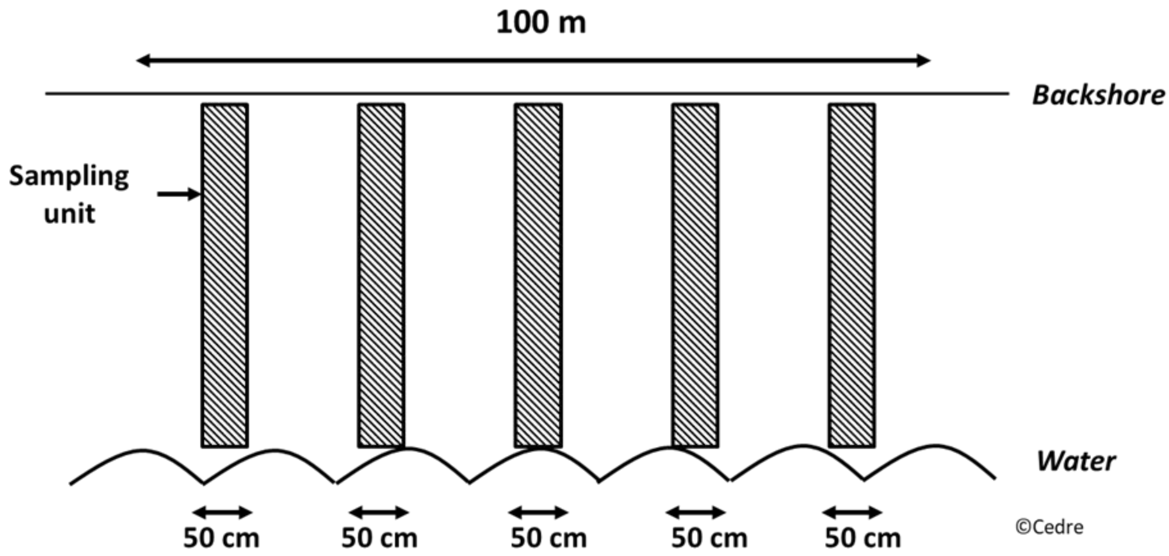
6.3.1 Sampling strategies

Two sampling strategies are proposed to integrate the constraints of both macrotidal and microtidal environments that exist on the European coastline. Both strategies have a sampling unit of five transects that are 50 cm wide, perpendicular to the waterline and are evenly distributed within a 100 m beach section (location 1, 10 m; location 2, 30 m; location 3, 50 m; location 4, 70 m; location 5, 90 m; Figure 6.3). This method allows a harmonisation with the beach litter monitoring programme and the possibility of comparing data between mesoplastic fragments / pellets and macro litter. It should be noted that the method uses transects (and not quadrats) to embrace the heterogeneous distribution of mesoplastic fragments and pellets on the beaches.

For each sampling, the same beach section (fixed GPS position) is used to collect mesoplastic fragments and pellets. In cases of fewer than five pellets or fewer than five mesoplastic fragments per 2.5 m (equivalent to less than 200 particles per 100 m), extending the transect width to 1 m is recommended in order to obtain more robust data.

It is important that the study area not be trampled prior to the sampling, as trampling can lead to particle burial in the sediment. Therefore, if the same beach section is used for this protocol and the macro litter monitoring programme, it is recommended that the protocol for pellets and mesoplastic fragments be conducted first.

Figure 6.3. Schematic depiction of the spatial sampling procedure.



Source: Centre of Documentation, Research and Experimentation on Accidental Water Pollution (Cedre).

6.3.1.1 Sampling strategy A – macrotidal environments

- The five transects are marked out on the beach (a specific custom-made tool can be used).
- In the five transects, the sampling is performed by scraping the top layer of sand (1 cm) using a flat trowel (Figure 6.4). All visible tidelines within the transects are sampled. They can be rinsed and shaken above the sampling area prior to the sampling, to release pellets/mesoplastics embedded inside them. To lighten the samples, pellets/mesoplastics are sieved on the beach with a metal sieve (1 mm) or a sieving bag (e.g. a vegetable bag with pore sizes of approximately 1 mm) (Figure 6.4). The advantages of sieving are the recovery of all pellets and mesoplastics, whatever their density, and the easy removal of the sand in the field.
- If pellets are observed within the five transects on the sand between these tidelines and on the backshore, these spots are also sampled.
- In the case of large bunches of seaweed occurring in the sampling section, seaweed can be shaken/cleaned to collect the pellets and mesoplastic fragments using a flat trowel.
- This sampling strategy can usually be applied in Atlantic areas with clear tidal action.

Figure 6.4. Photos of sampling using sampling strategy A and examples of tools that can be used for the sieving.

Sources: Photo credits: (a, b and c) Centre of Documentation, Research and Experimentation on Accidental Water Pollution (Cedre), (d) Wenneker et al. (2022) and (e) IOW.

6.3.1.2 Sampling strategy B – microtidal environments

- This sampling strategy applies to marine regions with low tidal action, such as the Baltic Sea. In these areas, a sand rake can be used to perform the sampling (e.g. Haseler et al., 2020; Figure 6.5). If it is not possible to use the sand rake, the method described above (sampling strategy A) can be used.
- In the five transects, the sand rake is used to collect the pellets by scraping the top layer (1 cm) of sand from the waterline to the back of the beach.
- A minimum sampling area of 25 m^2 is required (minimum of 5 m^2 per transect).

Figure 6.5. Photo of a sand rake that can be used in sampling strategy B.

Source: adapted from Haseler et al. (2020).

6.3.2 Samples storage

After the sampling, the samples from the five transects can be combined and stored in freezer bags or sieving bags at room temperature.

6.4 Lab protocol

6.4.1 Separation and identification

- Before the separation, samples are dried for 24 hours at 40 °C using an oven, or they can be air-dried over a sufficiently long period.
- Three analytical sieves (1 mm, 5 mm and 25 mm) are used to separate the pellets, mesoplastic and potential macro litter fractions. Natural debris and other litter types not considered in the monitoring (e.g. macroplastics, other microplastics) are removed at this step by visual sorting (Figure 6.6). After sieving, pellets / mesoplastic fragments are collected visually, and the remaining sample materials are put in a bucket of water in order to recover potentially overlooked pellets and mesoplastic fragments. Pellets and mesoplastics are then stored separately in labelled glass jars.
- The pellet fraction is weighed using a weighing device with an accuracy of 0.1 mg, and pellets are individually counted.
- The mesoplastic fraction is weighed. Mesoplastic fragments are then sorted and counted according to the six categories described in Section 6.1.1.1.

It is important that, if macro litter are accidentally collected during the sampling, they are added to the data from the beach macro litter monitoring programme if the two samplings are conducted on exactly the same beach section and on the same day. This is also valid for identifiable mesolitter included in the macro litter monitoring programme, such as cigarette butts and bottle caps. In any other case, these items are eliminated from the counting and the analysis.

The colour of pellets and mesoplastic fragments can also be assessed, in addition to the weight and the number, using the categories proposed for microlitter (see Section 7.4.4): (i) black, (ii) grey, (iii) white (including creams), (iv) red, (v) orange, (vi) yellow, (vii) green, (viii) blue (including cyan), (ix) purple (including violets), (x) pink (including magentas), (xi) brown (including tans), (xii) multicolour and (xiii) colourless.

The transparency of pellets and mesoplastics fragments may optionally be reported using the categories proposed for microlitter (see Section 7.4.5): (i) opaque and (ii) transparent/translucent.

Figure 6.6. (a) Separation of pellets and mesoplastics by sieving and (b) separate storage of fractions.

(a) (b)

Source: Centre of Documentation, Research and Experimentation on Accidental Water Pollution (Cedre).

6.4.2 Quality assurance / quality control

Spectroscopy analysis of 10 randomly selected pellets and 10 randomly selected mesoplastics from each sampling is performed to (i) confirm the plastic nature of collected particles and (ii) produce some additional polymer information. Different methods are available to perform these analyses including near-infrared spectroscopy (NIR), Raman spectroscopy and Fourier transform infrared (FTIR) spectroscopy.

6.4.3 Data extrapolation

This protocol allows the extrapolation of the collected data so that it can be aligned with the data from macro litter beach surveys, that is, the number and mass of items per 100 m. The value is obtained using the formula below:

$$N = n \times f$$

where *N* is the number or mass of items per 100 m of beach, *n* is the number or mass of items collected and *f* is the conversion factor used to extrapolate the results (see Table 6.1; e.g. if the five transects are each 50 cm wide and therefore have a total width of 2.5 m, the conversion factor is 40).

For each sampling, the mass of pellets and each type of mesoplastic are measured to provide additional information, that is, the mass of pellets or mesoplastics per 2.5 m.

Table 6.1. Examples of conversion factor used to extrapolate the results.

Number of transects	Width of transects (m)	Conversion factor to estimate the contamination for 100 m
5	0.5 (total sampled width = 2.5 m)	40 (100 m ÷ 2.5 cm)
5	1 (total sampled width = 5 m)	20 (100 m ÷ 5 m)

6.5 Data reporting

Tables 6.2 and 6.3 list the metadata and data reported for each survey.

Table 6.2. Data and metadata requested for each mesoplastic fragments and pellet survey.

Requested data
Beach code
Date
Start/end coordinates
Sampling tool
Sampling strategy (macrotidal/mesotidal or microtidal)
Sampled lengths (sum of the five transect widths)
Infrared results obtained on the 10 randomly selected pellets and 10 mesoplastics fragments
Total number of pellets calculated for 100 m (NPEL ([a]))
Total weight of pellets calculated for 100 m (WPEL ([a]))
Total number of mesoplastic fragments calculated for 100 m (NMESO ([a]))
Total weight of mesoplastic fragments calculated for 100 m (WMESO ([a]))
Total number of the different categories of mesoplastic fragments (see Section 6.1 for the description of the different categories) calculated for 100 m (NMESO_Frag ([a]), NMESO_Fil ([a]), NMESO_Film ([a]), NMESO_Foa ([a]), NMESO_Styr ([a]))
Total weight of the different categories of mesoplastic fragments (see part 6.1 for the description of the different categories) calculated for 100 m (WMESO_Frag ([a]), WMESO_Fil ([a]), WMESO_Film ([a]), WMESO_Foa ([a]), WMESO_Styr ([a]))

([a]) Variable names used by the EMODnet platform.

Table 6.3. Optional data for each mesoplastic fragments and pellet survey.

Optional data
Degree of urbanisation
Beach usage
Frequency of cleaning
Beach slope
Colour of the pellets samples from the five transects

Optional data
Colour of the mesoplastic fragments samples from the five transects

6.6 Level of maturity and cost-effort estimate

6.6.1 Level of maturity

This method has a low level of maturity, as the protocol has only been tested in a limited number of pilot studies.

6.6.1 Cost–effort assessment

The cost of monitoring is low/medium, as it does not require a high level of expertise or high-cost equipment.

The effort is medium as the time to perform the sampling is generally between 1 hour and 4 hours and the lab analysis of one sample takes between 1 hour and 5 hours, with one or two dedicated people.

7 Microlitter

7.1 Introduction

Marine microlitter is defined as a size subcategory of Marine Litter, which is, according to UNEP, 'any persistent, manufactured or processed solid material discarded, disposed of or abandoned in the marine and coastal environment' (UNEP, 2021). Microlitter is the size subcategory considered marine litter with a length of its maximum dimension below 5 mm. This chapter is focused on criterion MSFD D10C2 which states, 'The composition, amount and spatial distribution of microlitter on the coastline, in the surface layer of the water column, and in seabed sediment, are at levels that do not cause harm to the coastal and marine environment' according to Commission Decision (EU) 2017/848.

Microlitter monitoring programmes and assessments within the MSFD (Article 8) have mainly focused on the surface layer of the water column during the last 6-year reporting cycle (2012-2018) (Ruiz-Orejón et al., 2021; Tornero et al., 2023). Improvements in sampling and analytical methods for marine microlitter have developed intensively since the previous version of the MSFD guidance (Galgani et al., 2013), with significant advances for different environmental matrices. However, there are still discrepancies in the consideration of the properties of microlitter (e.g. Hartmann et al., 2019; Koelmans et al., 2022; Primpke et al., 2022), and sampling and analytical methods are not yet sufficiently harmonised.

Marine microlitter consists of small pieces of litter of common material types including plastic, metal, glass and paper. Microplastics are usually the most frequent component of microlitter in the environment; however, the proportions and distribution of different types of material will be influenced by the sources and physical conditions of the area sampled. The sampling protocols proposed in this chapter focus mainly on microplastics and other materials that cannot be considered plastic according to the classic definition, but materials other than plastics can be found in the environment and can be sampled with the methods proposed in this chapter.

Harmonised microlitter monitoring methods are needed to generate comparable data that can be used to define baselines, analyse trends and develop threshold values in the future. Microlitter monitoring should also be functional, allowing the identification of geographical sources, potential accumulation areas and pathways, the enabling the assessment of trends and changes due to mitigation measures, and providing information that allows the risks facing selected marine species to be evaluated (de Ruijter et al., 2020).

7.2 Scope and key questions

The aim of this chapter is to provide guidance to Members States for the monitoring of microlitter in different marine compartments. According to criterion D10C2 under Commission Decision (EU) 2017/848 'microlitter shall be monitored in the surface layer of the water column and in the seabed sediment and may additionally be monitored on the coastline. Microlitter shall be monitored in a manner that can be related to point-sources for inputs (e.g. harbours, marinas, waste-water treatment plans, storm water effluents), where feasible.'

Microlitter represents an area of scientific research where several approaches have been developed in parallel. The MSFD TG ML has evaluated the existing approaches for monitoring microlitter and recommends harmonised methods to maximise the comparability and consistency of monitoring data among regions. In this chapter, recommendations for monitoring marine litter are provided based on the outcomes from research projects, national efforts and discussions within the TG ML, including established or draft guidelines from the RSCs (e.g. AMAP 2021; HELCOM, 2022a, 2022b; OSPAR, 2023; UNEP/MED, 2021).

7.3 Monitoring strategy for microlitter

7.3.1 Monitoring locations

Monitoring programmes should be consistent, coherent and comparable within marine regions and surveys. Given the high heterogeneity of the litter and microlitter distribution, the criteria for the survey site selection could have crucial effect on results (UNEP, 2016). A monitoring strategy for marine microlitter should consider sampling locations based on factors such as proximity to potential sources of litter and microlitter, including the contribution of riverine litter inputs, flow and/or sediment deposition rates.

The distribution of monitoring locations should represent the variation within sub-basins and should, where possible, integrate stations for the measurement of the state of the marine environment, the achievement of environmental targets and the effectiveness of measures (European Commission, 2020; Commission Decision

(EU) 2017/848). It is suggested that seafloor sediment sampling should preferentially include stations with known sediment deposition rates. For floating marine microlitter, a wide network of monitoring locations that considers offshore and coastal waters is recommended. The final number of monitoring locations and stations surveyed by each Member State depends on the heterogeneity across stations and areas, and how many subregions each Member State encompasses.

The location of sampling areas and stations for microlitter sampling should be based on data needs within a coordinated strategy. If such stations appear to be compatible with sampling for other purposes (e.g. sediment sampling for contaminant monitoring), then this provides opportunities for cost-efficient approaches and enables the use of readily available metadata.

7.3.2 Frequency and timing of monitoring

The frequency of monitoring for microlitter in the different environmental compartments is still under discussion. It is suggested that monitoring frequency should be determined on the basis of further analysis of for example, sampling methods, variance in microlitter concentrations and local conditions. It is also proposed that different frequencies be considered in the case of parallel investigations (e.g. target and measure monitoring versus state monitoring stations).

Sampling frequency should be based on expected variabilities and on the number of data points needed to derive trends. Monitoring of the surface layer of the water column may be especially subject to variability in the measured amounts and distributions of litter, including microlitter. This is linked to seasonal variations in oceanographic and anthropogenic factors (e.g. Wang et al., 2020; Carretero et al., 2022). The organisation of monitoring must be flexible enough to take the variability of environmental conditions into account.

7.4 Microlitter parameters

The recommended monitoring methodologies in this chapter provide information about artificial polymer particles (microplastic), number-based counts, size, morphology, colour, transparency and polymer type. This information is expected to be crucial for source attribution purposes and for relating the microlitter to possible ecotoxicological effects (de Ruijter et al., 2020).

7.4.1 Count

There are different methods for counting microlitter items, with the most commonly used being based on optical detection with microscopy or instrumental spectroscopic analysis, where the resource effort increases with decreasing particle size (e.g. Sridhar et al., 2022). Most monitoring and analytical methods, in particular automated methodologies, enable the reporting of single-particle data. This is recommended, as it enables thorough data analysis across different parameters.

Analytical methods, such as pyrolysis gas chromatography mass spectrometry techniques, which provide only mass-based, targeted polymer concentrations in a given sample amount, are not recommended for microlitter monitoring, because they do not enable the analysis of key physical particle properties or the determination of particle number. These measurements alone do not provide sufficient data and parameters for MSFD D10C2 assessments; they can be used when they are complementary to number-based monitoring approaches, but cannot substitute them.

The reporting of dry weight (sediment and sand samples) or the amount of microlitter items per trawled area (samples from the surface water layer) of the samples is mandatory for calculating microlitter concentrations as particle count per kg of dry weight or water area (m^2), according to Commission Decision (EU) 2017/848. Reporting the amount of microlitter items per volume (m^3) is also additionally recommended for samples from the surface water layer.

At this stage, no recommendation for estimating mass data from particle data is given. The development of conversion algorithms for converting particle counts into weight based on polymer composition and particle size/volume is in process. Furthermore, complementary mass measurements of specific polymer concentrations could be employed.

7.4.2 Size classes

Particle size is the main attribute for microlitter classification. Microlitter is defined as particles that are < 5 mm in their maximum length. Size classes are introduced to reflect the heterogeneity of the methodologies used, while the operational limits linked to the sampling of microlitter in different environmental matrices may limit the harmonisation and comparison of size classes. Therefore, sampling and analysis of the microlitter size classes will depend on the combination of the matrix, sampling technique and instrumental approach employed.

The size of particles, measured as the length in their longest dimension, should be recorded according to the size classes presented in Table 7.1.

Table 7.1. Microlitter size classes – recommended and optional measurements in the different matrices

Size range classes (µm)	Surface layer of the water column	Seafloor sediment	Beach sand
1000 – 4999	Recommended	Recommended	Recommended
300 – 999	Recommended	Recommended	Recommended
100 – 299		Recommended	Recommended
50 – 99		Optional	Optional
20 – 49		Optional	Optional
< 20		Optional	Optional

Source: Column 1 adapted from SeaDataNet BODC Vocab Library (undated-*a).*

7.4.3 Morphological classes

The term morphology is used here to describe the structure or shape of the microlitter items. The morphology of all identified particles should be recorded according to the morphology classes presented in Table 7.2.

Table 7.2. Microlitter morphology classes.

Morphology class	Definition
Filaments	Slender thread-like microlitter particles, that is, it also covers fibres and threads
Fragments	Irregularly shaped hard microlitter particles with broken-off edges that may be rounded or angular
Films	Microlitter particles derived from sheets or thin films
Foams	Flexible microlitter particles in which material cells are all o partly intercommunicating ([a]), including expanded polystyrene (EPS) and extruded polystyrene (XPS) foams
Pellets/granules/beads	Microlitter particles that are spherical, flat on one side or cylindrical in shape

([a]) Adapted from ISO/TR 20342-7:2021.

Source: Adapted from SeaDataNet BODC Vocab Library (undated-b).

It is under discussion if microbeads should be reported as a different morphology class or identified in the data set under the morphology ‘granules’, given their (smaller) dimensions/size than pre-production resin pellets. Pellets and granules are also being evaluated regarding whether they should be categorised individually. In addition, it has to be considered that film and foam might not be identified due to the restrictions/limitations of different instruments (e.g. FTIR) or protocols, especially within the smaller size fractions.

Fibres with a length of > 5000 µm are considered mesolitter and are, therefore, excluded from the data analysis for microlitter. Fibres of < 5000 µm should be recorded under the filaments class (see Table 7.2).

7.4.4 Colour classes

Visual determination of the microparticle colour is the most commonly used method for the assessment of this parameter. However, colour perception has a high degree of observer subjectivity in visual determination, which affects the accuracy and objective classification of the colour of particles.

Instrumental colour determination of particles can provide objective colour determination, but with a significant increase in the cost–effort of the analysis. Visual determination based on colour spaces (e.g. RGB; cyan, magenta, yellow and black (CMYK); CIELAB) may facilitate the reduction of subjectivity with limited increase in the cost-effort (Martí et al., 2020). Some of these colour spaces consider the lightness and hues of colours, which could facilitate the empirical determination and harmonisation of colour analysis, but further studies are needed to provide robust methods.

Weathering and discolouration modify the colours of litter and microlitter particles toward yellowish/brownish colours, which could provide information on the ageing of the particles. However, particle ageing should be considered as an additional property and is beyond the scope of this guidance. Therefore, only the colours determined during the analysis process should be considered here, based on the use of basic colour information. The colour of microlitter particles may be optionally reported according to the classes presented in Table 7.3.

Table 7.3. Microlitter colour classes.

Colour classes	Comment
Black	
Grey	
White	Including cream
Red	
Orange	
Yellow	
Green	
Blue	Including cyan
Purple	Including violet
Pink	Including magenta
Brown	Including tan
Multicolour	Particles made up of two or more colours
Colourless	Particles without added dyes, pigments and/or other additives (e.g. carbon black in rubbers), excluding particles that gain natural colours during their production (e.g. acrylonitrile butadiene styrene (ABS) goes a natural pale yellow colour during production).

7.4.5 Transparency classes

Transparency is a microlitter property that classifies particles according to the degree to which light passes through them, regardless of their colour. This attribute may optionally be reported according to the EMODnet microlitter transparency classes presented in Table 7.4.

Table 7.4. Microlitter transparency classes.

Transparency class	Definition
Opaque	Term that qualifies microlitter particles that do not allow the light to travel through them
Transparent / translucent	Term that qualifies microlitter particles that allow light to travel through them either almost unaltered (transparent) or with some diffraction (translucent, translucid or semi-transparent)

Source: Adapted from SeaDataNet *BODC Vocab Library* (undated-c).

7.4.6 Microlitter material and polymer types

To ensure the accuracy and quality of the collected data, instrumental confirmation of material identification is required when microlitter items have been visually identified using direct observation, microscopy or staining techniques (e.g. Nile Red). However, providing instrumental confirmation for all collected microlitter items is time-consuming and economically costly. The time and cost levels associated with these analyses can vary significantly depending on the method used and the robustness of the data generated. To help reduce time and cost within microlitter monitoring, it is recommended that a representative subset of at least 10 % of particles (with a minimum of 20 particles per sample unless there are fewer than 20 particles in the sample) potentially identified as synthetic items is analysed. These particles should cover the range from 100 µm to 1000 µm in a sample. The selection of particles integrated into the subset should be representative of different size categories and morphologies.

It is suggested that the polymer types be aligned with the list presented in Table 7.5; a short list of prioritised synthetic polymers that are predominantly found in environmental samples, which must be reported when present in a sample, should also be created.

Table 7.5. Polymer types for data reporting.

	Polymer type name	Examples of materials
Artificial synthetic and semi-synthetic polymers	Acrylonitrile based	Acrylonitrile butadiene styrene (ABS), polyacrylonitrile (PAN)
	Cellulose based	Cellulose acetate (CA), cellulose nitrate (CN)
	Polyamide based	All types of polyamide (PA), such as various nylons
	Polycarbonate based	Polycarbonate (PC)
	Polychlorinated polymers	Polyvinyl chloride (PVC), chlorinated polyethylene, various chlorinated polymers
	Polyester based	Polyethylene terephthalate (PET), all other types of polyesters
	Polyethylene based	Includes high-density polyethylene (HDPE), low density polyethylene (LDPE), and copolymers with a major polyethylene (PE) fraction including ethylene-vinyl acetate copolymer (EVA)
	Polyfluorinated polymers	Polytetrafluoroethylene (PTFE)
	Polymeth(ester)acrylate based	All types of polymeth(ester)acrylate (PM(ester)A)
	Polypropylene based	Polypropylene (PP) and copolymers with a major PP fraction
	Polystyrene based	Polystyrene (PS) and copolymers with a major PS fraction
	Polyurethane based	All types of polyurethane (PUR)
	Varnish/paint particles	If different from PM(ester)A
	Other plastics	Polyether ether ketone (PEEK), polyoxymethylene (POM), polyvinyl acetate (PVA),

	Polymer type name	Examples of materials
		polylactic acid (PLA), polyhydroxyalkanoate (PHA)
	Other semi-synthetic polymers	Rayon
Other microlitter items	Rubbers, automotive	Styrene butadiene rubber (SBR), tyre wear
	Other rubbers	Includes ethylene propylene diene monomer rubber (EPDM), silicone, nitrile rubbers and natural rubbers
	Other microlitter materials	Metal, glass

Source: Adapted from SeaDataNet *BODC Vocab Library (*undated-d).

7.5 Sampling protocols

7.5.1 Floating microlitter

7.5.1.1 Technical requirements: sampling device, sample volume, replicates and on-board sample processing

To support inter-comparability among monitoring programmes, it is recommended that the sampling of microlitter in the surface layer of the water column be carried out using a manta trawl (e.g. Gago et al., 2016; Galgani et al., 2013; UNEP/MAP, 2021). For feasibility reasons, it is also acceptable to use pump systems, but the use of manta trawls typically covers an area of several thousand square meters, making it more representative than the few cubic meters of water generally collected using pumping methods.

Manta nets with a mesh size of 300 µm are recommended to support the harmonisation and comparability of floating microlitter data among monitoring programmes and the potential inter-comparability of results between the size ranges of the different environmental matrices recommended (see Section 7.4.2). Particles with sizes below the net mesh size are under-represented and, therefore, should not be considered in the analysis (see Table 7.1).

The manta net should be deployed from the side of the ship/vessel, away from the wake zone, using a spinnaker boom, A-frame or crane. Sampling should be conducted at a constant speed ranging from 0.5 knots to 3 knots. The maximum speed should not exceed 4 knots to 4.5 knots and the trawling duration may vary from 10 minutes to 30 minutes, depending on *in situ* conditions (e.g. weather, wave action, algae presence, maritime traffic), striving for a total sample volume of at least 100 m^3. For the estimation of filtered water volume, the use of a volume flow meter is recommended. Alternatively (only for nets), the calculation of filtered water volume can be applied.

After sampling, the net should be rinsed with seawater from the outside to concentrate the sample in the cod-end. The cod-end is then removed, and its contents are transferred to pre-labelled glass or metal jars using metal sieves with a significantly smaller mesh size than the net used for sampling, and funnels, tweezers and filtered seawater.

When pump systems are used for sampling, the total sample volume must be adjusted according to the minimal cut-off size (i.e. it is recommended that volume of 100 m^3 be sampled with a lower cut-off size of 300 µm, 10 m^3 be sampled with a lower cut-off size of 100 µm or at least 1 m^3 be sampled with a lower cut-off size of 20 µm).

Samples should be stored in glass or metal containers. It is recommended that samples be stored at a low temperature (maximum temperature of 6 °C or frozen ([23])) to stop biological processes. Alternatively, a conservation additive might be used if it can be proved not to impact any of the parameters that are to be measured subsequently.

([23]) The influence of a freezing cycle on particle integrity should be investigated if samples are frozen.

7.5.1.2 *Recording of metadata, sampling parameters and the sampling protocol*

Table 7.6 lists the metadata and Table 7.7 lists the basic parameters (Table 7.7) that should be recorded during the sampling of sea surface microlitter.

Table 7.6. Metadata – floating sea surface microlitter (valid for the overall monitoring approach)

EMODnet identifier	Specification	Remarks
Instrument (Standard terms from L05 or L22 SeaDataNet vocabularies)	Sampling device used	
Mesh_size	Mesh size	If applicable
Net_opening	Manta sampling width and height	If applicable

Source: Adapted and modified from Vinci et al. (2017).

Table 7.7. Sampling parameters – floating sea surface microlitter

EMODnet identifier	Specification
Cruise	Cruise
Station	Station name
SampleID	Sample ID
YYYY-MM-DDThh:mm:ss.sss	Date/time start
EventEndDateTime	Date/time end
Longitude/Latitude	Start coordinates
EventEndLongitude /EventEndLatitude	End coordinates
SamplingEffort	Sample volume
SamplingEffort	Transect length (sampling effort)
Wind_speed	Wind speed
Wind_direction	Wind direction
WMO_Sea_State	World Meteorological Organization (WMO) sea state

Source: Adapted and modified from Vinci et al. (2017).

7.5.1.3 *Quality assurance / quality control measures for sea surface floating microlitter sampling*

To minimise background contamination, the following measures should be considered during sampling campaigns.

- Preventive contamination measures include the following.
  - Prioritise the use of glass and aluminium/metal materials, where possible, and avoid the use of synthetic materials.
  - Wash and rinse sampling devices before using to avoid cross-contamination. Manta trawl nets should be washed and rinsed, without the cod-end attached, from the outside of the net with seawater. Using filtered seawater (and a mesh size smaller than the lowest particle detection limit) that is lightly pressurised is recommended in order to facilitate the removal of particles from airborne contamination or cross-contamination. The cod-end of the net should also be washed and rinsed following the same procedure.
  - Pre-clean sample containers and instruments with filtered water (using a mesh size smaller than the lowest particle detection limit) and/or ethanol or isopropanol. Cover sample containers and instruments and protect them from cross-contamination until their use. The glassware may also be baked in a muffle at 500 °C to facilitate the removal of synthetic particles.
  - Minimise the presence of staff/crew/operators in the work area during the sampling process, where possible.

- Ensure that operators avoid wearing synthetic clothing and instead wear brightly coloured work clothes that allow operator-generated contamination to be easily detected in samples. Operators should also position themselves facing the wind while retrieving the sample and take care to avoid potential contamination sources during sampling and sample processing.
- Retrieve material from any device of synthetic polymer origin used during sampling. These comparative materials should be investigated for their polymer composition to enable the exclusion of clearly identified contamination from sampling devices.

- Field blank samples preparation includes the following.
    - Include a representative number of field blank samples to account for contamination during sampling and should reflect the specific contamination potential of each variable condition (e.g. weather conditions, operators wearing different clothes). Field blank samples are also useful for identifying, and then eliminating or mitigating, specific sources of microlitter contamination in the field. For homogeneous conditions during the sampling campaign, a 10 % proportion of field blank samples relative to the total number of water column microlitter samples is recommended. The minimum number should be at least three field blank samples.
    - Generate field blank samples using an empty glass/metal sampling jar, positioned next to the sample, and opened while retrieving the sample. For manta trawl net sampling, it is also recommended that a second type of field blank be collected that includes a filtered water rinse of the net. The resulting blank samples should be subjected to laboratory analysis in the same manner as the water column microlitter samples.

7.5.2 Microlitter in seafloor sediments

7.5.2.1 Technical requirements: sampling device, sample volume, replicates and on-board sample processing

Sampling of seabed sediments can be performed using grabs or corer-based approaches (e.g. Van Veen grab, box corer, Gemax corer, Kajak corer). The aim of the sample is to collect microlitter recently deposited on the seafloor, with priority given to areas with a known and stable sedimentation rate. Therefore, samples are taken within the upper layer of the sediment by means of stainless steel equipment, with the depth depending on the sedimentation rate and other site characteristics. Samples should immediately be transferred to pre-cleaned and labelled glass or metal jars. For monitoring stations with a known sedimentation rate and the absence of bioturbation processes, the sample depth may be adjusted in order to consider the sedimentation of specific periods or assessment cycles.

The total sample volume is related to the sampling device. It is recommended that a minimum volume be retrieved that allows for replicate analyses and the determination of additional sediment-related parameters. It is also recommended to sample in replicates (two or three hauls) and to combine the resulting samples into a composite laboratory sample for further analysis (Bäuerlein et al., 2023).

Samples should be stored in glass, aluminium or metal containers, under light-absence conditions and be kept at low temperatures (e.g. cooling at 4 °C or freezing at -20 °C ([24])). The use of plastic storage bags or containers is to be avoided. The storage conditions depend on the storage time and the conditions during the sampling campaign and/or the schedule of the laboratory conditions.

7.5.2.2 Recording of metadata, sampling parameters and sampling protocol

Table 7.8 lists the metadata and Table 7.9 lists the basic parameters that should be recorded during the sampling of microlitter in seafloor sediments.

([24]) The influence of a freezing cycle on particle integrity should be investigated if samples are frozen.

Table 7.8. Metadata – seafloor sediment microlitter (valid for the overall monitoring approach)

EMODnet identifier	Specification	Remarks
Instrument (standard terms from L05 or L22 SeaDataNet vocabularies)	Sampling device used	
COREDIST/MINCDIST/MAXCDIST	Depth of sampled sediment layer	Below ground
Bot. depth	Water depth	Below surface

Source: *Adapted and* modified from Vinci et al. (2017).

Table 7.9. Sampling basic parameters – seafloor sediment microlitter

EMODnet identifier	Specification	Remarks
Cruise	Cruise	
Station	Station name	
SampleID	Sample ID	
YYYY-MM-DDThh:mm:ss.sss	Date/time of sampling	
Longitude/Latitude	Station coordinates	
SamplingEffort	Sample volume	
	Number of replicates	
	Weather condition	Optional
	WMO Sea State	Optional
Proportion_sand_size_particles/ Proportion_clay_size_particles/ Proportion_sylt_size_particles	Substrate	
	Bottom salinity	Optional
	Bottom temperature	Optional
	Bottom oxygen concentration	Optional
Proportion_organic_matter		Optional
Water_sampling_content		Optional

Source: *Adapted and* modified from Vinci et al. (2017).

7.5.2.3 Additional seafloor sediment parameters

The following additional parameters are relevant to the monitoring of microlitter in seafloor sediments.

- Mandatory parameters. Dry weight of sediment (grams, weight after drying at 105 °C, according to ISO 11465:1993 (2020).
- Optional parameters. Water content (%), carbonate content (%) and total organic carbon (%).

The inclusion of these parameters under a specific EMODnet identifier is under discussion. It is also under discussion whether organic content (%, to be determined by loss on ignition at 550 °C) should be mandatory parameter. It is recommended to include the grain size distribution according to sand (63-2000 µm, %) and clay and silt (2-63 µm, %).

7.5.2.4 Sampling quality assurance / quality control measures for microlitter in seafloor sediment

To minimise background contamination, the following measures should be considered within the sampling campaigns.

- Preventive contamination measures include the following.
    - Prioritise the use of glass and aluminium/metal materials, and avoid the use of synthetic materials.

- Pre-clean sample containers and instruments with filtered water (using a mesh size smaller than the lowest particle detection limit) and/or ethanol or isopropanol. Cover sample containers and instruments and protect them from cross-contamination until their use. The glassware may also be baked in a muffle at 500 °C to facilitate the removal of synthetic particles.
- Minimise the presence of staff/crew/operators in the work area during the sampling process, where possible.
- Ensure that operators avoid wearing synthetic clothing and instead wear brightly coloured work clothes that allow operator-generated contamination to be easily detected in samples. Operators should also position themselves facing the wind while retrieving the sample and take care to avoid potential contamination sources during sampling and sample processing.
- Retrieve material from any device of synthetic polymer origin used during sampling. These comparative materials should be investigated for their polymer composition to enable the exclusion of clearly identified contamination from sampling devices.

- Field blank samples preparation includes the following.
  - Include a representative number of field blank samples to account for contamination during sampling and should reflect the specific contamination potential of each variable condition (e.g. weather conditions, operators wearing different clothes). Field blank samples are also useful for identifying, and then eliminating or mitigating, specific sources of microlitter contamination in the field. For homogeneous conditions during the sampling campaign, a 10 % proportion of field blank samples relative to the total number of sediment samples is recommended. The minimum number should be at least three field blank samples.
  - Generate field blank samples using an empty glass/metal sampling jar, positioned next to the sample, and opened while retrieving the sample. Field blank samples should be subjected to laboratory analysis in the same manner as sediment samples.

7.5.3 Microlitter in beach sand

7.5.3.1 Technical requirements: sampling device, sample volume, replicates and sample processing

As microlitter on beaches is assumed to be highly variable, in terms of both its concentration in the target area and its depth distribution, it is not recommended that monitoring programmes aim to derive concentration trends. However, one-off sampling, including seasonally distributed point sampling, may enable the identification of the degree to which a beach has been exposed to litter.

Microlitter on beaches has been sampled at the strand line using the quadrat method or sampling a linear extension along the strand line (e.g. Frias et al., 2010; Álvarez-Hernandez et al., 2019; Bayo et al., 2019); however, there is currently no harmonised or widely used method for microlitter sampling on beaches.

Most of the previous studies have been conducted on sandy beaches, as this facilitates sampling of the microlitter. The vertical distribution of microlitter may be altered by the grain size of sandy beaches, where microlitter smaller than the sand grains can potentially infiltrate into lower layers. Furthermore, the distribution of microlitter particles on a beach is exposed to continuous disturbances that may affect the monitoring strategy and sampling (e.g. footprints, clean-up activities, rainfall).

Microlitter monitoring on sandy beaches may be combined with macro litter and mesolitter / pellet monitoring. For this purpose, microlitter sampling should be carried out first to avoid disturbing the distribution of microlitter in the study area. Subsequently, mesolitter / pellet and macro litter sampling can be conducted according to their respective protocols (see Chapter 3 'Beach macro litter' and 6 'Mesolitter fragments and pellets on the coastline'). Fibres with a length of > 5000 µm and pellets should be considered mesolitter and therefore excluded from the microlitter in beach sediment data analysis.

The areas of sampling should be distributed along the strand line, and optionally may be distributed to the supratidal zone in transects parallel to the coastline. It is suggested that a minimum of five replicate samples be collected from each transect, and sampling points should be separated by at least 5 m.

Samples should be taken within the upper 5 cm layer of the sediment using metal equipment (e.g. shovels, spoons) and subsequently transferred to pre-cleaned and labelled glass or metal jars. The use of plastic containers or other plastic materials should be avoided.

The storage of samples will depend mainly on the time until analysis and the humidity of the samples and replicates, but it is recommended that they be kept at a temperature below 6 °C.

7.5.3.2 Recording of metadata, sampling parameters and sampling protocol

There is no specific vocabulary for recording metadata and parameters of microlitter in beach sand. The following basic parameters should be recorded.

- Recommended. Beach name, sample ID, survey date, start and end coordinates, survey length and width, sampling device used, depth of the sampled sand, sample area (volume is also recommended), total sampled area, number of replicates, distance between replicates and sand granulometry.
- Optional. Weather and sea conditions, beach orientation, beach usage, beach access, beach slope, beach urbanisation degree, beach curvature, proximity to harbours, rivers and population centres, and beach-cleaning information (seasonality, frequency).

7.5.3.3 Quality assurance / quality control measures for beach microlitter sampling

To minimise background contamination, the following measures should be considered within the sampling campaigns.

- Preventive contamination measures include the following.
    - Prioritise the use of glass and aluminium/metal materials, and avoid the use of synthetic materials.
    - Pre-clean sample containers and instruments with filtered water (using a mesh size smaller than the lowest particle detection limit) and/or ethanol or isopropanol. Cover sample containers and instruments, and protect them from cross-contamination until their use. The glassware may also be baked in a muffle at 500 °C to facilitate the removal of synthetic particles.
    - Minimise the presence of staff/operators in the work area during the sampling process, where possible.
    - Ensure that operators avoid wearing synthetic clothing and instead wear brightly coloured work clothes that allow operator-generated contamination to be easily detected in samples. Operators should also position themselves facing the wind while retrieving the sample and take care to avoid potential contamination sources during sampling and sample processing.
- Field blank samples preparation includes the following
    - Include a representative number of field blank samples to account for contamination during sampling and should reflect the specific contamination potential of each variable condition (e.g. weather conditions, operators wearing different clothes). Field blank samples are also useful for identifying, and then eliminating or mitigating, specific sources of microlitter contamination in the field. For homogeneous conditions during the sampling campaign, a 10 % proportion of field blank samples relative to the total number of beach sediment samples is recommended. The minimum number should be at least three field blank samples.
    - Generate field blank samples using an empty glass/metal sampling jar, positioned next to the sample, and opened while retrieving the sample. Field blank samples should be subjected to laboratory analysis in the same manner as beach sediment samples.

7.6 Sample preparation protocols

To minimise the presence of natural biogenic particles and concentrate plastic particles for analysis, the preparation of samples for analysis often involves multiple steps, such as sieving, chemical digestion and density separation. However, it is important to consider the potential impact of sample treatment on synthetic polymers. Strong chemicals and high temperatures should be avoided to prevent damage to these materials.

It is important that the selection of treatment processes and methods be carefully controlled using contamination control and be validated using recovery tests with reference materials (see Section 7.6.1.2) to ensure the accuracy and reliability of the results.

7.6.1 Laboratory quality assurance / quality control

7.6.1.1 Laboratory contamination control measures

To ensure accurate and reliable laboratory analysis, it is crucial to take appropriate measures to minimise airborne contamination and cross-contamination, and maintain contamination control. Below are some recommended measures.

- Personal protection equipment made of natural materials, such as cotton laboratory coats, should be worn, and plastic fibre face masks should be avoided.
- Laboratory conditions should be well maintained by regular cleaning, regulated air circulation and minimised presence of staff. Clean rooms equipped with laminar flow chambers combined with fume hoods are recommended whenever possible.
- The use of glass and metal materials should be prioritised and the use of synthetic materials avoided during the analysis.
- Water and chemical solutions should be pre-filtered with a filter pore size significantly smaller than the minimum cut-off size of targeted particles in the samples.
- Glassware (e.g. beakers, flakes), instruments and filters should be pre-cleaned (including rinsing and annealing, depending on the filter material).
- Samples, working solutions, glassware and instruments should be covered or adequately stored throughout the sample processing to prevent contamination.
- The number of processing steps should be reduced as much as possible.
- Samples with reference materials (see Section 7.6.1.2) should be analysed in parallel with each sample series to ensure the accuracy and reliability of the results.

7.6.1.2 Blank samples and recovery tests

A relevant number of blank laboratory samples are to be analysed in parallel with each sample series. Combining field blank and laboratory blank samples is not recommended since the number of samples processed within one sample series may differ from the number of samples being representative of the field blank sample.

The number of microlitter particles detected within both field and laboratory blank samples is used to define the limit of detection (LOD) (mean + 3 × standard deviation of the particle concentration), according to MacDougall et al. (1980). Therefore, the LOD reflects the efficiency of the precautionary methods during sampling and sample processing by the laboratory. The LOD is reported within the data provided to EMODnet. Blank values are not subtracted from the results for sediment and sand samples. In this sense, for microlitter in sediment and sand samples, it is recommended that the blank results (field and laboratory) and the uncorrected sample results be reported.

A relevant number of reference samples with reference materials is to be analysed in parallel with each sample series. Reference samples reflect the efficiency of the laboratory protocol and are treated in the same manner and in all the same steps as the sediment and sand samples.

Reference samples should encompass real sediment samples that are spiked with a number of synthetic polymer particles that are representative of dominating size categories, morphologies and the polymer composition of the particles to be detected within the sediment samples. The number of added reference material particles is to be defined in the future. As the number of reference material particles will affect the resolution of the recovery rates, at least 50 reference particles for both fragments and fibres could be recommended, leading to a resolution of 2 %.

The recovery ratio (%) is calculated for re-detected added reference particles as the mean value accounting for different size categories, morphologies and polymer composition. It is recommended to include reference material containing three types of polymers with different densities, three morphologies and a similar size to the targeted lower cut-off size (i.e. 100 µm) of particles according to Cui et al. (2022). The mean recovery ratio is reported together with the data provided to EMODnet. It is under discussion if the results from sediment and sand samples will be corrected for recovery rates.

It is recommended that the aim be a minimum mean recovery of 80 % for particles > 100 µm, based on spiked sediments as described in the previous points. This recovery is known to be feasible when using techniques such as fluorescent reference particles to identify adsorption losses of microplastics in the extraction device, and rinsing with ethanol, for example, to recover these adsorbed microplastics.

7.6.2 Sample volume and weight for laboratory analyses

The sample volume for laboratory analyses is dependent on the sample composition, sample storage conditions and further sample processing methods. Field sediment samples should be homogenised by stirring with glass or metal spatulas or spoons. The volume for laboratory analyses is determined (e.g. by using a metal measuring spoon) and weighed in a pre-cleaned beaker using an analytical balance with a minimum accuracy of 0.1 mg.

For sediment samples, a second aliquot of the field sample is investigated for water content to determine the dry weight in parallel. Therefore, an aliquot of approximately 10 ml is transferred and weighed in an evaporating dish and dried at 105 °C. Following cooling to room temperature within a desiccator, the samples are reweighed, and the dry weight is calculated. Alternatively, the dry weight of sediment can be calculated from the weight difference derived through freeze drying.

7.6.3 Pre-sieving

Pre-sieving at the beginning of the laboratory processing procedure is suggested, using a series of cascading sieves with decreasing mesh sizes of 5000 µm, 1000 µm, 300 µm, and 100 µm (for sediments and sand). Sieves with mesh sizes of 5000 µm and 1000 µm are useful for separating the mesoplastics (> 5000 µm), large microplastic (1000-5000 µm) and small microplastic (< 1000 µm) classes, respectively. Note that the use of sieves of 300 µm and 100 µm can lead to the damage and/or loss of microplastics.

7.6.4 Sample digestion

Digestion protocols cover oxidative, enzymatic, alkaline or mixed treatments (Pfeiffer et al., 2020). For example, hydrogen peroxide 30 % is often used for oxidative digestion. The implementation of acid digestion is not recommended, as especially strong acids have been demonstrated to affect the physical properties of some synthetic polymers. The duration of the sample digestion depends on the selected digestion protocol.

The application of temperature (< 40 °C) and the stirring of the samples is an optional add-on within sample digestion. The application of temperatures of > 40 °C (> 50 °C when enzymatic digestion is applied) is to be avoided since it may damage certain synthetic polymers. Concerning sediment samples, the order of digestion and density separation (Section 7.6.5) may depend on the sample treatment protocol and particle analysis technique of the processing laboratory. As microplastics may be biofouled or partly entrapped in organic material in the sediment and sand, it is recommended that the sample digestion step be performed prior to the density extraction step.

After digestion, the digestion solution is rinsed-off over a sieve with a mesh size of the minimum size of targeted particles (300 µm is recommended for samples of the surface layer of the water column and 100 µm is recommended for sediment samples).

If particle dimensions are not determined for single particles, a size separation step with a sieving cascade encompassing at least mesh sizes of 100 µm (only applies to sediment samples), 300 µm and 1000 µm can be applied at this stage (smaller mesh sizes are optional).

7.6.5 Density separation

Density separation is a processing step that is highly recommended for any sediment samples and for water samples that contain high levels of inorganic particles. The choice of the density solution and the device used for density separation depends on the respective protocol applied. Commonly used density solutions include zinc chloride ($ZnCl_2$), sodium iodide (NaI), sodium polytungstate ($NaWO_4$), potassium carbonate (K_2CO_3), sodium bromide (NaBr) and potassium formate (HCO_2K), with a minimum density of 1.5 g/cm³. The application of solutions with densities of > 1.7 g/cm³ is recommended since this will distinctly improve the recovery rates of synthetic particles of higher material densities. The use of sodium chloride (NaCl) is not recommended, as several relevant synthetic polymers will not be recovered due to the low solution density.

In general, samples are introduced into the density separation solution, shaken or stirred for 10 minutes and left to settle for 24 hours. Shaking is a gentle method, which reduces the chance of damaging the microplastics. When using stirring, care must be taken to use gentle and short stirring movements to minimise the risk of damaging or fragmenting the microplastics. The supernatant suspension is rinsed thoroughly with filtered water and transferred onto the appropriate filters for the further particle identification technique to be used. Filters are left to dry in pre-cleaned glass Petri dishes. It is recommended that the density separation process be repeated at least once per sample.

7.6.6 Particle identification

The method used for the identification of synthetic particles depends on the availability of instrumentation at individual laboratories, and includes optical microscopic identification, spectroscopic approaches such as FTIR and Raman spectroscopy and staining approaches (e.g. Nile red staining) in combination with fluorescence microscopy. Particles are categorised according to number, size class, morphology, colour (optional), transparency (optional) and polymer composition (on at least a subset of 10 % or a minimum of 20 particles per sample). Filaments, including fibres with a length of > 5000 µm should be considered mesolitter and therefore excluded from microlitter data analysis. The minimum cut-off size for data to be reported is 100 µm for sediment samples and 300 µm for samples from the surface layer of the water column.

7.6.7 Synthetic polymer identification

The determination of polymer composition via FTIR, Raman spectroscopy or other spectroscopic techniques (e.g. quantum cascade laser spectroscopy) is mandatory for at least a subset of particles. Device settings and the minimum library match (%) attributed is to be recorded within the metadata provided to EMODnet. Spectral libraries utilised for polymer composition determination should integrate spectra from synthetic and organic components and weathered synthetic polymers. It is suggested that all processing laboratories agree on one or more spectral libraries for identification and/or to generate a dedicated combined FTIR and/or Raman spectra library for MSFD microlitter monitoring.

It is recommended that the polymer composition be analysed on a representative subset with a minimum of 10 % (preferably at least 20 particles per sample) of synthetic particles identified within the size categories from 100 µm to 1000 µm. The subset size for particles identified in smaller size categories is to be discussed. The particles integrated into the subset are to be selected representatively according to size categories and morphologies.

The identification of tyre wear particles, which are mainly composed of rubbers such as styrene butadiene rubber or butadiene rubber, by spectroscopic techniques is challenging, particularly if they contain black carbon components. Thermal analysis methods are commonly used to qualitatively analyse tyre wear particles. However, the quantification of these particles requires validated chemical markers which vary depending on the manufacturing processes or the age of the tyre, and may lead to underestimation of some tyre microparticles depending on the selection of these markers for environmental samples. Therefore, robust methods to identify tyre wear microparticles in environmental matrices need to be further discussed and developed.

7.7 Data reporting

Parameters are to be recorded according to EMODnet requirements. Data may also be reported through other data portals or databases that serve the needs of the Member State (e.g. the ICES Marine Environment Database (DOME)). The reporting to or harvesting of data through the ICES DOME is currently under discussion. Parameters and related attributes are under continuous development. Therefore, it is recommended that the latest tables and vocabularies be consulted online on the SeaDataNet portal ([25]).

Annex VI – 'List of sea surface floating microlitter parameters to be reported' and Annex VII – 'List of seafloor sediment microlitter parameters to be reported' comprise lists of parameters (mandatory and optional), EMODnet codes and descriptions, where available and suggestions for modifications or the integration of

([25]) See the SeaDataNet Vocabulary Library (https://vocab.seadatanet.org/search).

further parameters for microlitter in the floating surface layer and seafloor sediments, based on the EMODnet guidelines and formats (Vinci et al., 2021).

7.8 Level of maturity and cost–effort

7.8.1 Level of maturity

The three proposed methods differ in their degrees of maturity.

- Floating surface layer microlitter. The sampling protocol is considered mature and is applied in at least one region on a continuous basis.
- Microlitter in seafloor sediments. The sampling protocol is considered to be at an intermediate stage of maturity. Specific monitoring programmes are required.
- Microlitter in beach sand. The sampling protocol is not mature at this stage, and it is not applied continuously at any regional level. The protocol is restricted to medium-sized and fine-grained sandy beaches and has a wide variability between sampling sites. Specific monitoring programmes are required.

7.8.2 Cost–effort of monitoring

The cost of microlitter monitoring is influenced by several factors, including direct costs (e.g. salaries, materials, reagents) and indirect costs (e.g. instrumental analysis, vessels), which hinder the estimation of the cost of monitoring.

The cost and effort of microlitter monitoring are high compared with those of the monitoring of other marine litter size ranges, as this monitoring includes numerous sample preparation processes (e.g. digestion, density separation) and instrumental analyses (e.g. spectroscopic methods), and stringent QA/QC measures for sample contamination prevention and control measures. Enders et al. (2020) reported the effort to prepare microlitter samples from different environmental compartments, with a total effort – based on an 8-hour working day and up to eight samples treated in parallel – from 6 to 20 days, depending on the number of particles, the type of matrix (i.e. water, sediment) and the steps of the treatment applied.

8 Litter and microlitter ingested by biota and entanglement with litter

8.1 Introduction

This chapter focuses on the criteria reported in the new Commission Decision (Decision (EU) 2017/848) related to the impact of litter on marine biota: D10C3 'The amount of litter and microlitter ingested by marine animals is at a level that does not adversely affect the health of the species concerned' and D10C4 'The number of individuals of each species which are adversely affected due to litter, such as by entanglement, other types of injury or mortality, or health effects'. In the old Commission Decision from 2010 (Decision (EU) 2010/477), there was already the need to develop an indicator that included the amount and composition of litter ingested by marine animals, but there was not a clear link to the health of the animals. As no single species can provide full coverage of all Europe's marine sectors and each marine litter category (from macro to micro), a range of species is needed to monitor marine litter impact.

Given their propensity to ingest litter, their wide distribution and the large range of habitats used during their life, two species are already validated for monitoring ingested litter. The northern fulmar, *Fulmarus glacialis* (Linnaeus, 1761) was chosen as an indicator for the northern European waters (van Franeker et al., 2011), while sea turtles, in particular the loggerhead species *Caretta caretta* (Linnaeus, 1758), were chosen as an indicator for the Mediterranean basin (Matiddi et al., 2011, 2017). For those two species, threshold values have been suggested, while for most other taxa the indicators are still not mature. For assessing the impact of microplastic fish are becoming good candidate bioindicators (UNEP/MAP, 2019; Bray et al., 2019; Matiddi et al., 2021, Valente et al., 2022) even if no individual species has yet been chosen. Moreover, mussels have been investigated by different authors as bioindicator for microplastics (Li et al., 2018; Lusher et al., 2017; Bessa et al., 2019).

Entanglement in marine litter, defined as 'any marine organism wrapped, trapped or stuck in marine litter including fishing gear lost or abandoned' (Silvestri et al., 2021), has been reported to occur worldwide in various species, causing injuries and death, and protocols for monitoring have been developed within the EU projects MEDREGION (Silvestri et al., 2021) and INDICIT II (Loza et al., 2021).

As stated by Decision 2010/477/EU and Decision (EU) 2017/848, knowledge of the impacts of litter on marine life should be improved, especially regarding species affected, impacts on health, standardisation of methods and determination of thresholds. For this purpose, acquiring knowledge on the entanglement of marine organisms in litter for criterion D10C4, the 'impacts of litter on marine life' is also needed. This guidance should be considered for monitoring purposes and the methods described here are thought to apply the necessary degree of accuracy. Other methods are possible but their cost, time and complexity should be evaluated.

8.2 Scope and key questions to be addressed

Existing methods for monitoring marine biota litter ingestion and entanglement that fulfil the requirements of the MSFD are evaluated and reported here in a harmonised way. The methods provided in this chapter can be applied to assess the impact of litter on biota in Regional Seas to ensure comparability of results. The ingestion of marine macro litter and microlitter, and entanglement of marine organisms and the use of plastic litter as nesting material, are considered for inclusion in monitoring guidelines to assess impact. Explanation of different sections building starting from the previous knowledge, are reported in the following bullet points.

- In the North Sea, an indicator is available that expresses the impact of marine litter (the OSPAR ecological quality objective (EcoQO)). It measures ingested litter in northern fulmar and is used to assess temporal trends, regional differences and compliance with a set target for acceptable ecological quality in the North Sea area (van Franeker et al., 2011; OSPAR Commission, 2015a). The combined protocol proposed here can be used for seabirds in general and applied in most north-east-Atlantic countries, where the threshold value was calculated from near-pristine Canadian Arctic data (van Franeker et al., 2021).

- After some consideration and a pilot study conducted by Italian researchers, the experts of the TG ML have chosen the sea turtle *Caretta caretta*, (Linnaeus, 1758) as the target species for monitoring litter ingested by marine organisms in the Mediterranean Sea (Matiddi et al., 2011, 2017; Galgani et al., 2013). The protocol has been improved and made available in several languages (INDICIT consortium, 2018). All stages of manipulations during necropsy and two scenarios for threshold values were reported by Matiddi et al. (2019).

- Protocols for the analysis of marine litter in stranded marine mammals were developed at the International Whaling Commission (IWC) workshop and recently reviewed (IJsseldijkl et al., 2019). The methodology has been harmonised by the Agreement on the Conservation of Cetaceans of the Black Sea, Mediterranean Sea and Contiguous Atlantic Area (ACCOBAMS) and by the Agreement on the Conservation of Small Cetaceans of the Baltic, North-East Atlantic, Irish and North Seas (ASCOBANS).
- The assessment of microlitter ingestion in biota (birds, fish and invertebrates) can be incorporated into the provided protocols even if none of the assessment methods can be considered completely mature at this stage.
- Fish seem to be suitable organisms to be used as bio-indicators of microlitter ingestion and the present protocol comprises the INDICIT II EU project deliverable that considers the results of previous EU projects and scientific literature on this topic (Matiddi et al., 2021). Currently, none of the many candidate fish species have yet been chosen for monitoring microlitter ingestion but many are already investigated and proposed (Bray et al., 2019; Valente et al., 2022;2023).
- A protocol on microlitter ingestion by benthic filter-feeding organisms, such as mussels, oysters, and clams in shallow coastal waters (water depth < 5 m) is proposed.
- Ingestion protocols for invertebrates such as crustaceans, shellfish, worms or zooplankton are not included in this report.
- The monitoring protocols developed to assess the entanglement of megafauna (sea turtles and mammals) and sessile benthic organisms are provided as an easy tool for comparing standardised data and understanding the impact of marine litter on the marine environment, either globally or on a local scale. The proposed protocols are the outputs of the MEDREGION (Silvestri et al., 2021) and INDICIT II (Loza et al.,2021) EU projects.
- In addition, a harmonised protocol for assessing the use of plastic litter as nesting material and associated entanglement mortality in bird breeding colonies, sea turtles and seals is proposed for immediate application.

Key questions are still open and other aspects are crucial issues for further research, and as a result some options are not currently suitable for recommendation for large-scale monitoring applications at this stage. The following points summarise the key open questions that need further development.

- Monitoring of ingestion does not directly reflect a correlation with the health of the species concerned, though this is included in the Commission Decision (EU) 2017/848; only one proxy has been proposed, which is for the loggerhead turtle, where the weight of litter vs food of the gut content is compared (Matiddi et al., 2019).
- The impact of ingested marine litter is most frequently sublethal in effect rather than lethal. Sublethal effects are not easily detected and are difficult to distinguish from impacts resulting from other pollutants. To understand the implication of marine litter ingestion on animal conservation more studies are needed.
- Until now, threshold values have been validated only for norther fulmars (*Fulmarus glacialis*) (van Franeker et al., 2021), and loggerhead turtles (*Caretta caretta*) (Matiddi et al; 2019), while a possible fish GES scenario has been proposed (Matiddi et al., 2021).
- The first definition of microplastic as 'all particles less than 5 mm in diameter' (Arthur et al., 2009) did not define a lowest limit and originated different biases in data comparisons. For MSFD purposes, microlitter is defined as particles of < 5 mm in their maximum length, fixing the lowest limit for monitoring litter in biota as 100 µm.
- To assess the impact of marine litter on both megafauna and benthic organisms by entanglement, it is necessary to quantify the number of individuals of each species that are adversely affected. To do this, the population of a given species present in a specific area and the proportion of entangled animals should be known. Currently, it is not possible to determine this kind of information with certainty, and for this reason an assessment can be made using the frequency of occurrence as a percentage (FO%) of entanglement per region/area and per year (Silvestri et al., 2021).

8.3 Protocol for litter ingestion by seabirds

8.3.1 Protocol name

MSFD protocol for the monitoring of litter ingested by seabirds (Procellariiformes, like fulmars or shearwaters).

8.3.2 Protocol description

The methodology of this protocol follows the OSPAR (EcoQO) methods for monitoring litter items in the stomachs of northern fulmars (*Fulmarus glacialis*). The stomach contents of birds beached or otherwise found dead are used to measure trends and regional differences in marine litter. Background information and the technical requirements are described in detail in documents related to the fulmar EcoQO methodology. A pilot study evaluating methods and potential sources of bias was conducted by van Franeker and Meijboom (2002). Bird dissection procedures, including parameters for age, sex and cause of death, have been specified by van Franeker (2004). Further OSPAR EcoQO details were given by OSPAR Commission (2008, 2010a, 2010b, 2015a, 2015b), van Franeker and the SNS Fulmar Study Group (2011) and van Franeker et al. (2011).

8.3.3 Related marine compartments

Seabirds such as fulmars or shearwaters mostly feed at or near the surface of the sea. Therefore, the water column and especially the water surface are the marine compartment addressed when quantifying litter in the stomachs of fulmars. The plastics in fulmar stomachs mostly consist of mesoplastics (0.5–2.5 cm) and large microplastics (1–5 mm), with a small fraction of macroplastics (> 2.5 cm).

8.3.4 Technical requirements

Bird corpses are stored frozen until analysis. Standardised dissection methods for fulmar corpses have been published in a dedicated manual (van Franeker, 2004) and are internationally calibrated during regular workshops. Stomach content analyses and methods for data processing and presentation of results are described in detail by van Franeker and Meijboom (2002) and were updated in later reports. The methodology has been published in peer-reviewed scientific literature (van Franeker et al., 2011; van Franeker and Law, 2015). For context, some of the methodological information is repeated here in a condensed form.

During dissection, a full series of data is recorded to determine sex, age, breeding status, likely cause of death, origin, and other issues. Age, the only variable found to influence litter quantities in stomach contents, is largely determined on the basis of the development of sexual organs (size and shape) and the presence of the Bursa of Fabricius (a gland-like organ positioned near the end of the gut which is involved in the immune systems of young birds; it is well developed in chicks, but disappears within the first year of life or shortly after). Further details are provided by van Franeker (2004).

After dissection, the stomachs of birds are opened for analysis. Fulmar stomachs have two units: initially, food is stored and starts to digest in a large glandular stomach (the *proventriculus*), after which it passes into a small muscular stomach (the *gizzard*) where the remains of harder prey can be processed through mechanical grinding. To achieving cost-effective monitoring, the contents of the proventriculus and gizzard are combined, but optional separate recordings should be considered where possible.

Stomach contents are carefully rinsed in a sieve with a 1 mm mesh and then transferred to a Petri dish for sorting under a binocular microscope. The 1 mm mesh is used because smaller meshes become easily clogged with mucus from the stomach wall and with food remains. Analyses using smaller meshes were found to be extremely time-consuming and particles smaller than 1 mm are very rare in fulmar stomachs, contributing little to plastic mass. Should the method be applied to other, small species, such as storm petrels or phalaropes, a smaller mesh size may need to be considered.

If oil or chemical pollutants are present, these may be sub-sampled and weighed before rinsing the remainder of the stomach contents. If sticky substances hamper further processing of the litter objects, hot water and detergents can be used to rinse the material clean prior to further sorting and counting under a binocular microscope.

8.3.4.1 *Litter categories – source related information*

In the fulmar protocol, stomach contents are sorted into the categories shown in Table 8.1, and this categorisation is followed for monitoring marine litter ingestion in seabirds.

Table 8.1. Categories for the classification of items for monitoring marine litter ingestion in biota.

Biota categories for the contents of the digestive tract			
PLA	Plastic	Acronym	All plastic or synthetic items. Note the number of particles and the dry mass for each category
IND	Pellets	ind	Industrial plastic granules (usually cylindrical but oval, spherical or cubical shapes exist)
	Probab ind?	pind	Suspected industrial, used for tiny spheres (glassy, milky, etc.) (i.e. microbeads)
USE	Sheet	she	Remains of sheet from bags, cling-foil, agricultural sheets, rubbish bags, etc.
	Thread	thr	Threadlike materials, pieces of nylon wire, net-fragments, woven clothing, etc.; includes balls of compacted material
	Foam	foam	All foamed plastics, polystyrene foam, foamed soft rubber (as in mattress filling), PUR used in construction, etc.
	Fragments	frag	Fragments, broken pieces of thicker type of plastics; can be a bit flexible but not like sheetlike materials
	Other	Poth	Any other items, including elastics, dense rubber, cigarette filters, balloon pieces, soft air gun bullets and objects. Specific items should be described.
RUB	Other rubbish	Acronym	Any other non-synthetic consumer wastes. Note the number of particles and (in principle) the dry mass for each category
RUB	Paper	pap	Newspaper, packaging and cardboard. Includes multilayered material (e.g. Tetra Pak pieces) and aluminium foil
	Kitchen food	kit	Human food remains (galley waste) such as onions, beans, chicken bones, bacon, seeds of tomatoes, grapes, peppers, melons, etc.
	Other rubbish	rubvar	Other various rubbish, such as processed wood, pieces of metal, metal airgun bullets, lead shot and paint chips. Describe
	Fishhook	hook	Fishing hook remains (not for hooks on which longline victims were caught)
POL	Pollutants (industrial/chemical waste)	Acronym	Other non-synthetic industrial or shipping wastes. Note the number of items and the mass per category (wet mass for paraffin)
POL	Slag/coal	slag	Industrial oven slags (looks like non-natural pumice) or coal remains

	Oil/tar	tar	Lumps of oil or tar (also note as *n* = 1 and *g* = 0.0001 g if other particles are smeared with tar but cannot be sampled separately)
	Paraf/chem	chem	Lumps or soft mush of unclear paraffin, waxlike substances (not stomach oil); if needed, estimate mass by subsampling
	Feather lump	confea	Lump of feathers from excessive preening of fouled feathers (n = 1 with dry mass) (not meaning a few of their own feathers, which is normal)
FOO	Natural food	foo	Various categories, depends on the species studied and the aims of study
NFO	Natural non food	nfo	Anything natural that cannot be considered normal nutritious food for the individual

Source: Adapted from Galgani et al. (2013).

The fulmar categorisation of stomach contents is based on the general 'morphs' of plastics (sheet like, thread like, foamed, fragment, other) or other general rubbish or litter characteristics. This is because particles cannot be unambiguously linked to specific objects in most cases. Where this is possible, in the notes on datasheets, the items should be described and assigned a litter category number using the *Joint List of Litter Categories for Marine Macrolitter Monitoring* developed by the TG ML group (Fleet et al., 2021).

For each litter category/subcategory an assessment is made of the:

- incidence (percentage of investigated stomachs containing litter);
- abundance by number (average number of items per individual);
- abundance by mass (weight in grams, accurate to fourth decimal place per individual).

Due to the potential variations in annual data, it is recommended that 'current levels' be noted as the average for all data from the most recent 5-year period, in which the average is the population average and includes individuals that were found to have zero litter in their stomachs.

As indicated, EcoQO data presentation for northern fulmars is for the combined contents of glandular (proventriculus) and muscular (gizzard) stomachs. The results for all age groups should be combined except for those chicks and fledglings, which should be dealt with separately. Potential bias from age structure in samples should be checked regularly.

8.3.4.2 Size range

In the fulmar monitoring scheme, stomach contents are rinsed over a sieve with a 1 mm mesh prior to further categorisation, counting and weighing. The size of plastics monitored is thus ≥ 1 mm. Unpublished data on particle size details in stomachs of fulmars show that a smaller mesh size would not be useful because smaller items would have passed into the gut.

In the OSPAR Commission fulmar EcoQO approach, the focus is on the mass of each litter category, rather than on the size of individual particles. However, the litter Descriptor of the MSFD makes a distinction between macro litter and microlitter particles, the latter defined as objects where the largest dimension is < 5 mm. Both size groups are common in seabird stomachs. For comparative purposes it is therefore useful to know the proportions of microlitter and macro litter found in seabird stomachs. Whether this assessment of particle size is incorporated into standard monitoring methods or it is evaluated on a more incidental basis will depend on practical and financial considerations. In the current fulmar project, particle size assessment is not standard procedure (particle number and combined mass per litter category only give average size information), but a dedicated study is currently assessing the exact sizes of all particles in a large number of samples from different locations and periods. This dedicated detailed work can be repeated at appropriate time points.

In the seabird studies it is standard to filter stomach contents over a 1 mm sieve, which largely ignores the potential presence of microplastics of < 1 mm in size. In fulmar stomachs, objects of such sizes seem extremely rare, but could potentially be present in gut material in the intestines as a result of the break-up of larger items in the stomach or from secondary (passive) ingestion during zooplankton or fish consumption.

8.3.4.3 Spatial coverage

Dead birds are collected from beaches or from accidental mortalities; they are often long-line victims and fledglings killed on roads, for example (for the methodology, see van Franeker, 2004).

8.3.4.4 Survey frequency

Continuous sampling is required. A sample size of 40 birds or more is recommended for a reliable annual average for a particular area. However, years with low sample sizes can be used in the analysis of trends as the standard trend analyses are based on individual birds and not on annual averages. For reliable conclusions on changes or stability in ingested litter quantities, data over periods of 4–8 years (depending on the category of litter) are needed (van Franeker and Meijboom, 2002). In the OSPAR Commission approach (OSPAR 2015a) recent trends are evaluated over all individuals investigated over the most recent 10 years of data.

8.3.4.5 Maturity of the tool

The method is mature and in use. The OSPAR Commission (2015a, 2015b) has made specific guidelines outlining the requirements of the agreed OSPAR monitoring of plastic ingestion in fulmars in the North Sea. The formal OSPAR requirements use a categorisation of stomach contents that quantifies only the number and mass of the main plastic categories (industrial, user, and their combined total).

8.3.4.6 Regional applicability of the tool

The tool is applicable to the MSFD marine regions where fulmars occur; the Greater North Sea, the English Channel and the Celtic Sea For similar seabird species, including any of the tubenose family, the methodology can follow this protocol. This could, for example, be applied to shearwater species occurring further south in the Atlantic or in the Mediterranean Sea.

8.3.5 Estimation of costs

A cost estimate for fulmar biota monitoring can be based on the current level of funding available for the monitoring project in the Netherlands. This currently amounts to approximately EUR 60000 annually, largely dedicated to personnel costs (based on contract rates by Wageningen University and Research, the Netherlands). This concerns the time invested in coordinating the collection programme by volunteers and other groups (ca. EUR 20000), the lab dissections, stomach analyses and data analysis of approximately 40-50 birds annually (ca. EUR 20000); and formal report writing and production and associated post reporting activities (ca. EUR 20000). Material costs for transports and lab disposables are minor in the Netherlands, but are occasionally higher if providing volunteer groups with materials such as freezers. The actual field work in this approach is conducted without cost by volunteer beach bird surveyors or other people/organisations regularly surveying beaches. Their reward is provided by the coordinator, who spends a considerable part of her effort on providing good reports to the participants about the programme’s outcomes (through reports, the web page, individual contacts).

In the Dutch programme no funds are allocated to assisting other countries, integrating data analysis or report writing for the OSPAR Commission (e.g. for its intermediate assessments). These tasks are considered incidental and are funded separately. Costs for separate national programmes may be reduced significantly if integration of analyses and reporting by a single lead partner is more structurally arranged and financially supported.

8.3.6 Quality assurance / quality control

The methodology referred to in this tool is based on an agreed OSPAR methodology which has been developed over a number of years with the ICES and the OSPAR Commission and which has received full quality assurance through publication in peer-reviewed scientific literature (van Franeker et al., 2011; Van Franeker and Law 2015). The EcoQO methodology has been fully tested and implemented on northern fulmars (*Fulmarus glacialis*), including those from several North Atlantic and Pacific populations (e.g. Mallory, 2008; Provencher et al., 2009; Nevins et al., 2011; Avery-Gomm et al., 2012, 2018; Kühn and van Franeker, 2012; Bond et al., 2014; Donnelly-Greenan et al., 2014; Trevail et al., 2015; Herzke et al., 2016; Poon et al., 2017; Terepocki et al., 2017), allowing wide spatial comparisons of marine litter in European waters and other North Atlantic and Pacific regions. All methodological details can be applied to other tubenose seabirds (Procellariiformes) with no or very minor modifications. Trial studies have been conducted using shearwaters from the more southern parts of the North Atlantic and Mediterranean, but currently it has proved too complicated to obtain a good regional spread in annual samples. In other seabird families, methods may have to be adapted, as stomach morphology, foraging ecology, and regurgitation of indigestible stomach contents differ and can affect methodological approaches.

8.3.6.1 Trend assessment

In the fulmar assessment, the statistical significance of trends in ingested litter, that is, plastics, is based on linear regression of ln-transformed data for the mass of litter (of a chosen category) in individual stomachs against their year of collection. Recent trends are defined as being derived from all data over the most recent 10-year period. The fulmar assessment focuses on trend analyses for industrial plastics, user plastics and their combined total. Generalised linear model (GLM) procedures using annual frequencies of occurrence were recently applied for modelling expected compliance with the OSPAR target in the future.

8.4 Protocol for litter ingestion by sea turtles

8.4.1 Protocol name

MSFD protocol for the monitoring of litter ingested by sea turtles (*Caretta caretta*) and MSFD protocol for sampling litter excreted by live sea turtles (faecal pellet analysis) (optional).

8.4.2 Protocol description

The gastrointestinal contents of dead loggerhead sea turtles *Caretta caretta* (Linnaeus, 1758) are used to measure trends and regional differences in marine litter ingestion.

The original methodologies were first proposed in Italy and incorporated into the MSFD guidelines (Matiddi et al., 2011; Galgani et al., 2013), and then later applied along the Spanish (Domenech et al., 2018), French (Darmon and Miaud, 2016) and Italian (Camedda et al., 2014) coasts and validated by Matiddi et al. (2017). Finally, the protocol was consolidated in the framework of the European project INDICIT (project number G.A. 11.0661/2016/748064/SUB/ENV.C2) and harmonised with the Specially Protected Areas / Regional Activity Centre (SPA/RAC) protocol (INDICIT consortium, 2018). The procedures for dead sea turtle dissection, including the analysis of ingested litter and possible scenarios for thresholds, have been specified in detail and published as a video tutorial by Matiddi et al. (2019). The protocol proposes the collection of a series of basic and optional parameters. The basic parameters correspond to the minimum parameters fundamental to monitor criterion D10C3 based on the occurrence of litter ingestion and the quantity of ingested litter in sea turtles. The optional parameters allow for the acquirement of more knowledge on the impacts of litter ingestion on an individual’s health.

8.4.3 Related marine compartments

Caretta caretta feeds in the water column and on the seafloor. Therefore, these two marine compartments are addressed when quantifying litter in the gastrointestinal tract of loggerhead turtles. The ingested plastics mostly consist of macroplastics, while mesoplastics and microplastics could generally be considered as created through the breaking up of macroplastics during feeding activities.

8.4.4 Technical requirements

As the loggerhead sea turtle (*Caretta caretta*) is a protected species, only authorised people can handle live and dead animals or parts of them. Upon finding an animal, its management and recovery should be reported and coordinated with the responsible authorities. Note that a Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) permit is required if a specimen or sample has to be sent/received.

To minimise risks of infectious diseases such as zoonosis, sanitary precautions for the handling of dead or live wild animals must be followed.

8.4.4.1 Protocol for application in the case of finding a dead sea turtle

Based on initial observations and ideally while still at the place of discovery, some data should be recorded (an observation sheet is provided in Annex VIII – 'Observation sheet for litter ingestion by sea turtles').

A photo of the animal should be taken before any manipulation.

The specimen's body condition level should be reported on the following scale: 1 (alive), 2 (fresh – dead recently), 3 (partially decomposed – internal organs are still in good condition), 4 (advanced decomposition – skin scales are raised or lost) or 5 (mummified – part of the skeleton or part of the body are missing) (Figure 8.1). For level 1, litter can be extracted from the analysis of faeces in a rescue centre. Levels 2 and 3 are adequate for litter ingestion analysis from necropsies. Level 4 allows the measurement of biometric data and assessment of the presence/ absence of ingested plastic (for the evaluation of the frequency of occurrence of litter ingestion (or prevalence, expressed as a percentage – FO %) and entanglement. Level 5, for which individuals have usually lost the gastro-intestinal material, the analysis of litter ingestion is not possible.

Figure 8.1. Specimen's body condition level

Source: Modified from Matiddi et al. (2019).

The circumstances of the animal should be noted based on four categories: stranded (animal found on the beach or on the shoreline); bycatch/fisheries (animal captured actively by fishers, for example ingestion of a hook, trapped in a net, brought back by fishers); found at sea (animal discovered on the sea surface); dead at the recovery centre (the animal arrived alive, but died during its recovery).

The animal should be transported to an authorised service centre for necropsy. In cases where the body is too decomposed for this, the integrity of the digestive tract should be assessed before disposal at the licensed contractor. If the necropsy cannot be carried out immediately after recovery, the carcass should be frozen at – 16 °C, in the rehabilitation facility.

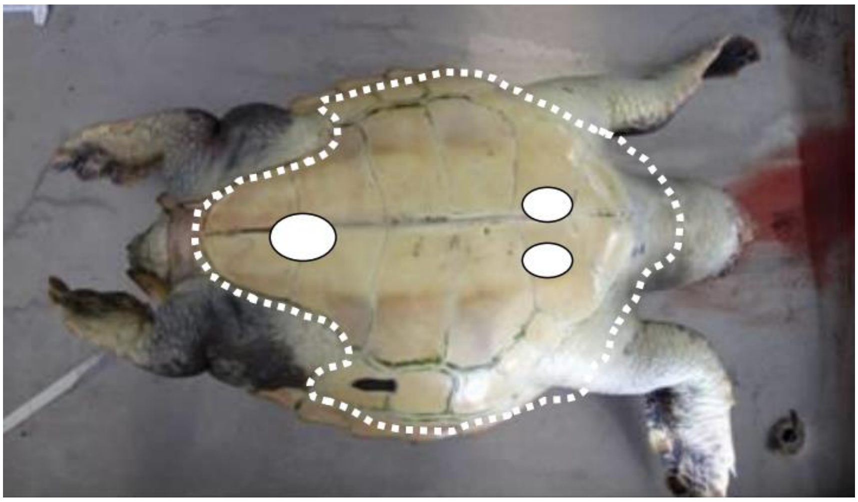
Before the necropsy operation, morphometric measurements should be collected.

The standard curved carapace length (CCL) (notch to tip) (Bolten, 1999) is mandatory, while other measurements are optional (e.g. curved carapace width, weight).

External examination of the animal should be conducted, including inspecting the oral cavity for the possible presence of foreign material. To remove and separate the plastron from the carapace, an incision should be made on the outside edge, as shown by the dashed line in Figure 8.2.

The ligament attachment of the pectoral and pelvic girdle should be cut once the inside of the animal is accessed, as indicated in the white circles in Figure 8.2. Qualitative evaluation of the trophic status of the animal should be made, including the atrophy of the pectoral muscles (none, moderate, severe), and the fat thickness in the articular cavities and on the coelom membrane (abundant, normal, low, none).

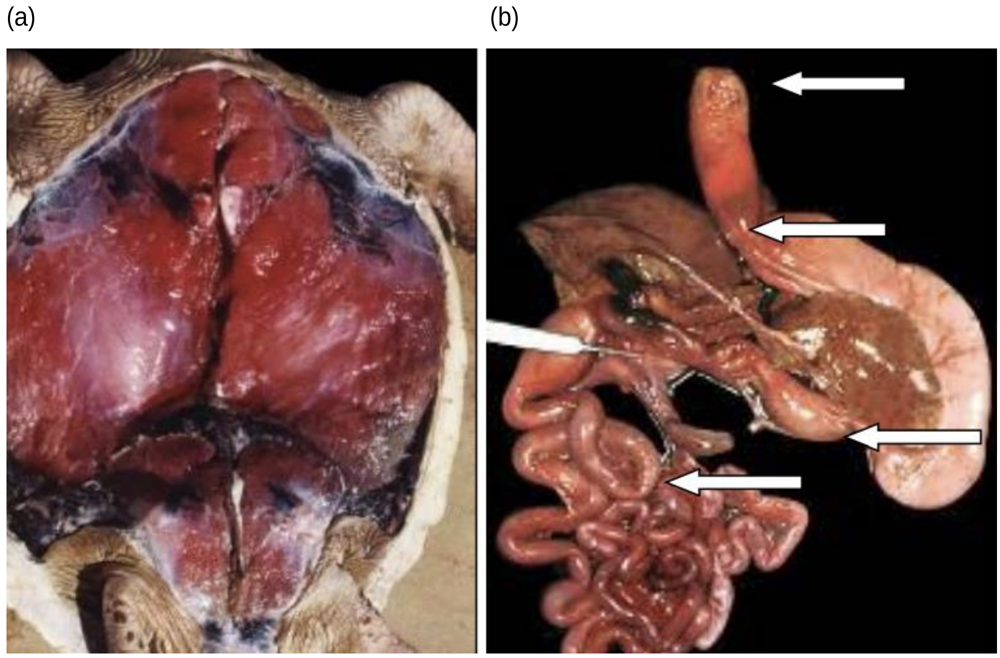
Figure 8.2. Cutting line (dashed line) and location of main plastron ligaments (ovals) in a turtle



Source: Modified from Wyneken (2001).

Removal of the pectoral muscles and the heart should expose the gastrointestinal system (GI) (Figure 8.3(a)). The different portions of the GI should be isolated by means of plastic clamps, fixed on the oesophagus proximal to the mouth, on the oesophageal valve, on the peg and on the cloaca, as close as possible to the orifice, as indicated by the arrows in Figure 8.3(b). The entire GI should be removed and placed on the examination surface. This is easier if done by at least two operators: one person keeps the animal lying on its side, while the other separates the ligaments of the different organs and the membranes of the carapace by extracting the GI from the animal. The sex of the animal should be recorded. The three parts of the GI (oesophagus, stomach, intestines) should be separated, affixing additional clamps at the cut edges to prevent spillage of the contents.

Figure 8.3. (a) The ventral pectoral and pelvic musculature, which covers most of the internal organs and must be removed to expose the peritoneal cavity and (b) a different portion of the sea turtle GI



NB: In part (b), arrows indicate location of clamps.

Source: Modified from Wyneken (2001).

The following sampling procedure of GI contents can be applied to any section of the GI (oesophagus, stomach, intestines).

The section of the GI should be observed and any ulcers or any lesions caused by hard plastic items should be recorded.

The contents should be inspected for the presence of any tar, oil, or particularly fragile material that must be removed and treated separately. The liquid portion, mucus and the digested unidentifiable matter should be removed, by washing the contents with freshwater through a 1mm filter mesh, followed by a rinse of all the material collected by the filter using 70 % alcohol and finally by another rinse in freshwater. The retained content should be enclosed in plastic bags or pots, labelled and frozen, not forgetting to note the sample code and corresponding section of the GI. The contents can then be sent for analysis.

Note that if the contents are stored in liquid fixative, a note must be taken of the compound and the percentage of dilution, which should be communicated to the staff in charge of further analysis.

For the analysis of GI contents, the organic component should be separated from any other items or material (marine litter). The fraction of marine litter should be analysed and categorised according to the shape of the items by using a stereomicroscope (Figure 8.4, Table 8.2). Detailed information on categorisation of marine litter of this type is provided by the INDICIT consortium (2018) and Matiddi et al. (2019).

Table 8.2. Classification of marine litter items plus food remains and natural non-food remains.

Type	Code	Description
Industrial plastic	IND PLA	Industrial plastic granules, usually cylindrical but also sometimes oval, spherical or cubical shapes
Use sheet	USE SHE	Remains of sheet, from bags, cling film, agricultural sheets, rubbish bags, etc.
Use thread	USE THR	Threadlike materials, pieces of nylon wire, net fragments, woven clothing, etc.
Use foam	USE FOA	All foamed plastics, polystyrene foam, foamed soft rubber (as in mattress filling), etc.
Use fragment	USE FRAG	Fragments, broken pieces of thicker types of plastics; can be a bit flexible, but not like sheetlike materials
Other use plastics	USE POTH	Any other type of plastics, including elastics, dense rubber, cigarette filters, balloon pieces and soft airgun bullets
Litter other than plastic	OTHER	All non-plastic rubbish and pollutants
Natural food	FOO	Natural food for sea turtles (e.g. pieces of crabs, jellyfish, algae)
Natural no food	NFO	Anything natural that cannot be considered normal nutritious food for sea turtles (stone, wood, pumice, etc.)

Source: *Adapted from* INDICIT (2018).

Figure 8.4. Examples of marine litter categories: (a) IND PLA, plastic pellets and granules, (b) USE SHE, materials such as plastic bags, agricultural sheets or plastic foil, (c) USE THR, ropes, filaments and other threadlike materials, (d) USE FOA, such as polystyrene foam or foamed soft rubber, (e) USE FRA, fragments of hard plastic material, (f) USE POTH, any other plastic items, including elastics, dense rubber, balloon pieces and soft airgun bullets, (g) OTHER, all non-plastic marine litter, such as cigarette butts, newspapers, rubbish and hard pollutants and (h) FOO, remains of the turtle's natural diet

Source: Matiddi et al. (2019).

The fraction of marine litter and the organic fraction should be dried at room temperature or in an oven at 35 °C for 12 hours. Both fractions should be weighed, including individually weighing the different categories of items identified within the marine litter fraction.

8.4.4.1.1 Extraction of data

Abundance by mass (weight in grams, accurate to second decimal place) is the main information that is useful for monitoring programmes.

Other information that is useful for research and impact analysis includes, the colours of litter items; the volume of litter; the different types of litter; the incidences of different litter in the oesophagus; intestine and stomach; and the incidence and abundance by number per litter category. Other uses of the data set are reported by INDICIT consortium (2018) and Matiddi et al. (2017, 2019).

8.4.4.1.2 Size range

Litter should be ≥ 1 mm (stomach contents are rinsed over a 1 mm mesh sieve).

It is optional to separate microlitter items (1–5 mm) from mesolitter and macro litter items; it is possible to superpose a sieve of 5 mm mesh on the 1 mm sieve.

8.4.4.1.3 Spatial coverage

Dead sea turtles are collected from beaches or at sea; they are often collected because of accidental mortalities, that is, they are victims of longline fishing (bycatch) or of boat collisions, for example.

8.4.4.1.4 Survey frequency

Continuous sampling is required. A sample size of 50 turtles or more is recommended for generating annual averages for the chosen assessment area. For reliable conclusions on change or stability in ingested litter quantities, data over periods of 3–6 years are needed.

8.4.4.1.5 Maturity of the tool

The tool is mature at this stage. Specific monitoring programmes are required. The INDICIT consortium collected more than 1 000 data records, from the international established network, and various countries (Spain, France, Italy) are carrying out national monitoring programmes.

8.4.4.1.6 Regional applicability of the tool

The tool is applicable to the MSFD marine regions where loggerhead sea turtles (*Caretta caretta*) occur, in particular the Mediterranean Sea countries and a part of the Atlantic east coast, but not the Black Sea.

8.4.4.2 Optional protocol for application for sampling litter excreted by live sea-turtles (faecal pellet analysis) in the case of finding a specimen alive

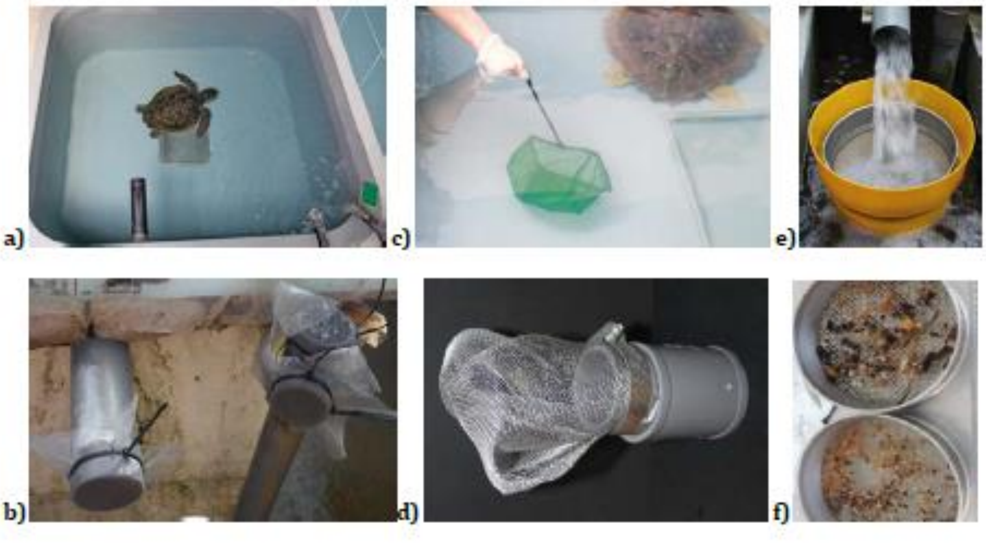
To ensure homogeneity of approaches and allow the comparability of turtles and regions over time, the collected faeces will be analysed only for the individuals remaining in the rescue centre for a minimum of 1 month (Figure 8.5(a)). The faeces are collected for 2 months after the arrival of the individual.

At the rehabilitation facility, the morphologic parameters should be recorded, and the animal placed in the rehabilitation tanks. The standard CCL, notch to tip (Bolten, 1999) is mandatory, while other measurements are optional (e.g. curved carapace width, weight). In most cases, the observed standard time for GI transit is approximately 1.5 months after the first evacuation. The faeces should be sampled from the tank for the entire period of hospitalisation. A 1 mm filter should be placed in all the discharge tubes of the tank (Figure 8.5(b)).

The water tank should be controlled daily by filtering water through the 1 mm mesh sieve according to the following method:

- collect the faeces manually with a 1 mm mesh dip net (Figure 8.5(c));
- put a flexible 1 mm mesh collector in the drain tube (Figure 8.5(d));
- place a rigid 1 mm mesh sieve under the drain (Figure 8.5(e)).

Figure 8.5. Sequence of faeces sampling: (a) the turtle is placed in an individual tank, (b) 1 mm mesh sieves are placed in discharge tubes, (c) a 1 mm dip net for handling faeces, (d) collector with 1 mm mesh placed in discharge tube to filter the water tank (e) a 1 mm mesh rigid sieve down discharge tube to filter the water tank and (f) a sample collected in a rigid sieve



Source: INDICIT consortium (2018).

The digested part of the faeces should be removed by washing the sample with freshwater through 1 mm filter mesh and drying the retained fraction at room temperature. To analyse the litter content and identify the different categories, the same approach as that used for the dead turtle stomach content should be followed and using a similar template.

8.4.5 Estimation of costs

Monitoring activities should be conducted by institutes or rescue centres already authorised and equipped for turtle recovery and necropsy.

A cost estimate for sea turtle litter monitoring is difficult to estimate due to the different national network organisations and the local salary of the involved people. Considering only the average time spent collecting the sample, performing the necropsy, and identifying and analysing the ingested marine litter, monitoring will require:

- at least 50 samples for a country in each subregion;
- two people for 2 days for each sample (200 person-days);
- 3–6 years of monitoring.

8.4.6 Quality assurance / quality control

The previous gaps in QA/QC due to the lack of long-term monitoring programmes have been filled by scientific results in recent years (Camedda et al., 2014; INDICIT consortium, 2018; Matiddi et al., 2011; 2017; 2019).

Specific long-term monitoring programmes are required.

8.5 Protocol for litter ingestion by marine mammals

8.5.1 Protocol name

MSFD protocol for the monitoring of litter ingested by marine mammals (cetaceans and pinnipeds).

8.5.2 Protocol description

The methodology of this protocol follows the methods described in the literature based on the work of responsible bodies for the monitoring of microlitter, mesolitter and macro litter ingested by marine mammals, such as the IWC and ACCOBAMS/ASCOBANS. The amount of macro litter and microlitter in marine mammals can be used to measure trends and regional differences in marine macro litter and microlitter in EU waters and to monitor the impacts of anthropogenic litter on marine mammals and their habitat.

8.5.3 Related marine compartments

Marine mammals hunt and feed in all compartments of the sea: at the surface, in the water column and close to the seafloor (deep-diver cetacean species). Furthermore, they prey on fish of different size classes based on different feeding habits (ranging from filter-feeding species to top predators). As marine litter is affecting marine mammals, no matter in which aforementioned compartments they occur, they all need to be addressed when it comes to macro litter and quantifying litter in the GI tract of marine mammals.

8.5.4 Technical requirements

For marine mammals, impacts from marine litter can be divided into (i) those arising from entanglement in macro litter (see Section 8.8), which can result in injury, drowning or strangulation, and (ii) those arising from ingestion of microlitter and macro litter (both direct and secondary from prey), which can have no effects or having severe direct effects, such as blockage of the digestive tract, suffocation, starvation due to a perceived feeling of satiation and inflammation or even perforation due to sharp objects (Unger et al., 2017; Fossi et al., 2018a). This section focuses on the effects from ingestion. Sublethal impacts include injury, compromised feeding and digestion, associated impacts on malnutrition, disease, reduced reproduction, growth and longevity and generally reduced fitness (McCauley and Bjorndal, 1999; Katsanevakis, 2008; Moore et al., 2013; Werner et al., 2016). While individual strandings provide indications of the range of pathology that can occur, the evaluation of the frequency and severity of impacts of marine litter on cetaceans is complicated. It can be assumed that the number of unrecorded cases is high since devitalised individuals in particular die offshore without reaching the coastline and being available for necropsies. Depending on the presence of a well-established stranding network, the sample size, and thus the detection rate, is low (with only 0–6.2 % of cetacean carcasses recovered from the sea out of the total of estimated mortalities).

A list of suggested species for the monitoring of ingested litter will not be provided here. However, the most representative ones from an ecosystem perspective and from their state of conservation (International Union for the Conservation of Nature (IUCN) – Red List of Threatened Species) should be considered. These may include deep diver cetacean species (*Physeter macrocephalus, Ziphius cavirostris*), coastal and pelagic odontocetes (*Tursiops truncatus, Phocoena phocoena, Stenella coeruleoalba, Delphinus delphis*), mysticetes (e.g. *Balaenoptera physalus, Megaptera novaeangliae*) and several pinniped species.

Ingestion of plastic litter have been documented in over 60 % of all cetacean species, with species employing a variety of feeding techniques in different compartments of the water body (Baulch and Perry, 2014; Kühn et al., 2015; Fossi et al., 2018b). Items ingested are most commonly plastic and range in size from small fragments (< 5 mm) to large plastic items and netting. Pathology can range from no discernible impact to complete obstruction of the digestive tract. When analysing species reported to have ingested marine litter, 50 out of 86 species (58.1 %) had at least one case of ingestion documented (relative to the number of species rather than in terms of the number of individuals being necropsied). Baulch and Perry (2014) stated that a relatively low number of stranding networks are currently established for collecting data on the rates of marine litter ingestion. More recently, Fossi et al. (2018b) published a compressive assessment of more than 86 papers on the impact of the ingestion of marine litter on a variety of cetacean species (Table 8.3).

Table 8.3. Number of cetacean species with documented records of ingested marine litter

	Family	Species total (n)	Ingestion	
			n	%
Baleen whales (*Mysticeti*)	Balaenidae	4	2	50
	Neobalaenidae	1	1	100
	Eschrichtiidae	1	0	0
	Balaenopteridae	8	5	62.5
Toothed whales (*Odontoceti*)	Physeteridae	1	1	100
	Kogiidae	2	2	100
	Ziphiidae	22	14	63.6
	Pontoporiidae	1	1	100
	Monodontidae	2	1	50
	Phocoenidae	7	4	57.1
	Delphinidae	37	19	51.4
	Total	86	50	58.1

Source: Adapted from Fossi et al. (2018b).

The study of microplastic ingestion by cetaceans is a challenging task due to (i) the handling of large volumes of gut contents in particular for large cetaceans, and (ii) the limitation in the availability of precise sample handling when it comes to the avoidance of secondary pollution (Philipp et al., 2020, 2021).

8.5.4.1 *Current existing protocols for and approaches to the analysis of the impacts of marine litter in stranded organisms*

Protocols for the analysis of marine litter in stranded marine mammals were developed at a workshop hosted by the IWC in 2013; these protocols were recently reviewed according to the existing protocols for other marine taxa (Lusher et al., 2017a, 2018; Fossi et al., 2018b, 2020). A new multidisciplinary approach has also recently been proposed by Corazzola et al. (2021).

In situ examination of entangling and ingested debris and associated traumatic injuries is essential for revealing the pathologic impacts of fishing gear and debris on cetaceans. Impacts can include laceration, amputation and constriction-related injuries externally, and/or blockage, strangulation, ulceration, impaction, emaciation and rupture internally (Unger et al., 2017). Evidence of chronic effects (e.g. emaciation) or prior trauma from entanglement and debris interaction, where material is no longer present, can also be identified as suspected through clinical or post-mortem examinations by scientists. Furthermore, the potential chemical exposure should also be evaluated, which can be accompanied by gross or histologic changes due to the transfer of additives and priority pollutants sorbed from the plastic into the tissues (Rochman et al., 2013; Fossi et al., 2016). Based on the protocols developed during the IWC workshop, recommended procedures are given in the following section for assessing marine litter impacts in stranded cetaceans.

The methodology proposed in this document has already been integrated into the related protocol that was developed by a joint ACCOBAMS and ASCOBANS workshop on harmonisation of the best practices for necropsy of cetaceans and for the development of diagnostic frameworks (Padua, Italy, 24–25 June 2019).

8.5.4.2 *Recommended diagnostic approach*

To evaluate possible impacts caused by ingestion, a standardised methodology and a classical differential diagnostic approach need to be applied to ensure the comparability of the information collected.

- Investigation of possible traumas, chemical exposure and other sequelae related to the exposure should be conducted.
- Analysis of their role in contributing to morbidity and mortality in the context of other potential causes, such as infectious or non-infectious diseases, nutritional status and other possible ecologies, should be conducted. If a full differential diagnostic approach is not feasible, the documentation of marine litter presence, either external or internal, is still very important. Most studies focus on macroplastics since they are visible and easily accessible. Nevertheless, efforts should also be made to document microplastic occurrence, especially to monitor trends in secondary pollution from prey species.

All necropsies of stranded marine mammals should include the following components, as appropriate.

- Necropsy and reporting. This should include descriptions, sketches, images, measurements, collection, and preservation of entanglement/debris and affected body part(s). The entire gastrointestinal tract should be opened and examined. Standard cetacean necropsy protocols should be followed (McLellan et al., 2004; Pugliares et al., 2007; Moore and Barco, 2013). In the case of microplastic investigations special care needs to be taken to keep the risk of contamination as low as possible.
- Item characterisation. If possible, the object should be named as rope, net, packaging, a cigarette butt or other anthropogenic material. Furthermore, the size (measurement on side) and shape (image analysis of digital photographs) are of importance. If applicable, it is advisable to identify the polymer type of plastics by either Raman spectroscopy or FTIR. All pieces of evidence should be identified using established techniques (Browne et al., 2010) to narrow down the sources and pathways. This information is important for engagement with the relevant industries and sectors, such as plastics and fishing, to establish solutions for minimising the risk of additional litter input into the marine environment.
- Confirmatory diagnostics. To document the presence and the type of items ingested and entangled in, and possible impacts on the animals, further analyses should be undertaken as practical and indicated. This includes histopathology, imaging, analytical chemistry, blood tests and organ function tests. It would be advisable to provide resources to develop techniques for identifying particles of plastic in the tissues of animals. Criteria for the assignation of the degree of confidence of findings (e.g. quality of data) of ingestion or entanglement contributing to or causing morbidity

and mortality have been published and should be applied (Moore et al., 2013). The chain of custody documentation should be maintained as required if applicable.

- Training and database creation. Training designed for specific countries and regions, and establishing a global database and ensuring its maintenance would both enhance the understanding of these problems and help to establish solutions to avoid marine debris input and subsequent impacts.
- Categorisation of contents. The categorisation of GI contents is based on the general morphology of plastic items found, that is, sheetlike, filament, foamed, fragment or other (see list given in Table 8.1). In most cases, smaller fragments will not be unambiguously related to a defined item. However, if possible, items should be described and assigned to a litter category number using the *Joint List of Litter Categories for Marine Macrolitter Monitoring* developed by the MSFD TG ML (Fleet et al., 2021).

For each litter category/subcategory an assessment is made of the:

- incidence (percentage of investigated stomachs containing litter);
- abundance by number (average number of items per individual);
- abundance by mass (weight in grams, accurate to third decimal place).

8.5.4.3 Litter categories – source related information

Categorisation of ingested litter items is essential for understanding their source, distribution, and impact on marine mammals. For marine mammal analyses, stomach contents are sorted into the same categories given above for seabirds (Section 8.3). Following the protocol for seabirds and sea turtles, abundance by mass (weight in grams, accurate to third decimal place) is the main information of use for a standardised monitoring programme. Other information that is useful for research and impact analysis includes, the colour of items, the volume, the different types of litter; the incidences of litter in the oesophagus, intestine and stomach; and incidence and abundance by number per litter category.

8.5.4.4 Size range

Litter should be ≥ 1 mm (stomach contents are rinsed over a 1 mm mesh sieve).

8.5.4.5 Spatial coverage

Dead marine mammals are collected from beaches or at sea; they are often a result of accidental mortalities such as mass stranding (Unger et al., 2016) of bycatch in fishing gear (e.g. victims of longline fishing) or of boat collisions. If not available, the establishment of a national stranding network should be pushed forward and connected at the international level. Furthermore, to establish stranding networks in different countries, it would be advisable to draw on the expertise of countries that already have a stranding network. This helps to keep the data collected consistent and allow for analysis on a global scale.

8.5.4.6 Survey frequency

Continuous sampling is required. A minimum sample size (for the identified species) per year and season must be established in order to draw reliable conclusions on trends or stability in ingested litter quantities.

8.5.4.7 Maturity of the tool

The tool is not mature at this stage. Specific monitoring programmes are required.

8.5.4.8 Regional applicability of the tool

The tool is applicable to the MSFD marine regions where marine mammals suitable for monitoring occur, the Greater North Sea, the English Channel, the Celtic Seas, and the Mediterranean Sea.

8.5.5 Estimation of costs

Owing to the lack of dedicating monitoring programmes at the national level, the cost of monitoring litter on marine mammals is difficult to estimate at this stage. The costs are also related to the dimensions of the species analysed, the proximity to the laboratory where analysis/dissection is carried out, and the cost of disposal of the carcasses. Cost to be intended per single marine mammal stranding networks in an assessment area and monitoring programmes can be integrated with National stranding monitoring, where available.

8.5.6 Quality assurance / quality control

There is a lack of QA/QC due to a lack of monitoring programmes. The data available are poor quality and based on only a few years (Baulch and Perry, 2014; Kühn et al., 2015 Lusher et al., 2015, 2018; Fossi et al., 2018b, 2020; Corazzola et al., 2021). Only in some cases is it possible to analyse a large time series retrospectively (Unger et al., 2017).

8.5.6.1 Trend assessment

Specific long-term monitoring programmes are required.

8.5.6.2 Target definitions

Specific targets have to be developed, for example, based on the OSPAR Commission recommendation for seabirds (see Section 8.3).

8.6 Protocol for microlitter ingestion by fish

8.6.1 Protocol name

MSFD protocol for the monitoring of microlitter ingested by marine fish.

8.6.2 Protocol description

The methodology of this protocol follows the INDICIT II EU project guidelines for monitoring microlitter particles in the stomachs of marine fish. Background information and technical requirements are described in detail by Matiddi et al. (2021), where the main literature on this topic is also reported. A pilot study evaluating methods and potential sources of bias was conducted during the INDICIT II project by ISPRA (Italy), FRCT (Portugal), CNR-IAS (Italy), EPHE (France), INSTM (Tunisia), HCMR (Greece), EOMAR-ULPGC (Spain), PAU DEKAMER (Turkey), UNIVPM (Italy) and with the results to be published in peer-reviewed scientific literature.

8.6.3 Related marine compartments

Recent studies have highlighted that the feeding habits of different fish species influence microlitter ingestion rates (Lopes et al., 2020) and the analytical methods needed for particle identification (Bianchi et al., 2020). Moreover, the distribution of microlitter items in the marine environment varies according to their shape, size and chemical composition (Palazzo et al., 2021), while several environmental factors (e.g. waves, tides, and currents) on different geographical scales contribute to defining different accumulation pathways for different marine litter types (Angiolillo et al., 2021).

As a result, more than one fish species must be selected for describing the microlitter contamination of the marine environment (Valente et al., 2022; 2023). Some considerations based on previous experiences and recent studies confirm that different fish species are needed to assess all three marine habitat compartments (benthic, demersal, pelagic).

8.6.4 Technical requirements

Samples should be collected and assessed directly on board, checking the fish for any disease and ensuring that any fish showing signs of net feeding or regurgitation are rejected (by checking in the mouth). To avoid any bias due to the regurgitation of plastic items caused by the expansion of the swim bladder, it is recommended to reject all fish with an everted stomach (Figure 8.6) or completely empty stomach (Lusher et al., 2017). All individuals should be rinsed with ultrapure water and frozen upon collection. Samples collected at a fish market or shop are not allowed. Fish can be stored frozen until analysis.

Standardised dissection methods for fish and stomach analysis have been published by the INDICIT II project in dedicated guidelines (Matiddi et al., 2021), and these are summarised here.

Figure 8.6. (a) A normal fish stomach and (b) an everted fish stomach, unsuitable for analysis

(a) (b)

Source: Valente, T.

To reduce the possible variability in microlitter ingestion due to differences in the feeding behaviour of fish during different life stages (e.g. juveniles/adults), choosing comparable individuals (e.g. similar size and/or life stage for the species) is suggested.

Several methods and protocols have previously been applied to assess microplastic ingestion by fish (Lusher et al., 2017). The most accurate procedures involve the digestion of the entire gastrointestinal tract with its content (Bianchi et al., 2020), typically by using potassium hydroxide (KOH) (Box 8.1) or hydrogen peroxide (H_2O_2) (Box 8.2).

Box 8.1. Digestion steps using KOH

Th digestion steps according to Rochman et al. (2015) (modified) are as follows:

— add KOH (10 % weight/volume, 3 × tissue volume) to a beaker;

— optionally, incubate samples using a hot plate, hot bath or oven (≤ 40 °C) to increase digestion speed;

— optionally, neutralise the digestate before filtration by adding 1 M citric acid solution (Thiele et al., 2019);

— use a blank sample to test for possible ambient contamination by adding a similar volume of 10 % KOH as that used in the samples to a beaker without samples (follow the protocol as normal).

The use of enzymes or other methods to degrade bio-organic materials are not reported due to their high costs and the procedural complexity, but they are considered viable alternatives.

It should be noted that both KOH and H_2O_2 could affect plastic particle structures, morphology and colour. For this reason, water baths should be maintained at no more than 40 °C and digestion should not proceed for more than 5 days. It is recommended that the temperature and time of digestion be reduced based on the organic digestion rate. The use of other reagents is possible, but their potential to cause plastic corrosion should be pre-assessed before analysis (Bianchi et al., 2020; Valente et al., 2022).

Box 8.2. Digestion steps using H_2O_2

The digestion steps according to the MEDSEALITTER project (modified) are as follows:

— for each gram of GI, gradually add 20 ml of H_2O_2 (15 %) into the beaker. Use two aliquots if the GI is ≤ 2 g or more aliquots if the GI is ≥ 2 g;

— optionally, add HNO_3 up to 5 % to increase tissue degradation (Bianchi et al., 2020);

— Incubate samples using a hot plate, hot bath or oven (≤ 40 °C), adding supplementary 15 % H_2O_2 when evaporation occurs, until all organic matter is digested (see Section 8.6.4);

— Add 100 ml of distilled H_2O and stir using a magnetic stirrer;

— Use a blank sample to test for possible ambient contamination by adding a similar volume of 15 % H_2O_2 as that used in the samples to a beaker without samples (follow the protocol as normal).

To standardise the data, pre-filter the solution through a 100 μm sieve, under a laminar flow cabinet, collecting all the material by washing the sieve with ultrapure water. Carefully check the sieve for any possible micro particles remaining. Using a vacuum pump, filter the material retained by the sieve onto a glass fibre membrane, Anodisc or other membrane (i.e. silver, gold) with a mesh size of < 100 μm. Rinse the glass funnel above the membrane with ultrapure water. Place the membrane into a glass Petri dish and cover with a glass top.

Place the Petri dish in a clean cupboard to dry the membrane at room temperature. Detect the number and position of the fibres on the membrane using a stereomicroscope, before opening the dish to avoid airborne contamination during the counting of the fibre microparticles. Note the position of the particles that should be checked. Detect all the other types of microliter items under the stereomicroscope.

The polymer identification is a very important step to distinguish synthetic polymers from any remaining items of natural origin (e.g. organic fibres) and is included in the new Commission Decision (Decision (EU) 2017/848). For example, organic and inorganic particles derived from a natural diet (fish scales or bones, crustacean exoskeletons, etc.) can often be confused with plastics. Spectroscopy techniques offer the most robust polymer identification for suspected microplastic particles, but this requires expensive equipment and is a time-consuming activity that needs personnel with high level of expertise.

Particles of uncertain origin and composition that are longer than 1 mm can be tentatively identified as microplastics using an optical microscope or a hot needle test. However, a minimum of 10 % of the collected items should be analysed and verified using FTIR, Raman spectroscopy (Galgani et al., 2013) or other suitable spectroscopic techniques (e.g. quantum cascade laser spectroscopy).

Textile fibres are ubiquitous, and many laboratories are not well equipped to completely avoid this secondary source of contamination. According to the MSFD TG ML (Galgani et al., 2013), secondary contamination must not exceed 10 % of the results. Avio et al. (2020) proposed that if the blank is contaminated, microlitter items with similar characteristics (shape, colour, polymer type, size) should be excluded from the results (i.e. the specific microlitter type found in the blank control, should be subtracted from the same specific microlitter type value in the samples of the same batch). Some steps to reduce airborne contamination are reported in Box 8.3.

Box 8.3. How to reduce airborne contamination

The following guidelines are useful to limit levels of contamination:

— close the window and reduce personnel in the laboratory;

— during the procedure of dissection and filtration, process samples under a laminar flow cabinet or glove box (Torre et al., 2016);

— keep the 100 µm sieve clean and protected from air pollution;

— during stereomicroscopy observation of the membrane, cover Petri dishes with a glass dish, cover the stereomicroscope and perform any manipulation under the cover (Torre et al., 2016);

— dress only in cotton clothing;

— use only glass and metal labware, where possible;

— clean all equipment with ultrapure water before each sample analysis;

— perform a blank control at every step, and place a damp filter paper in a Petri dish in the working area to assess any airborne contamination;

— adjust field results according to a blank subtraction approach (Avio et al., 2020).

8.6.4.1 Litter categories – source related information

Even if the new Commission Decision (Decision (EU) 2017/848) only asks for the categorisation of microlitter items comprising artificial polymers, a better categorisation is proposed for data comparison and source identification. A specific template for data collection is proposed in Annex IX – 'Template for data collection for microlitter ingestion by fish' with basic and optional information. Fibres are ubiquitous and generally represent 70-90 % of the total number of microlitter items extracted from fish, but they are not always composed of synthetic material (Avio et al., 2020). Fibres are thought to originate primarily from textiles, and it is currently under discussion if they should be placed in a separate category to filaments (e.g. fishing line) (See also Section 7.4.3). It is also yet to be decided whether beads are to be reported as a single category or included in the category 'granules', with the (smaller) dimension compared with resin pellets. Pellets and granules are also being evaluated as to whether they should be categorised individually.

The following categories, which are based on those first proposed by Kovac Viršek et al. (2016) and later modified by Matiddi et al. (2021), should be used for microlitter ingested by fish.

- Filament. This is a threadlike artificial polymer element that is elongated, generally derived from the fragmentation of fishing gear fragmentation.
- Fibre microparticle, only from textile. This can be short or long, with different thicknesses and colours. It can be made of artificial polymer, be semi-synthetic or be made from natural materials (e.g. wool, cotton, rayon). Note that it is under discussion if fibre microparticles should be categorised in a separate category from filaments.
- Film-layer, foil. This appears in irregular shapes. Compared with a fragment, it is thinner and more flexible. It is derived from sheets or thin films.
- Fragment. This is rigid and thick, with sharp crooked edges and an irregular shape. It can come in a variety of different colours.
- Pellet. This is only from industrial origin. It is usually flat on one side and can be of various colours, be in an irregular or round shape, and is normally bigger in size, around 5 mm in diameter.
- Granule. This comes in a spherical shape, in comparison with a pellet. A granule has a regular round shape and usually a smaller size, around 1 mm in diameter. It appears in natural colours (white, beige, brown). Note that it is under discussion if granules should be categorised in a separate category from pellets.

- Foam. This is flexible microlitter particles in which material cells are all or partly intercommunicating (ISO/TR 20342-7:2021). It most often comes from large particles of plastic foam (including expanded polystyrene and extruded polystyrene foams).

8.6.4.2 Extraction of data

To collect comparable data across different European countries, the INDICIT consortium developed a specific dataset with optional and mandatory information to be collected (Annex IX – 'Template for data collection for microlitter ingestion by fish'). While the main information to be reported is the number of fish with at least one ingested item out of the total number of fish samples, all the other required information is useful for research purposes and for analysis of impact on animal health.

8.6.4.3 Size range

Different definitions of microlitter and microplastics have been proposed:

- 'all sorts of small manmade particles, less than 5 mm in two of the three dimension or diameter, that pass t[h]rough a 5 mm mesh screen but are retained by a lower one';
- 'all sorts of small particles of plastic less than 5 mm in two of the three dimension or diameter that pass through a 5 mm mesh sieve but are retained by a lower one', proposing to fix the lower limit for micro items at 100 µm.

To harmonise sample collection and data comparison, microlitter is defined as particles of < 5 mm in the maximum length, excluding fibres **of ≥** 5 mm. The lower limit for monitoring microlitter in biota is fixed to 100 µm.

Alternative size classes have been proposed by Valente et al. (2019) and Matiddi et al. (2021), where the lowest limit is harmonised according to the BASEMAN proposal (Frias et al., 2018) for monitoring microplastic in sediments (100 µm), and the size classes from 330 µm up to 5mm (330 µm ≤ x < 1 mm; 1 mm ≤ x < 5mm) are comparable with data coming from microplastic sea surface monitoring, using manta trawls (Galgani et al., 2013).

To maintain harmonisation within the chapter 7 microliter of this guidance, the size classes proposed are modified as follow (Table 8.4)

Table 8.4. Proposed size classes for marine litter monitoring

Size classes	From	To
Size class 1	1000 µm	4999 µm
Size class 2	300 µm	999 µm
Size class 3	100 µm	299 µm

Source: Modified from Valente et al. (2019) and Matiddi et al. (2021).

8.6.4.4 Spatial coverage

To date, it has not been possible to identify a single target species that is representative for all the MSFD marine waters. Many target species have been proposed for the Mediterranean Sea (Fossi et al., 2018a; UNEP/MAP SPA/RAC, 2018; Bray et al., 2019), deep-water habitats (Alomar and Deudero, 2017; Valente et al., 2019), the Atlantic Ocean (Herrera et al., 2019; Pereira et al., 2020) and the North Sea (Kühn et al., 2020). A wide intercomparison of the Mediterranean Sea, the Black Sea, the eastern Atlantic Ocean and the northern European seas should be planned.

8.6.4.5 Survey frequency

Continuous sampling is required even if differences in ingestion rate in respect of different seasons should be considered. The number of sampling stations must be representative of the entire area assessed (e.g. national sub-region). The number of collected specimens per sampling station must not be lower than 30 individuals per species, to combine the right levels of effort and statistical analysis (Di Giacomo and Koespell, 1986). Assessment areas and sampling stations should be planned locally according to the heterogeneity of the RSCs.

For very clean areas (i.e. scarce microplastic sources of pollution), it is necessary to increase the number of fish to 50 individuals. Considering that three environmental compartments (i.e. benthic, demersal and pelagic) should be investigated for each area, at least 90 individuals (30 individuals × 3 species) per sampling station must be collected.

8.6.4.6 Maturity of the tool

The tool is not mature at this stage. Specific monitoring programmes are required.

To reduce possible variability in microlitter ingestion due to the variation in the feeding behaviour of fish during different life stages (e.g. juveniles versus adults), it is suggested that comparable individuals be chosen, fixing the fish size around the size of first maturity. However, more studies are needed to investigate the relationship between microlitter ingestion and the ontogenetic stages of different species.

8.6.4.7 Regional applicability of the tool

The tool is applicable to all the MSFD marine regions.

8.6.5 Estimation of costs

To reduce costs associated with sampling, it could be possible to collect samples from ongoing monitoring programmes, such as fish stock assessments cruises (e.g. MEDITS, SOLEMON, ICES-DATRAS, etc.). EU DCF surveys could be used as a platform to conduct sampling of the target species.

8.6.6 Quality assurance / quality control

Specific long-term monitoring programmes are required.

Background contamination is one of the major issues affecting the reliability of ingested microlitter quantification (Prata et al., 2021). It is therefore necessary to reduce airborne contamination with some specific procedures. For example, samples must be processed under a laminar flow cabinet or glove box (Torre et al., 2016). Similarly, during stereomicroscopy observation of the membrane, Petri dishes must be covered by a glass dish. Whenever possible, only glass and metal labware must be used. A blank control must be performed at every step.

Following Avio et al. (2020), field results should be adjusted according to a blank-subtraction approach, where microlitter items with similar characteristics (shape, colour, polymer type, size) should be excluded from the results (i.e. the specific microlitter type found in the blank control should be subtracted from the same specific microlitter type value in the sample in the same batch).

8.7 Protocol for microlitter ingestion by mussels

8.7.1 Protocol name

MSFD protocol for the monitoring of microlitter ingested by mussels.

8.7.2 Protocol description

The methodology of this protocol follows the methods described in the literature for monitoring microlitter items (< 5 mm) in mussels. The microlitter content in mussel body can be used to measure trends (spatial and temporal) and regional differences in marine microlitter.

8.7.3 Related marine compartments

The tool is proposed for application for benthic filter-feeding mussels, such as blue mussels, oysters and clams in shallow coastal waters (water depth of < 5 m). Therefore, the water column and the seafloor compartments of the marine environment are addressed when quantifying microlitter in the tissue of different mussel species.

8.7.4 Technical requirements

Microlitter in mussels has been investigated in a number of studies (Van Cauwenberghe et al., 2015; Lusher et al., 2017; Karlsson et al., 2017; Catarino et al., 2018; Li et al., 2018; Phuong et al., 2018; Waite et al., 2018; Reguera et al., 2019) and previous European projects focused on harmonising methods to use this organism in microplastic monitoring (Bessa et al., 2019). To date, however, there is no agreed protocol for sampling and subsequent laboratory analyses. Compared with the monitoring of motile marine animals, the monitoring of microlitter in mussels is advantageous, because mussels can be used and sampled with low logistic and financial efforts. Alternatively, where mussels are abundant over long periods, exposure in cages is unnecessary, and mussels can be sampled directly from the sea.

The following species are proposed as potential indicator species that cover the North and Baltic Seas, the north-east Atlantic Ocean, the Mediterranean and the Black Sea: blue mussels (*Mytilus edulis* (L.)), Mediterranean mussels (*Mytilus galloprovincialis* (L.)) and European flat oysters (*Ostrea edulis* (L.)). Further species can be considered for microlitter monitoring (i.e. the Baltic clam (*Limecola balthica* (L.))), which is an infaunal bivalve, living buried in the mud or silt and extending its siphons to the bottom surface. Through the siphons it feeds on organic matter on the sediment surface. Therefore, monitoring microplastics in *Limecola balthica* can provide information about microplastic ingestion from the sediment surface.

It is recommended that mussels be deployed in cages for 3–4 weeks (Catarino et al., 2018) to achieve a steady state of microlitter concentrations in the mussels between feeding and excretion. The cages should be fixed to the sea bottom, and the positions are recorded by means of a GPS. It is recommended that the cages not be marked with buoys, as this can lead to removal of the cages by fishers.

The mussels should be from natural populations from the region where the monitoring is being conducted. The depth should lie between 3 m and 5 m for blue mussels, and each cage should contain five to six specimens, which should subsequently be pooled for analyses in the laboratory (Lusher et al., 2017). A larger number of individuals is necessary for mussel species smaller than blue mussels. To cover the small-scale spatial variability of microlitter concentrations in seawater and mussels, there should be at least three replicate cages at each location.

Ideally, water adjacent to the cages should be sampled and subsequently analysed for microlitter in parallel to the mussels. Parallel sampling allows for bivariate correlation analyses between concentrations in the two compartments. A significant good positive correlation would provide evidence that the mussels are appropriate indicators of microlitter pollution in ambient seawater. It is recommended that the sample water be sampled at least two times during deployment (i.e. at the beginning and at the end of the deployment period). Water sampling is done using a vacuum filter pump and a micro-fibre filter (grade GF/D, 2.7 μm) from onboard a boat (Lusher et al., 2017). The suggested volume of filtered water is approximately 1000 l, which should ensure a sufficiently high abundance of microplastic particles.

After deployment, the mussels are sampled and transported to the laboratory in a cool and moist state. In the laboratory, the size of the individual mussels is determined, the shells and the byssus filaments are removed, and the wet weight of each mussel pool, consisting of the tissue of several individuals, is determined (accurate to the fourth decimal place). Afterwards, the tissue is frozen, pending digestion of the samples. Alternatively, directly after transport to the laboratory, the mussels can be frozen pending further treatment and analysis.

Sample treatment and analyses follow the recommendations of Lusher et al. (2017), who performed investigations on microplastics in blue mussels in Norwegian marine waters. In the laboratory, the water filtrates and pooled mussel tissue (i.e. of five to six individuals for blue mussels) are treated with 10 % KOH solution in glass jars. The glass jars are incubated in an oscillation incubator at 60 °C and 145 rpm for 24 hours. Subsequently, vacuum filtration is carried out using glass fibre filters. Afterwards, the filters are dried at room temperature for 72 hours prior to analyses with a FTIR spectrometer.

Enzymatic digestion is a viable alternative to 10 % KOH (von Friesen et al., 2019), but the protocol could be more expensive and not possible for all users.

It is recommended that FTIR spectroscopy be used for analyses, but in the absence of a FTIR spectrometer, other methods, such as Raman spectroscopy or the Nile red method (Maes et al., 2017), can be applied. For every step of sample transport and treatment, blank samples have to be taken to account for contamination, and all lab equipment should be rinsed threefold with filtered water to minimise contamination.

For each litter category, an assessment is made of the:

- abundance of litter items (average number per individual);
- abundance by mass (average number per weight in grams of mussel pools, accurate to the fourth decimal place).

Owing to potential temporal variations, it is recommended to describe 'current levels' as the average for all data from a location from the most recent 5-year period, in which the average is the sample average, also including subsamples that were found to contain no microlitter.

8.7.4.1 Litter categories

The following categories should be used for microlitter ingested by mussels, which are based on those first proposed by Kovac Viršek et al. (2016) for sea surface monitoring and latter modified by Matiddi et al., 2021:

- Filament. This is a threadlike artificial polymer element that is elongated, generally derived from the fragmentation of fishing gear fragmentation.
- Fibre microparticle, only from textile. This can be short or long, with different thicknesses and colours. It can be made of artificial polymer, be semi-synthetic or be made from natural materials (e.g. wool, cotton, rayon). Note that it is under discussion if fibre microparticles should be categorised in a separate category from filaments.
- Film-layer, foil. This appears in irregular shapes. Compared with a fragment, it is thinner and more flexible. It is derived from sheets or thin films.
- Fragment. This is rigid and thick, with sharp crooked edges and an irregular shape. It can come in a variety of different colours.
- Pellet. This is only from industrial origin. It is usually flat on one side and can be of various colours, be in an irregular or round shape, and is normally bigger in size, around 5 mm in diameter.
- Granule. This comes in a spherical shape, in comparison with a pellet. A granule has a regular round shape and usually a smaller size, around 1 mm in diameter. It appears in natural colours (white, beige, brown). Note that it is under discussion if granules should be categorised in a separate category from pellets.
- Foam. This is flexible microlitter particles in which material cells are all or partly intercommunicating (ISO/TR 20342-7:2021). It most often comes from large particles of plastic foam (including expanded polystyrene and extruded polystyrene foams).

8.7.4.2 Size range

Microlitter is classified into large microlitter (1–5 mm) and small microlitter (< 1 mm). Previous studies revealed maxima in the size distribution of microplastics in mussels of well below 100 µm (Phuong et al., 2018). However, it is costly and difficult to detect microplastic particles of < 50 µm. Therefore, it is recommended that the lower size limit of microlitter in mussels be set to 50 µm.

8.7.4.3 Spatial coverage

For the selection of sampling locations, it is recommended that positions in shallow coastal waters that are remote from any significant sources of microlitter, such as harbours or effluents from waste-water treatment plants, be chosen. This ensures that microlitter concentrations reflect the background, are spatially representative of the water body and, therefore, can be used for comparisons with thresholds and for trend analyses.

8.7.4.4 Survey frequency

Deployment of mussel cages should be done once a year and at the same time of the year, being outside the spawning season for mussels. In temperate regions (i.e. the North Sea, the Baltic Sea and the north-east Atlantic Ocean), monitoring should ideally be performed at the end of summer. In the Mediterranean, sampling should be carried out in spring. Monitoring at the same temporal intervals avoids autocorrelation and bias in trend analyses, in turn evoked by seasonality in the growth and in the feeding rates of mussels.

8.7.4.5 Maturity of the protocol

The protocol is not mature at this stage. Specific monitoring programmes are required. For harmonisation of protocols, methods of trend analyses are recommended to follow statistical analyses applied for the OSPAR EcoQO 'northern fulmar'.

8.7.4.6 Regional applicability of the protocol

The protocol is applicable in coastal waters. The selection of species should be optimised for regional comparison. Wherever possible, overlapping species must be chosen in adjacent areas.

8.7.5 Estimation of costs

The most significant costs arise from sample digestion and clean-up, and from FTIR analyses. The overall estimated costs for one FTIR sample amount up to 1 person-day.

8.7.6 Quality assurance / quality control

The methodology needs to be further developed. At present, there is a considerable lack of QA/QC due to the non-existence of long-term monitoring programmes. The mussel microlitter studies mentioned above have generated only a small amount of data, mostly representing 'snapshots' and which do not currently allow for trend analyses.

8.7.6.1 Trend assessment

Due to the lack of maturity of the tool, specific long-term monitoring programmes have to be developed, generating the sufficiently long time series necessary for trend analyses.

8.8 Entanglement of sea turtles and marine mammals

Different methodologies could be used for monitoring the rate of entanglement. Stranding and photo identification networks or drones are some examples of ways to obtain data on entangled marine animals. The main reason for the lack of data is that in Europe, a great part of marine megafauna (all sea turtles, certain seabirds, and marine mammals) are protected species and their handling require specific permits from national/regional authorities in accordance with applicable regulations. It is challenging to engage stakeholders in data sharing without established conventions and specific agreements. For this reason, the best way to collect data is from official stranding networks or recovery centres, using the same networks involved in the collection of data on marine litter ingestion by sea turtles.

In general, stranding networks make a continuous and almost homogeneous efforts year by year, creating an important source of data about marine fauna threats and impacts. A multitude of professionals and experts are engaged in this process (veterinarians, biologist, environmental authorities, etc.). Multiple parameters are collected to describe the circumstances of each stranding event. Rescue centres are usually associated with or coordinated by stranding networks, so detailed and accurate data about each stranded animal are usually collected.

Another way to collect data is through the activity of citizen science (online platforms), where images are collected by the general public or environmental organisations. This kind of data are not homogeneous, and it is necessary to involve experts to check and catalogue the information from images.

Two protocols are presented to collect data on the entanglement of sea turtles and marine mammals:

- the protocol for the collection of entanglement data from stranding networks or recovery centres (the standard protocol);
- the protocol for the collection of entanglement data from citizen science, with data and images collected by the general public and environmental organisations (the social media protocol).

8.8.1 Entanglement data on sea turtles and marine mammals gathered from stranding network or recovery centres

8.8.1.1 *Protocol name*

MSFD protocol for the monitoring of entanglement of sea turtles and marine mammals from stranding network or recovery centres.

8.8.1.2 *Protocol description*

This protocol to assess entanglement of sea turtles and marine mammals gathered from stranding network or recovery centres was drafted after the TG ML meeting on harm, held at Berlin in 2019, and the Joint ACCOBAMS and ASCOBANS Workshop on harmonisation of the best practices for necropsy of cetaceans and for the development of diagnostic frameworks, which was held in Padua, Italy, on 24-25 June 2019. Several steps were carried out to update this entanglement monitoring protocol and related assessments during the implementation of two European projects: the MEDREGION project (Silvestri et al., 2021) and the INDICIT II project (Loza et al., 2021), the protocols for the collection of data were defined and the collected data were aggregated.

The aim of this protocol is to provide an easy tool for comparing harmonised data and comprehending the impact of marine litter on the marine environment, either globally or on a local scale.

The main points of this protocol are the:

- homogeneous effort;
- data quality (collected by experts);
- small spatial scale of data, depending on the number of stakeholders involved.

Using this protocol, it is possible to collect homogeneous data to assess the impact of marine litter on marine organisms. Two kind of data could be obtained on marine megafauna: (i) the general data (number of stranded/registered animals per year, number of entangled animals per year), to allow the (FO %) of entanglement per region/area and per year to be obtained, and (ii) the individual data (details of the stranding event, characterisation of the litter, impact of the litter on individuals) to allow analysis of the percentage of marine litter items causing entanglement in marine fauna, and the main injuries and impacts caused by entanglement.

8.8.1.3 *Related marine compartments*

This protocol primarily focuses on sea turtles and marine mammals; therefore, the water column and especially the water surface or the seafloor are the marine compartments addressed when quantifying entanglement for:

- sea turtles (mainly water surface):
    - loggerhead sea turtle – *Caretta caretta* (mainly oceanic habitats);
    - leatherback sea turtle – *Dermochelys coriacea* (oceanic habitats);
    - green sea turtle – *Chelonia mydas* (neritic habitats);
- marine mammals (water surface and water column):
    - common bottlenose dolphin – *Tursiops truncatus*;
    - striped dolphin – Stenella coeruleoalba;
    - common dolphin – *Delphinus delphis*;
- other species.

8.8.1.4 *Technical requirements*

In Europe, many marine megafauna (all sea turtles, certain seabirds, and marine mammals) are protected species, and the operations described below will require a permit according to the national regulations, including related to animal welfare. Furthermore, health precautions should always be taken regarding zoonosis risks.

Upon finding of a specimen (either live or dead), the authorised staff should proceed to make an external examination of the specimen at the time and place of discovery, or after being hospitalised (live) or stored (dead) in authorised facilities. The data sheets proposed in Annex X – 'Data sheet for recording individual-specific data for entanglement of sea turtles and marine mammals', Annex XI – 'Data sheet for recording of entanglement data on sea turtles and marine mammals' and Annex XII – 'Data sheet for recording general data (frequency of occurrence as a percentage) for sea turtles and marine mammals' are designed mainly with boxes to be ticked to help in recording the requested data. In order to complete the data collection, it is recommended to attach any available post-mortem and/or hospitalisation veterinarian report to the sea turtle entanglement data sheet. Taking pictures is essential for documenting the level of impact of entanglement by litter. The pictures should be carefully codified and stored. For better identification of the categories of impact, participants at the TG ML meeting in Berlin on 21-22 May 2019 recommended the preparation of a photographic atlas, which might be continuously updated thanks to the contribution of European teams in charge of sea turtle monitoring.

8.8.1.4.1 Data collectors (stakeholders)

Stakeholders could be all kinds of organisations/institutions in charge of the stranding networks in a region/country, under environmental authority permits, which make homogeneous efforts over time and include trained staff available for data collection (veterinarians, biologists, public staff with environmental backgrounds, experts, trained volunteers, etc.).

Stakeholders are composed of the following groups:

- local/regional/national stranding networks (coordinated by environmental authorities);
- public/private rescue centres in charge of or associated with stranding networks;
- public/private research institutions in charge of stranding networks (under official permits);
- NGOs managing stranding networks under official permits;
- other organisations involved in or collaborating with stranding networks and rescue centres or involved in marine animal colony monitoring (e.g. of seabirds, seals).

Two kinds of data have been included in the standard protocol:

General data (required from each stakeholder). These are used to obtain the FO% of entanglement per region and per year. They include the following:

- area covered (by the stakeholder);
- number of total stranded/registered animals per year;
- number of entangled animals per year.

Individual data and entanglement data. These are used to obtain accurate and extended information on the impact of entanglement on marine fauna. Specific data from each litter typology should be obtained to identify the main types of litter involved in entanglement per region, spatial and temporal variations, the taxa and species affected, and the impact generated.

Several parameters must be collected from each entangled individual; these are split into four sections.

- Stranding even characterisation. This covers date, location, circumstance, etc.
- Individual characteristics. This covers, size, sex, conservation status (if dead), etc.
- Litter characterisation. This is used to classify and characterise the litter involved.
- Litter impact. This is the parameter 'impact severity' developed by the INDICIT II consortium, based on the effect of injuries/lesions caused by entanglement on animal viability.

All data are described in the data sheets disposed in Annexes X, XI and XII.

Entanglement or bycatch?

Assessing the frequency of entanglement in marine organisms relies on the ability to distinguish between bycatch in fishing gear and entanglement (Kühn et al., 2015; Ryan, 2018). Our ability to distinguish between bycatch in fishing gear and entanglement is low. It is challenging to differentiate between active gear and ghost gear for most entanglement events. Certain marine organisms, when caught in active fishing gear, can tear it off, attempting to free themselves; other will move on after being released by fishermen who voluntarily cut the gear. In both cases, these animals may continue to move over long distances with bits of gear entangled around their bodies (Asmutis-Silvia et al., 2017). For this reason, one of the main obstacles encountered when trying to integrate data is distinguishing entanglement in marine litter from bycatch in active fishing gear. When an animal is found entangled in fishing gear, it is difficult to identify the real origin of the event, that is, if the animal has interacted with the fishing gear whilst it was actively in use, or, if the gear was discarded or lost before the interaction with the animal.

To solve this problem, the INDICIT II Consortium decided to establish adequate definitions of entanglement and other related concepts.

- Marine litter (UNEP, 2021). items that have been deliberately discarded, unintentionally lost or transported by winds and rivers, into the sea and onto beaches.
- Ghost gear. Any fishing gear that has been abandoned, lost or discarded in the sea. There are many reasons why fishing gear can be lost or abandoned, including severe weather, snags beneath the surface, conflict with other gear, interaction with other vessels and intentional discard when no other options are available.
- Entanglement (INDICIT II proposal). The process of being wrapped, trapped or stuck in marine litter.
- Bycatch (European Commission). The inadvertent catch of organisms that were not specifically targeted by a fishing operation (e.g. non-target fish species, marine mammals, seabirds) that are either discarded or landed for commercial sale.
- Doubtful cases. When the item trapping the animal is not present or it is not possible to ensure the distinction between entanglement in marine litter and bycatch in active fishing gear. (These cases should be also registered and included in the databases).
- Accidental catch in active structures. The process of being wrapped, trapped or stuck in anthropogenic structures disposed at sea for any other uses than fishing activities (e.g. anchoring structures, signalling structures).

The INDICIT II consortium decided to establish several criteria to help distinguish entanglement in marine litter from bycatch in active fishing gear.

- Criteria to identify entanglement in marine litter.
    - Litter from land-based sources. This covers packing straps, plastic bags, heavy-duty sacks, etc.
    - Degradation of materials. Degraded material indicates that the item is not suitable for use or has not been used for a long time. Therefore, it should be considered litter.
    - Biofouling attached. The presence of attached biota indicates that the item has not been used for a considerable time period. For this reason, active fishing gear rarely present biota attached, except in aquaculture gear.
    - Medium/small animals (turtles, seabirds, seals, small cetaceans) trapped in large fishing gear. Fishers are unlikely to discard a whole piece of large gear due to the bycatch of medium/small animals, and medium/small animals are not strong enough to pull large fishing gear.
    - Mix of different fishing gear or/and other marine litter. Several materials mixed together indicate that they have been circulating for a long time on the surface and are therefore considered litter.
    - Morphology distortion observed on the animal. This is caused by long-term entanglement.

- Criteria to identify bycatch in active fishing gear.
    - Animals clearly caught by the fishing gear. This covers animals accidentally caught during active commercial or recreational fishing, or directly sent/delivered by fishers due to being bycatch found in their own gear.
    - Ingested hook. These animals are bycatch that are then released after cutting the line.
    - Heavy animals (whales) trapped in large fishing gear. Fishers could discard a whole piece of gear if a large/heavy animal is caught. In addition, large/heavy animals are strong enough to pull large fishing gear.
    - Accessory structures of fishing gear (excluding ropes and buoys attached to pots). Animals could be trapped when the gear is working or when it is not, but either involves a direct interaction with active fishing gear.
- Criteria to identify doubtful cases.
    - Animal with typical injuries (flipper lacerations, throttle, etc.) but no material present. Injuries could be caused by active fishing gear or by entanglement in marine litter. In these cases, local scientific expertise could support the identification, or the case could be included as doubtful if distinction cannot be assured.
    - The item trapping the animal is difficult to identify as fishing gear.
    - Any other doubtful case that could not be solved by the rest of criteria (e.g. animal trapped on clean and non-degraded net).
- Criteria to identify accidental catch in active structures (not related to fishing activity).
- Animals entangled in any other structure that are at sea but not related to fisheries (e.g. anchoring structure nets to keep algae blooms, jellyfish protection nets, shark protection nets).

8.8.1.4.2 Extraction of data

According to criterion D10C4, to assess the impact that marine litter has on large marine animals getting caught in it, it is necessary to quantify the number of individuals of each species adversely affected by litter. Therefore, comprehensive data on the population of a species in a specific area and the number of animals affected by entanglement are needed. However, in practice, it is not possible to have this information with any certainty. Therefore, general data, such as the number of stranded or entangled animals per year, is used as a proxy to estimate the FO% in a region or area per year. Each region needs to be analysed separately, and caution should be taken when considering variations in the frequency of occurrence from main threats like accidental capture, entanglement, boat collisions, and human interaction. In Table 8.5 and 8.6 are reported two examples of how to report the data.

Moreover, with individual data (details of the stranding event, characterisation of litter, impact of litter on individuals), it is possible to analyse the percentage of specific litter typologies that affect marine fauna, and the main injuries and impacts caused by entanglement.

Table 8.5. Example of the assessment of entanglements per year

Area	Total number of individuals (total individuals stranded or registered in the area covered)	Number entangled (total individuals affected by entanglement)	FO%
A1	500	200	40
A2	300	60	20
A3	100	10	10

Table 8.6. Example of the assessment of megafauna affected by marine litter per year

Area	Number entangled (total individuals affected by entanglement)	Fisheries and aquaculture, *N* (%)	Land based, *N* (%)	Both sources, *N* (%)	Unknown, *N* (%)
A1	200	100 (50%)	50 (25%)	20 (10%)	30 (15%)
A2	60	27 (45%)	11 (18%)	3 (5%)	19 (32%)
A3	10	2 (20%)	5 (50%)	1 (10%)	2 (20%)

8.8.1.4.3 Litter Categories – source related information

The main categories of debris reported to cause entanglement are proposed based on the INDICIT II litter typologies updated using *Joint List of Litter Categories for Marine Macrolitter Monitoring* by Fleet et al. (2021).

8.8.1.4.4 Size range

The size of litter causing entanglement can range from 10 cm up to several metres or square metres.

8.8.1.4.5 Spatial coverage

Dead and live sea turtles or marine mammals are collected from beaches or at sea; they are often collected because of stranding events, sea observations or accidental captures during fishing operations. All the European countries (and non-European countries such as Tunisia and Turkey); have official stranding networks that collect data reports on stranded animals throughout the whole year.

8.8.1.4.6 Survey frequency

Continuous sampling is required. A minimum sample population size for the year and the period of sampling should be established to ensure reliable conclusions after the development stage of a possible indicator.

8.8.1.4.7 Maturity of the tool

This tool is not mature at this stage. Specific monitoring programmes are required.

Moreover, important advances have been achieved by the INDICIT II project, where most of the data records collected on entanglement by the INDICIT II consortium were on loggerhead turtle (*N* = 2332; 97.53 %). To date, the most accurate data were collected since 2017. Moreover, important bases have been created, and most stakeholders have updated and harmonised their databases and incorporated most of the important parameters described in the INDICIT II – standard protocol for entanglement. Therefore, evaluation of GES scenarios and indicators' constraints could be established more accurately in the next MSFD implementation cycle. Data on other species, such as green and leatherback turtles or other taxa (cetaceans, seals and seabirds), are very interesting and could be collected during the next few years following the standard protocol developed by the INDICIT II consortium.

8.8.1.4.8 Regional applicability of the tool

The tool is applicable to the MSFD marine regions.

8.8.1.5 Estimation of costs

The costs of the monitoring of sea turtles and marine mammals entangled in litter can be integrated within stranding and rehabilitation monitoring programmes. Most of these programmes already monitor the ingestion of debris for both live and dead individuals. It can also be mutualised with other programmes, such as the oceanographic and fishery observation campaigns.

Costs estimates depend on the country, the network organisation, the local cost of materials, and the skills and salaries of the involved staff on the local level.

In general, it is proposed that one or two experts on marine litter and marine fauna be involved as focal points in each country/region to coordinate data collection from stakeholders, harmonise the classification of litter involved in entanglement (review pictures, identify new litter typologies, etc.) and establish connections with national authorities to facilitate the transfer of data for MSFD assessment. To estimate this, costs should be calculated based on an average of 8 hours for two employees in each county/region.

Specifically for entanglement, the inclusion of pictures of individuals in the stranding protocols is the best way to achieve accurate databases, which could be reviewed by experts on marine litter to harmonise and avoid confusion in litter classification. Some tools may support data collection, in particular when a turtle is observed at sea or found stranded or as bycatch, for example phone apps or online platforms (e.g. RedPROMAR app, developed by the Canary Islands government, or ObsEnMer which offers a collaborative platform managed by Cybelle Planèete in France), allowing citizen or institutions (NGOs, rescue centres, stranding networks) to post pictures with date and GPS location.

8.8.1.6 Quality assurance / quality control

There is a lack of QA/QC due to a lack of previous dedicated monitoring programmes. The data available have been reported to be poor and based on non-standardised collection of data (Votier et al., 2011; Barreiros and Raykov, 2014; Kühn et al., 2015; Lawson et al., 2015; Werner et al., 2016; Duncan et al., 2017; Claro et al., 2018; Anastasopoulou and Fortibuoni, 2019). In general, standard data collected from different regions (and stakeholders) by the INDICIT II project (*N* = 2391 entangled animals) are diverse and disperse, with important differences between species and time periods included. The most accurate data on loggerhead turtle were collected from 2017 to date. Moreover, important bases have been created and most stakeholders have updated and harmonised their databases and incorporated most of the important parameters described in the standard protocol for entanglement. Therefore, evaluation of GES scenarios and indicators' constraints could be established more accurately in the next MSFD implementation cycle.

8.8.1.6.1 Trend assessment

Specific long-term monitoring programmes are required.

8.8.2 Entanglement data on sea turtles and marine mammals gathered from activity of citizen science

8.8.2.1 Protocol name

MSFD protocol for the monitoring of entanglement of sea turtles and marine mammals from activity of citizen science.

8.8.2.2 Protocol description

The aim of this protocol is to collect data from citizen science to increase the official data coming from stranding networks. The use and the integration of these kind of data will be decided by the competent authorities responsible of the national data collection. This protocol can be used by environmental organisations, people who travel with sailboats or fishers. During travelling or fishing activities, these groups can find entangled marine animals and collect data and information on the phenomenon. Regarding stranded animals, citizens must inform local authorities and will be followed the protocol of *"Entanglement of sea turtles and marine mammals from stranding networks or recovery centres".*

Regarding this protocol, the main points are the:

- large spatial scale of data;
- non-homogenous effort;
- lack of usefulness for indicator monitoring;
- requirement for experts to analyse images.

8.8.2.3 Related marine compartments

The water column and especially the water surface or the seafloor are the marine compartments addressed when quantifying the entanglement of sea turtles and marine mammals. This protocol is primarily focused on the following sea turtles and marine mammals:

- sea turtles (mainly surface waters):
- loggerhead sea turtle – *Caretta caretta* (mainly oceanic habitats);
- leatherback sea turtle – *Dermochelys coriacea* (oceanic habitats);
- green sea turtle – *Chelonia mydas* (neritic habitats);
- marine mammals (water surface and water column):
    - common bottlenose dolphin – *Tursiops truncatus;*
    - striped dolphin – Stenella coeruleoalba;
    - common dolphin – Delphinus delphis;
- other species.

8.8.2.4 Technical requirements

In order to collect data through citizen science, it is necessary to organise specific training sessions explaining the problem of the impact of litter on marine fauna, the definition of entanglement, how to distinguish between entanglement and bycatch, and how to use the criteria described in the 'protocol of entanglement from stranding network or recovery centres (see Section 8.8.1):

- criteria for identifying entanglement in marine litter;
- criteria for identifying bycatch in active fishing gear;
- criteria for identifying doubtful cases;
- criteria for identifying accidental catches in active structures (non-related to fishing activity).

Annex XIII – 'Entanglement observation sheet – sea turtles and marine mammals' provides the data collection tool used by environmental organisations or the general public. It is also possible to produce an app containing the same information reported in Annex XIII. An image storage tool is essential to better identify and classify the litter causing entanglement as experts on marine litter could use images to evaluate further details: animal size, litter size, size relationship between the litter and the entangled animal and even the impact of the entanglement (main injuries, animal status, etc.). The pictures of each stranding event are essential to improve the description of the event and collect relevant information that is not registered in the moment.

8.8.2.4.1 Extraction of data

It is not possible to obtain the FO% of entanglement or the percentage of marine litter that affect megafauna from data collected by citizen science, because there are no data on the total number of individuals of a given species present in a given area. For this reason, the entanglement of sea turtles and marine mammals' data from activity of citizen science are considered additional information on the phenomenon.

A specific protocol for conducting images searches regarding entanglement on social media and online platforms has been developed by the INDICIT II project (entanglement protocol – social media review). This protocol could be used by experts within each MSFD implementation cycle to improve indicator criteria and verify litter typologies.

8.8.2.4.2 Litter categories – source related information

The main categories of litter reported to cause entanglement are proposed in Annex XI.

8.8.2.4.3 Size range

The size of the litter causing entanglement can range from 10 cm up to several metres or several square metres.

To facilitate data collection on litter size, the INDICIT II consortium has developed a Litter reference size, which could also be used as a reference for animal size.

8.8.2.4.4 Spatial coverage

The spatial coverage depends on the area covered by the observers at seas (citizens or experts) or the accidental captures during fishing operations.

8.8.2.4.5 Survey frequency

Continuous sampling is required. A minimum sample population size for the year and the period of sampling should be established to ensure reliable conclusions after the development stage of this possible indicator.

8.8.2.4.6 Maturity of the tool

Specific monitoring programmes are required.

The INDICIT II project has found important source of data on entanglement on social media and online platforms. Images of 415 entangled individuals were found and analysed from these sources (data from 2003 to 2021).

The review of these images concluded the following.

- The definitions and criteria developed by the INDICIT II consortium to distinguish entanglement from bycatch are very useful in most of the cases, mainly when images are present. However, larger animals, such as leatherback turtles and large cetaceans, present more difficulties when trying to distinguish entanglement from bycatch.
- The list of litter typologies established by the INDICIT II consortium is appropriate for monitoring the litter entanglement of marine fauna. However, the list could be reduced, based on the taxon and the region, to facilitate data collection by stakeholders.
- Important differences have been found regarding litter entanglement in relation to taxon and sea turtle species; this is probably caused by different behaviours and habitat uses.
- Entanglement was more frequently observed in sea turtles, with entanglement of loggerhead turtle being the most abundant (N = 333). Therefore, loggerhead turtles could be proposed as an indicator to monitor entanglement in oceanic habitats in the Atlantic and Mediterranean Basins.
- Few data on green turtles were found on social media (N = 11), but, depending on the standard data, this species could be proposed for use in monitoring neritic / coastal habitats.
- There is a lack of data on loggerhead turtles in the OSPAR region (only 17 cases were found) in relation to the rest of the regions.
- The parameter of impact severity developed by the INDICIT II consortium (based on the effect injuries caused by entanglement have on animal viability) could be used to measure the impact of entanglement and to identify specific litter typologies that potentially induce greater impacts on the animals.

8.8.2.4.7 Regional applicability of the tool

The tool is applicable to the MSFD marine regions.

8.8.2.5 Estimation of costs

Activities of citizen science do not generate costs. Moreover, one or two experts on marine litter and marine fauna could be involved as focal points in each country/region to coordinate data collection on online platforms, harmonise the classification of litter involved in entanglement (review pictures, identify new litter typologies, etc.), and establish connections with national authorities to facilitate the transfer of data for MSFD assessment. These experts could be the same as those proposed for the standard data.

8.8.2.6 Quality assurance / quality control

There is a lack of QA/QC due to a lack of previous dedicated monitoring programmes.

Moreover, the INDICIT II project has found important sources of data on entanglement on social media and online platforms. Images of 415 entangled individuals were found and analysed (data from 2003 to 2021). A specific protocol (entanglement protocol – social media review) has been developed to harmonise data collection on images collected by citizen science (social media and online platforms). This protocol could be used by experts within each MSFD implementation cycle to improve indicator criteria and verify litter typologies.

8.8.2.6.1 Trend assessment

Specific long-term monitoring programmes are required.

8.9 Entanglement in seabird colonies

8.9.1 Protocol name

MSFD protocol for the monitoring of plastic litter as nesting material in seabird breeding colonies and associated entanglement mortality.

8.9.2 Protocol description

Seabirds are apex predators in marine ecosystems and are particularly vulnerable to entanglement in plastics and other marine litter (Votier et al., 2011). Seabirds such as northern gannets (*Morus bassanus*), shags (*Phalacrocorax aristotelis*) or kittiwakes (*Rissa tridactyla*) tend to incorporate marine litter, much of it originating in fisheries, into their nests, at times resulting in entanglement. Depending on the regional occurrence and distribution of breeding colonies, the nesting materials of different species can be assessed for marine litter. In addition, the associated entanglement mortality can be studied. Ideally both components should be assessed in combination. The share of plastic items in nests of certain species of bird can be used as an indicator of the amount of litter in the natural environment in the vicinity of their breeding sites and to assess entanglement risk of animals. The associated entanglement mortality can serve as an indicator of the direct harm caused by the incorporation of marine litter into the nests of breeding colonies.

A protocol has been developed for the survey of plastic litter as nesting material and associated entanglement in seabirds. These surveys of breeding colonies can serve as a powerful indicator regarding inflicted mortality for seabirds due to marine litter. Negative effects can be documented rather easily and clearly compared with the often more indirect and sublethal effects of plastic ingestion, for example.

Another advantage is that a lot of seabird colonies are already regularly surveyed in many European countries to record the number of breeding pairs and/or breeding success. Thus, a protocol on entanglement in marine litter might potentially be filled out alongside existing investigations without too much extra effort.

8.9.3 Related marine compartments

The litter is collected by seabirds for nest construction in the surroundings of the colonies on beaches and the sea surface.

8.9.4 Technical requirements

First, (part of) a colony should be selected that is easily surveyed from fixed viewpoint(s) and for which the borders of the study section or plot(s) can be easily described. If only a part of a colony is monitored, this should be representative of the whole colony and comprise at least 5–10 % of all nests (at least several tens of nests). Subsampling a representative plot can allow the calculation of pollution and entanglement for an entire colony, but this is also a function of frequency of occurrence. If the frequency of occurrence of marine litter and entanglement is low, a large number of nests needs to be monitored to be able to accurately monitor trends.

GPS and ground marks should be used to fix the viewpoint(s) from which observations will be made and ensure that the spot(s) can be easily found again in later years for continued monitoring.

Photographs should be taken and the exact borders of the study plot documented. In principle, an area fully defined by ‘natural’ borders should be selected, so it is easily reproducible.

A decision should be made on standard dates on which surveys should be conducted. For plastic as nesting material, one survey is recommended and for entanglement (at least) three surveys per breeding season are recommended. The dates and numbers of surveys need to be documented to supply information on the observational effort. This may allow for subsequent corrections of entanglement rates. Litter as nesting material and as entanglement should be recorded alongside each other.

For entanglement, the first survey should be made prior to or at the beginning of the breeding season, to distinguish new entanglement victims from old entanglement victims still present from the previous year.

The second survey should be conducted during the peak of the breeding season to record the maximum number of apparently occupied nets (AON) and the respective total number of breeding birds for all species in the monitoring plot(s) and for the entire colony. Here, both entanglement and plastic as nesting material should be recorded. The latter enables the calculation of nest litter rates in relation to all active nests within each plot and for the whole colony.

The third survey should be conducted shortly after the fledging of the chicks at the end of the breeding season to receive an estimate of the minimum total number of birds that died of entanglement during the breeding season. Intermediate counts may refine the picture. The surveys for entanglement and nest litter may be combined with surveys of breeding numbers and success.

For the surveys, binoculars or a telescope of fixed type and magnification should be used (standardising the likelihood of observing details in nest structures). When the location and accessibility of the colonies allow, *in situ* observations can be made provided breeding birds are not disturbed.

A detailed count should be made of the number of nests in the study plot and this should be documented with (digital) photographs whenever possible. This helps to ensure consistent monitoring of plots regarding the number of breeding birds, the occurrence of plastic as nesting material, the categorisation of different litter types and the entanglement rates.

A detailed count should be made of the nests that contain visible marine synthetic litter, documenting pollution by using digital photographs whenever possible. The nest litter rate (frequency of occurrence) is assessed as the number of nests containing visible litter divided by the overall number of nests in the study plot.

During in situ counts, it is possible to record the number of items of litter in each nest (e.g. using five classes: 0, 1-5, 6-10, 11-20 and more than 20 items).

Depending on the situation, attempts should be made to specify details of different types of litter – for example, specifying strings, ropes, net (remains), sheets, packaging, fragments or other types – using the standard MSFD categorisation of litter items based on the MSFD TGML *Joint List of Litter Categories for Marine Macrolitter Monitoring* (Fleet et al., 2021). Attempts should also be made to identify sources of litter, for example, fishing, shipping or recreational. To classify the amount of plastic per nest, a four-step system was designed that could be applied from distant observation points (Table 8.7). For the litter category net and net rests, a slightly different approach is used, as it is impossible to distinguish between net rests in a single nest from a distance.

Table 8.7. Classification of categories of litter in the nests of northern gannets

Class	Nets / net rests	String/rope/packaging
0	No nets or net rests in the nest	No string/rope/packaging in the nest
1	Up to one third of the nest is made up of net rests	1–5 pieces per nest
2	One third to two thirds of the nest is made up of net rests	6–10 pieces per nest
3	More than two thirds of the nest is made up of net rests	> 10 pieces per nest

A detailed count should be made of the birds visibly entangled. All species affected should be recorded separately. In mixed colonies, species that do not use plastic as nesting material themselves regularly become entangled in the litter used by other species. For example, common guillemots (*Uria aalge*) frequently become entangled in the litter used by gannets. The age (adults, immature or chick) and status (if alive or dead) of the species should be recorded. Entanglement should be documented using (digital) photographs whenever possible. Ideally, these counts should be conducted at standard dates, which need to be defined.

The impact level from litter in nests is assessed as the number of dead or dying animals (specified for species and age classes) divided by the overall number of breeding birds in the study plot (entanglement mortality rate). The number of live birds that are cut loose and released should be specifically recorded as such, but should be included in the totals for individuals mortally entangled, because without human intervention they would have died. In general, the extrapolation of entanglement victims and the entanglement mortality rates have to take direct and indirect losses into account. For example, if a parent gannet or guillemot dies due to entanglement, the brood will usually fail, resulting in the death of the chick. Thus, these indirect victims have to be added to the number of chicks observed to be entangled. Moreover, the number of adults entangled is related to the number of breeding birds in a given colony. For entangled chicks, the number has to be related to the number of chicks. Therefore, the average breeding success can be used as a proxy for the number of chicks present in the colony. To calculate the latter, the average breeding successes of gannets, guillemots and kittiwakes (~ 0.7 chicks per pair), fulmars (~ 0.4 chicks per pair) and shags (~ 1.4 chicks per pair) can be derived based on the long-term seabird monitoring programme data from the United Kingdom (JNCC, 2020).

8.9.4.1 Example monitoring survey

In a colony of 1000 breeding pairs (AON) of gannets, 500 nests are surveyed for entanglement (50 %). The 1000 pairs would produce 700 chicks on average (calculated as 1000 × 0.7 chicks per pair). Ten adult birds and 10 chicks are observed to be entangled. Another 7 chicks (10 × 0.7 chicks per pair) are added due to the death of a parent. The extrapolated number of dead adults would be 20 (2 × 10) and the number of dead chicks 34 (2 × 17) as only 50 % of the colony was surveyed. The entanglement mortality rate for adults would be 20 victims / 2000 breeding adults × 100 = 1.0 %. The entanglement mortality rate for chicks would be 34 victims / 700 chicks × 100 = 4.9 %.

Where colonies are intensively surveyed for population monitoring, entanglement rates can also be compared with the number of breeders, the number of chicks, the breeding success, etc.

However, sometimes, three or more surveys over the breeding season may not be possible. In these cases, a survey at the peak of the breeding season to record the number of breeding birds and active nests is needed for both the nest litter rate and the entanglement mortality rate. For the latter another survey shortly after the fledging of the majority of the chicks in the colony is required. This can supply an estimate of the minimum total number of birds killed by entanglement.

If possible, these surveys should be conducted in a number of different plots to provide a measure of local variability (known to be high, for example, in neighbouring shag colonies in France (Cadiou et al., 2011)).

These surveys can be conducted easily without entering study plots and without disturbance or with minimal disturbance of breeding birds. As a general rule for repeated monitoring, it is not recommendable to collect nest structures after the breeding season to quantify proportions of litter included. In many cases, nests are multi-year structures, and removal may negatively affect the breeding of the nest owners and their neighbours in the next season owing to extra efforts to construct a new nest, disputes with neighbours over remaining nests and materials, or the quality of the nest affecting breeding success. This type of work is only recommended as incidental effort in dedicated research projects.

8.9.4.2 Litter categories – source related information

There are issues to be aware of in interpreting results from this type of monitoring.

Different seabird species have different ranges from colonies when looking for nesting material and may use different types of litter as nesting material depending on their species and location.

The litter in nests of northern gannets (e.g. Montevecchi 1991; Votier et al., 2011; Bond et al., 2012) originates exclusively from the sea, whereas kittiwakes also pick up litter from land to use as nesting material (e.g. Clemens and Hartwig, 1993; Hartwig et al., 2007). Gathering litter from land may also apply to cormorants and shags.

Votier et al., (2011) stated that gannets seem to prefer certain types of plastics, such as synthetic ropes, for building nests, relative to the proportions of them found on adjacent beaches. This apparent selectivity needs to be considered if seabirds are used as indicators for measuring trends in certain types of litter.

8.9.4.3 Size range

Detection of all visible litter particles from microlitter to mega litter is possible, with the focus being on macro litter.

8.9.4.4 Spatial coverage

This protocol is designed for application in breeding colonies of seabirds.

8.9.4.5 Survey frequency

In general, well-built nests are found during incubation and during the rearing period. The nest may frequently be more or less destroyed by the young. To investigate the frequency of occurrence of marine litter, the best period is during incubation at the peak of the breeding season (see Section 8.9.4). To determine the entanglement rate, at least another survey after fledging is required (see Section 8.9.4). As standard procedure, (at least) three surveys for entanglement are recommended (before breeding season, at peak breeding season and after fledging).

8.9.4.6 Maturity of the tool

The tool is not fully mature at this stage. It has been tested and shown to produce sufficient and robust data. Based on the protocols used in previous studies and the requirements of the MSFD, a standard protocol has been developed by various international experts working in the field. The protocol is applicable to a wide range of seabird colonies with justifiable effort and can produce reliable and comparable data.

8.9.4.7 Regional applicability of the protocol

This protocol can be applied in all regions where suitable seabird breeding colonies exist. This covers large parts of the north-east Atlantic Ocean, including the North Sea, Celtic Sea, the Irish Sea and the English Channel, where northern gannets breed. It could also be used in waters such as the Mediterranean Sea, the Baltic Sea or the Black Sea, which are breeding areas for species such as cormorants and shags that build litter into their nests.

8.9.5 Estimation of costs

In cases where this protocol can be applied alongside other monitoring or in existing studies (on breeding pairs/success, or any study involving capture/banding of adults and/or chicks), there may be no additional cruise costs required. If dedicated monitoring is carried out just for this reason, 1–3 days (or more) of cruise to the colony with 1–3 days (or more) of fieldwork will be needed; a driver for the boat is also required. At regularly worked colonies, multiple surveys each year are possible. The estimated costs for the monitoring of nest litter and entanglement based on the long-term experience at the seabird colony on Helgoland are presented in Table 8.8. The equipment costs are low, consisting of binoculars/telescopes, which, in many cases, will be part of existing field equipment.

Table 8.8. Overview of workloads for and financial costs of future monitoring of nest litter and entanglement at the northern gannet colony on Heligoland.

Work step	Annual workload (hours/year)	Annual costs (euro/year)	Initial costs (euro)
Equipment (notebook, GPS, binoculars, telescope with zoom eyepiece, tripod, digital single-lens reflex camera with zoom)			11200.00

Work step	Annual workload (hours/year)	Annual costs (euro/year)	Initial costs (euro)
Mapping of nests in the gannet colony on Heligoland (occurrence, amount and type of litter, survey and documentation of entangled birds)	80	3372.80	
Data processing (litter and entanglement)	100	4216.00	
Data analysis (litter and entanglement)	70	2951.20	
Preparation of a short report	50	2108.00	
Committee and public relation work	25	1054.00	
Project meetings	25	1054.00	
Material expenses		500.00	
Travel expenses		1000.00	
Net sum		16256.00	
10 % overhead		1475.60	
19 % value added tax		3369.00	2128.00
Gross sum		21100.60	13328.00

NB: For the calculation of costs, an hourly net cost of EUR 42.16 was used.

8.9.6 Quality assurance / quality control

Having two observers (or even more than two) count independently can produce error estimates. The methodology has been tested using replicate analyses and shows a certain variation between observers. The protocol applied can supply comparable and reproducible data on entanglement rates and nest litter.

8.9.6.1 Trend assessment

Data analysis and trend assessments can be carried out by time-series analyses (found in most statistic packages).

One problem is the longevity of plastic litter in nests as in many locations these materials may persist for many years if they are not blown or washed away by storms, rain and flooding or taken away by humans.

As a result, nests may contain the plastic litter of several breeding seasons, and trends in the indicator values may show delays and thus have functionality for assessing long-term rather than short-term trends. Finally, as indicated variability scales in the indicator need to be assessed (e.g. Cadiou et al., 2011).

8.10 Entanglement on benthic organisms

8.10.1 Protocol name

MSFD protocol for the monitoring of entanglement and other interactions between litter and benthic organisms.

8.10.2 Protocol description

Seafloor imagery technology allows researchers to quantify the abundance and distribution of litter on the seafloor using a standardised approach and, at the same time, to describe and quantify its interactions with and impact on marine organisms. This methodology is increasingly being used because it consists of a non-destructive sampling technique, with many operating hours and direct observation *in situ*. It is suitable for marine protected areas and sensitive habitats and can provide high-resolution data (depending on the optical device) on marine litter. It can be applied effectively at various depths and to all sea bottom types, including complex rocky habitats, where some litter (especially some ALDFG) may be found in abundance.

This protocol is based on peer reviewed international papers (i.e. Galgani et al, 2013, 2018; Melli et al, 2016; Consoli et al, 2018a, 2018b, 2019; Angiolillo, 2019; Angiolillo et al, 2021). It was developed by considering (i) the Italian MSFD protocol (MATTM/ISPRA, 2020) for the monitoring of coralligenous and mesophotic / deep rocky reefs and (ii) the MEDREGION protocol (Silvestri et al., 2021).

8.10.3 Criteria for choosing the survey areas

The protocol could be applied to different areas of investigation, and should primarily be used in areas where the presence of sensitive benthic habitat, such as coralligenous, mesophotic and cold-water coral (CWC) habitats and deep-sea sponge ground, is known. The habitat should be sufficiently extensive, and the visibility conditions (transparency of the water) in the area should make the investigation possible. In addition, areas should be selected to be representative of different environmental conditions in the sub-region and of impacts of different intensities.

8.10.4 Protocol for investigation

The protocol is based on video-imagery techniques and can be carried out through scuba diving in shallow areas or TUCs, ROVs, AUVs and submersibles for deeper waters.

Each methodology applied should be able to provide controlled sampling, precise data on geographical position and depth, high-definition video, and reference points to use as a metric scale to measure the field of view. In each area of investigation, investigators should:

- Acquire morpho-bathymetric data on the seafloor morphology;
- acquire visual data (high-definition and georeferenced videos/photos) along transects where monitoring activities are conducted;
- processing data to assess the extension and condition of the habitat, the litter abundance and the impact on benthic species.

8.10.4.1 Acquisition of morpho-bathymetric data on the survey area

The acquisition of morpho-bathymetric data should be performed using a multibeam echo sounder (MBES), preferably a hull-mounted one capable of acquiring backscatter data. Bathymetric and morphological data have to provide a high level of detail on the seabed sections of interest (digital terrain model (DTM) at the best possible resolution: cells of 1 m × 1 m, or smaller, in the order of centimetres). The use of the MBES is to be considered a priority for monitoring in the coralligenous / mesophotic / CWCs habitats.

8.10.4.2 Acquisition of visual data

Based on detailed morpho-bathymetric data, 3 investigation sites should be identified in each area, and preferably at a distance of no less than 500 m from each other. ROV exploratory paths should be conducted at each site, within which 3 transects will be identified. These transects should be 200 m long and spaced no less than 50 m. The position of the transects should represent the extension (horizontally and vertically), continuity, and the bathymetric range within which the habitat is included. The surveys should be carried out using a georeferenced remote platform (acquiring high-definition photos or videos). Each video and photographic survey should be recorded in line with the WGS84 datum (expressed in decimal degrees to the fifth decimal place: DD.DDDDD°).

The start of the dive is defined as the moment at which the ROV (or other cameras/ vehicles) dives in the seawater. The end of the dive is defined as the moment at which ROV is at surface/on the deck. The start of the transect is defined as the moment at which the ROV is at the bottom and the end of the transect is when ROV leave the bottom (off the bottom).

The survey area is defined by the video transect width and length. The inspected surveyed area results from multiplying the transect length by the visual field (width) of the video. The visual field can be estimated from the laser pointers scale in the video images. The estimation of litter abundance and litter interaction requires the measurement of the surveyed area.

ROVs (or other cameras/vehicles) should be moved along linear transects, in continuous recording mode, at a constant slow speed (e.g. < 1.5 nm/s) and at a constant height from the bottom (e.g. < 1.5 m), thus allowing for adequate illumination and facilitating the taxonomic and litter identification. Each video transect is analysed through the imaging technique, using the start and end times of the transect at the bottom as references. A visual census of megabenthic species and litter items has to be carried out along the complete extent of each 200 m long transect, including its width (visual field). The survey area inspected can be calculated by multiplying the transect length by its visual field (i.e. 50 cm visual field × 200 m long transect = 100 m^2 of bottom surface covered per transect). The visual field can be estimated using the laser pointers scale in the video images. The estimation of litter abundance and litter interaction requires the measurement of the area surveyed.

8.10.4.3 Procedures for analysing georeferenced video transects and required parameters

8.10.4.3.1 Location and extent of the habitat

The transect of 200 m has to be positioned on a map at a scale of 1:1500 or 1:2000. The presence of hard or soft bottoms, the presence of structuring species and the extent of the habitat should be reported.

8.10.4.3.2 Condition of the habitat and marine litter

For each video transect, the following parameters must be recorded.

- The extent of hard bottom, calculated as percentage of total bottom extent and showing the type of substratum (rocky reefs, biogenic reefs, etc.), should be calculated.
- Species richness (considering only conspicuous megabenthic sessile organisms), that is, the total number of sessile hard bottom megabenthic taxa should be recorded, identified at the lowest taxonomic level possible.
- The number of colonies or individuals of each structuring species (see Annex XIV – 'List of structuring species') should be counted, and the density of each structuring species should be computed for the transect area of the hard-bottom surface (number of colonies per square metre, or number of individuals per square metre).
- Marine litter should be recorded and counted, in order to obtain information about type, abundance and occurrence. Each item should be classified based on the litter type, following the *Joint List of Litter Categories for Marine Macrolitter Monitoring* (Fleet et al., 2021). The total abundance and occurrence of litter items per transect and the abundance and occurrence per each main category of litter should be recorded. The abundance should be expressed as counts of litter items per area surveyed (number of items per square kilometre), considering the entire transect length and width (in a constant field of view of the camera). When it is not possible to estimate the area surveyed (e.g. when lasers are not available), the unit in which marine litter should be expressed is items per unit length (items per kilometre) (mandatory). In the case of points of accumulation, where it is not possible to count the single items, these will be identified as 'litter hotspots'. These can be expressed as number of litter hotspots per kilometre (mandatory), and also as number of litter hotspots per square kilometre (recommended) or number of litter hotspots per survey.
- For each category of marine litter counted and identified, it must also be indicated whether or not it entangles/covers (entanglement) benthic organisms and, in positive cases, which species and how many organisms are involved. The percentage of colonies entangled in lost fishing gear or other marine litter should be calculated for each structuring species. The interactions recorded relate only to macrofauna identified through visual observations, no further investigation on microfauna is required. If it is not possible to identify the organisms at the species level, taxa should be reported or at least the group (gorgonians, coral, sponge, etc.).
- Any type of additional information should be recorded for each item in respect of interaction and impact.
- All data from each video transect should be entered on the seafloor litter monitoring sheet template (Annex XV – 'Seafloor litter monitoring sheet'), adapted within the framework of the MSFD.

8.10.5 Related marine compartments

The seafloor is the marine compartment addressed when quantifying entanglement and interaction with benthic organisms.

8.10.6 Technical requirements

Given that surveys might be performed using video-imagery techniques through scuba diving or ROV classes with different equipment, or other more sophisticated instruments, it is very important to record any extra camera/vehicle characteristics and instrumentation to ensure harmonisation among and between the teams performing surveys.

To record the interaction of litter with biota, the following conditions are suggested:

- the video survey should allow the recording of the precise position of items;
- the reference points to use as a metric scale for measuring the field of view are recommended;
- noting the camera information is recommended.

ROVs (or other camera/vehicle) should be equipped with the following items:

- an underwater acoustic tracking position system (USBL), to provide detailed geographical and depth positions of the ROV along the transects;
- an automatic depth system (auto depth);
- a compass;
- a high-definition video camera or digital camera (at least 1920 pixels × 1080 pixels);
- a high-definition digital camera (optional);
- laser beams at a known distance, to be used as a metric scale (at least two lasers).

8.10.6.1 Extraction of data

According to criterion D10C4, to assess the impact that marine litter has on benthic organisms through entanglement, it is necessary to quantify the number of individuals of each species adversely affected by litter. To do that, values should be known for the population of species present in a given area and the number of entangled organisms. However, in practice, this information is limited to a very small number of studies (Angiolillo and Fortibuoni, 2020): data on entanglement of benthic species is mainly qualitative, and studies are often limited and heterogeneous in space. The effects of marine litter on marine communities and habitats remain poorly known. There is not enough data on the variation in entanglement rates among species, species vulnerability and the frequency of interactions with different marine litter types. Very few studies put fishing effort, bycatch and the entanglement rate into relation to assess their impacts and obtain information on possible implications in terms of populations (Enrichetti et al., 2019, 2020). Moreover, there is a gap in knowledge on recreational fishing impact, which could significantly affect benthic assemblages.

To address the D10C4 criterion for benthic species a first assessment could be made using data on FO%, considering the number of entangled colonies/individuals of a target species in relation to the total number of colonies/individuals of that species.

Moreover, based on litter data, it is possible to analyse the percentage of marine litter (number and type) that affects marine fauna, the percentage of structuring species affected by entanglement and the main injuries caused by entanglement.

8.10.6.2 Litter categories – source related information

The main categories of litter reported to cause entanglement are proposed according to the *Joint List of Litter Categories for Marine Macrolitter Monitoring* (Fleet et al., 2021) (Annex XI).

The identification and correct categorisation of litter items should be facilitated by the use of photos when images will be analysed in the post-processing step. Unknown litter or items that are not on the list should be noted in the appropriate 'other item box' and descriptive detail and/or the source should be included.

8.10.6.3 Size range

All macroscopic litter items > 2.5 cm (longest dimension) should be identified and counted in each transect.

8.10.6.4 Spatial coverage

The tool is applicable to the MSFD marine regions.

8.10.6.5 Survey frequency

Monitoring is based on an opportunistic approach, taking advantage of any survey occurring at any time when the protocol can be applied. Data will then be collected when possible, planning the reporting to fit with the MSFD implementation cycle, on the basis of this occurring every 6 years.

8.10.6.6 Maturity of the tool

The method is mature and in use.

8.10.6.7 Regional applicability of the tool

The tool is applicable to all MSFD marine regions where shallow sea and deep-sea monitoring activities are established.

8.10.7 Estimation of costs

The costs related to seafloor monitoring surveys can vary widely based on the instrument used (scuba, ROV, submersible). There are no additional costs for the application of the protocol. The identification and quantification of interaction with biota is carried out through the post-processing of the videos acquired. Moreover, the protocol may be opportunistically mutualised with other regular surveys (monitoring in marine protected areas, offshore platforms, etc.) or programmes on biodiversity.

8.10.8 Quality assurance / quality control

The adoption of a common protocol will lead to a significant level of standardisation among the countries that apply it as their sampling strategy. Data on litter on the shallow seafloor are collected through protocols already validated for benthic species (Clean Atlantic, AMAre European projects and Plastic Buster Marine Protected Areas (PBMPA), RAMOGE).

8.10.8.1 Trend assessment

Data analysis and trend assessments can be carried out using time-series analyses. Data series have been collected by former oceanographic campaigns using ROVs in European waters; however, a dedicated study is necessary to make them available for MSFD purposes. Furthermore, no standard dedicated protocol was in use at the time of sampling, in particular for deep waters.

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Chapter 8 'Litter ingested by biota and entanglements with litter'

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List of abbreviations and definitions

ACCOBAMS	Agreement on the Conservation of Cetaceans of the Black Sea, Mediterranean Sea and Contiguous Atlantic Sea
ALDF	Abandoned, lost or otherwise discarded fishing gear
AMAP	Arctic monitoring and assessment programme
AON	Apparently occupied nets
ASCOBANS	Agreement on the Conservation of Small Cetaceans of the Baltic, North East Atlantic, Irish and North Seas
AUV	Autonomous underwater vehicle
BITS	Baltic International Trawl Survey
MSFD CIS	Marine Strategy Framework Directive common implementation strategy
CPR	Continuous plankton recorder
CSO	Civil society organisation
CCL	Curved carapace length
ICES DATRAS	ICES – Database of Trawl Surveys
D10	MSFD Descriptor 10 marine litter
DAD	Dive against debris
DCF	Data Collection Framework
ICES DOME	ICES – Marine Environment database
DSLR	Digital single-lens reflex camera
DTM	Digital terrain model
EcoQO	Ecological quality objective (OSPAR)
EEA	European Environment Agency
EMODnet	European Marine Observation and Data Network
FLM	Floating litter monitoring
FMML	Floating marine macro litter
FO%	Frequency of occurrence as a percentage
FTIR	Fourier transform infrared spectroscopy
GES	Good environmental status
GESAMP	Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (IMO)
GI	Gastrointestinal system; oesophagus, stomach, intestines
GIS	Geographic information system
GOV	Grande ouverture verticale
HELCOM	Baltic Marine Environment Protection Commission – Helsinki Convention
IBTS	International Bottom Trawl Survey
ICES	International Council for the Exploration of the Sea
ILC	Inter-laboratory comparison
INSPIRE	Infrastructure for Spatial Information in the European Community
ISO	International Organization for Standardization

IWC	International Whaling Commission
JRC	Joint Research Centre
LOD	Limit of detection
MEDITS	Mediterranean International Trawl Survey
MEDREGION	Support Mediterranean Member States towards the implementation of the Marine Strategy Framework Directive new GES decision and programmes of measures and contribute to regional/sub-regional cooperation
µFTIR	Micro-Fourier transform infrared spectroscopy
MBES	Multibeam echo sounder
MPA	Marine protected area
MSFD	Marine Strategy Framework Directive (Directive 2008/56/EC)
NGO	Non-Governmental Organisation
NOAA	National Oceanic and Atmospheric Administration
OSPAR	Convention for the Protection of the Marine Environment of the North-East Atlantic
QA/QC	Quality assurance / quality control
QUASIMEME	Quality Assurance of Information on Marine Environmental Monitoring in Europe
RGB	Red, green and blue
ROV	Remote operated vehicle
RSC	Regional Sea Convention
TG ML	Technical Group on Marine Litter under the Marine Strategy Framework Directive
TUC	Towed underwater camera
TVL	Large TV beam trawl
TVS	Small TV beam trawl
UAV	Unmanned Aerial Vehicles
UAS	Unmanned Aircraft System
UN	United Nations
UNEP	United Nations Environment Programme
UPS	Uninterruptible power supply
USBL	Underwater acoustic tracking position system
WFD	European Water Framework Directive
WG DIKE	Working Group on Data, Information and Knowledge Exchange under the MSFD
WMO	World Meteorological Organization

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Annexes to Chapter 3 'Beach macro litter'

Annex I. Survey site (beach) identity form (A1)

(Survey site: a beach or a selection of a large beach chosen for placing one or more sampling units) (26)

Name of the survey site (A1) .... Date of record ....

Code of the survey site (A1) .... Country ....

Contact person .... Email ....

Total length of the coast/beach: .... (m)

.... Latitude *(central point)* ....

.... Longitude *(central point)* ....

Urbanisation degree: ☐ Urban ☐ Semi-urban ☐Remote/Natural

Back of the beach: ☐ Cliffs ☐ Dunes ☐ Rocks ☐ Forest ☐ Bush

☐ Crops ☐ Fields ☐ Built-up area ☐ Road ☐ Other (specify) ....

Is there any development behind the beach? ☐ No ☐ Yes

Description of the development behind the beach: ....

Looking from the beach to the sea, what direction is the beach facing*: ☐ N ☐ E ☐ S ☐W

Coastline curvature: ☐ Linear ☐ Concave ☐ Convex ☐ Sinusoidal

Beach substrate (% coverage): .... of sand .... of pebbles .... of rocky coast

Objects in the sea that influence the currents *(e.g. pier, reef, etc.)*: ....

Beach slope: ☐ Level ☐ Gentle slope ☐ Moderate slope ☐ Steep slope

Beach access: ☐ Pedestrian ☐ Vehicle ☐ Boat

Primary beach usage *(e.g. tourism and recreation, fishing, etc.)*: .... ☐ Seasonal ☐ Whole year around

Secondary beach usage *(e.g. tourism and recreation, fishing, etc.)*: .... ☐ Seasonal ☐ Whole year around

Estimated number of persons using the beach on average: winter.......... spring.......... summer.......... autumn..........

Any other noteworthy information ....

....

(e.g. an otherwise remote and unvisited location may be subject to an annual surfing competition that results in a 'pulse' of litter).

*you may tick one or two boxes

(26) To be filled out once and updated if necessary.

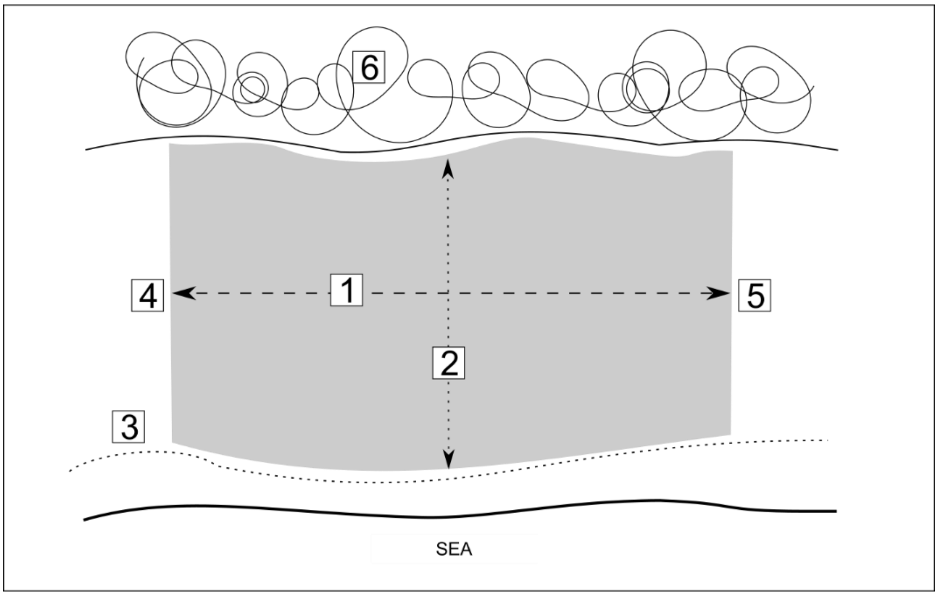
Annex II. Sampling unit identity form (A2)

(Sampling unit: a fixed stretch of coast, typically 100 m long, covering the area from the water edge to the black of the beach) ([27])

Code of the survey site (A1).. Date of record..

Name of the sampling unit (A2).. Code of the sampling unit (A2)..

Contact person.. Email..



1: Sampling unit length

2: Sampling unit width

3: Edge of the water

4 and 5: GPS coordinates of the sampling unit

6: Back of the beach

Sampling unit length *(measured along the beach curve at the mid-point between the water edge and the back of the beach)*:.............. (m)

Sampling unit width ([28]) *(perpendicular to the shoreline line; measured at the mean water level in areas with small tidal amplitude and mean high tide level for areas with high tidal amplitude)* .... (m)

GPS coordinates start..

GPS coordinates end ....

Direction of the prevailing winds*: ☐ N ☐ E ☐ S ☐W

Direction of the prevailing water currents*: ☐ N ☐ E ☐ S ☐W

([27]) To be filled once and updated if necessary.

([28]) Defined as the distance between the water edge and the back of the beach (base of dunes, cliff, vegetation line or human artefacts) and measured at half its length.

Name of the nearest town: ....

Distance of the town from to the sampling unit: .... (km)

Position of the town in relation to the sampling unit*: ☐ N ☐ E ☐ S ☐W

Size of the residential population of the nearest town:..

Food/drink outlet near the sampling unit: ☐ No ☐ Yes

Distance of the food/drink outlet from to the sampling unit:.. (km)

Position of the food/drink outlet from to the sampling unit*: ☐ N ☐ E ☐ S ☐W

Present all year round: ☐ Yes ☐ No, please specify the months. ....

Name of the nearest harbour: ....

Distance of the harbour from to the sampling unit: .... (km)

Position of the harbour in relation to the sampling unit*: ☐ N ☐ E ☐ S ☐W

Type of shipping using the harbour: ☐ Passenger ☐ Merchant ☐ Fishing ☐ Military

☐ Recreational ☐ All kinds ☐ Other (specify): ....

Name of the nearest river mouth:..

Distance of the nearest river mouth from to the sampling unit:.. (km)

Position of the river mouth in relation to the sampling unit*: ☐ N ☐ E ☐ S ☐W

Distance of the nearest wastewater or stormwater discharge point from to the sampling unit:.............................. (km)

Position of the wastewater or stormwater discharge point in relation to the sampling unit*: ☐ N ☐ E ☐ S ☐W

Distance of the nearest shipping lane from to the sampling unit:.. (km)

Position of the shipping lane in relation to the sampling unit*: ☐ N ☐ E ☐ S ☐W

Estimated traffic density:..*(n. of ships/year)*

Type of shipping using the shipping lane: ☐ Passenger ☐ Merchant ☐ Fishing

☐ Military ☐ Recreational ☐ All kinds ☐ Other

*you may tick one or two boxes

Annex III. Marine litter monitoring survey form (A3)

(Survey: the process of recording data related to a sampling unit at a given time) ([29])

Code of the survey site (beach) (A1) .... Date of survey ....

Code of the sampling unit (100m) (A2) .... Name of surveyor 1: ....

Code of the survey: .... Name of surveyor 2: ....

Other information: .... Name of surveyor 3: ....

.... Name of surveyor 4: ....

Length of the surveyed sampling unit ([30]): ....(m)

Date of the last known cleaning action: ....

Weather conditions during the date of the surveys ☐ Wind ☐ Rain ☐ Snow ☐ Ice

☐ Fog ☐ Sand storm ☐ Exceptionally high tide ☐ Other ....

Deviations from the sampling protocol: *(e.g. transect length reduction or displacement of the transect, sampling outside the expected period, sub-sampling)* ....

....

Motivation *(e.g. extreme weather events, flooding, new infrastructures in place)* ....

Special circumstances that could have caused an unusual occurrence of litter in terms of abundance and/or type: *(e.g. clean-up days, cleaning machine tracks, beach party or competition, cargo losses nearby, extreme weather conditions)* ....

Entangled animals ☐ No ☐ Yes How many .... ☐ Alive ☐ Dead

☐ Bird ☐ Turtle ☐ Fish ☐ Mammal ☐ Other .... Sex (if known) .... Age (if known) ....

Nature of the entanglement and type of litter ....

....

Any other noteworthy information ....

....

([29]) To be filled out during each survey in a specific sampling unit.

([30]) The actual length surveyed, which may differ slightly from the suggested 100 m recorded in the sampling unit identity form (A2) (Annex II). Measured along the beach curve at the mid-point between the water's edge and the back of the beach.

Litter data form

** Items to be recorded also if smaller than 2.5 cm*

SUP: Single Use Plastic, FG: Fishing Gears

J-CODE	SUP/FG	NAME	COUNT
ARTIFICIAL POLYMER MATERIALS			
J220		plastic sheeting from greenhouses	
J221		plastic irrigation pipes	
J222		other plastic items from agriculture	
J90		plastic flower pots	
J223		trays for seedlings of foamed plastic	
J46	FG	plastic oyster trays	
J45	FG	plastic mussels/oyster mesh bags, net sack, socks	
J47	FG	plastic sheeting from mussel culture (Tahitians)	
J102		plastic flip-flops	
J136		footwear made of plastic – not flip flops	
J40		plastic gloves (household/dishwashing, gardening)	
J41		plastic gloves (industrial/professional applications)	
J252		single-use plastic gloves	
J69		plastic hard hats/helmets	
J256		foamed plastic insulation including spray foam	
J89		plastic construction waste (not foamed insulation)	
J8	SUP	plastic drink bottles >0.5 l	
J7	SUP	plastic drink bottles ≤ 0.5 l	
J224	SUP	plastic food containers made of foamed polystyrene	
J21*	SUP	plastic caps/lids drinks	
J225	SUP	plastic food containers made of hard non-foamed plastic	
J1	SUP	plastic 4/6-pack yokes & six-pack rings	
J226	SUP	cups and cup lids of foamed polystyrene	
J227	SUP	cups and lids of hard plastic	
J228	SUP	plastic cutlery	

J-CODE	SUP/FG	NAME	COUNT
J229	SUP	plastic plates and trays	
J230	SUP	plastic stirrers	
J231	SUP	plastic straws	
J30	SUP	plastic crisps packets/sweets wrappers	
J31	SUP	plastic lolly & ice-cream sticks	
J85	FG	plastic commercial salt packaging	
J58	FG	fish boxes – foamed polystyrene	
J57	FG	fish boxes – hard plastic	
J92	FG	plastic bait containers/packaging	
J60*	FG	plastic fishing light sticks / fishing glow sticks incl. packaging	
J62	FG	plastic floats for fishing nets	
J59	FG	plastic fishing line	
J54	FG	plastic nets and pieces of net > 50cm	
J53	FG	plastic nets and pieces of net 2.5 cm ≥ X ≤ 50 cm	
J232	FG	plastic string and filaments exclusively from dolly ropes	
J233	FG	other plastic string and filaments exclusively from fishery	
J234	FG	plastic tangled nets and rope without dolly rope or mixed with dolly rope	
J235	FG	plastic tangled dolly rope	
J61	FG	other plastic fisheries related items not covered by other categories	
J42	FG	plastic crab/lobster traps (pots) and tops	
J44	FG	plastic octopus pots	
J70		plastic shotgun cartridges	
J11		plastic beach use related body care and cosmetic bottles and containers	
J12		plastic non-beach use related body care and cosmetic bottles and containers	
J95	SUP	plastic cotton bud sticks	
J29		plastic combs/hair brushes/sunglasses	
J98		plastic diapers/nappies	

J-CODE	SUP/FG	NAME	COUNT
J236		other plastic personal hygiene and care items	
J96	SUP	plastic sanitary towels/panty liners/backing strips	
J144	SUP	plastic tampons and tampon applicators	
J97		plastic toilet fresheners	
J237	SUP	plastic wet wipes	
J253		plastic single-use face-mask	
J211		other plastic medical items (swabs, bandaging, adhesive plasters etc.)	
J100*		plastic medical/ pharmaceuticals containers/tubes/ packaging	
J99		plastic syringes/needles	
J9		plastic bottles and containers of cleaning products	
J15		plastic engine oil bottles & containers >50cm	
J14		plastic engine oil bottles & containers 2.5 cm ≥ ≤ 50 cm	
J17		plastic injection gun containers/cartridges	
J16		plastic jerry cans	
J22*		plastic caps/lids chemicals, detergents (non-food)	
J23*		plastic caps/lids unidentified	
J24*		plastic rings from bottle caps/lids	
J13		other plastic bottles & containers (drums)	
J3	SUP	plastic shopping/carrier/grocery bags	
J101		plastic dog/pet faeces bag	
J5	SUP	the part that remains from tear-off plastic bags	
J36		other plastic heavy-duty sacks	
J238		plastic mesh bags for vegetable, fruit and other products	
J4	SUP	small plastic bags	
J91*		plastic biomass holder from sewage treatment plants and aquaculture	
J18		plastic crates, boxes, baskets	
J65		plastic buckets	
J93		plastic cable ties	

J-CODE	SUP/FG	NAME	COUNT
J84		plastic CDs & DVDs	
J67		plastic sheets, industrial packaging, sheeting	
J64		plastic fenders	
J68		fibreglass items	
J63		plastic floats/buoys other source than fishing or not known	
J239		other foamed plastic items and fragments not made of foamed polystyrene	
J257*		foamed plastic packaging	
J83		fragments of foamed polystyrene > 50cm	
J82		fragments of foamed polystyrene 2.5 cm ≥ ≤ 50 cm	
J80		fragments of non-foamed plastic > 50cm	
J79		fragments of non-foamed plastic 2.5cm ≥ ≤ 50cm	
J240		other identifiable foamed plastic items	
J241		other identifiable non-foamed plastic items	
J166		plastic paint brushes	
J28		plastic pens and pen lids	
J49		plastic rope (diameter more than 1cm)	
J242		plastic string and cord (diameter less than 1cm) not from dolly ropes or unidentified	
J66		plastic strapping bands	
J43		plastic tags (fishing, shipping, farming and industry)	
J87		plastic masking/duct/packing tape	
J88		telephone	
J72		plastic traffic cones	
J86		plastic fin trees (from fins for scuba diving)	
J243		plastic remains of fireworks	
J32*		plastic toys and party poppers	
J27*	SUP	tobacco products with filters (cigarette butts with filters)	
J26		plastic cigarette lighters	
J25		plastic tobacco pouches / plastic cigarette packet packaging	

J-CODE	SUP/FG	NAME	COUNT
J19		plastic vehicle parts	
RUBBER			
J127		rubber boots	
J133		rubber condoms (incl. packaging)	
J131*		rubber band (small, for kitchen/household/post use)	
J248		rubber sheet	
J134		other rubber pieces	
J249		rubber belts	
J125*	SUP	rubber balloons	
J126		rubber balls	
J250		rubber inner-tubes	
J251		rubber tyres	
CLOTH/TEXTILE			
J137		clothing	
J138		shoes & sandals made of leather and/or textile	
J141		cloth textile carpet & furnishing	
J140		hessian sacks/packaging	
J143		sails, canvas	
J145		other textiles	
J139		cloth textile backpacks & textile bags	
PAPER/CARDBOARD			
J150		paper cartons/Tetrapak milk	
J151		paper cartons/Tetrapak (non-milk)	
J244		paper cups	
J245		paper food trays, food wrappers, drink containers	
J246		paper cotton bud sticks	
J247		other paper containers	
J147		paper bags	
J148		cardboard boxes	

J-CODE	SUP/FG	NAME	COUNT
J156		paper fragments	
J154		paper newspapers & magazines	
J158		other paper items	
J155		paper tubes and other pieces of fireworks	
J152		paper cigarette packets	
PROCESSED/WORKED WOOD			
J159		wooden corks	
J165		wooden ice-cream sticks, chip forks, chopsticks, toothpicks	
J164		wooden fish boxes	
J163		wooden crab/lobster pots	
J162		wooden crates, boxes, baskets for packaging	
J172		other processed wooden items > 50cm	
J171		other processed wooden items 2.5 cm ≥ ≤ 50 cm	
J160		wooden pallets	
J167		wooden fireworks & matches	
METAL			
J194		metal cables	
J175		metal drinks cans	
J176		metal food cans	
J181		metal tableware (e.g. plates, cups & cutlery)	
J184		metal lobster/crab pots	
J182*		metal fisheries related weights/sinkers, and lures	
J180		metal appliances (refrigerators, washers, etc.)	
J187		metal drums & barrels	
J174		metal aerosol/spray cans	
J188		other metal cans	
J190		metal paint tins	
J178*		metal bottle caps, lids & pull tabs from cans	

J-CODE	SUP/FG	NAME	COUNT
J195*		metal household batteries	
J177		metal foil wrappers, aluminium foil	
J199		other metal pieces > 50cm	
J198		other metal pieces 2.5cm ≥ ≤ 50cm	
J186		metal industrial scrap	
J191		wire, wire mesh, barbed wire	
J179		metal disposable BBQs	
J193		metal vehicle parts / batteries	
J130		wheels with metal hub	
GLASS/CERAMICS			
J204		glass ceramic construction materials (bricks, tiles, cement)	
J203		glass and ceramic tableware (plates/cups/glasses)	
J207		ceramic or glass octopus pots	
J200		glass bottles	
J201		glass jars	
J208		pieces of glass/ceramic (glass or ceramic fragments ≥ 2.5 cm)	
J205		glass fluorescent light tube	
J202		glass light bulbs	
J219		other ceramic items	
J210		other glass items	
CHEMICALS			
J216		unidentified generally dark-coloured oil-like chemicals	
J217		unidentified generally light-coloured paraffin-like chemicals	
J218		unidentified chemicals	
FOOD WASTE			
J215		organic food waste	

J-CODE	SUP/FG	NAME	COUNT

ADDITIONAL DATA AND NOTES

Annexes to Chapter 4 'Floating marine macro litter'

Annex IV. Beaufort wind force scale

Beaufort wind force scale

Beaufort grade	Description	Wind speed (knots)	Wind speed (km/h)	Specifications for use at sea
0	Calm	<1	<1	Sea like a mirror
1	Light air	1 – 3	1 – 5	Ripples with appearance of scales are formed, without foam crests
2	Light breeze	4 – 6	6 – 11	Small wavelets still short but more pronounced; crests have a glassy appearance but do not break
3	Gentle breeze	7 – 10	12 – 19	Large wavelets; crests begin to break; foam of glassy appearance; perhaps scattered white horses
4	Moderate breeze	11 – 16	20 – 28	Small waves becoming longer; fairly frequent white horses
5	Fresh breeze	17 – 21	29 – 38	Moderate waves taking a more pronounced long form; many white horses are formed; chance of some spray
6	Strong breeze	22 – 27	38 – 49	Large waves begin to form; the white foam crests are more extensive everywhere; probably some spray
7	Near gale	28 – 33	50 – 61	Sea heaps up and white foam from breaking waves begins to be blown in streaks in the direction of the wind; spindrift begins to be seen
8	Gale	34 – 40	62 – 74	Moderately high waves of greater length; edges of crests break into spindrift; foam is blown in well-marked streaks in the direction of the wind
9	Strong gale	41 – 47	75 – 88	High waves; dense streaks of foam in the direction of the wind; sea begins to roll; spray affects visibility
10	Storm	48 – 55	89 – 102	Very high waves with long overhanging crests; resulting foam is in great patches and is blown in dense white streaks in the direction of the wind; on the whole the surface of the sea takes on a white appearance; rolling of the sea becomes heavy; visibility affected
11	Violent storm	56 – 63	103 – 117	Exceptionally high waves; small- and medium-sized ships might be lost to view behind the waves for a long time; sea is covered with long white patches of foam; the edges of the wave crests are blown into foam everywhere; visibility affected
12	Hurricane	≥64	≥118	The air is filled with foam and spray; sea is completely white with driving spray; visibility very seriously affected

Source: Adapted from Maritime Integrated Decision Support Information System on Transport of Chemical Substances (2020), 'Beaufort scale' (https://midsis.rempec.org/en/tools-guides/beaufort-scale).

Annex V. Douglas scale – state of the sea

Douglas scale – state of the sea

Douglas degree	Description	Average Height of the wave (m)	Description according to the swell
0	Calm (glassy)	No wave	No swell
1	Calm (rippled)	0.00 – 0.10	Very low (short or low wave)
2	Smooth	0.10 – 0.50	Low (long and low wave)
3	Slight	0.50 – 1.25	Light (short and moderate wave)
4	Moderate	1.25 – 2.50	Moderate (average and moderate wave)
5	Rough	2.50 – 4.00	Moderate rough (long and moderate wave)
6	Very rough	4.00 – 6.00	Rough (short and high wave)
7	High	6.00 – 9.00	High (average and high wave)
8	Very high	9.00 – 14.00	Very high (long and high wave)
9	Phenomenal	>14.00	Confused (wavelength and height indefinable)

Source: Adapted from Word Meteorological Organization, (2019), Manual on codes, Volume I.1 – International Codes – Annex II to the WMO Technical Regulations – Part A – Alphanumeric codes, WMO, No. 306, pp. 326, Geneva, (https://library.wmo.int/idurl/4/35713).

Annex VI. List of sea surface floating microlitter parameters to be reported

Label / column header	Concept ID	Use	Comments
Cruise		metadata/mandatory (ODV Default)	
Station		metadata/mandatory (ODV Default)	
Type		metadata/mandatory (ODV Default)	The suggestion is to use type 'B'. From manual: 'B' for bottle profile data. For time series and trajectories set to 'B' for small (<250) row groups
YYYY-MM-DDThh:mm:ss.sss		metadata/mandatory (ODV Default)	Start date/time. Format must be adapted to the date value (for example YYYY-MMDDThh:mm is second are not available)
Longitude [degrees_east]		metadata/mandatory (ODV Default)	start point coordinates
Latitude [degrees_north]		metadata/mandatory (ODV Default)	start point coordinates
LOCAL_CDI_ID		metadata/mandatory (ODV Default)	
EDMO_code		metadata/mandatory (ODV Default)	EDMO_CODE of the data centre distributing the data (the one connected to the CDI service)
Bot. Depth [m]		metadata/mandatory (ODV Default)	Field empty if no data
MinimumObservation Depth [m]	MINWDIST	mandatory in ODV micro-litter	
MaximumObservation Depth [m]	MAXWDIST	mandatory in ODV micro-litter	
SampleID:INDEXED_TEXT	SAMPID01	mandatory in ODV micro-litter	
SamplingEffort [Km or L]	LENTRACK/ VOLWBSMP	mandatory in ODV micro-litter	The amount of effort expended during an event. It can be the survey distance from the beginning point in kilometres or a filtered volume in litres
Net_opening [cm]	MTHWDTH1	mandatory in ODV micro-litter	Net opening of the instruments used. This information is needed for the calculation of the covered surface in cm (e.g. diameter of the Ocean Pack RACE filtering 'cakes' or bongo/manta net opening)
Mesh_size [micrometres]	MSHSIZE1	mandatory in ODV micro-litter	Mesh size of the filtering surface (e.g. manta or bongo net, filtering 'cakes' of OceanPack RACE...) in µm

Microlitter_Type:IND EXED_TEXT	MLITTYPW	mandatory in ODV micro-litter	Type of the item (H01 SDN vocabulary)
Microlitter_Size:INDE XED_TEXT	MLITSIZW	mandatory in ODV micro-litter	Size classes (H03 SDN vocabulary)
Microlitter_Count [Dimensionless]	MLITCNTW	mandatory in ODV micro-litter	Number of items collected. It's the official mandate from MSFD to provide the count of collected microplastics.
EventEndDateTime [YYYY-MMDDThh:mm:ss.sss]	ENDX8601	additional/optional	End date/time
EventEndLongitude [degrees_east]	ENDXXLON	additional/optional	End point coordinates. Either End Lat/Lon or SamplingEffort are mandatory
EventEndLatitude [degrees_north]	ENDXXLAT	additional/optional	End point coordinates. Either End Lat/Lon or distance are mandatory.
Microlitter length	NEW	additional/optional	
Microlitter_Weight [g]	MLDWWD01	additional/optional	Weight of the collected items, not mandatory. Information in grams
Microlitter_Shape:INDEXED_TEXT	MLITSHPW	additional/optional	Shape of the item (H02 SDN vocabulary)
Microlitter_Color:IN DEXED_TEXT	MLITCOLW	additional/optional	Colour classes (H04 SDN vocabulary)
Microlitter_Transparency:INDEXED_TEXT	MLITROPW	additional/optional	Transparency classes (H06 SDN vocabulary)
Microlitter_Polymer_type:INDEXED_TEXT	MLITPOLW	additional/optional	Polymer type of the micro-litter (H05 SDN vocabulary)
WMO_Sea_State [Dimensionless]	WMOCSSXX	additional/optional	Sea conditions following the Douglas scale
Wind_direction [degT]	EWDAZZ01	additional/optional	Direction relative to true north from which the wind is blowing
Wind_speed [m/s]	WSBZZ01	additional/optional	Sustained speed of the wind (distance moved per unit time by a parcel of air) parallel to the ground at a given place and time.
Sampling_protocol	SAMPPROT	additional/optional	The name of, reference to, or description of the method or protocol used to produce the sample

NB: ODV, Ocean Data View; SDN, SeaDataNet.
Source: Modified from Vinci et al. (2017).

Annex VII. List of seafloor sediment microlitter parameters to be reported

Label/column header	Concept id	Use	Comments
Cruise		metadata/mandatory (ODV Default)	
Station		metadata/mandatory (ODV Default)	
Type		metadata/mandatory (ODV Default)	The suggestion is to use type 'B'. From manual: 'B' for bottle profile data. For time series and trajectories set to 'B' for small (<250) row groups
YYYY-MM-DDThh:mm:ss.sss		metadata/mandatory (ODV Default)	Start date/time. Format must be adapted to the date value (for example YYYY-MMDDThh:mm is second are not available)
Longitude [degrees_east]		metadata/mandatory (ODV Default)	start point coordinates
Latitude [degrees_north]		metadata/mandatory (ODV Default)	start point coordinates
LOCAL_CDI_ID		metadata/mandatory (ODV Default)	
EDMO_code		metadata/mandatory (ODV Default)	EDMO_CODE of the data centre distributing the data (the one connected to the CDI service)
Bot. Depth [m]		metadata/mandatory (ODV Default)	Field empty if no data
MinimumObservation Depth [m]	MINWDIST	mandatory in ODV micro-litter	
MaximumObservation Depth [m]	MAXWDIST	mandatory in ODV micro-litter	
SampleID:INDEXED_TEXT	SAMPID01	mandatory in ODV micro-litter	
Microlitter_Type:IND EXED_TEXT	MLITTYPW	mandatory in ODV micro-litter	Type of the item (H01 SDN vocabulary)
Microlitter_Size:INDE XED_TEXT	MLITSIZW	mandatory in ODV micro-litter	Size classes (H03 SDN vocabulary)
Microlitter_Count [Dimensionless]	MLITCNTW	mandatory in ODV micro-litter	Number of items collected. It's the official mandate from MSFD to provide the count of collected microplastics.
EventEndDateTime [YYYY-MMDDThh:mm:ss.sss]	ENDX8601	additional/optional	End date/time

EventEndLongitude [degrees_east]	ENDXXLON	additional/optional	End point coordinates. Either End Lat/Lon or SamplingEffort are mandatory
EventEndLatitude [degrees_north]	ENDXXLAT	additional/optional	End point coordinates. Either End Lat/Lon or distance are mandatory.
Microlitter length	NEW	additional/optional	
Microlitter_Weight [g]	MLDWWD01	additional/optional	Weight of the collected items, not mandatory. Information in grams
Microlitter_Shape:INDEXED_TEXT	MLITSHPW	additional/optional	Shape of the item (H02 SDN vocabulary)
Microlitter_Color:IN DEXED_TEXT	MLITCOLW	additional/optional	Colour classes (H04 SDN vocabulary)
Microlitter_Transparency:INDEXED_TEXT	MLITROPW	additional/optional	Transparency classes (H06 SDN vocabulary)
Microlitter_Polymer_type:INDEXED_TEXT	MLITPOLW	additional/optional	Polymer type of the micro-litter (H05 SDN vocabulary)
WMO_Sea_State [Dimensionless]	WMOCSSXX	additional/optional	Sea conditions following the Douglas scale
Wind_direction [degT]	EWDAZZ01	additional/optional	Direction relative to true north from which the wind is blowing
Wind_speed [m/s]	WSBZZ01	additional/optional	Sustained speed of the wind (distance moved per unit time by a parcel of air) parallel to the ground at a given place and time.
Sampling_protocol	SAMPPROT	additional/optional	The name of, reference to, or description of the method or protocol used to produce the sample

NB: ODV, Ocean Data View; SDN, SeaDataNet.

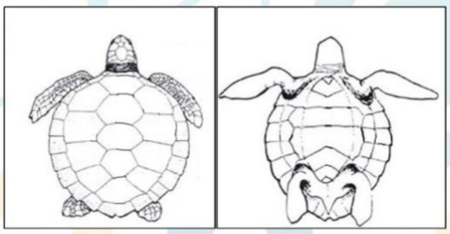
Source: Modified from Vinci et al. (2017).

Annex VIII. Observation sheet for litter ingestion by sea turtles

APPENDIX 1 – OBSERVATION SHEET 1/2

OBSERVATION SHEET - Litter ingestion by sea turtles	
COLLECTOR:	**LOCAL CODE:**
INSTITUTION:	
CONTACT:	
Discovery circumstances:	
SPECIES	☐ Caretta caretta ☐ Dermochelys coriace ☐ Chelonia mydas ☐ Other
INDIVIDUAL TAG	Tag number: Electronic chip N°:
INDIVIDUAL CODE:	- - - - - CC RR (Region) YY MM DD nº
DATE OF DISCOVERY (yyyy/mm/dd):	
LOCATION:	**X CORD :** **Y CORD :**
CIRCUMSTANCES	☐ By-catch/Fishery ☐ Stranding ☐ Dead at rescue centre ☐ Found at sea ☐ Other ☐ NR
BY-CATCH ENGINE CAUSE	☐ Longline ☐ Trawl ☐ Drift net ☐ Fishing rod ☐ Other ☐ NR
CAUSE OF DEATH/ STRANDING	☐ Bycatch/Fisheries ☐ Entanglement in debris ☐ Ingestion of litter ☐ Anthropogenic trauma ☐ Natural trauma ☐ Natural disease ☐ Oils ☐ Healthy ☐ Other ☐ NR
ENTANGLEMENT TYPE	☐ Active ☐ Passive ☐ NR
LITTER CAUSING ENTANGLEMENT	☐ Net pieces ☐ Monofilament lines ☐ Rope/s ☐ Plastic bags ☐ Raffia ☐ Other ☐ NR
PICTURES ☐	Picture names :
Animal body condition:	
CONSERVATION STATUS	☐ 1 - Alive ☐ 2 - Fresh ☐ 3 - Partially ☐ 4 - Advanced ☐ 5 - Mummified ☐ NR
HEALTH STATUS (Plastron shape)	☐ Poor (concave) ☐ Fair (plane) ☐ Good (convex) ☐ NR
MAIN INJURIES	☐ No injuries ☐ Fracture ☐ Amputation ☐ Sectioning ☐ Abrasion ☐ Other
AFFECTED PARTS	☐ Flipper ( ) ☐ Carapace ☐ Neck ☐ Head ☐ Plastron ☐ Other
FAT RESERVES	☐ Thin ☐ Fat ☐ Normal ☐ NR
Biometric measurements:	

Curved measurements (0,01cm)		Straight measurements (0,01cm)	
CCLst	cm	*SCLst*	cm
CCLmax	cm	*SCLmax*	cm
CCLmin	cm	*SCLmin*	cm
CCW	cm	*SCW*	cm
CPL	cm	*SPL*	cm
CPW	cm	*SPW*	cm



WEIGHT (0,01kg)	
NOTES AND REMARKS (Discovery and Animal conditions):	

Project number : 11.0661/2016/748064/SUB/ENV.C2

Source: Extracted from INDICIT consortium (2018), Monitoring Marine Litter Impacts on Sea Turtles – Protocol for the collection of data on ingestion and entanglement in the loggerhead turtle (*Caretta caretta* Linnaeus, 1758), deliverable D2.6 of the Indicit II project, European Commission, Brussels.

APPENDIX 1 – OBSERVATION SHEET 2/2

INDIVIDUAL CODE:	CC -	RR (Region) -	YY -	MM -	DD -	n°

Extraction of ingested litter

PROTOCOL	☐Necropsy
ARRIVAL DATE / / **DEAD DATE** / /	
FAT RESERVES	☐Thin ☐Fat ☐Normal
Please describe :	
VISCERAS STATUS *(note the presence of any infection, suspect colour, fluid effusion, perforation, presence of oil, etc.):*	
DIGESTIVE TRACT *(note the presence of any infection, suspect colour, fluid effusion, perforation, presence of oil, etc.):*	
TURTLE BEHAVIOUR AND TREATMENTS:	

Capacities of digestive tract section and gut content

	FULL				EMPTY			
	mass	Vol (V1)	vol (V0)	V1-V0	mass	Vol (V1)	vol (V0)	V1-V0
ESOPHAGUS								
STOMACH								
INTESTINES								

Marine debris measurements

	ESOPHAGUS		STOMACH		INTESTINE	
	DRY MASS	NUMBER	DRY MASS	NUMBER	DRY MASS	NUMBER
IND. PLA						
USE SHE						
USE THR						
USE FOA						
USE FRAG						
USE POTH						
Other non plastic						
FOO (nat. Food)						
NFO (nat. no food)						
TOTAL						

	TOTAL DEBRIS		NUMBER OF ITEMS		NUMBER OF ITEMS	
	dry mass		micro (1-5mm)		white transparent	
	number of items		meso (5-25mm)		dark coloured	
	volume		macro (>25mm)		light coloured	

INDICIT

Project number : 11.0661/2016/748064/SUB/ENV.C2

Source: Extracted from INDICIT consortium (2018), Monitoring Marine Litter Impacts on Sea Turtles – Protocol for the collection of data on ingestion and entanglement in the loggerhead turtle (*Caretta caretta* Linnaeus, 1758), deliverable D2.6 of the Indicit II project, European Commission, Brussels.

Annex IX. Template for data collection for microlitter ingestion by fish

Annex adapted from Matiddi et al. (2021).

Below is a template for data collection (all reported data are fictitious)*.

ID (XX_Yy_zzz)	species (*Genus species*)	country	location	date (dd/mm/yyyy)	latitude	longitude	fishing gear	length (cm)	weight (g)	liver (g)	gonads (g)	sex (M/F/ND)	stomach (g)	intestine (g)	GI (g)	occurrence (0/1)	items (N)	fibers (N)	filaments (N)	fragments (N)	granules (N)	pellets (N)	films (N)	foams (N)
IT_Sc_001	*Scomber colias*	Italy	Anzio	07/08/2019	41,211617	12,696367	net	26,8	150,5	1,2	0,1	F	1,6	4,2	5,8	1	3	0	1	2	0	0	0	0
IT_Sc_002	*Scomber colias*	Italy	Anzio	07/08/2019	41,211617	12,696367	net	28,1	167,5	1	0,8	M	2,8	4,3	7,1	1	2	0	1	1	0	0	0	0
IT_Sc_003	*Scomber colias*	Italy	Anzio	07/08/2019	41,211617	12,696367	net	27	140,7	1,1	0,1	M	2,3	3,4	5,7	1	1	0	1	0	0	0	0	0
IT_Sc_004	*Scomber colias*	Italy	Anzio	07/08/2019	41,211617	12,696367	net	27,3	158,1	0,7	0,6	M	2,4	3,2	5,6	0	0	0	0	0	0	0	0	0
IT_Sc_005	*Scomber colias*	Italy	Anzio	07/08/2019	41,211617	12,696367	net	27,3	152,2	1,2	1,7	M	3,9	2,7	6,6	0	0	0	0	0	0	0	0	0
IT_Mb_001	*Mullus barbatus*	Italy	Anzio	09/08/2019	41,545867	12,221433	trawl	23,4	162,4	0,1	0,5	F	3,9	5,4	9,3	0	0	0	0	0	0	0	0	0
IT_Mb_002	*Mullus barbatus*	Italy	Anzio	09/08/2019	41,545867	12,221433	trawl	24,2	192,9	0,1	0,3	M	2,7	3,5	6,2	1	5	0	1	4	0	0	0	0
IT_Mb_003	*Mullus barbatus*	Italy	Anzio	09/08/2019	41,545867	12,221433	trawl	24,1	182,7	0,1	0,0	F	2,9	5,4	8,3	0	0	0	0	0	0	0	0	0
IT_Mb_004	*Mullus barbatus*	Italy	Anzio	09/08/2019	41,545867	12,221433	trawl	24,5	187,0	0,2	0,2	F	3,8	4,6	8,4	0	0	0	0	0	0	0	0	0
IT_Mb_005	*Mullus barbatus*	Italy	Anzio	09/08/2019	41,545867	12,221433	trawl	24,6	169,8	0,2	0,3	M	2,6	2,8	5,4	1	2	0	1	1	0	0	0	0
IT_Mb_006	*Mullus barbatus*	Italy	Anzio	09/08/2019	41,545867	12,221433	trawl	21,0	117,1	0,1	0,2	F	2,1	1	3,1	0	0	0	0	0	0	0	0	0

The key for Excel sheet 1 (for fish) is as follows.

- ID. Sample identification code. It must be unique, reporting at least information on the origin country and the species. The suggested format is XX_Yy_zzz, where XX = country initials, Yy = acronym of the species and zzz = progressive number. More complex structures are allowed, as long as they are specified.
- Species. Binomial name of the species (in the form *Genus species*).
- Country / location / date / latitude / longitude / fishing gear. Data on the origin of the sample.
- Length/weight/liver*/gonads*/sex*/stomach*/intestine*/GI (* indicates optional information). Fish biometric parameters, namely length (total length), weight (total wet weight (recording if fresh or defrosted)), liver (liver wet weight), gonads (gonads wet weight), sex (F, female; M, male; ND, not determined), stomach (full stomach wet weight), intestine (full intestine wet weight) and GI (full gastrointestinal wet weight (stomach + intestine)). Total length must be reported to the nearest mm; weight measures must be reported to the nearest 0.1 g.
- Occurrence. 0, Absence; 1, presence. Data on the occurrence of microlitter in the gastrointestinal tract.
- Items. Total number of microlitter items in the gastrointestinal tract.
- Fibres/filaments/fragments/granules/pellets/films/foams. Total number of microlitter items for each category.

* Reported data are fictitious.
**Optional information

ID (XX_Yy_zzz)	species (*Genus species*)	organ (S/I)	ML (XX_Yy_zzz(w))	count	category	size (μm)	size class (1/2/3)	color	opacity (T/O)	polymer
IT_Sc_001	*Scomber colias*	S	IT_Sc_001(1)	1	filament	1625	1	blue	T	nylon
IT_Sc_001	*Scomber colias*	S	IT_Sc_001(2)	2	fragment	847	2	black	O	polypropylene
IT_Sc_001	*Scomber colias*	I	IT_Sc_001(3)	3	fragment	849	2	black	O	polypropylene
IT_Sc_002	*Scomber colias*	I	IT_Sc_002(1)	1	filament	2077	1	blue	O	nylon
IT_Sc_002	*Scomber colias*	I	IT_Sc_002(2)	2	fragment	1075	1	blue	O	polystyrene
IT_Sc_003	*Scomber colias*	S	IT_Sc_003(1)	1	filament	666	2	red	T	polyethylene
IT_Mb_002	*Mullus barbatus*	S	IT_Mb_002(1)	1	filament	655	2	blue	T	polypropylene
IT_Mb_002	*Mullus barbatus*	S	IT_Mb_002(2)	2	fragment	157	3	blue	T	polyethylene
IT_Mb_002	*Mullus barbatus*	I	IT_Mb_002(3)	3	fragment	629	2	blue	T	polyethylene
IT_Mb_002	*Mullus barbatus*	I	IT_Mb_002(4)	4	fragment	138	3	green	O	polyethylene terephthlate
IT_Mb_002	*Mullus barbatus*	I	IT_Mb_002(5)	5	fragment	256	3	red	O	polyvinylchloride
IT_Mb_005	*Mullus barbatus*	I	IT_Mb_005(1)	1	filament	184	3	blue	T	polypropylene
IT_Mb_005	*Mullus barbatus*	I	IT_Mb_005(2)	2	fragment	425	2	blue	O	polypropylene

The key for Excel sheet 2 (for items) is as follows.

- ID. Sample identification code (see key for Excel sheet 1 for details).
- Species. Binomial name of the species (in the form *Genus species*).
- Organ (optional). I, intestine; S, stomach. Tract of the digestive system in which the item was found.
- ML. Microlitter item identification code. It must be unique, reporting the ID and a progressive number that identifies the item.
- Count. Cumulative number of items found in a sample.
- Category. Microlitter category.
- Size (optional). Particle diameter.
- Size class. 1 (1 mm–5 mm), 2 (330 μm–1 mm) or 3 (100 μm–330 μm).
- Colour. Particle colour.
- Opacity. O, opaque; T, transparent.
- Polymer. Polymer identity ascertained through spectroscopy.

Annex X. Data sheet for recording individual-specific data for entanglement of sea turtles and marine mammals

Database – Individual data

PARAMETER	DESCRIPTION	OPTIONS
Country Code	Member state code, enter the value 'IT' for Italy, 'ES' for Spain etc.	
Region / Location	Area of work or location name	
Latitude	Latitude of discovery of the animal in the reference system WGS84 decimal degrees of the centroid	
Longitude	Longitude of discovery of the animal in the reference system WGS84 decimal degrees of the centroid	
Year	Year of discovery of the species in YYYY format	
Month	Month of discovery of the species in the format 1-12	
Day	Day of discovery of the species in the format 1-31	
Individual Code	Specify the animal identification code. For example: 'two letters for the country"_"two letters for the location (e.g. region or institution)'_"YY" _ 'MM"_"DD"_"chip number".	
ID_Report	The Institute's own report number (Test Report)	
Discovery circumstance	Circumstance of discovery. Enter one of the values from the list. The 'stranded' value includes all animals. NOTE: For example, if the specimen was found on the beach and the specimen shows evident signs of a previous capture that caused its death (found with hook and line), this field must be marked with 'stranded' and in the 'Circumstance of dead' column the value 'ByCatch' must be entered.	*Stranded* = Found at the beach *ByCatch* = Caught and delivered by fishermen *Dead RC* = Dead at the Rescue Center *At sea* = Found at sea Unknown = Unknown Other = other
Pictures	Yes No Unknown	Yes: Pictures collected No: Pictures no collected Unknown: No data about pictures

PARAMETER	DESCRIPTION	OPTIONS
Species	Specify the scientific name of the species found. In case of a species not present in the list, specify ‘Other’. Enter one of the values from the list	TT = *Tursiops truncatus* SC = *Stenella coeruleoalba* DD = *Delphinus delphis* CC = *Caretta caretta* DC = *Dermochelys coriacea* CM = *Chelonia mydas* Other = Other species
Other_species	If the ‘Species’ field has been completed with ‘Other species’, specify the name of the species here.	
Conservation _Status	Specify whether the animal was found alive or dead and the state of decomposition. Enter one of the values from the list. If the specimen dies at the Rescue Center after being collected alive and hospitalized, mark this field with ‘2’ and mark the ‘Discovery’ field with ‘DeadRC’.	*Level 1*: Alive *Level 2:* Fresh (dead recently) *Level 3:* Partially decomposed (internal organs are still in good condition) *Level 4:* Advanced decomposed (skin scales are raised or lost) *Level 5:* Mummified (part of the skeleton or part of the body are missing)
Health_Status	State of health at death or at the time of recovery. Enter one of the values from the list.	Poor Fair Good
Injuries	Major injuries. Enter one of the values from the list.	- No injuries (there are no injuries or are not visible) - Abrasion (skin erosion) – Slightly cuts – Deep cuts – Bone fracture (carapace or flipper bones) - Amputation - Throttle - Other
Circumstance of dead	In case of dead animal specify the cause otherwise enter ‘None’. Enter one of the values from the list.	- *None*: alive – *Entanglement*: animal dead entangled in marine litter – *Bycatch*: animal captured by active fishing gears or they accessory structures – *Doubtful:* interaction with fishing gears but difficult to distinguish between entanglement in marine litter or bycatch in active gears – *Accidental catch in marine structures*: animal entangled in other structures disposed at sea and not related with fisheries (anchoring, signals, etc.) – *Ingestion*: marine litter ingested – *Natural predation*: usually shark attack – *Anthropic* aggressions – Boat collision – *Illness*: a variety of illness symptoms (Ex. buoyancy, eye infections, etc.) – *Unknown*: No data about the event

PARAMETER	DESCRIPTION	OPTIONS
Length (CCLst / TL)	Length Measure, expressed in cm, of the Standard Curved Carapace Length (CCL) for reptiles or of the total length of the animal for mammals.	
Weight	Weight in kg of the specimen (2 decimal places). Enter 999 if the data is not available	
Gender	Specify the gender of the specimen. Enter one of the values from the list	M = Male F = Female NA = not detected or not determined
TAG	If a tag already exists, specify the number (N°. Indicate the presence and number of electronic chips. Otherwise, note NO.	

NB: WGS84, World Geodetic System 1984.

Annex XI. Data sheet for recording of entanglement data on sea turtles and marine mammals

Database - entanglement data

PARAMETER	DESCRIPTION	OPTIONS
Country Code	Member state code, enter the value 'IT' for Italy, 'ES' for Spain etc.	
Individual Code	Specify the animal identification code. For example: 'two letters for the country"_"two letters for the location (e.g. region or institution)'_"YY" _ 'MM"_"DD"_"chip number".	
Litter categories (updated according to Fleet *et al*. (2021)	Specify the category of entangled litter. In case of multiple material, mark the various categories on different lines. Take into account Fleet et al., (2021).	- Nets (J45, J53, J54) - Fishing lines (J59) – String and cords (J232, J233, J242) – Ropes (J49) - Floats (J62) – Buoys (J63) – Fenders (J64) – Pots, tops, traps (J42, J44, J163, J184, J207) –Aquaculture related (J45, J46, J47) –Other fishing related (J57, J58, J60, J61, J92, J164, J170, J182) –Plastic bags (J3, J4, J5) – Mesh bags (J238) – Heavy-duty sacks (J36, J85) – Strapping bands (J66) – Food packing (J21, J22, J23, J24, J30, J31, J224, J225, J245) – Plastic sheeting greenhouse (no code) –Textile (J137, J138, J139, J145) – Medical – hygienic care (J40, J41, J133, J144, J211, J252) – Recreational related (J32, J125, J126, J155, J167) –Other land-based (J43, J87, J93, J131, J194, J239) – Unknown = no information – Other = other material not listed
Source	Specify the source of the litter. Enter one of the values from the list. In case of multiple sources, mark the various sources on different lines.	- Fishing and maritime sources – Aquaculture sources – Land-based sources – Unknown = not identifiable
Affected body part	Specify the part of the body that is entangled. Enter one of the values from the list. Mark all the entangled parts, even if multiple: in the case of multiple body parts, mark the various parts on different lines.	- Head – Neck – Front flipper (R/L) – Rear flipper (R/L) – Dorsal fin – Caudal fin – Carapace – Whole body – Other parts of the animal (e.g. plastron, eyes, nostrils, etc.) – Unknown
Remarks	Notes and comments	

Annex XII. Data sheet for recording general data (frequency of occurrence as a percentage) for sea turtles and marine mammals

Database - general data (FO%)

PARAMETER	DESCRIPTION	OPTIONS
Area covered	Region covered	
Organisation/institution name (stakeholder)	Stakeholder in charge of animal strandings and data collection	
Year/period	Year or period of time covered	YYYY
Total number of individuals registered	Total number of stranded / registered individuals	#
Number of entangled animals	Number of entangled individuals	#
FO**%**	Frequency of occurrence (in %)	%

Annex XIII. Entanglement observation sheet – sea turtles and marine mammals

NOTE: ENTANGLEMENT/BYCATCH:

– Entanglement means any marine organism wrapped, trapped, or stuck in marine litter including fishing gear lost or abandoned.

– Bycatch means the unwanted fish and other marine organisms trapped by commercial fishing nets during fishing for a different species.

DATE OF DISCOVERY: ____________________

PERSON (name, institution): ____

COORDINATE (Google Maps) (Name location: __________________________________)

Cord LONG__________________

Cord LAT __________________

SPECIES

☐ Sea turtles: ☐*Caretta caretta* ☐*Dermochelys coriacea* ☐*Chelonia mydas*

☐ Marine mammals: ☐*Tursiops truncatus* ☐*Stenella coeruleoalba* ☐*Delphinus delphis*

☐ Other (specify) ____________

DISCOVERY CIRCUMSTANCES

☐ Stranded ☐ Floating at sea ☐ Dead at RC ☐ Captured by fishers

STATE OF ANIMAL

☐Live animal ☐ Dead animal: ☐ Fresh ☐ Partially ☐ Advanced ☐Mummified

ANIMAL SIZE:

CCLst (turtles): ___________cm

Total Length (cetaceans): ______________cm

Weight: _________kg

SEX

☐ Female ☐ Male ☐ Unknown

AFFECTED BODY PART: (multiple choice)

☐ Head

☐Neck

☐ Carapace

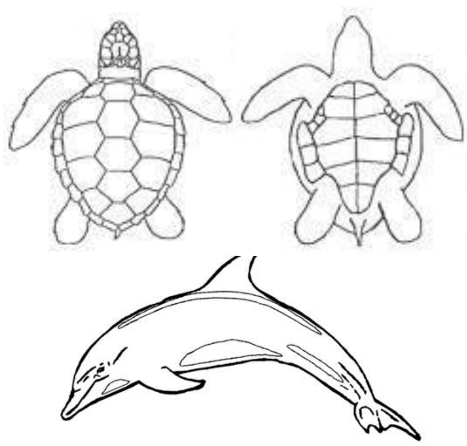
☐ Front flipper ☐R ☐L

☐ Rear flipper ☐R ☐L

☐ Other fins ☐Caudal ☐Dorsal

☐ Whole Body

☐ Other (specify) ____________



TYPE OF LITTER THAT CAUSING ENTANGLEMENT

☐ Fishing net ☐ Fishing line ☐ Rope, string, and cord ☐ Buoys, floats and fenders

☐ Pots, tops, and traps ☐ Other fishing related (lures, weight, etc.)

☐ Shopping Bags ☐ Mesh vegetable bags ☐ Heavy-duty sacks ☐ 4/6-pack yokes, six-pack rings

☐ Strapping bands ☐ Plastic sheeting greenhouse

☐ Food packing ☐ Clothing / rags ☐ Recreational related

☐Other (specify) ______________

LITTER SIZE REFERENCE:

☐ Finger (<10cm) ☐ hand (10-20cm) ☐ elbow (20-40cm) ☐ arm (40-60cm) ☐ half-body (60-100cm)

☐ 1 person (100-200cm) ☐ 2 people (200-400cm) ☐ 2-6 people (400-1000cm) ☐ >6 people (>10m)

Measured size: ___________________

PHOTO

☐ General view ☐ Individual ☐ Litter causing entanglement ☐ Main injuries

NB: possibly include an object of known size in the photo (e.g. coin or debit card, centimetre). If not possible, try to estimate size of the litter (cm): ______________

NOTE/COMMENTS:

Annex XIV. List of structuring species

Phylum	Class	Taxon
Porifera	Demospongiae	*Axinella cannabina*
Porifera	Demospongiae	*Axinella polypoides*
Porifera	Demospongiae	*Calyx nicaeensis*
Porifera	Demospongiae	*Spongia lamella*
Porifera	Demospongiae	*Sarcotragus foetidus*
Cnidaria	Hydrozoa	*Errina aspera*
Cnidaria	Anthozoa	*Acanthogorgia hirsuta*
Cnidaria	Anthozoa	*Antipathella subpinnata*
Cnidaria	Anthozoa	*Antipathes dichotoma*
Cnidaria	Anthozoa	*Bebryce mollis*
Cnidaria	Anthozoa	*Callogorgia verticillata*
Cnidaria	Anthozoa	*Cladocora caespitosa*
Cnidaria	Anthozoa	*Corallium rubrum*
Cnidaria	Anthozoa	*Dendrophyllia cornigera*
Cnidaria	Anthozoa	*Dendrophyllia ramea*
Cnidaria	Anthozoa	*Ellisella elongata*
Cnidaria	Anthozoa	*Eunicella cavolinii*
Cnidaria	Anthozoa	*Eunicella singularis*
Cnidaria	Anthozoa	*Eunicella verrucosa*
Cnidaria	Anthozoa	*Leptogorgia sarmentosa*
Cnidaria	Anthozoa	*Lophelia pertusa*
Cnidaria	Anthozoa	*Madrepora oculata*
Cnidaria	Anthozoa	*Paramuricea clavata*
Cnidaria	Anthozoa	*Paramuricea macrospina*
Cnidaria	Anthozoa	*Savalia savaglia*
Cnidaria	Anthozoa	*Viminella flagellum*
Cnidaria	Anthozoa	*Villogorgia bebrycoides*
Cnidaria	Anthozoa	*Parantipathes larix*

Phylum	Class	Taxon
Cnidaria	Anthozoa	*Leiopathes glaberrima*
Bryozoa	Gymnolaemata	*Myriapora truncata*
Bryozoa	Gymnolaemata	*Pentapora fascialis*

Annex XV. Seafloor litter monitoring sheet

Database - transect

Parameter	Description
CountryCode	Code of the Member State, e.g. insert the value 'IT' for Italy
SiteID	Survey site code
TransectID	Transect code
TransetctName	Name of the transect
Year	Year of sampling in YYYY format
Month	Sampling month in the format 1-12
Day	Sampling day in 1-31 format
StartTime	Hours-minutes-seconds of the beginning of the transect in the format HH: MM: SS as shown in the video
EndTime	Hours-minutes-seconds of the end of the transect in the format HH: MM: SS as shown in the video
Transect HBID	Code of the hard bottom transect section
LatitudeStart	Latitude in the reference system WGS84 decimal degrees of the starting point of the hard bottom transect
LongitudeStart	Longitude in the reference system WGS84 decimal degrees of the starting point of the hard bottom transect
LatitudeEnd	Latitude in the reference system WGS84 decimal degrees of the end point of the hard bottom transect
LongitudeEnd	Longitude in the reference system WGS84 decimal degrees of the end point of the hard bottom transect
StartTimeHB	Hours-minutes-seconds of the start of the hard bottom along the transect in the format HH: MM: SS as reported in the video. A separate line in the transect sheet corresponds to each hard bottom section
EndTimeHB	Hours-minutes-seconds of the end of the hard bottom along the transept in the format HH: MM: SS as reported in the video. A separate line in the transect sheet corresponds to each hard bottom section
TotalTimeHB	Total time in which the hard bottom is present along the transept in the format HH: MM: SS equal to start time hard bottom (HB) – end time HB
Habitatmapfile	Name of the GIS file that contains the polygon (s) of the location and extension of the habitat.
GISfile	Name of the GIS file that contains the polyline relating to the ROV route along the transect. In the table of attributes of the GIS file, for each polyline, the transect

Parameter	Description
	code must be reported
Videofile	Name of the video file associated with the transect produced by ROV.
Remarks	Note

NB: WGS84, World Geodetic System 1984.

Database - megabenthos

Parameter	Description
CountryCode	Code of the Member State, e.g insert the value 'IT' for Italy
TransectID	Transect code
Videofile	Name of the video file associated with the transect produced by ROV in which the species in question is included.
Phylum	Phylum of the structuring species (s)
Classes	Class of the structuring species
SpecieS	Name of the structuring species, enter one of the values from the list in the sheet Annex XIV
SpecieNS	Genus species of the non-structuring species
TipologySpecies	Indicate whether the species is structuring or not.
SpecNumber	Number of colonies / individuals for each structuring species
SpecAbundance	Specific abundance of each structuring species, expressed in number of colonies / individuals per m^2. Enter numeric value, do not enter units of measure. If the transect include also soft bottom, the density have to be calculated by referring only to the hard substrate
EntanglementPerc	Percentage of colonies / individuals of structuring species evidently affected by the presence of fishing gear or other litter (entanglement). Format 0-100
Remarks	Note

Database - marine litter type

Parameter	Description
CountryCode	Code of the Member State, e.g. insert the value 'IT' for Italy
TransectID	Transect code
Depth	Depth in meters at which the marine litter is found
DebTypeCode	Code of marine litter, enter one of the values from the Joint List of Macrolitter Categories (Fleet et al., 2021).

DepTypeDes	Description of marine litter, enter one of the values from the Joint List of Macrolitter Categories (Fleet et al., 2021)
DebNumber	The number of the marine litter type indicated in the 'DebTypeCode' field and found at the depth indicated in the 'Depth' field
Entanglement	Indicate if there are organisms entangled in marine litter (Yes / No).
TAXA	Taxonomic denomination at the lowest level (preferably at the species level) of organism entangled
N_ind	Number of organisms entangled in marine litter of that species
Remarks	Note

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