



Monitoring the presence and effects of marine litter in Mediterranean MPAs: the Plastic Busters MPAs protocols

PREPARED BY

THE INTERREG MED PLASTIC BUSTERS MPAs PROJECT

Interreg 
Mediterranean



PLASTIC BUSTERS
MPAs

<https://plasticbustersmpas.interreg-med.eu>

Document Information

Document	D.5.2.1 Monitoring the presence and effects of marine litter in Mediterranean MPAs: the Plastic Busters MPAs protocols
Version:	V01
Date:	21/06/2022
Authors:	Fossi M.C, Anastasopoulou, A., Alomar, C., Bains, M., Caliani, I., Consoli, P., Deudero, S., Galgani, F., Kaberi H., Panti, C., Romeo, T., Tsangaris, C., Zeri, C., Vlachogianni, T.
Contributors:	Angiolillo, M., Casini, S., Campani, T., Cillari T., D'Alessandro, M., Galli, M., Concato, M., Limonta, G., Pedà, C., Scotti, G.

Document Information

This document (Deliverable 5.2.1) is a compilation of all the protocols that should be applied in order to elaborate a comprehensive diagnosis of the presence and effects of marine litter in Mediterranean MPAs.

Approvals

Date	Partner
20/4/2022	M.C. Fossi/UNISI (Project Scientific Coordinator, Task Leader)
21/07/2022	M.C. Fossi/UNISI (Project Scientific Coordinator)

Document History

Version	Date	Comments & Status	Authors
V00	20/04/2019	First draft	UNISI, MIO-ECSDE
V01	21/07/2022	Finalized texts	MIO-ECSDE, UNISI

Citation

Fossi M.C, Anastasopoulou, A., Alomar, C., Bains, M., Caliani, I., Consoli, P., Deudero, S., Galgani, F., Kaberi H., Panti, C., Romeo, T., Tsangaris, C., Zeri, C., Vlachogianni, T. 2022. **Monitoring the presence and effects of marine litter in Mediterranean MPAs: the Plastic Busters MPAs protocols**. Interreg Med Plastic Busters MPAs project (D.5.2.1).

Table of contents

1. INTRODUCTION	6
1.1 <i>Marine litter a lurking threat in Mediterranean MPAs.....</i>	6
1.2 <i>The Plastic Busters MPAs project in a nutshell</i>	6
1.3 <i>Definitions and policy context</i>	7
1.4 <i>About this document</i>	9
2. METHODOLOGY FOR MONITORING MACROLITTER ON BEACHES	10
2.1. <i>Site selection.....</i>	11
2.2. <i>Frequency and timing of surveys</i>	11
2.3. <i>Sampling unit.....</i>	11
2.4. <i>Litter size classes to be surveyed</i>	13
2.5. <i>Litter items classification and quantification.....</i>	13
2.6. <i>Litter items removal and disposal.....</i>	13
2.7. <i>Materials and equipment</i>	14
2.8. <i>Additional considerations</i>	14
2.9. <i>Survey sheets</i>	15
3. METHODOLOGY FOR MONITORING MICROLITTER IN BEACH SEDIMENTS.....	25
3.1. <i>Survey site selection.....</i>	26
3.2. <i>Frequency and timing of surveys</i>	26
3.3. <i>Sampling unit.....</i>	27
3.4. <i>Microlitter size classes to be surveyed.....</i>	28
3.5. <i>Litter analysis and classification</i>	28
3.6. <i>Reporting units</i>	29
3.7. <i>Materials and equipment</i>	29
3.8. <i>Survey sheets</i>	30
4. METHODOLOGY FOR IDENTIFYING MARINE LITTER HOTSPOTS ON BEACHES	31
4.1. <i>Introduction</i>	32
4.2. <i>Methodological approach</i>	32
5. METHODOLOGY FOR MONITORING MACROLITTER ON THE SEA-SURFACE WITH VISUAL OBSERVATION BY SMALL- AND MEDIUM-SIZED VESSELS	34
5.1. <i>Site selection.....</i>	35
5.2. <i>Frequency and timing of surveys</i>	35
5.3. <i>Sampling unit and sample size</i>	35
5.4. <i>Visual observation process</i>	36
5.5. <i>Litter size classes to be surveyed</i>	37
5.6. <i>Litter classification and quantification</i>	37
5.7. <i>Materials and equipment</i>	37
5.8. <i>Recording sheets.....</i>	39
6. METHODOLOGY FOR MONITORING MICROLITTER ON THE SEA-SURFACE USING MANTA NET TOWS....	46
6.1. <i>Site selection.....</i>	47
6.2. <i>Frequency and timing of surveys</i>	47
6.3. <i>Sampling unit and sample size</i>	47
6.4. <i>Sample processing and size classification.....</i>	49
6.5. <i>Sample analysis</i>	51
6.6. <i>Expression of the results</i>	52

6.7.	<i>Materials and equipment</i>	52
6.8.	<i>Recording sheets</i>	54
7.	METHODOLOGY FOR MONITORING MACROLITTER ON THE SEAFLOOR WITH BOTTOM TRAWL SURVEYS	
	56	
7.1.	<i>Site selection</i>	57
7.2.	<i>Frequency and timing of surveys</i>	57
7.3.	<i>Sampling unit and sample size</i>	57
7.4.	<i>Trawling operation</i>	57
7.5.	<i>Litter size classes to be surveyed</i>	58
7.6.	<i>Litter classification and quantification</i>	58
7.7.	<i>Materials and equipment</i>	59
7.8.	<i>Recording sheets</i>	60
8.	METHODOLOGY FOR MONITORING MACROLITTER ON THE SEAFLOOR WITH VISUAL SURVEYS WITH SCUBA/SNORKELLING (SHALLOW COASTAL WATERS, 0–30M)	69
8.1.	<i>Site selection</i>	70
8.2.	<i>Frequency and timing of surveys</i>	70
8.3.	<i>Sampling unit</i>	70
8.4.	<i>Litter size classes to be surveyed</i>	71
8.5.	<i>Litter classification and quantification</i>	71
8.6.	<i>Materials and equipment</i>	72
8.7.	<i>Sampling & recording sheets</i>	73
9.	METHODOLOGY FOR MONITORING MACROLITTER ON THE SEAFLOOR WITH ROV – DEEP SEA	80
9.1.	<i>Site selection</i>	81
9.2.	<i>Frequency and timing of surveys</i>	81
9.3.	<i>Sampling unit</i>	81
9.4.	<i>ROV operation</i>	81
9.5.	<i>Litter size classes to be surveyed</i>	81
9.6.	<i>Litter classification and quantification</i>	82
9.7.	<i>Materials and equipment</i>	82
9.8.	<i>Recording sheets</i>	83
10.	MONITORING PRESENCE AND IMPACT OF MARINE LITTER IN BIOTA: THE PLASTIC BUSTERS MPAS APPROACH	85
10.1	<i>Monitoring the presence and effects of marine litter in biota: the Plastic Busters MPAs approach</i> 86	
10.2	<i>The marine litter impact on biodiversity: candidate biondicators selection</i>	86
10.3	<i>The threefold monitoring approach</i>	87
11	METHODOLOGY FOR MONITORING PRESENCE AND EFFECTS OF MARINE LITTER IN INVERTEBRATES ...	91
11.1	<i>Sampling approaches</i>	92
11.2	<i>Frequency and timing of sampling</i>	93
11.3	<i>Sample size</i>	93
11.4	<i>Tissues collection</i>	93
11.5	<i>Litter size classes to be surveyed</i>	95
11.6	<i>Litter analysis, classification and quantification</i>	95
11.7	<i>Analysis of plastic tracers and PBTs</i>	96
11.8	<i>Biomarkers analysis</i>	97
11.9	<i>Materials & Equipment</i>	98
11.10	<i>Sampling & recording sheets</i>	100

12	METHODOLOGY FOR MONITORING PRESENCE AND EFFECTS OF MARINE LITTER IN FISH	104
12.1	<i>Sampling approaches</i>	105
12.2	<i>Frequency and timing of surveys</i>	105
12.3	<i>Sample size</i>	105
12.4	<i>Tissues collection</i>	105
12.5	<i>Litter size classes to be surveyed</i>	107
12.6	<i>Litter analysis and classification</i>	107
12.7	<i>Analysis of plastic tracers and PBTs</i>	111
12.8	<i>Biomarkers analysis</i>	111
12.9	<i>Materials & Equipment</i>	113
12.10	<i>Sampling & recording sheets</i>	115
13	METHODOLOGY FOR MONITORING PRESENCE AND EFFECTS OF MARINE LITTER IN SEA TURTLES	119
13.1	<i>Species sampling</i>	120
13.2	<i>Description of investigated turtle and biometric measurements</i>	120
13.3	<i>Conservation/health status of the organism</i>	121
13.4	<i>Protocol for dead sea turtles</i>	123
13.5	<i>Protocol for live sea turtles</i>	125
13.6	<i>Litter analysis and classification</i>	129
13.7	<i>Analysis of plastic tracers and PBTs</i>	131
13.8	<i>Biomarkers analysis</i>	131
13.9	<i>Materials & Equipment for sampling</i>	133
13.10	<i>Sampling & recording sheets</i>	135
14	METHODOLOGY FOR MONITORING PRESENCE AND EFFECTS OF MARINE LITTER IN SEABIRDS	142
14.1	<i>Species sampling</i>	143
14.2	<i>Data to be recorded</i>	143
14.3	<i>Conservation/health status of the organism</i>	143
14.4	<i>Protocol for dead seabirds</i>	144
14.5	<i>Protocol for live seabirds</i>	145
14.6	<i>Litter analysis and classification</i>	148
14.7	<i>Analysis of plastic tracers and PBTs</i>	150
14.8	<i>Biomarkers analysis</i>	150
14.9	<i>Materials & Equipment for sampling</i>	152
14.10	<i>Sampling & recording sheets</i>	154
15.	METHODOLOGY FOR MONITORING PRESENCE AND EFFECTS OF MARINE LITTER IN MARINE MAMMALS	160
15.1	<i>Sampling strategies and analyses</i>	161
15.2	<i>Protocol for dead animals</i>	161
15.3	<i>Protocol for free-ranging marine mammals</i>	167
15.4	<i>The threefold approach in marine mammals</i>	171
15.5	<i>Litter analysis and classification</i>	171
15.6	<i>Analysis of plastic tracers and PBTs</i>	174
15.7	<i>Biomarkers analysis</i>	174
15.8	<i>Materials & Equipment for sampling</i>	176
15.9	<i>Sampling & recording sheets</i>	179
16.	REFERENCES	185

1. Introduction

1.1 Marine litter a lurking threat in Mediterranean MPAs

The Mediterranean Sea is one of the areas most affected by marine litter worldwide. Marine litter - any persistent, manufactured or processed solid material- is found lying on the shores, as well as floating anywhere from the surface to the bottom of the sea. Even in pristine environments of the Mediterranean, such as coastal and marine protected areas (MPAs), marine litter is building up, threatening habitats and species. Impacts vary from entanglement and ingestion, to bio-accumulation and bio-magnification of toxic substances released from litter items, facilitation of introduction of invasive species, damages to benthic habitats, etc. MPA managers stand at the forefront of this issue, and admittedly they lack the tools, knowledge, and often the resources to effectively tackle it. As a result, the achievement of the conservation goals set is hampered.

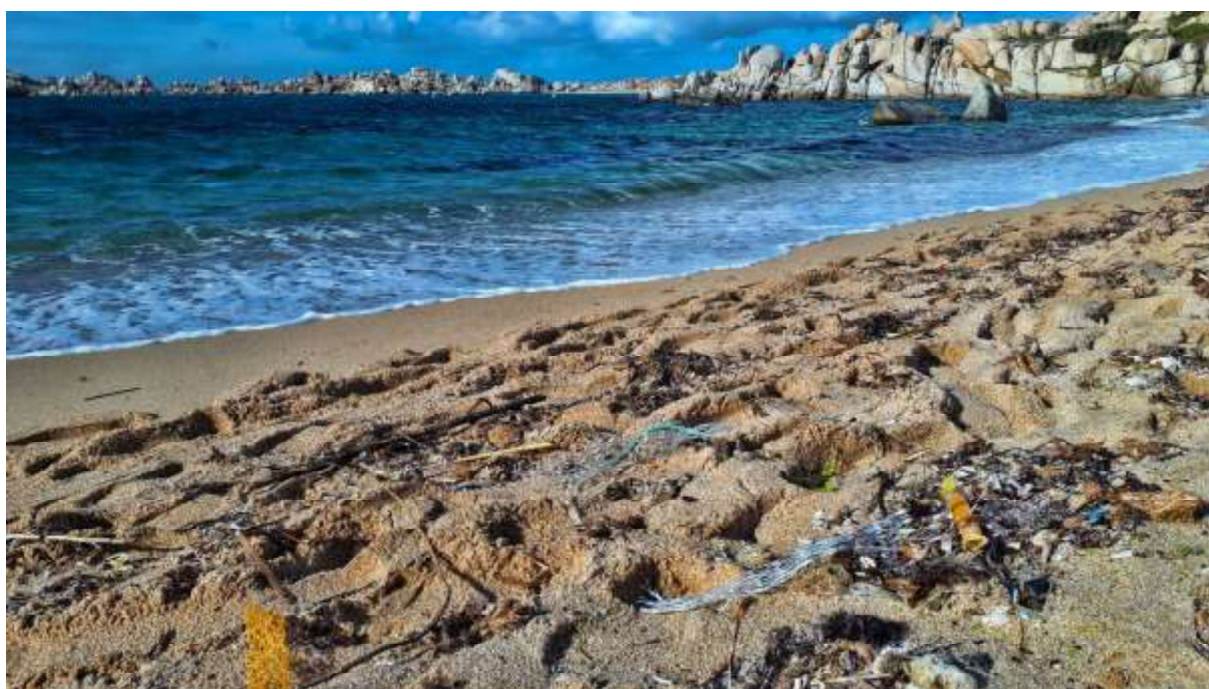


Figure 1-1. Marine litter a lurking threat in Mediterranean MPAs (Photo © Th. Vlachogianni).

1.2 The Plastic Busters MPAs project in a nutshell

The 4-year-long Interreg Med Plastic Busters MPAs project aimed at contributing to biodiversity protection and preservation of natural ecosystems in pelagic and coastal marine protected areas (MPAs), by defining and implementing a harmonized approach against marine litter. The project entailed actions that addressed the entire management cycle of marine litter, from monitoring and assessment to prevention and mitigation, as well as actions to strengthen networking between and among pelagic and coastal MPAs.

Plastic Busters MPAs consolidated Mediterranean efforts against marine litter by:

- Assessing the impacts of marine litter on biodiversity in MPAs and identifying marine litter ‘hotspot’ areas;
- Defining and testing tailor-made marine litter surveillance, prevention and mitigation measures in MPAs;
- Developing a common framework of marine litter actions for Interreg Mediterranean regions towards the conservation of biodiversity in Mediterranean MPAs.

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



Figure 1-2. The Plastic Busters MPAs project in a nutshell.

1.3 Definitions and policy context

Within this document, marine litter is defined as any persistent, manufactured or processed solid material discarded, disposed of, or abandoned in the marine and coastal environment. Marine litter can be classified in size classes as follows: macrolitter refers to items larger than 25 mm in the longest dimension, mesolitter to items between 5 mm to 25 mm, and microlitter to items ranging from 1 µm to 5 mm. This latter size class is sometime further broken down into large microlitter ranging from 1 mm to 5 mm and microplastic, from 1 µm to 1 mm in size.

The main legislative frameworks related to marine litter monitoring are the EU Marine Strategy Framework Directive – MSFD (2008/56/EC, 2010/477/EC, 2017/848/EC) and the Barcelona Convention Ecosystem Approach (COP19 IMAP Decision IG.22/7, UNEP/MED WG.450/3, June 2018) (see Box 1.1 and Box 1.2).

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

Marine Litter within the EU MSFD

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

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

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

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

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

Criteria D10C3 - Secondary: The amount of litter and micro-litter ingested by marine animals is at a level that does not adversely affect the health of the species concerned.

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

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

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

Marine Litter and the Barcelona Convention Ecosystem Approach

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

IMAP Common Indicator 22:

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

IMAP Common Indicator 23:

Trends in the amount of litter in the water column including micro plastics and on the seafloor.

IMAP Candidate Indicator 24:

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

1.4 About this document

The overarching aim of this document is to provide an operational protocol for implementing the Plastic Busters MPAs harmonized marine litter monitoring approach and assess the presence and effects of marine litter in pelagic and coastal Mediterranean MPAs with special emphasis on marine species, including endangered ones (cetaceans, sea turtles, birds, endangered sharks, etc.). In this respect, this document is a compilation of all the protocols that should be applied in order to elaborate a comprehensive diagnosis of the marine litter problem in Mediterranean MPAs.

This document takes stock of all recent advances made by the EU MSFD Technical Group on Marine Litter and the Barcelona Convention CORMON Group. Furthermore, this document capitalizes on the outcomes of relevant projects such as the IPA-Adriatic DeFishGear project, the EU-funded INDICIT project and the Interreg Med marine litter related projects, namely the MEDSEALITTER, AMARE and ACT4LITTER.



Photo © Th. Vlachogianni



5. Methodology for monitoring MACROLITTER on the sea-surface with visual observation by small- and medium-sized vessels

This document describes the methodological approach for monitoring macrolitter on the sea surface. It has been compiled based on the related methodologies developed within the IPA-Adriatic DeFishGear, the Interreg Med MEDSEALITTER projects and the 2022 MSFD TGML Updated Guidance on Monitoring of Marine Litter in European Seas, while taking into account the results from the Plastic Busters MPAs testing phase.

PREPARED BY

THE INTERREG MED PLASTIC BUSTERS MPAs PROJECT



5.1. Site selection

The monitoring of floating marine macrolitter by human observers is a methodology indicated for transects in selected areas. The selected areas should include:

- ▶ Low density areas (e.g. open sea);
- ▶ High density areas (e.g. close to ports);
- ▶ Other selected areas e.g. in estuaries, in the vicinity of cities, in local areas of touristic, recreational or commercial traffic.

Incoming currents from neighbouring areas or outgoing currents should be considered.



Figure 5-1. Floating macrolitter (Photo © Th. Vlachogianni).

5.2. Frequency and timing of surveys

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

- ▶ Autumn: October
- ▶ Spring: April

5.3. Sampling unit and sample size

The survey area is defined by the transect width and length. The transect width recommended to be used for small-scale vessels is 3 m on each side of the boat (6 m in total if two observers are deployed) and for medium-scale vessels 5 m on each side of the boat (10 m in total if two observers are deployed). The transect length should correspond approximately to 1 h of observation for each survey with a boat speed of 4-6 knots.

There is no agreed minimum sampling effort for obtaining a representative sample size and representative area coverage per survey campaign that can be extrapolated to all regions and/or density of litter situations for offshore and coastal waters, however for a transect width of 10 m, 15-30 h of effort have been recommended for monitoring an adequate sample size, while for a for a transect width of 6 m, 34-56 h of effort have been recommended (Aguilar et al., 2019).

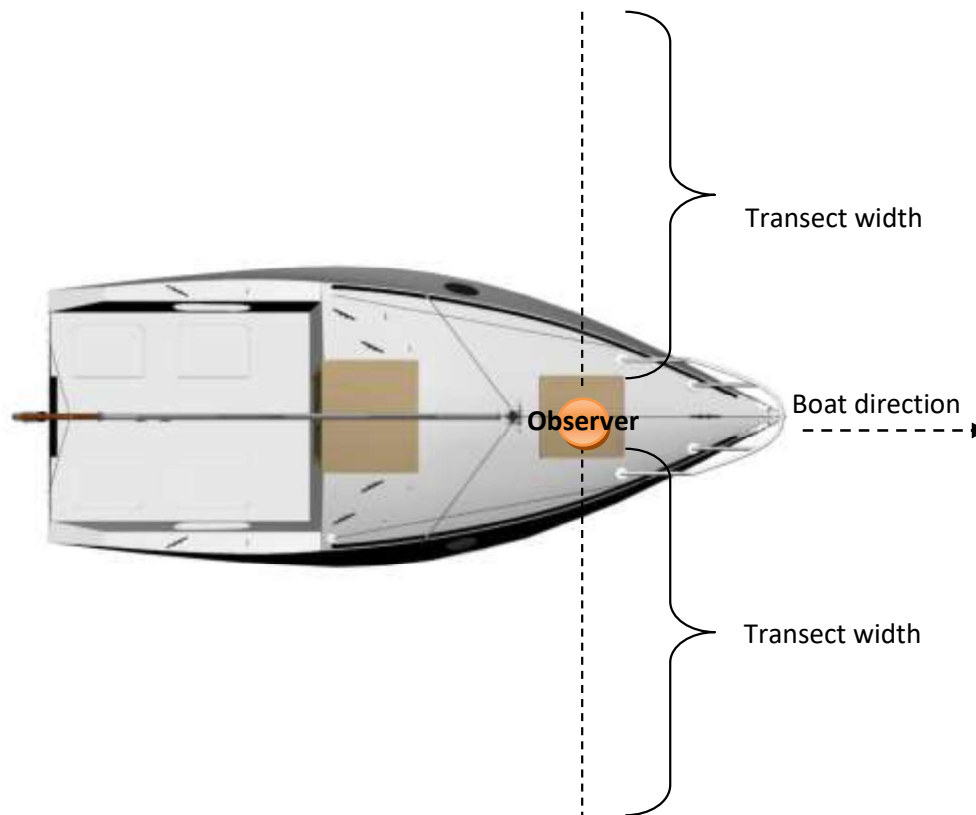


Figure 5-2. Schematic representation of observation position and transect width on a vessel.

5.4. Visual observation process

The observation should be made with naked eyes and binoculars can be used to confirm the litter sightings. A GPS is used to record the track of the monitored transect, to mark the beginning and the end of transect and indicate the position of the sighted objects. A telescopic fishing rod should be used in order to set the strip width.

The observation transect width should be set at 6 to 10 meters for small-sized vessels and medium-sized vessels respectively while the speed of the boat should not be higher than 4-6 knots. The observation, quantification and identification of floating litter items must be made by two dedicated observers who do not have other duties at the same time. The transect length should correspond approximately to 1 h of observation for each survey. The ideal location for observation is often the bow area of the boat. The observation direction must be perpendicular to the boat track (see figure below). The surveyors should conduct the survey from the glare-free side of the vessel and avoid the hours of the day when the sun is low on the horizon (sunrise and sunset), since visibility is not good enough due to glare and/or reflection. The surveys should be performed with sea state smaller or equal to 2 at the Beaufort scale.

5.5. Litter size classes to be surveyed

Litter items larger than 2.5 cm (in the longest dimension) should be monitored and reported. Given that visual observation will not permit the exact measuring of object sizes, the following size range classes should be reported for each recorded litter item:

- A. 2.5 cm-5 cm
- B. 5 cm-10 cm
- C. 10 cm-20 cm
- D. 20 cm-30 cm
- E. 30 cm-50 cm
- F. 50 cm - 100 cm
- G. >100 cm

5.6. Litter classification and quantification

All items observed on the survey area should be classified by type, according to the 'Joint List of Marine Litter Items Categories' prepared by the MSFD Technical Group on Marine Litter (MSFD TG ML) in close collaboration with EU Member States and the Regional Sea Conventions (Fleet et al., 2021). The manual for applying the Joint List classification system provides detailed information on how to classify litter items and a complementary photo guide helps the surveyors identify and categorise the litter items ([Online Photo Catalogue of the Joint List of Litter Categories](#)). Data should be entered on the sheet while being observed.

Unknown litter or items that are not on the survey sheet should be noted in the appropriate "other item" category. A short description of the item should then be included on the survey sheet. If possible, digital photos should be taken of unknown items so that they can be identified later and, if necessary, be added to the survey sheet.

Furthermore, the occurrence of groups of floating litter items should be recorded along with their location as these could provide useful information with regards to accumulation areas. Ideally, each item in the group should be identified and recorded.

The unit in which litter will be assessed on the sea surface will be 'number of items' and it will be expressed as counts of litter items per square kilometer (litter items/km²). In order to compute the exact surveyed area, GPS coordinates must be recorded regularly (every min) to obtain an accurate measurement of the travelled transect. A handheld GPS unit might be handy in this respect.

5.7. Materials and equipment

The following items are necessary to carry out floating litter surveys:

- ▶ Telescopic fishing rod;
- ▶ Digital camera;
- ▶ Binoculars;
- ▶ Hand-held GPS unit;
- ▶ Extra batteries (ideally rechargeable batteries);
- ▶ Clipboard for the surveyor;
- ▶ Recording sheets (printed on waterproof paper);
- ▶ Pencils;
- ▶ First aid kit (to include sunscreen, bug spray, drinking water).

References

Aguilar, A., et al., 2019. Common monitoring protocol for marine litter. Interreg Med MEDSEALITTER.

Fossi, M.C, Vlachogianni, T., Anastasopoulou, A., Alomar, C., Bains, M., Caliani, I., Campani, T., Casini, S., Consoli, P., Cillari T., D'Alessandro, M., Deudero, S., Galgani, Galli M., F., Kaberi H., Panti, C., Pedà, C., E. Romeo, T., Scotti, G., Tsangaris, C., Zeri, C., 2019. Toolkit for monitoring marine litter and its impacts on biodiversity in Mediterranean MPAs. Interreg Med Plastic Busters MPAs project (D.3.3.2).

IPA-Adriatic DeFishGear, 2014. Methodology for Monitoring Marine Litter on the sea surface (macro-debris >2.5 cm).

5.8. Recording sheets

Monitoring MACROLITTER on the Water Surface
Data Sheet

Location name	
Location ID	
Country	
Surveyor Name	
e-mail address	
Date of survey	

VESSEL CHARACTERISTICS		
Vessel name		<i>Name of the vessel</i>
Type of vessel		<i>Type e.g. research, fishing, hired, regular ferry etc.</i>
Vessel length and weight		<i>Length of the vessel (metres) Gross weight of the vessel (tonnes)</i>

VISUAL SURVEY TRANSECT DETAILS			
Latitude/longitude start			<i>Recorded as nnn.nnnnn degrees at the start of the sample unit</i>
Latitude/longitude end			<i>Recorded as nnn.nnnnn degrees at the end of the sample unit</i>
Coordinates system			<i>Datum and coordinate system employed</i>
Vessel speed			<i>Average ship speed in knots</i>
Observation height			<i>Observation elevation above the sea</i>
Distance covered			<i>Total distance covered by the transect (m)</i>
Time start/end			<i>Time over which the survey took place</i>
Surface covered			<i>Area covered by the vessel (km²)</i>

ENVIRONMENTAL PARAMETERS - OBSERVATION DETAILS			
Wind speed			<i>Recorded in (Beaufort)</i>
Wind direction	<input type="checkbox"/> N <input type="checkbox"/> E <input type="checkbox"/> S <input type="checkbox"/> W		<i>Tick more than one boxes e.g. for SE wind</i>
Sea surface salinity			<i>Expressed in ‰ when reporting</i>
Viewing quality			<i>Good/Moderate/Poor ; in the latter two case state cause (e.g. fog)</i>
Sea state			<i>Expressed in accordance with the Douglas Sea Scale (0-9)</i>
NOTES			
.....			

SITE CHARACTERISTICS		
Nearest river name		<i>Name of nearest river</i>
Nearest river distance		<i>Distance to the nearest natural input (river or stream) (kilometers)</i>
Nearest river position	<input type="checkbox"/> N <input type="checkbox"/> E <input type="checkbox"/> S <input type="checkbox"/> W	<i>Position of river mouth in relation to survey area</i>
Nearest major fishery		<i>Name of the nearest major fishery (named by type)</i>
Nearest major fishery distance		<i>Distance to the nearest major fishery (kilometers)</i>
Nearest major fishery position	<input type="checkbox"/> N <input type="checkbox"/> E <input type="checkbox"/> S <input type="checkbox"/> W	<i>Position of the nearest major fishery in relation to survey area</i>
Nearest town		<i>Name of nearest town</i>
Nearest town distance		<i>Distance to the nearest town (kilometers)</i>
Nearest town position	<input type="checkbox"/> N <input type="checkbox"/> E <input type="checkbox"/> S <input type="checkbox"/> W	<i>Position of the nearest town in relation to survey area</i>
Population size of this town		<i>No of inhabitants</i>
Additional features of the town	<input type="checkbox"/> Residential <input type="checkbox"/> Tourist <input type="checkbox"/> Residential & tourist	<input type="checkbox"/> Winter <input type="checkbox"/> Spring <input type="checkbox"/> Summer <input type="checkbox"/> Autumn <i>Indicate the main characteristic of the town, residential or touristic town; in case of the later indicate the high season peak</i>
Name of the nearest beach		<i>Name of the nearest beach</i>
Distance to nearest beach		<i>Distance to the closest coastline (kilometers)</i>
Position of the nearest coast	<input type="checkbox"/> N <input type="checkbox"/> E <input type="checkbox"/> S <input type="checkbox"/> W	<i>Position of the closest coastline in relation to survey area</i>
Nearest shipping lane distance		<i>Distance to the nearest shipping lane (kilometers)</i>
Estimated traffic density		<i>Recorded in number of ships/year</i>
Vessel type		<i>Indicate the type of vessels that mainly use it e.g. merchant ships, etc.</i>
Position of the shipping lane	<input type="checkbox"/> N <input type="checkbox"/> E <input type="checkbox"/> S <input type="checkbox"/> W	<i>Position of shipping lane in relation to survey area</i>
Name of the nearest harbor		<i>Name of nearest harbor</i>
Distance to nearest harbor		<i>Distance to the closest harbor (kilometers)</i>
Harbor position	<input type="checkbox"/> N <input type="checkbox"/> E <input type="checkbox"/> S <input type="checkbox"/> W	<i>Position of the nearest harbor in relation to survey area</i>
Type of harbor		<i>Based on the types of vessels visiting the harbor</i>
Size of harbor		<i>Record the number of ships that reach the harbor per year</i>
Nearest discharge of waste water distance		<i>Distance to the closest waste water discharge point(kilometers)</i>
Position of nearest discharge point	<input type="checkbox"/> N <input type="checkbox"/> E <input type="checkbox"/> S <input type="checkbox"/> W	<i>Position of nearest discharge points in relation to survey area</i>
Type of waste water discharge	<input type="checkbox"/> Industrial <input type="checkbox"/> Municipal <input type="checkbox"/> Other	<i>Indicate type of waste water discharged</i>

Joint List of Marine Macrolitter Items

* To be recorded also if smaller than 2.5 cm

J-CODE	SUP/FG	NAME	ITEMS 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	
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 ≥ ≤ 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	

J-CODE	SUP/FG	NAME	ITEMS COUNT
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	
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 \geq \leq 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	
J84		plastic CDs & DVDs	
J67		plastic sheets, industrial packaging, sheeting	
J64		plastic fenders	
J68		fibre glass items	
J63		plastic floats/buoys other source than fishing or not	

J-CODE	SUP/FG	NAME	ITEMS COUNT
		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	
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	
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 \geq \leq 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	
J195*		metal household batteries	
J177		metal foil wrappers, aluminium foil	
J199		other metal pieces > 50cm	
J198		other metal pieces 2.5cm \geq \leq 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 \geq 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	



6. Methodology for monitoring MICROLITTER on the sea-surface using manta net tows

This document describes the methodological approach for monitoring microlitter on the sea surface. It has been compiled based on the related methodologies developed within the IPA-Adriatic DeFishGear project and the 2022 MSFD TGML Updated Guidance on Monitoring of Marine Litter in European Seas, while taking into account the results from the Plastic Busters MPAs testing phase.

PREPARED BY

THE INTERREG MED PLASTIC BUSTERS MPAs PROJECT



6.1. Site selection

Given the high heterogeneity of litter distribution, the criteria for the survey sites selection could have crucial effect on results. The selection of the monitoring sites depends on the purpose and the methodology of monitoring, and can be made on the basis of certain characteristics of interest (i.e. MPAs of different scale such as large, medium or small) or through a random selection of survey sites.

For large scale MPAs, comprising of pelagic areas, sites of high and low microlitter accumulation should be surveyed.

For medium and small scale MPAs, confined to coastal waters around and in between small islands, an adequate number of sampling sites should be selected, based on the morphology and orientation of the island (shape, presence of inlets and gulfs, etc.) in order to cover all parts around the islands (N, S, E, W).

6.2. Frequency and timing of surveys

At least two survey campaigns, one in autumn and one in early spring should be carried out. If possible, avoid periods with intense zooplankton blooms. The proposed campaign periods are:

- ▶ Autumn: October
- ▶ Spring: April

6.3. Sampling unit and sample size

Manta nets are the most common tools for sampling floating mesolitter and microlitter in the surface layer of the water column. It is recommended that manta net should have mesh size of 333 μm and be equipped with a flow meter. The sampling should be performed at low wind conditions (0-2 Beauforts) which can be recorded by a portable anemometer or by ship's instruments. The manta net should be towed for 30 min at a vessel speed that is maintained at less than 3 knots and both the start and end position should be recorded with GPS as well as the track.



(a) Correct



(b) Wrong

Figure 6-1. Images illustrating the proper and improper positioning of the manta net.



Figure 6-2. All tows should be conducted from the ship's side and beyond the ships' wake (Photo © Th. Vlachogianni).



Figure 6-3. After completion of each tow, the net should be washed thoroughly from the outside with filtered seawater (Photo © Th. Vlachogianni).

After completion of each tow, the net should be washed thoroughly from the outside with filtered seawater (<300 μ m) using the ship's hose in order to collect all particles in the cod-end. The sample collected in the cod-end should then be rinsed with seawater on a <300 μ m metallic sieve and transferred in glass jars with seawater. Any natural debris items, such as leaves, twigs, seaweed etc., should be rinsed separately above the sieve and removed from the sample. The samples should be stored in 70% ethanol solution for further analysis and a limited number of samples should be kept frozen to perform chemical analysis.



(a)



(b)

Figure 6-4. Sample on the sieve (a), cleared of any natural debris and transferred in glass jars (b).



(a)



(b)

Figure 6-5. Sample rich in seaweeds before (a) and after (b) separation.

6.4. Sample processing and size classification

The litter collected is classified in three size classes:

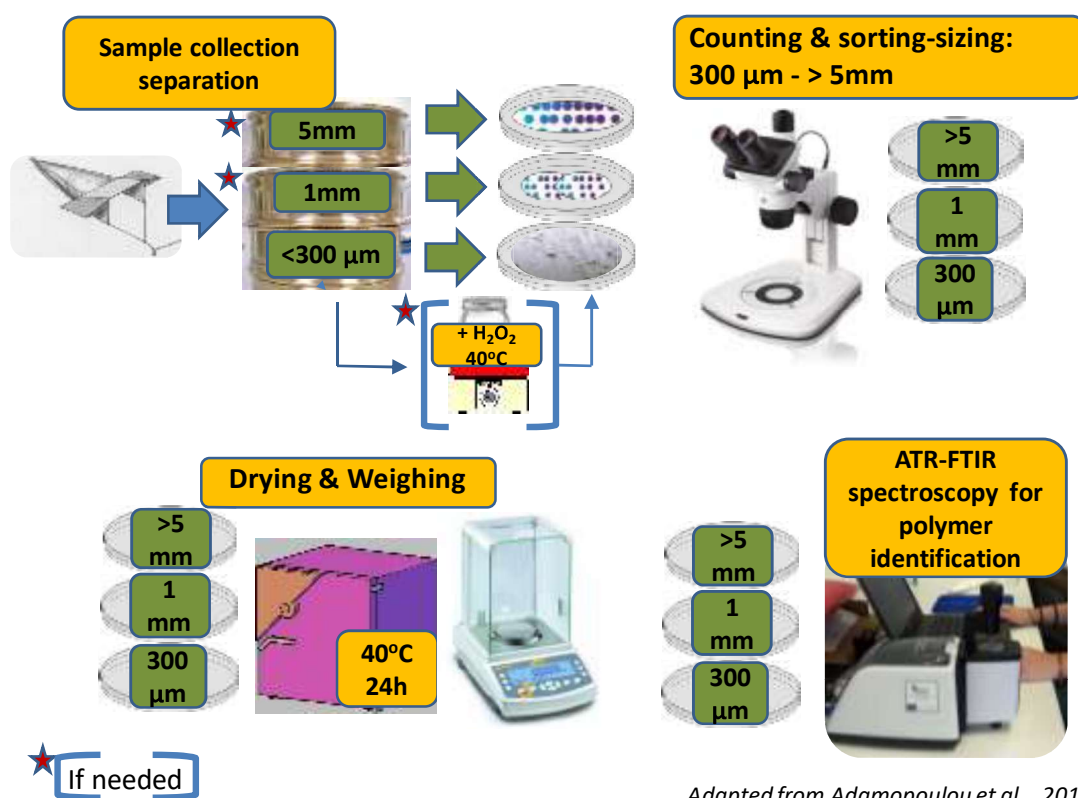
- ▶ Mesolitter (5 mm-25 mm)
- ▶ Large microlitter, LML (1mm-5mm)
- ▶ Small microlitter, SML (300µm – 1mm)

In case of samples poor in natural particles and organic material, transfer the sample into a petri dish and observe under a stereomicroscope. Measure the particles' longest dimension using an image analysis software, count and classify into the 3 sizes classes. For the determination of weight, transfer the characterized MPs into three pre-weighted petri dishes according to size classes, dry at 40°C and weigh.

For samples rich in natural particles and organic material, the successive sieving as described below is helpful for the separation of the plastic particles but does not substitute the size characterization process with an image analysis software.

- ▶ Wet sieving and separation into 3 size classes: Pour the sample through a stacked arrangement of 5mm, 1mm, and 0.3 mm stainless steel mesh sieves. Accordingly, the litter items are classified in three size classes: small microlitter SML (300µm – 1mm), large microlitter LML (1mm-5mm), mesolitter (5 mm-25 mm).

- ▶ Mesolitter (5 mm-25 mm): Visually inspect the sample on the sieve, transfer and count only plastics in pre-weighed Petri dish. Dry at 40°C and weigh to determine the mass of mesoplastics.
- ▶ LML (1mm – 5mm): Visually inspect the sample on the sieve, transfer and count the LML (1 mm-5 mm) particles in pre-weighed Petri dishes. Dry at 40°C and weigh to determine the mass of LMLs.
- ▶ SML (0.3mm -1mm): Collect the sample from the sieve with deionised water and filter through pre-weighed GF/C filters (pore size 1.2 µm). Dry the filters at 40°C for 24 hours and weigh. Determine the mass of small microlitter particles (SML mass). Examine the filter under a stereomicroscope and count SML particles.
- ▶ In case of high natural organic matter content in the samples (LML or SML) a step of peroxide digestion precedes filtration: Add 15% hydrogen peroxide (H₂O₂) with 1:1 (sample:H₂O₂) volume ratio and boil on a hot plate (approx.40°C) until the digestion is complete (no natural organic material should be visible). Collect the digested material with deionised water and continue with filtration, drying and mass determination.



Adapted from Adamopoulou et al., 2015

Figure 6-6. Schematic representation of the various steps of processing floating mesolitter and microlitter samples.

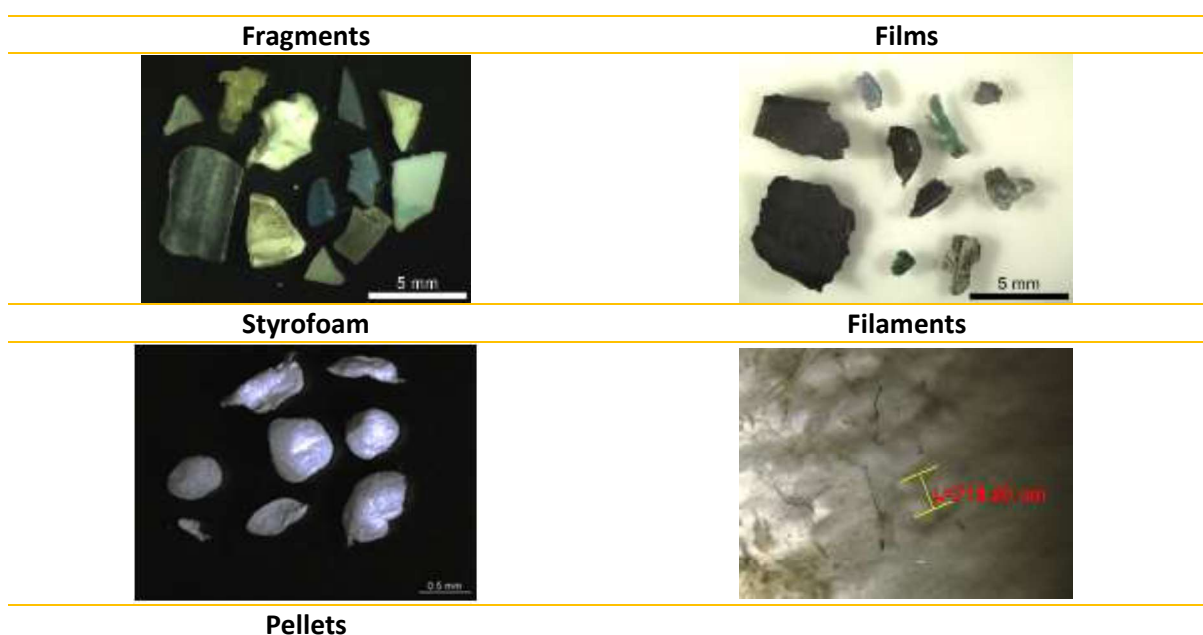
In all cases, for an accurate determination of the size of plastic particles, the particles collected in the petri dishes should be measured in their longest dimension under a stereoscope using an image analysis software, and then they should be classified in the three size classes (Meso, LMP, SMP). This is important because filaments and elongated particles may pass the sieves.



Figure 6-7. A microlitter sample (Photo © Th. Vlachogianni).

6.5. Sample analysis

Microplastics sorted, counted and characterized by **type** on the basis of the following categories: pellet, fragment (granule, flake), fibre, film, filaments, microbeads, foam (expanded polystyrene-PS), in line with the MSFD TGML guidelines. The most common **colours** of microplastics identified are the following: black, blue, white, transparent, red, green, multicolour, other. For the identification of the **polymer type** it is recommended to use an ATR-FTIR spectrometer or Raman spectroscopy or Pyrolysis-Gas chromatography-mass spectroscopy (Py-GCMS). FT-IR spectroscopy is mostly used in microplastic studies and in particular ATR-FTIR is considered fast, low cost and adequate for analyzing particles >300 µm, in size like the ones collected with manta nets.



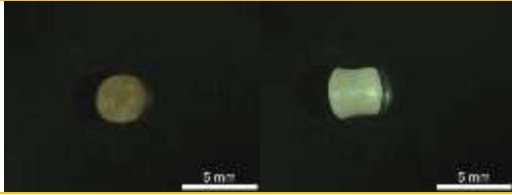


Figure 6-8. Examples of microlitter particles as seen under the stereomicroscope.

All surfaces should be clean. The glassware needs to be rinsed thoroughly with purified water. Samples should be covered with foil paper during the analysis. A glove bag should be used as working area for sample rinsing and filtrations. Petri dishes should be covered with glass lids during observation under stereomicroscope. Procedural blank samples should be used in all steps and items similar to those found in blank samples excluded, as they should be considered airborne contamination. Samples are to be kept in Petri dishes for long-term storage.

6.6. Expression of the results

Microlitter counts (N) are reported as follows:

- ▶ N per km² or N per m², based on the start - end transect coordinates and the dimensions of the manta net mouth.
- ▶ N per Km³ or N per m³, based on flow meter indication and relevant formula.

Microlitter mass is reported as follows:

- ▶ g per km² or g per m²
- ▶ g per Km³ or g per m³

6.7. Materials and equipment

Sampling equipment

- ▶ Manta net with wings and cod end (mesh size: 330 µm)
- ▶ Oceanographic flowmeter
- ▶ Submersible water pump with a hose (for rinsing the net) or other equipment for net rinsing
- ▶ GPS
- ▶ Glass jars with caps or plastic bottles (500 ml) (one or more per each sample; when on the sea is a lot of sea grass, than you need 2 - 3 plastic bottles per sample)
- ▶ Sample container – cool box
- ▶ Screw driver
- ▶ Sieve (max 0.3 mm mesh size; preferable with smaller mesh size)
- ▶ Large bowl or washbasin (to prevent spillage of sample when emptying cod-end; 5 l <)
- ▶ Tap/fresh water source (tap/hose/squirt bottle)
- ▶ Squirt bottles 2 x (one for water; one for alcohol)
- ▶ Tweezers (longer)
- ▶ Metal spoon
- ▶ Funnel (∅ 20 cm)
- ▶ Latex gloves without powder
- ▶ 70 % ethanol
- ▶ Waterproof marker, vellum paper and pencil

Sample separation equipment

- ▶ Stereomicroscope (min. 80x zoom; recommended also: transmission light with dark field, polarisation contrast and ring light)
- ▶ Object glasses (marked – number of a sample, date of analysis)
- ▶ Micro tweezer and tweezer
- ▶ Glass petri dishes
- ▶ Lab coat
- ▶ 70 % ethanol
- ▶ 3 Sieves with mesh sizes: 5mm; 1mm; 0.3 mm or smaller
- ▶ Squirt bottle 2x (one for distilled water; one for alcohol)
- ▶ Latex gloves without powder
- ▶ Filtered water or distilled water
- ▶ Analytical laboratory scale
- ▶ Multiwell plate provided by NIC for storing the microlitter particles

Monitoring MICROLITTER on the Water Surface Data Sheet

Location name	
Location ID	
Country	
Surveyor Name	
e-mail address	
Date of survey	

VESSEL CHARACTERISTICS		
Vessel name		<i>Name of the vessel</i>
Type of vessel		<i>Type e.g. research, fishing, hired, regular ferry etc.</i>
Vessel length and weight		<i>Length of the vessel (metres) Gross weight of the vessel (tonnes)</i>

MANTA NET TRANSECT DETAILS			
Latitude/longitude start			<i>Recorded as nnn.nnnnn degrees at the start of the sample unit</i>
Latitude/longitude end			<i>Recorded as nnn.nnnnn degrees at the end of the sample unit</i>
Coordinates system			<i>Datum and coordinate system employed</i>
Vessel speed			<i>Average ship speed in knots</i>
Distance covered			<i>Total distance covered by the transect (m)</i>
Time start/end			<i>Time over which the survey took place</i>

ENVIRONMENTAL PARAMETERS - OBSERVATION DETAILS		
Wind speed		<i>Recorded in (Beaufort)</i>
Wind direction	<input type="checkbox"/> N <input type="checkbox"/> E <input type="checkbox"/> S <input type="checkbox"/> W	<i>Tick more than one boxes e.g. for SE wind</i>
Sea surface salinity		<i>Expressed in ‰ when reporting</i>
Viewing quality		<i>Good/Moderate/Poor ; in the latter two case state cause (e.g. fog)</i>
Sea state		<i>Expressed in accordance with the Douglas Sea Scale (0-9)</i>
NOTES		
.....		

SITE CHARACTERISTICS		
Nearest river name		Name of nearest river
Nearest river distance		Distance to the nearest natural input (river or stream) (kilometers)
Nearest river position	<input type="checkbox"/> N <input type="checkbox"/> E <input type="checkbox"/> S <input type="checkbox"/> W	Position of river mouth in relation to survey area
Nearest major fishery		Name of the nearest major fishery (named by type)
Nearest major fishery distance		Distance to the nearest major fishery (kilometers)
Nearest major fishery position	<input type="checkbox"/> N <input type="checkbox"/> E <input type="checkbox"/> S <input type="checkbox"/> W	Position of the nearest major fishery in relation to survey area
Nearest town		Name of nearest town
Nearest town distance		Distance to the nearest town (kilometers)
Nearest town position	<input type="checkbox"/> N <input type="checkbox"/> E <input type="checkbox"/> S <input type="checkbox"/> W	Position of the nearest town in relation to survey area
Population size of this town		No of inhabitants
Additional features of the town	<input type="checkbox"/> Residential <input type="checkbox"/> Tourist <input type="checkbox"/> Residential & tourist	<input type="checkbox"/> Winter <input type="checkbox"/> Spring <input type="checkbox"/> Summer <input type="checkbox"/> Autumn Indicate the main characteristic of the town, residential or touristic town; in case of the later indicate the high season peak
Name of the nearest beach		Name of the nearest beach
Distance to nearest beach		Distance to the closest coastline (kilometers)
Position of the nearest coast	<input type="checkbox"/> N <input type="checkbox"/> E <input type="checkbox"/> S <input type="checkbox"/> W	Position of the closest coastline in relation to survey area
Nearest shipping lane distance		Distance to the nearest shipping lane (kilometers)
Estimated traffic density		Recorded in number of ships/year
Vessel type		Indicate the type of vessels that mainly use it e.g. merchant ships, etc.
Position of the shipping lane	<input type="checkbox"/> N <input type="checkbox"/> E <input type="checkbox"/> S <input type="checkbox"/> W	Position of shipping lane in relation to survey area
Name of the nearest harbor		Name of nearest harbor
Distance to nearest harbor		Distance to the closest harbor (kilometers)
Harbor position	<input type="checkbox"/> N <input type="checkbox"/> E <input type="checkbox"/> S <input type="checkbox"/> W	Position of the nearest harbor in relation to survey area
Type of harbor		Based on the types of vessels visiting the harbor
Size of harbor		Record the number of ships that reach the harbor per year
Nearest discharge of waste water distance		Distance to the closest waste water discharge point(kilometers)
Position of nearest discharge point	<input type="checkbox"/> N <input type="checkbox"/> E <input type="checkbox"/> S <input type="checkbox"/> W	Position of nearest discharge points in relation to survey area
Type of waste water discharge	<input type="checkbox"/> Industrial <input type="checkbox"/> Municipal <input type="checkbox"/> Other	Indicate type of waste water discharged