

free litter at



ADVANCING TOWARDS LITTER - FREE
ATLANTIC COASTAL COMMUNITIES
BY PREVENTING AND REDUCING
MACRO AND MICRO LITTER

D2.2.1. HARMONISED SAMPLING GUIDELINES TO MONITOR MICROPLASTICS IN DIFFERENT MATRICES

WORK PACKAGE 2: ASSESSING AND MONITORING MICRO AND MACRO
LITTER CONTAMINATION. ACTIVITY 2: IMPROVED MICROPLASTICS
MONITORING, TASK 2: HARMONISED SAMPLING GUIDELINES TO
MONITOR MICROPLASTICS IN DIFFERENT MATRICES (WATER, SEDIMENTS
AND BIOTA)



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|--------------------------|--|
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1. BACKGROUND

Primary and secondary microplastics are increasingly becoming the focus of concern due to their pervasive nature in the environment (Kamel et al., 2024). Despite the numerous studies already published on microplastic monitoring, there is still an urgent need to establish harmonised guidelines for the collection, quantification and reporting of plastic particles, so that comparisons among different locations/studies can be made and effective actions can take place (Bessa et al., 2019; Bhardwaj et al., 2024; Borja & Elliott, 2021).

Microplastic monitoring involves several steps that must be fully optimised and validated to obtain reliable reports on the presence and characterisation of microplastics, thereby contributing to baseline assessments, for instance, within the framework of the Marine Strategy Framework Directive (MSFD) (Galgani et al., 2023). However, monitoring micro-litter remains a major challenge, as its small size makes it harder to detect, being more pervasive and associated with numerous potential impacts that are still poorly understood (Ramos et al., 2024). Over recent years, growing efforts have focused on improving the detection and monitoring of micro-litter pollution, with attempts to standardise sampling, sample processing and analytical methods (Bessa et al., 2019; OSPAR, 2024). However, further work is still required to strengthen monitoring capabilities through harmonised protocols.

In this context, Work Package 2 (WP2) Activity 2 of the Free LitterAT project aims to propose harmonised guidelines for sampling microplastics in the aquatic environment across multiple matrices— including water, sediment and biota. Activity 2 also aims to propose optimised protocols for sample processing and preparation for microplastic analysis and to test, enhance, and refine both current and innovative methods used for microplastic quantification and characterisation (WP2 A2 D2.2.3; WP2 A2 D2.2.4). These developments will contribute to obtaining validated, comparable and reproducible data on microplastic contamination in the aquatic environment, supporting the establishment of robust baselines in line with the MSFD.

This Deliverable addresses the complexities and methodological challenges of micro-litter sampling. It suggests harmonised guidelines for sampling procedures across different environmental matrices, outlining appropriate strategies (including



equipment, volumes and number of replicates) specific to different aquatic environments (marine, estuarine and freshwater) and sample types (water, sediment and biota). It also indicates relevant data to record during sampling and highlights organisms that may serve as bioindicators of microplastic contamination. Additionally, this Deliverable includes a dedicated section focused on quality assurance and control to minimise contaminations and to ensure the reliability and comparability of results across studies. The content of this Deliverable is built upon the European Union's Guidelines on Marine Litter Monitoring in European Seas [with inputs by the MSFD Technical Group on Marine Litter (Galgani et al., 2023)] which address marine waters, seafloor and coastal sediments, and biota. These are complemented by other official guidelines, such as those from GESAMP (GESAMP, 2019), focusing on marine waters, seabed sediments and biota, and from the OSPAR Microplastic Expert Group (OSPAR, 2024) with emphasis on seafloor sediments, marine surface waters and biota. In addition it is supported by a comprehensive literature review (Bessa et al., 2019; Gago et al., 2019; Gunaalan et al., 2024; Jamšek et al., 2024; Lusher et al., 2017; Michida et al., 2023; Nayebi et al., 2023; Pasquier et al., 2022; Poli et al., 2024; Prata et al., 2024; Ugwu et al., 2024) and inputs from the practical expertise gained by the project team (Almeida et al., 2023; Espincho et al., 2024; Moura et al., 2025; Rivoira et al., 2020; Rodrigues et al., 2023; Rodrigues et al., 2019; Silva et al., 2022) and its partners (D2.2.2. State of the Art - Microplastic Sampling Methods in Natural Water Bodies) to include also matrices not mentioned in the referred documents. By encompassing diverse environmental matrices, recognising current methodological limitations and proposing harmonised guidelines, this Deliverable introduces a novel and comprehensive framework to enhance microplastic monitoring capacity and comparability.



2. WATER SAMPLING

Water sampling procedures vary according to water type (marine, freshwater or brackish) and environmental setting (open, coastal and enclosed waters and rivers). Consequently, sampling procedures must be adapted across the different environments and the selection of the sampling strategy and equipment should be done according to the specific sampling objectives.

The guidelines presented below describe three main procedures (net-based, pump-based and bulk sampling) that can be applied across different aquatic environments. The net-based sampling is size selective, retaining only microplastics larger than the mesh size of the net (Poli et al., 2024). While net-based sampling is effective for capturing larger microplastic, pump-based systems are better suited for capturing smaller microplastic and fibres (Ugwu et al., 2024). Bulk sampling, in contrast, is volume selective, as it does not allow for a collection of a large volume (Poli et al., 2024), but could be the most appropriate option in certain environments or contexts.

The information presented in the following sections as well as the suggested procedures, was gathered from the team experience (Espincho et al., 2024; Rodrigues et al., 2023; Rodrigues et al., 2019), guidelines from MSFD (Galgani et al., 2023), GESAMP (GESAMP, 2019) and OSPAR (Ospar, 2024), literature survey (Bhardwaj et al., 2024; Gago et al., 2019; Gunaalan et al., 2024; Michida et al., 2023; Nayebi et al., 2023; Pasquier et al., 2022; Poli et al., 2024; Ugwu et al., 2024), and also adapted from the Deliverable of WP2, Activity 2, Task 1: “Literature Review – State of the Art: Microplastic sampling methods in natural water bodies”.

To facilitate practical use, Section 2 - Water sampling- is divided according to the different sampled environments: Marine open waters; Marine coastal and estuarine waters; and River and lake waters. Although the sampling methods may be similar across environments, some sections are intentionally repeated so that each protocol can be read and applied independently, allowing users to access the relevant procedure without needing to consult other sections.



2.1. Marine open waters

When sampling in open waters, the large spatial scales and potential low microplastics concentrations require methods that can cover large areas or that filter substantial volumes of water to obtain representative samples (Ugwu et al., 2024). The choice between net-based or pump-based sampling depends on the research objectives (Poli et al., 2024), whether for quantifying large microplastics in surface waters, in a specific water depth or investigating the vertical microplastics distribution throughout the water column (Gunaalan et al., 2024).

2.1.1. Sampling using nets

Net sampling in marine open waters targets, in general, the collection of floating or suspended particles, including microplastics and plankton, from both the surface water or from a specific water depth. The most commonly used nets include manta, neuston and bongo nets, with equipment selection depending on the study objectives and field constraints. Manta and neuston nets are designed for surface sampling of floating microplastics, whereas the bongo net allows sampling of the water column through oblique tows, allowing the collection of suspended particles at different depths. Mesh size selection depends on the study objectives, however mesh sizes of 300–333 µm are most commonly used, as they represent a compromise between capture efficiency and the risk of clogging.

Net-based sampling might have some limitations, including the underestimation of small particles, particularly fibres that may pass through the mesh, the risk of secondary microplastic contamination from air exposure, net materials, or tow ropes and uncertainty in sampled water volumes due to flowmeter inaccuracies, clogging or variable towing conditions. These potential limitations should be considered when interpreting net-sampling results. Further information on the advantages and disadvantages of microplastic sampling methods in natural water bodies is available in *Deliverable 2.2.2 – Literature review: State of the art – Microplastic sampling methods in natural water bodies*.



EQUIPMENT AND MATERIAL

1. Sampling nets
 - a. Manta trawl – surface sampling
 - b. Neuston net – surface sampling
 - c. Bongo net – surface and water column
2. Flowmeter
3. Metal sieve
4. Funnels
5. Tweezers
6. Glass or metal sample containers
7. Cool box
8. Labels and markers
9. GPS device

NOTE:

- a. Neuston nets can capture the ocean surface layer in wavy conditions (Michida et al., 2023)
- b. Manta nets can be best used in still waters (Michida et al., 2023)

SAMPLING PROCEDURE

1. Net deployment
 - Deploy the net from the side of the ship/vessel, away from the wake zone
 - Use appropriate deployment systems (e.g., spinnaker boom, A-frame, crane)
 - The depth depends on the net used:
 - Manta net – up to 25 cm
 - Neuston net - up to 50 cm
 - Bongo net – up to 25 cm (surface) or up to 200m (water column)
2. Sampling parameters



- **Sample volume:** Minimum of 100 m³ (use a flowmeter to measure collected volume), although volume should be adjusted to ensure samples representativeness to evaluate microparticles contamination
- **Towing speed:** Maintain a constant speed between 0.5 and 3 knots (recommended ca. 2 knots whenever possible, adjusting as needed according to turbidity and wave conditions)
- **Towing duration:** 10–30 minutes, depending on local hydrodynamics and vessel speed
- **Towing area:** In linear or circular trajectories, depending on site conditions (register the coordinates using a GPS)
- **Replicates per site:** Ideally 3 (distance between replicates depends on the sampling area)

NOTE: Replicates should be collected as independent tows conducted under comparable environmental conditions. To avoid resampling water disturbed by the vessel, replicate tows should not be performed along the exact same track immediately after one another. Whenever possible, replicates should be conducted along parallel transects, positioned up-current or upwind of previously sampled water. Tow conditions should be kept as consistent as possible among replicates. Start and end GPS coordinates, towing time, vessel speed, environmental conditions and filtered water volume should be recorded for each replicate.

3. Post-sampling

- Rinse the net from the outside with seawater
- Carefully remove the cod-end
- Transfer the collected sample to a glass or metal container using a metal sieve with significantly smaller mesh size than the net used for sampling (using funnels, tweezers and filtered seawater)

SAMPLE STORAGE

- Store in fully closed glass or metal containers
- Keep samples at 4 °C and without light for short-term storage (≤1 month)
- Optionally (if it does not compromise microplastic integrity)
 - Add a conservation additive



- Freeze at -20 °C

2.1.2. *Sampling with pump*

Pump-based sampling is used to collect water samples at various depths, from a few centimetres below the surface to different levels of the water column. This method allows precise control over both sampled depth and sampled volume. However, pump-based sampling also presents some potential limitations, including lower particle concentrations compared with net-based sampling, a high risk of filter clogging when large volumes of water or particle-rich samples are processed through limited filter areas, and the potential fragmentation of microplastics into smaller particles due to shear stress generated by pump blades. Further information on the advantages and disadvantages of microplastic sampling methods in natural water bodies is available in *Deliverable 2.2.2 – Literature review: State of the art – Microplastic sampling methods in natural water bodies*.

EQUIPMENT AND MATERIAL

1. Peristaltic or diaphragm pump
2. Tubing
3. Flowmeter
4. Filters (different porosities) and filter holder
5. Tweezers
6. Glass or metal sample containers
7. Labels and markers
8. GPS device

SAMPLING PROCEDURE

1. Preparation
 - Install the filter on the filter holder, connect with a tube to the pump and connect another tube to collect water
 - Place the pump after the filter holder so that water passes through the filter before it gets to the pump



2. Pump deployment

- Place the tube of the filter holder just below the surface or at the desired depth, avoiding the wake zone
- Avoid sampling near sediment plumes or turbulent areas

3. Sampling parameters

- **Sample volume:** Use a flowmeter
 - 100 m³ for a lower cut-off filter size of 300 µm
 - 10 m³ for lower cut-off filter size of 100 µm
 - 1 m³ for lower cut-off filter size of 20 µm
(mentioned volumes are suggestions; volume should be adjusted to ensure samples representativeness to evaluate microparticles contamination)
- **Stationary sampling:** Register the coordinates with the GPS
- **Replicates per site:** Ideally 3 (distance between replicates depends on the sampling area)

NOTE: Check for air bubbles and flow interruptions

4. Post-sampling

- Collect the filter with the tweezers to the sample container
- Rinse the pump filter holder with deionised water before placing a new filter

SAMPLE STORAGE

- Store the filters in fully closed glass or metal containers at room temperature (ca. 20 °C) in a dry area without light
- If possible, store the filters in an excicator



2.2. Marine coastal and estuarine waters

When sampling in marine coastal and estuarine waters, tidal dynamics strongly influence water circulation and particle distribution. Therefore, the timing of sampling should be carefully selected in relation to the tidal cycle, in accordance with the specific objectives of the study.

2.2.1. Sampling using nets

Net sampling in marine coastal and estuarine waters targets, in general, the collection of floating or suspended particles, including microplastics and plankton, from both the surface water or from a specific water depth. The most commonly used nets include manta, neuston and bongo nets, with equipment selection depending on the study objectives and field constraints. Manta and neuston nets are designed for surface sampling of floating microplastics, whereas the bongo net allows sampling of the water column through oblique tows, allowing the collection of suspended particles at different depths. Mesh size selection depends on the study objectives, however mesh sizes of 300–333 μm are most commonly used, as they represent a compromise between capture efficiency and the risk of clogging.

Net-based sampling might have some limitations, including the underestimation of small particles, particularly fibres that may pass through the mesh, the risk of secondary microplastic contamination from air exposure, net materials, or tow ropes and uncertainty in sampled water volumes due to flowmeter inaccuracies, clogging or variable towing conditions. These potential limitations should be considered when interpreting net-sampling results. Further information on the advantages and disadvantages of microplastic sampling methods in natural water bodies is available in *Deliverable 2.2.2 – Literature review: State of the art – Microplastic sampling methods in natural water bodies*.



EQUIPMENT AND MATERIAL

1. Sampling nets
 - a. Manta trawl – surface sampling
 - b. Neuston net – surface sampling
 - c. Bongo net – surface and water column
2. Flowmeter
3. Metal sieve
4. Funnels
5. Tweezers
6. Glass or metal containers
7. Cool box
8. Labels and markers
9. GPS device

NOTE:

- a. Neuston nets can capture the water surface layer in wavy conditions (Michida et al., 2023)
- b. Manta nets can be best used in still waters (Michida et al., 2023)

SAMPLING PROCEDURE

1. Net deployment
 - Deploy the net from the side of the ship/vessel, away from the wake zone
 - Use appropriate deployment systems (e.g., spinnaker boom, A-frame, crane)
 - The depth depends on the net used
 - Manta net - up to 25 cm
 - Neuston net - up to 50 cm
 - Bongo net - up to 25 cm (surface) or up to 200m (water column)
2. Sampling parameters



- **Sample volume:** Minimum of 100 m³ (use a flowmeter to measure collected volume), although volume should be adjusted to ensure samples representativeness to evaluate microparticles contamination
- **Towing speed:** Maintain a constant speed between 0.5 and 3 knots (recommended ca.2 knots)
- **Towing duration:** 10–30 minutes, depending on local hydrodynamics and vessel speed
- **Towing area:** In linear or circular trajectories, depending on site conditions (register the coordinates using a GPS)
- **Replicates per site:** Ideally 3 (distance between replicates depends on the sampling area)

NOTE: Replicates should be collected as independent tows conducted under comparable environmental conditions. To avoid resampling water disturbed by the vessel, replicate tows should not be performed along the exact same track immediately after one another. Whenever possible, replicates should be conducted along parallel transects, positioned up-current or upwind of previously sampled water. Tow conditions should be kept as consistent as possible among replicates. Start and end GPS coordinates, towing time, vessel speed, environmental conditions and filtered water volume should be recorded for each replicate.

3. Post-sampling

- Rinse the net from the outside with seawater/ estuarine water
- Carefully remove the cod-end
- Transfer the collected sample to a sample container (glass or metal) using a metal sieve with significantly smaller mesh size than the net used for sampling (using funnels, tweezers and filtered seawater)

SAMPLE STORAGE

- Store in fully closed glass or metal containers
- Keep samples at 4 °C without light for short-term storage (≤1 month)
- Optionally (if it does not compromise microplastic integrity)
 - Add a conservation additive
 - Freeze at -20 °C



2.2.2. Bulk sampling

Bulk sampling consists of collecting surface or low-depth water column samples, using manual submersion methods such as grab sampling. This technique is not size selective, as all microplastics present in the collected water volume may be sampled, but is volume selective, with the container capacity determining the amount of water collected (Poli et al., 2024). For that reason there can be a high variability between replicates due to the small volumes typically collected, a lower particle concentrations compared with net-based sampling, and practical difficulties associated with transporting large volumes of bulk water samples to the laboratory. Further information on the advantages and disadvantages of microplastic sampling methods in natural water bodies is available in *Deliverable 2.2.2 – Literature review: State of the art – Microplastic sampling methods in natural water bodies*.

EQUIPMENT AND MATERIAL

1. Glass or metal containers
2. Labels and markers
3. GPS device

SAMPLING PROCEDURE

1. Sample collection
 - Submerge sample container manually a few centimetres below the water surface or at a defined depth ahead of the vessel's bow wave
2. Sampling parameters
 - **Sample volume:** Depends on purpose (e.g., 1 L per replicate)
 - **Stationary sampling:** Register the coordinates using a GPS
 - **Replicates per site:** 3 (equally separated in the site being surveyed, distance between depends on sampling area)
3. Post-sampling
 - If using individual bottles for each replicate, store them directly



- If reusing the same bottle, transfer each sample to a clean glass or metal container
 - Rinse the sampling bottle with deionised water between replicates to avoid cross-contamination

SAMPLE STORAGE

- Store in fully closed glass or metal containers
- Keep samples at 4 °C without light for short-term storage (≤1 month)
- Optionally (if it does not compromise microplastic integrity)
 - Add a conservation additive
 - Freeze at -20 °C

2.2.3. Sampling with pump

Pump-based sampling is used to collect water samples at various depths, from a few centimetres below the surface to different levels of the water column. This method allows precise control over both sampled depth and volume. However, pump-based sampling also presents some potential limitations, including the lower particle concentrations compared with net-based sampling, high risk of filter clogging when large volumes of water or particle-rich samples are processed through limited filter areas and the potential fragmentation of microplastics into smaller particles, due to shear stress generated by pump blades. Further information on the advantages and disadvantages of microplastic sampling methods in natural water bodies is available in *Deliverable 2.2.2 – Literature review: State of the art – Microplastic sampling methods in natural water bodies*.



EQUIPMENT AND MATERIAL

1. Peristaltic or diaphragm pump
2. Tubing
3. Flowmeter
4. Filters (different porosities) and filter holder
5. Glass or metal sample containers
6. Labels and markers
7. GPS device

SAMPLING PROCEDURE

1. Preparation

- Install the filter on the filter holder, connect with a tube to the pump and connect another tube to collect water
- Place the pump after the filter holder so that water passes through the filter before it gets to the pump

2. Pump deployment

- Place the tube of the filter holder just below the surface or at the desired depth, avoiding the wake zone
- Avoid sampling near sediment plumes or turbulence areas

3. Sampling parameters

- **Sample volume:** Use a volume flowmeter
 - 100 to 500 L for a lower cut-off filter size of 300 μm
 - 50 to 200 L for lower cut-off filter size of 100 μm
 - 10 a 100 L for lower cut-off filter size of 20 μm
(mentioned volumes are suggestions; volume should be adjusted to ensure samples representativeness to evaluate microparticles contamination)
- **Stationary sampling:** Register the coordinates (area cannot be estimated)



- **Replicates per site:** Ideally 3 (distance between replicates depends on the sampling area)

NOTE: Check for air bubbles and flow interruptions

4. Post-sampling

- Collect the filter with the tweezers and transfer to sample container
- Rinse the pump filter holder with deionised water before placing a new filter

SAMPLE STORAGE

- Store the filters in fully closed glass or metal containers at room temperature (ca. 20 °C) in a dry area without light
- If possible, store the filters in an exicator

2.3. River and lake waters

2.3.1. Sampling using nets

Net sampling in rivers targets floating or suspended material such as microplastics and zooplankton from the surface water or from a specific water depth. The most commonly used nets include manta, neuston and bongo nets, with equipment selection depending on the study objectives and field constraints. Manta and neuston nets are designed for surface sampling of floating microplastics, whereas the bongo net allows sampling of the water column through oblique tows, allowing the collection of suspended particles at different depths. Mesh size selection depends on the study objectives, however mesh sizes of 150, 300 or 500 µm are most commonly used, as most studies target plankton.

Net-based sampling might have some limitations, including the underestimation of small particles, particularly fibres that may pass through the mesh, the risk of secondary microplastic contamination from air exposure, net materials, or tow ropes and uncertainty in sampled water volumes due to flowmeter inaccuracies, clogging or



variable towing conditions. These potential limitations should be considered when interpreting net-sampling results. Further information on the advantages and disadvantages of microplastic sampling methods in natural water bodies is available in *Deliverable 2.2.2 – Literature review: State of the art – Microplastic sampling methods in natural water bodies*.

EQUIPMENT AND MATERIAL

1. Sampling nets
 - a. Manta trawl – surface sampling
 - b. Neuston net – surface sampling
 - c. Bongo net – surface and water column
2. Flowmeter
3. Metal sieve
4. Funnels
5. Tweezers
6. Glass or metal sample containers
7. Cool box
8. Labels and markers
9. GPS device

SAMPLING PROCEDURE

1. Net deployment
 - Deploy the net from the side of the ship/vessel, away from the wake zone
 - Use appropriate deployment systems (e.g., spinnaker boom, A-frame, crane)
 - The depth depends on the net used
 - Manta net – up to 25 cm
 - Neuston net - up to 50 cm
 - Bongo net - up to 25 cm (surface) or up to 200m (water column)



2. Sampling parameters

- **Sample volume:** Minimum of 100 m³ (use a flowmeter to measure collected volume), although volume should be adjusted to ensure samples representativeness to evaluate microparticles contamination
- **Towing speed:** Maintain a constant speed between 0.5 and 3 knots (recommended ca. 2 knots)
- **Towing duration:** 10–30 minutes, depending on local hydrodynamics
- **Towing area:** In linear or circular trajectories, depending on site conditions (register the area surveyed using the GPS)
- **Replicates per site:** Ideally 3 (distance between replicates depends on the sampling area)

NOTE: Replicates should be collected as independent tows conducted under comparable environmental conditions. To avoid resampling water disturbed by the vessel, replicate tows should not be performed along the exact same track immediately after one another. Whenever possible, replicates should be conducted along parallel transects, positioned up-current or upwind of previously sampled water. Tow conditions should be kept as consistent as possible among replicates. Start and end GPS coordinates, towing time, vessel speed, environmental conditions and filtered water volume should be recorded for each replicate.

3. Post-sampling

- Rinse the net from the outside with river/estuarine water
- Carefully remove the cod-end
- Transfer the collected sample to a sample (glass or metal) container using a metal sieve with significantly smaller mesh size than the net used for sampling (using funnels, tweezers and filtered seawater)

SAMPLE STORAGE

- Store in fully closed glass or metal containers
- Keep samples at 4 °C without light for short-term (≤1 month)
- Optionally (if it does not compromise microplastic integrity)
 - Add a conservation additive
 - Freeze at -20 °C



2.3.2. Bulk sampling

Bulk sampling consists of collecting surface or low-depth water column samples, using manual submersion methods such as grab sampling. This technique is not size selective, as all microplastics present in the collected water volume may be sampled, but is volume selective, with the container capacity determining the amount of water collected (Poli et al., 2024). For that reason there can be a high variability between replicates due to the small volumes typically collected, a lower particle concentrations compared with net-based sampling, and practical difficulties associated with transporting large volumes of bulk water samples to the laboratory. Further information on the advantages and disadvantages of microplastic sampling methods in natural water bodies is available in *Deliverable 2.2.2 – Literature review: State of the art – Microplastic sampling methods in natural water bodies*.

EQUIPMENT AND MATERIAL

1. Glass or metal containers
2. Labels and markers
3. GPS device

SAMPLING PROCEDURE

1. Sample collection
 - Submerge sample container manually a few centimetres below the water surface or at a defined depth ahead of the vessel's bow wave
2. Sampling parameters
 - **Sample volume:** Depends on purpose (e.g., 1 L per replicate)
 - **Stationary sampling:** Register the coordinates (area cannot be estimated)
 - **Replicates per site:** 3 (equally separated in the site being surveyed, distance between depends on the sampling area)
3. Post-sampling



- If using individual bottles for each replicate, store them directly
- If reusing the same bottle, transfer each sample to a clean glass or metal container
 - Rinse the sampling bottle with deionised water between replicates to avoid cross-contamination

SAMPLE STORAGE

- Store in fully closed glass or metal containers
- Keep samples at 4 °C without light for short-term (≤ 1 month)
- Optionally (if it does not compromise microplastic integrity)
 - Add a conservation additive
 - Freeze at -20 °C

2.4. Sampling data

During the sampling campaign, it is suggested to register the following data:

1. Sample ID
2. Date
3. Sampling point
4. Geographical location (sampling coordinates; start and end points where applicable)
5. Sampling depth
6. Sampling device /filter mesh size
7. Water volume collected (L) or flowmeter reading
8. Number of replicates
9. Distance between replicates (m)
10. Water temperature (°C)
11. pH
12. Salinity (PSU)
13. Dissolved oxygen (mg L⁻¹)
14. Weather condition
15. Tidal stage (where applicable)



3. SEDIMENT SAMPLING

Sediment sampling procedures vary according to the sediment zone (e.g., seabed, intertidal, supratidal, or riverine) and the environmental setting (e.g., marine, estuarine, or freshwater systems). Sampling approaches must be adapted to local conditions, including sediment type, accessibility and tidal influence. The selection of sampling strategies and equipment should therefore be guided by the specific monitoring objectives and the targeted sediment compartment.

The guidelines suggested distinguish between subtidal environments (e.g., seabed, subtidal and riverbed sediments) and exposed environments (e.g., intertidal, supratidal and riverbank sediments), as these zones differ in terms of sampling constraints.

The information and suggested procedures presented in the following sections were compiled from the team's experience (Almeida et al., 2023; Rivoira et al., 2020), guidelines from MSFD (Galgani et al., 2023), GESAMP (GESAMP, 2019) and OSPAR (OSPAR, 2024), as well as from a literature survey (Bhardwaj et al., 2024; Jamšek et al., 2024; Nayebi et al., 2023; Prata et al., 2024).

3.1. Subtidal sediments

Sampling bed sediment from marine, riverine or estuarine environments targets the upper sediment layer to evaluate microplastic contamination levels, while minimizing disturbance from bioturbation or resuspension processes.

EQUIPMENT AND MATERIAL

1. Metal shovel or spoon
2. Corer-based tools (e.g., Box corer, Gemax corer, Kajak corer)
3. Sampling quadrat
4. Glass or metal sample containers
5. Labels and markers
6. GPS device



SAMPLING PROCEDURE

1. Sampling strategy

- **Frequency:** One-off or seasonally distributed point sampling
- **Site selection:** Prioritize areas with known and stable sedimentation rates

2. Sampling parameters

- **Area sampled:** Record total monitored area (km²) and sampled site area within it (m²)
- **Sampling depth:** Upper 5 cm (can be adjusted based on sedimentation rate and absence of bioturbation)
- **Sample volume:** Depends on sampling device - minimum for replicate analysis and determination of additional sediment-related parameters
 - Regarding weight it can be up to 1-2 Kg - should be adjusted to ensure samples representativeness to evaluate microparticles contamination
- **Replicates per site:** 3-5 (equally spaced)

SAMPLE STORAGE

- Store in glass or metal containers
- Keep samples at low temperature (e.g., 4 °C or frozen at -20 °C)
- Store in the dark and in a dry environment



3.2. Exposed sediments

Sampling sediment in beaches, riverbanks or estuarine intertidal areas environments targets the upper sediment layer to evaluate microplastic contamination levels.

EQUIPMENT AND MATERIAL

7. Metal shovel or spoon
8. Corer-based tools (e.g., Box corer, Gemax corer, Kajak corer)
9. Sampling quadrat
10. Glass or metal sample containers
11. Labels and markers
12. GPS device

SAMPLING PROCEDURE

1. Sampling layout

- **Beach:** Along the strand line and optionally the supratidal zone, in transects parallel to the coastline
- **Riverbanks:** Along the strand line in transects parallel to the riparian zone
- **Estuarine intertidal area:** Define and register total area to be monitored (km²) and specific sampled site area (m²). Recommended: 100 m² area per site

NOTE: In beach and river sites, sampling points should be separated by at least 5 m

2. Sampling parameters

- **Sampling depth:** Upper 5–10 cm sediment layer (for river margin and mudflat a deeper sampling is possible — record exact depths)
- **Sample volume:** Depends on sampling device - minimum for replicate analysis and determination of additional sediment-related parameters (regarding weight it can be up to 1 Kg) -- -- should be adjusted to ensure samples representativeness to evaluate microparticles contamination



- **Replicates per site:** Ideally 5 (minimum 3) per transect or site sampled, equally separated

SAMPLE STORAGE

- Store in glass or metal sample containers
- Keep samples at low temperature (e.g., 4 °C or frozen at -20 °C)
- Store in the dark and in a dry environment

3.3. Sampling data

During the sampling campaign, it is suggested to register the following data:

- | | |
|-------------------------------------|--|
| 1. Sample ID | 5. Geographical location (sampling coordinates) |
| 2. Date | 6. Sampling device |
| 3. Sampling point | 7. Number of replicates |
| 4. Depth of the sampled sediment | 8. Distance between replicates (m) |

Complementarily, the following data can be recorded:

- Weather and local conditions
- Sampling site orientation, usage, access, slope, urbanisation degree and curvature
- Proximity to harbours, rivers, estuary or sea and population centres
- Cleaning information (seasonality, frequency)



4. BIOTA SAMPLING

This section outlines harmonised procedures for sampling different taxonomic groups—ranging from plankton to fish—across marine and freshwater environments. Methodologies consider both direct collection from the environment and, when applicable, acquired from markets, ensuring traceability. Sampling can be one-off or seasonally distributed, depending on the study purpose. The information presented in the following sections as well as the suggested procedures, was gathered from the team experience (Espincho et al., 2024; Moura et al., 2025; Rivoira et al., 2020; Rodrigues et al., 2023; Silva et al., 2022), guidelines from MSFD (Galvani et al., 2023), GESAMP (2019) and OSPAR (2024), and literature survey (Bessa et al., 2019; DiGiacomo & Koepsell, 1986; Galvani et al., 2013; Hermsen et al., 2018; Lusher et al., 2017; Martínez Abraín, 2014; Underwood et al., 2017).

NOTE: Samples acquired from markets are not allowed for environmental studies under MSFD-related monitoring, where organisms must be collected directly from the environment.

4.1. Taxonomic group

EQUIPMENT AND MATERIAL

1. For zooplankton
 - a. Plankton nets (150, 300 or 500 μm)
 - b. Flowmeter
2. For bivalves:
 - a. Hand scrappers
3. For crustaceans, echinoderms, cephalopods
 - a. Traps
4. For teleost
 - a. Trawl net
5. Glass or metal sample containers
6. Labels and markers



SAMPLING PROCEDURE

Table 1 summarises the recommended sampling approaches by taxonomic group for biota commonly used in microplastic monitoring studies. The table provides representative examples for each group and indicates appropriate sampling methods (Lusher et al., 2017). These approaches aim to ensure sample representativeness while considering ethical constraints and logistical feasibility.

TABLE 1: EXAMPLES OF SAMPLING PROCEDURES BY TAXONOMIC GROUP

| Group | Examples | Sampling method |
|-------------|-------------------------------------|---|
| Crustaceans | Crab | Grab by hand, traps, or acquired from market ^a |
| Echinoderms | Sea urchin, sea cucumber | Grab by hand or acquired from market ^a |
| Bivalves | Mussel, oyster | Grab by hand or acquired from market ^a |
| Cephalopods | Octopus | Use traps or acquired from market ^a |
| Teleost | Sardine, seabass | Trawl or acquired from fish market ^a |
| Zooplankton | Fish larvae, copepods, chaetognaths | Plankton net ^b |

^a. Must know location and date of capture

^b. Register sampled area (km²)

Number of individuals per species is influenced by the research question, budget, logistics and available resources, with larger numbers generally improving accuracy and statistical robustness (Underwood et al., 2017). For biota monitoring, existing guidelines



for fish and bivalves recommend the analysis of at least 50 individual organisms per species and sampling site (Galgani et al., 2013) however it might not be always feasible or ethically appropriate (*e.g.*, in the case of protected species).

In this context, a sample size of 30 individuals is often adopted in ecological field studies as a pragmatic compromise, as it is generally considered sufficient to support statistical inference and hypothesis testing. (Martínez Abrain, 2014). For fish, a sample size of 30 individuals provides a high probability (95 %) of detecting microplastics contamination when the expected frequency of occurrence is low (5 %) (DiGiacomo & Koepsell, 1986). In practice, the project team has been analysing a sample size of 30 individuals to characterise microplastic contamination in oysters, in line with these considerations (Moura et al., 2025).

Nevertheless, the suitability of a sample size of 30 depends strongly on the variability and contamination level of the study population, as well as on the aim of the study. High variance among individuals or low-contamination areas, may require larger number of individuals to detect meaningful differences with adequate statistical power. To optimise the sampling design, a pre-survey or pilot study is therefore recommended to assess variability and determine the most appropriate number of individuals for the research objectives (Martínez Abrain, 2014).

In addition to the number of individuals, organism size and life stage should also be considered, as these biological traits may influence microplastic ingestion, retention and accumulation. Therefore, individuals should preferably be selected within a comparable size range and at the same life stage or, when feasible, maturity status.

SAMPLE STORAGE

- Store in glass or metal containers
- Keep at 4 °C or frozen at –20 °C (recommended) until further analysis
- Conservation additives may be used, if they do not compromise microplastics integrity
- Store in the dark and in a dry environment

NOTE:

- a. The time between collection and storage should be as short as possible



- b. When organisms are collected for detection of microplastics in the gastrointestinal tract, precautions must be taken to avoid the organ's eversion

4.2. Sampling data

During the sampling campaign, it is suggested to register the following data:

1. Sample ID
2. Date
3. Sampling point
4. Geographical location (sampling coordinates; start and end points where applicable)
5. Sampling device/method
6. Number of replicates
7. If acquired in the market: location and date of capture

Complementarily, the following data can be recorded:

- Weather and local conditions
- Sampling site usage, access, slope, urbanisation degree and curvature
- Proximity to harbours, rivers, estuary or sea and population centres
- Cleaning information (seasonality, frequency)



5. SAMPLING QUALITY ASSURANCE AND QUALITY CONTROL

5.1. Sampling quality assurance

To reduce contamination and ensure the reliability of results being obtained.

5.1.1. *Material and equipment*

1. Use appropriate material

- Glass or metal containers and tools
- Avoid plastic materials

NOTE: If plastic devices are used register polymer type

2. Pre-clean sampling items

- Wash all containers and instruments before use with
 - Filtered seawater for marine sampling
 - Deionised water for river and estuarine sampling
 - Optionally: Clean with ethanol or isopropanol where suitable
- Keep sample containers and instruments covered after cleaning

3. Rinse sampling devices before each use

- For marine sampling
 - Rinse nets externally with seawater
 - Rinse the cod end separately
- For river/estuarine sampling
 - Rinse equipment thoroughly with deionised water

5.1.2. *Operator practices*

- Minimize the presence of operators in the sampling area
- Wear bright, non-synthetic clothing
- Face the wind when sampling



5.2. Sampling quality control

To ensure sample integrity and detect potential contamination, field blanks should be systematically collected and processed.

5.2.1. Field blanks

1. Collect representative blanks

- Recommended proportion is 10 % of the total number of samples, with a minimum of 3
- Field blanks should reflect variable contamination risks (*e.g.*, weather, operator clothing, device used)

2. Prepare a field blank

- Use an empty glass or metal sampling container
- Position the container next to the sample and open it during sample retrieval, closing it tightly afterwards
- Collect the filtered/deionised water used to rinse the sampling equipment prior to sampling in a glass or metal container and store it as a sample



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